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Oral complications of Type 1 diabetes mellitus in a non-smoking population

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Abstract

Type 1 diabetes mellitus (T1DM) is a complex metabolic condition that results in hyperglycemia due to insulin deficiency (Daneman, 2006). Diabetes has a range of effects on almost every system in the body including the kidneys, the eyes, the cardiovascular system, the genito-urinary system, the gastro-intestinal system and the nervous system (Daneman, 2006). The effects of this condition are widespread and have a significant impact both on life expectancy and the quality of life of individuals suffering from diabetes (Scottish Diabetes Survey Monitoring Group, 2011). The impact of diabetes on oral health has been investigated over many decades, however, the conclusions have been varied and study design has not always been adequate (Mealey et al., 2006; Khader et al., 2006; Chávarray et al., 2009). Research presented in this thesis is largely the result of a cross-sectional clinical study examining the oral cavities of non-smoking T1DM patients, funded by the Chief Scientist Office of the Scottish Government. The clinical part of the study took place between January 2006 and May 2009 in Glasgow Dental Hospital.

Chapter one provides an introduction and narrative review on the subject of T1DM, periodontal disease, and the various other reported oral manifestations of diabetes mellitus. The methods for measuring general and oral health related quality of life outcomes are also discussed. Chapter one reveals some of the inadequacies of studies investigating the link between T1DM and oral disease to date and contextualises the studies presented in this thesis.

Chapter two presents the main periodontal findings of a large cross-sectional study. 112 non-diabetic subjects and 203 subjects with type 1 diabetes were examined. 203 diabetic patients were divided into well controlled and poorly controlled groups based on their average blood sugar levels over the previous two years. 169 were poorly controlled. (PCD). Those with T1DM, (especially those with poor glycaemic control) had a greater extent and severity of periodontitis than those without diabetes. There was also some evidence that never smoking T1DM patients were more likely to have periodontal disease than non-diabetic subjects. The odds ratio (OR) was 1.43 [0.74 to 2.75] ($p = 0.29$) for all T1DM patients and 1.58 [0.75 to 3.33] ($p = 0.23$) for PCD.

This difference remained even after the multivariable analysis took into account age, gender and lifestyle including: body mass index of the subject; whether they had smoked in the past; whether they attended a dentist; their level of education and how deprived the area they lived in was.

Chapter three presents an analysis of the impact of age, HbA1c, and duration on the expression of periodontal disease in T1DM subjects. Cross-tabulations and multivariable logistic regression analysis was performed on the periodontal data from T1DM subjects and non-diabetic subjects in order to determine the relationship between age, HbA1c and duration, and periodontitis. Diabetic subjects developed periodontitis at a younger age than non-diabetes subjects. This will represent a significant impact on life time dental service provision for subjects affected at a young age. The relationship between HbA1c and severe periodontitis is not a simple one. It is possible that unknown factors confound the relationship between glycaemic control and periodontitis. There was no relationship between duration of diabetes and periodontitis when age was controlled for.

Chapter four presents the results of a small study investigating biomarkers of bone turnover in patients with and without T1DM and in patients with and without periodontitis. Patients with T1DM had higher levels of osteoprotegerin an osteoprotective molecule that normally leads to a reduced propensity for bone loss. T1DM patients were also shown to have reduced levels of biomarkers of bone formation (osteocalcin). It is possible that a reduced capacity for bone repair and regeneration may account for the increase levels of periodontitis seen in T1DM. Further prospective studies would be required to confirm this hypothesis.

Chapter five investigated the level of caries and oral mucosal abnormalities in T1DM. There was little difference in caries indicators or in oral mucosal lesions between the groups. There was no difference in the bacterial microflora and in the level of resistance to antibiotics found in this cohort. T1DM patients, however, did have an increase in the symptoms of dry mouth, an increased density of candida colonisation and reduced salivary flow rates.

Chapter six reports the data derived from the oral health questionnaire, including the Oral Health Impact Profile -14 (OHIP-14) and the Audit of Diabetes Dependent Quality of Life (ADDQOL©). Patients with T1DM, despite having increased levels of periodontal disease, reduced salivary flow rates and increased symptoms of xerostomia did not have higher OHIP scores by any measure. The reasons for this apparently negligible impact of oral disease or oral health related quality of life are discussed. The OHIP-14 was shown to have construct validity in this population although the correlations were relatively weak and the differences were small. It is possible that patients with T1DM do not consider the impact of their oral health to be a significant problem in light of their other on-going medical issues. This finding requires further in-depth investigation of the psychology behind this apparent reduced impact.

This is the first study of its kind to examine the oral and dental health of non-smoking type 1 diabetic patients. The conclusions from the clinical data support the view that patients with T1DM should be targeted with oral and dental health advice. Encouragingly the prevalence of periodontitis was lower in well controlled diabetic subjects suggesting that the effect of T1DM on the oral cavity can be ameliorated by good glycaemic control even though logistic regression analysis did not show a linear relationship. It is important that health professionals work together in order to prevent and manage the oral complications of T1DM in the same way that there are preventive and screening programmes for other diabetic complications. The pathogenesis behind the increased prevalence and severity of periodontal disease in T1DM requires further study.

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Declaration

This thesis is the original work of the author

Douglas Paul Robertson BDS(Hons), MFDS RCSEd, FHEA

List of Abbreviations

16s DNA – 16 subunit deoxyribonucleic acid

16s RNA – 16 subunit ribonucleic acid

AACE - American Academy of Clinical Endocrinologists

ABB - *A. actinomycetemcomitans*-binding B cell

ACCORD – Action to Control Cardiovascular Risk in Diabetes

ADA - American Diabetes Association

ADDQOL - Audit of Diabetes Dependent Quality of life

ADHS - Adult Dental Health Survey

AGE - Advanced glycation end-products

ANOVA - Analysis of variance

AWI - Average weighted impact

BCD – Better Controlled Diabetic

BMI - Body Mass Index

BMS - Burning mouth syndrome

BOP - Bleeding on probing

BPE - Basic periodontal examination

BSA - Bovine Serum Albumin

BSP - British Society of Periodontology

CAL - Clinical attachment loss

CD4+ T Lymphocytes- Cluster of Differentiation 4 positive T Lymphocytes

CD8+ T Lymphocytes - Cluster of Differentiation 8 positive T Lymphocytes

CD14 - Cluster of Differentiation 14

95% CI – 95 percent Confidence Interval

CLSI - Clinical Laboratory Standards Institute

CML - N-(carboxymethyl) lysine

CPD - Clinical Probing Depth

CpG DNA - Cytosine-Guanine Deoxyribonucleic acid

CPITN - Community Periodontal Index of Treatment Need

DCCT - Diabetes control and complications trial

DEPCAT - Deprivation category

DFS - Decayed and filled surfaces

DMFS - Decayed missing and filled surfaces

DMFT - Decayed missing and filled teeth

DQ6 -

DQ8 -

DQOL - Diabetes Quality of Life

DSQOLS - Diabetes Specific Quality of Life Scale

DVD - Digital Versatile Disc

EASD - European Association for the Study of Diabetes

EDTA - Ethylene Diamine Triacetic Acid

EIA – Enzyme Immunoassay

ELAM - Endothelial leukocyte adhesion molecule

ELISA – Enzyme Linked Immunosorbent Assay

ESBL - Extended-Spectrum Beta-lactamases

EURDIAB - European Diabetes Study Group

FDI - Federation Dentaire Internationale (World Dental Federation)

FFA - Free fatty acids

FOTI - Fibre-optic transillumination

GAD65 - Glutamic acid decarboxylase 65

GCF - Gingival crevicular fluid

GDP - General Dental Practitioner

GI - Gingival Index

GOHAI - Geriatric Oral Health Assessment Index

HbA1c - Haemoglobin A1c or Glycated Haemoglobin

HIV AIDS - Human Immunodeficiency Virus-Acquired Immunodeficiency syndrome

HLA - Human Leukocyte Antigen

hOPG – Human Osteoprotegerin

HPLC - High performance liquid chromatography

HRP - Horseradish peroxidase

HRQOL - Health related quality of life

IAA - Insulin Autoantibody

iC3b - inactivated C3b

ICA - Islet cell autoantibody

ICAM - Inter-Cellular Adhesion Molecule

ICIDH - International Classification of Impairments, Disabilities and Handicaps

ICTP – C-terminal telopeptide of type 1 collagen

IDDM - Insulin Dependent Diabetes Mellitus

IDF - International Diabetes Federation

IL-1 α - Interleukin 1 alpha

IL-1 β - Interleukin 1 beta

IL- 6 - Interleukin 6

IL- 6-(174) - Interleukin 6-(174)

IL- 11 - Interleukin 11

IL- 13 - Interleukin 13

IL- 17 - Interleukin 17

INF γ - Interferon gamma

IQR – Interquartile range

ITCP - C-terminal cross-linking telopeptide of type I collagen

KDa - KiloDalton

K2EDTA - Potassium (K2) Ethylene Diamine Triacetic Acid

LIF - Leukemia Inhibitory Factor

LPS - Lipopolysaccharide

MAP - Mitogen activated protein

MGI - Modified gingival index

MIC - Minimum inhibitory concentration

μ L – Microlitres

mm - millimetre

MMP - Matrix metalloproteinase

mRNA - Messenger ribonucleic acid

MRSA - Multi-Drug resistant *Staphylococcus aureus*

NALP1 - NACHT-LRR-PYD-containing protein-1

NDIP - National Dental Inspection Programme

NDS – Non-Diabetic Subjects

NF-KB - Nuclear factor-KappaB

NHS - National Health Service

NICE - National Institute for Health and Clinical Excellence

NIDDM - Non Insulin Dependent Diabetes Mellitus

NIH - National Institute for Health

Nm – nanometers

NOD - Nucleotide-binding domain, Leucine-Rich repeat containing protein

NOD mouse – Non-Obese Diabetic mouse model

NUG - Necrotising Ulcerative Gingivitis

NUP - Necrotising Ulcerative Periodontitis

OCIF - Osteoclastogenesis inhibitory factor

OHIP - Oral Health Impact Profile

OHIP-14 - Oral Health Impact Profile-14

OHIP-49 – Oral Health Impact Profile – 49

OHI-S - Simplified Oral Hygiene Index

OHRQoL - Oral health related quality of life.

OIDP - Oral Impact on Daily Performance

OPG – Osteoprotegerin

OR - Odds Ratio

OSM - Oncostatin-M

PAMP - Pathogen associated molecular patterns

PASW - Predictive analytics software

PBS – Phosphate buffered solution

PCD - Poorly Controlled Diabetic

PCR - Polymerase Chain Reaction

PGE₂ - Prostaglandin E2

P. gingivalis – Porphyromonas gingivalis

PI - Plaque Index

QLF - Quantitative Light Induced Fluorescence

QOL - Quality of life

RAGE - Receptor for advanced glycation end-products

RANK – Receptor Activator of Nuclear Factor Kappa B

RANKL – Receptor Activator of Nuclear Factor Kappa B Ligand

RNA - Ribonucleic acid

RPM – Revolutions per minute

SAPS - Secreted aspartyl proteinases

S-DD – Sensitive dependent on dosing

SDS - Scottish Diabetes Survey

sIg-A – Secretory Immunoglobulin A

SIGN – Scottish Intercollegiate Guidelines Network

SIMD - Scottish Index of Multiple Deprivation

SLE - Systemic Lupus Erythematosus

SP - Severe Periodontitis

SPSS - Statistical package for the Social Sciences

sRANKL – soluble Receptor Activator of Nuclear Factor Kappa B

SRM - Standardised response mean

Streptavidin-HRP - Streptavidin conjugated to horseradish-peroxidase

STROBE - Strengthening the Reporting of Observational Studies in Epidemiology

T1DM - Type 1 Diabetes Mellitus

T. Forsythia – Treponema Forsythia

TGF- β 1 – Transforming growth factor beta1

TIMP - Tissue inhibitor of metalloproteinases

TLR2 - Toll-like receptor 2

TLR4 - Toll-like receptor 4

TLR5 - Toll-like receptor 5

TLR9 - Toll-like receptor 9

TMB – tetramethylbenzidine

TNF- α - Tumour Necrosis Factor α

TRAF - TNF receptor associated factor

TTC – Triphenyltetrazoliumchloride

UKPDS - United Kingdom Prospective Diabetes Study

VNTR - Variable number of tandem repeats

WCD - Well Controlled Diabetic

WHO - World Health Organisation

1 Introduction

1.1 Diabetes Mellitus

1.1.1 Introduction

Diabetes mellitus is a heterogeneous group of metabolic conditions caused by either a lack of insulin, resistance to its effects, or both (Daneman, 2006). Sufferers universally experience hyperglycaemia as a result of the body's inability to maintain normal blood glucose levels through homeostatic mechanisms. Diabetes has been recognised for millennia and was, until the development of insulin therapy, a fatal disease (Banting et al. 1922). Now all types of diabetes mellitus are treatable with insulin or anti-diabetic drugs although long term complications remain high.

Diabetes mellitus is the fifth most common cause of death in the world and it is estimated that one in eight deaths (12.2%) among 20 to 79-year-olds were attributable to the condition in 2010 (International Diabetes Federation, 2009). Life expectancy is reduced, on average, by more than 20 years in people with Type 1 diabetes and by up to 10 years in people with Type 2 diabetes (Diabetes U.K., 2011).

The financial costs associated with diabetes are huge. It is currently estimated that 10 percent of the NHS budget in the United Kingdom is spent on diabetes and its complications. This represents £9 billion a year based on the 2007/2008 budget for the NHS of approximately £90.7 billion. In addition, one in ten people admitted to hospital have diabetes. In 2006, 28.4 million items to treat diabetes were prescribed at a cost of £561.4 million (Diabetes U.K., 2011). The impact of this chronic condition on individuals and society cannot be overstated.

1.1.2 Classification

Diabetes mellitus is classified by four distinct categories based on aetiopathogenesis although two main categories of diabetes make up the bulk of cases. Type 1 diabetes mellitus (T1DM) (previously known as insulin dependent diabetes mellitus (IDDM)) and Type 2 diabetes mellitus (previously known as non-insulin dependent diabetes mellitus (NIDDM)) account for 99.4% of all cases in Scotland. They are the predominant types in

all areas of the world (SDSMG, 2009; IDF, 2009). Other categories include gestational diabetes and other specific types of diabetes. The latter are made up of those associated with gene defects of pancreatic β cell function and insulin resistance; other syndromes associated with diabetes; diseases of the exocrine pancreas; and endocrinopathies and diabetes induced by drugs, chemicals or infective agents (Table 1-1) (Expert Committee on the Diagnosis and Classification of Diabetes mellitus, 2003; American Diabetes Association, 2011).

The above classification includes changes to reflect the aetiopathogenesis rather than the therapeutic implications of the groups. It also reflects the fact that there are a range of presentations, as well as therapeutic treatments, all of which can change with time, meaning that patients should not be classified according to these overlapping criteria. The terms insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus and their acronyms, IDDM and NIDDM, were therefore removed from the classification as a result of the confusion that their use generated. The terms type 1 and type 2 diabetes mellitus were retained, with Arabic numerals being used (Expert Committee on the Diagnosis and Classification of Diabetes mellitus, 2003) and this classification will be adopted within this thesis.

Type 2 diabetes mellitus includes the most prevalent form of diabetes, which results from insulin resistance, with or without a secretory defect. It primarily occurs with increasing age and is associated with genetic and environmental risk factors. Type 2 diabetes is commonly preceded by a long period of abnormal glycaemic control and is part of the metabolic syndrome associated with hypertension, dyslipidaemia and hyperglycaemia. The condition has a stronger genetic aetiology than T1DM although environmental factors such as diet, exercise, obesity and smoking will impact on the development of type 2 diabetes (Stumvoll et al., 2005).

Table 1-1 Classification of Diabetes Mellitus (American Diabetes Association, 2011)

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)
A. Immune mediated
B. Idiopathic
II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)
III. Other specific types
A. Genetic defects of β-cell function
1. Chromosome 12, HNF-1 α (MODY*3)
2. Chromosome 7, glucokinase (MODY2)
3. Chromosome 20, HNF-4 α (MODY1)
4. Mitochondrial DNA
5. Others
B. Genetic defects in insulin action
1. Type A insulin resistance
2. Leprechaunism
3. Rabson-Mendenhall syndrome
4. Lipotrophic diabetes
5. Others
C. Diseases of the exocrine pancreas
1. Pancreatitis
2. Trauma/pancreatectomy
3. Others
D. Endocrinopathies
1. Acromegaly
2. Cushing's syndrome
3. Glucagonoma
4. Pheochromocytoma
5. Hyperthyroidism
6. Somatostatinoma
7. Aldosteronoma
8. Others
E. Drug- or chemical-induced
F. Infections
G. Uncommon forms of immune-mediated diabetes
H. Other genetic syndromes sometimes associated with diabetes
IV. Gestational diabetes mellitus (GDM)

* Maturity onset diabetes of the young

1.1.3 Epidemiology of Type 1 Diabetes Mellitus

Diabetes mellitus affects 284.6 million people in the world today and this figure is predicted to continue to rise in the coming decades. The World Health Organisation (WHO) and the International Diabetes Federation (IDF) predict that by 2030 between 366

and 438 million individuals will be suffering from diabetes. The burden of this major cause of morbidity and mortality falls on the developing world (Wild, 2004; International Diabetes Federation, 2009). In 2009 there were 2.6 million diabetic patients in the United Kingdom. It is estimated that this figure will rise to over 4 million by 2025 (Diabetes UK, 2010). World-wide, type 2 diabetes mellitus accounts for 85-95% of all diabetes and in Scotland type 2 diabetes mellitus accounts for 87.4% of cases (SDSMG, 2009). The second largest group is T1DM which accounts for 5-15% of diabetic patients world-wide and 12% in Scotland. The other forms of diabetes mellitus listed in Table 1-1 account for less than 2% of all diabetic subjects world wide and 0.6 % in Scotland (IDF 2009, SDSMG, 2009).

The incidence of T1DM varies significantly between countries and racial groups, with countries such as Venezuela and China having an incidence of less than 0.1 children diagnosed per 100,000 per year. However, Northern European countries have much higher incidence. In Finland 40.1 children of every 100,000 are diagnosed per year (Diamond Project group, 2006).

The prevalence of T1DM has been increasing world-wide over the last several decades and a review of the Scottish Diabetes Surveys, over the six years since implementation, confirms that numbers are continuing to rise in Scotland (Figure 1-1). The absolute number of patients with T1DM continues to increase (22,597 in 2003; 27,367 in 2009). This is reflected in the rising incidence of T1DM in children over the last 30 years. It is known, from a series of studies that the incidence of T1DM has been increasing by 2-3% a year since 1968 (Patterson et al., 1983). T1DM is one of the most common long term conditions affecting children and adolescents in Scotland, which has one of the highest prevalence rates of T1DM in Europe. The average annual incidence for Scotland from 1984 to 1993 was 23.9/100,000 children. (Patterson et al., 1983; Rangasami et al., 1997). In the EURDIAB study of the incidence of T1DM in Europe most countries had incidence rates of 6-9/100 000 children. (Green et al., 1992)

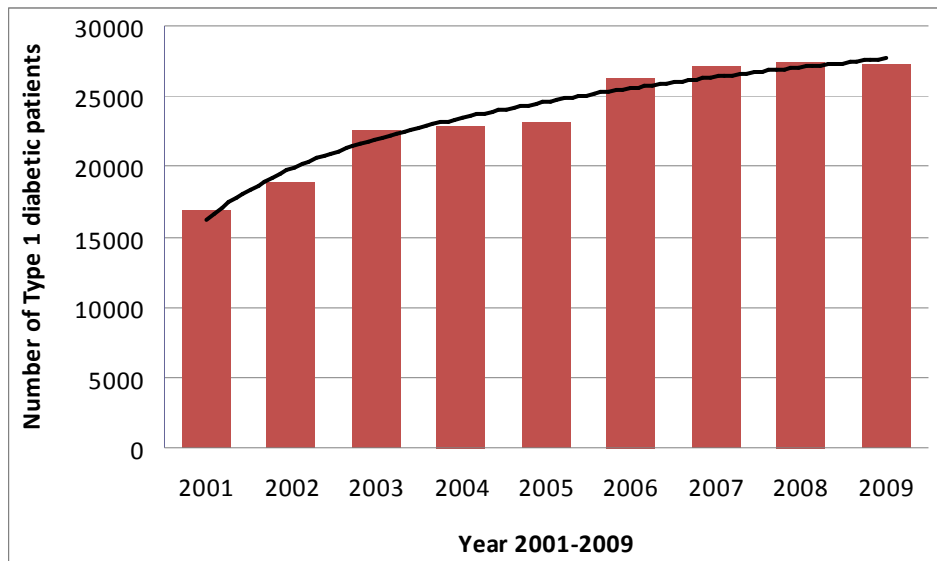


Figure 1-1 Number of patients with type 1 diabetes registered on the Scottish diabetes register (2001-2009)

1.1.4 Pathogenesis of Type 1 Diabetes Mellitus

T1DM is normally due to autoimmune destruction of the insulin producing cells of the pancreas (the beta cells of the islets of Langerhans). This results in an absolute deficiency in endogenous insulin production (Daneman, 2006). Specific destruction of the beta cells occurs when the islets of Langerhans are infiltrated with dendritic cells, macrophages and CD4+ and CD8+ T lymphocytes (Atkinson et al., 2001). As a result of the advanced stage of beta cell depletion, by the time symptoms appear, these cases have an acute onset and are prone to acute ketoacidosis. Ketoacidosis is a condition where ketone bodies build up in the blood as a result of lipolysis in the absence of the insulin required for glycolysis (Daneman, 2006). The majority of cases are diagnosed in the first two decades of life but a significant proportion of cases also present in young adults. There is no upper age limit which precludes a diagnosis of type 1 diabetes (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003).

1.1.4.1 Clinical Presentation

Type 1 diabetic patients normally present with the classic symptoms of polyuria, nocturia, enuresis, polydipsia, polyphagia, weight loss, lethargy, fatigue and abdominal pain (Daneman, 2006). When blood glucose levels are allowed to increase due to excess carbohydrate intake, or a lack of appropriate insulin therapy, acute hyperglycaemia and even diabetic ketoacidosis requiring emergency medical management will occur. Diabetic

ketoacidosis is a potentially fatal condition and was the cause of 39 deaths in 2009 due to hyperglycaemia in England and Wales (Office for National Statistics, 2009).

The short term implications of metabolic dysregulation of blood glucose can also lead to acute hypoglycaemia due to lack of carbohydrate intake, excess insulin or lack of preparation for increased physical activity. Acute hypoglycaemia leads to confusion, palpitations, sweating, tachycardia and loss of consciousness. Without medical intervention this may lead to death. Severe hypoglycaemia occurs with a frequency of about five to 50 episodes per 100 patient-years, dependent both on treatment approach and level of control achieved (Diabetes Control and Complications Trial Research Group, 1993; Daneman, 2006). This has implications for patient suitability for hazardous workplaces and even driving. There is also evidence for cumulative neurological damage and other tissue damage occurring during critically low levels of blood sugar. According to the Office for National Statistics 3-5 people die each year as a result of diabetic coma in England and Wales (Office for National Statistics, 2009). Doctors must tread a fine line between the desirability of near normal blood glucose levels and the increased risk of severe hypoglycaemic events as reported by the Diabetes Control and Complications Trial (DCCT). In this study strict blood glucose targets were met but the risk of serious hypoglycaemic events increased two to three fold. Three people died due to road traffic accidents during the DCCT study as a result of the driver becoming hypoglycaemic (DCCTRG, 1993).

Despite the seriousness of the acute complications that accompany a diagnosis of diabetes, the main threats to life and quality of life are the chronic complications associated with long term glucose and lipid dysregulation. Complications include microvascular and macrovascular disease, nephropathy and retinopathy as well as increased susceptibility to infection and possibly oral complications. Risk factors for the development of diabetic complications include the following: poor glycaemic control; early onset and long duration of type 1 diabetes; genetic predisposition; family history of diabetes-related complications or hypertension; smoking; obesity; sedentary lifestyle; hypertension; and hyperlipidaemia (Daneman, 2006). While T1DM is a significant risk factor for the development of these complications, optimum control of blood glucose levels can reduce the risk and slow the progression of complications (DCCTRG, 1993).

T1DM includes those cases currently attributable to an autoimmune process as well as those with an unknown aetiology. It does not include those forms of β -cell destruction or failure for which non-autoimmune-specific causes can be assigned, for example pancreatitis or cystic fibrosis (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003). Idiopathic type 1 diabetes is a heterogeneous group of conditions with no known aetiology. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. The environmental or genetic aetiopathogenesis of this type of diabetes mellitus is not well understood. The rate of beta cell destruction varies between individuals and can be rapid and complete or it can be a slower process whereby the patient, usually an adult, retains some residual beta cell function for some years. Although only a minority of patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian ancestry. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may come and go, although most will eventually require insulin therapy for survival (Imagawa et al., 2000; Pinera-Pilono et al., 2001).

Most cases of T1DM are characterised by the presence of one or more autoantibodies to islet cells (ICA), insulin (IAA), glutamic acid decarboxylase (GAD65) or to the tyrosine phosphatases IA-2 and IA-zx2 β . 85-90% of individuals have one or more of these autoantibodies present at diagnosis and these are present in the blood before acute symptoms appear (Atkinson et al., 2001, Pihoker, et al., 2005, A.D.A., 2009). The factor which initiates this cell mediated autoimmunity is not well understood although there are strong associations with HLA loci, strengthening the case for susceptible and resistant genotypes. Models in the Non-obese Diabetic (NOD) mouse and the Biobreed diabetes prone rat model indicate that Human leukocyte Antigen (HLA) is necessary, but not sufficient, for diabetes to develop (Ounissi-Benkhalha and Polychronakos, 2008). Eisenbarth originally proposed that all individuals had susceptibility to diabetes whether low or high. This has been further explained by an understanding of the risk conferred by HLA genotype and a range of other less important Insulin Dependent Diabetes Mellitus (IDDM) susceptibility genes (Eisenbarth et al., 1986; Ounissi-Benkhalha and Polychronakos, 2008).

1.1.4.2 Candidate genes influencing diabetic susceptibility

It is clear that there is a genetic element to the development of T1DM although the association is much less strong than in type 2 diabetes mellitus. Diabetes UK estimates that if a mother has the condition, the risk of the child developing T1DM is about 2% whereas if the father is diabetic then the risk to the child is estimated to be 8%. If both parents suffer from T1DM then the offspring will have a 30% chance of developing the condition (Diabetes UK, 2010).

For T1DM, the concordance rate for monozygotic twins from a number of studies has been estimated as 21-53%, with most estimates being between 30-50% (Ounissi-Benkalha and Polychronakos, 2008). Candidate gene studies and genome wide analysis have revealed that the most important loci are in the HLA class II region on chromosome 6p21. This region accounts for 50% of familial aggregation through protective and detrimental effects (Noble et al., 1996). HLA class II antigens are responsible for self versus non-self recognition and antigen presentation to CD4+ T cells. The DR and DQ genes have the greatest influence with certain combinations being of particular importance, for example DR4-DQ8 or DR3-DQ2 which are present in 90% of diabetic patients. Alternatively DR15-DQ6 are protective haplotypes which occur in only 1 % of type 1 diabetic patients but in 20% of the general population (Devendra et al., 2003, 2004). Recently a weak association has also been described between the ubiquitously expressed class I HLA antigens A and B (Nejentsev et al., 2007). The proposed mechanism by which these haplotypes confer susceptibility is either by poor binding of auto antigens in the case of HLA class II or through endogenous antigens synthesized in response to a viral trigger in the case of HLA class I. Both mechanisms may lead to compromised adaptive T cell self tolerance. Use of the transgenic mouse model has confirmed that mice expressing DR3 and DQ8 are susceptible to diabetes, shown by loss of immune tolerance to glutamic acid decarboxylase. Those mice expressing DQ6 were protected from this effect thus confirming its protective function (Abraham et al., 2000, 2001).

To date, candidate genes have been identified, which manifest their effects through defects in T cell maturation, activation and self tolerance leading to increased autoimmunity. In addition, genes controlling defects in antigen presentation and response to viral triggers leading to alterations in self/non self recognition have been described. It is possible that genetic defects in the developing immune system could account for other host mediated

tissue damage including periodontal disease (Ounissi-Benkalha and Polychronakos, 2008). Patients with T1DM are also prone to a range of other autoimmune conditions, and comorbidity is common for these diseases, including autoimmune thyroid disease, coeliac disease, rheumatoid arthritis and Addison's disease (Kordonouri et al., 2005; Skovbjerg et al., 2005; Daneman, 2006; Liao et al., 2009)

1.1.5 Environmental aetiology of Type 1 Diabetes Mellitus

While genetic susceptibility has been clearly shown it is thought that susceptible individuals require exposure to an initiating stimulus which begins the process of beta cell destruction. This would explain why genetically susceptible patients develop diabetes at different ages subsequent to exposure to the trigger at different times in their lives. Suggested environmental triggers include exposure to cow's milk, chemicals or viral infections, which may initiate the autoimmune process. Diagnosis of diabetes has been shown to follow seasonal variation, an observation which precludes a purely genetic basis for disease initiation (Moltchanova et al., 2009). It has also been shown that some migrant populations assume the risk profile of their new country of residence increasing the likelihood of an environmental trigger (Serrano-Rios et al., 1999). The association between T1DM and a viral trigger has long been hypothesised and recent evidence for a role of enterovirus has strengthened the case for viral involvement in susceptible patients (Nairn et al., 1999). Sera from 110 children in the age range 0-15 years was obtained shortly after the diagnosis of T1DM, and tested for the presence of enteroviral sequences by the polymerase chain reaction. One hundred and eighty-two controls tested were matched for age, geographical location and time of year. The authors found that a significantly greater number of children with diabetes had evidence of enteroviral RNA sequences compared with the non-diabetic subjects (27% of the patients with T1DM versus 4.9% non-diabetic subjects, $P < 0.005$). Sequence analysis showed that there was considerable variation in the sequences detected, although all appeared to be of the coxsackie/echovirus type. For a recent review of the putative viral aetiology of T1DM the reader is referred to Roivainen and Klingel (2010).

1.1.6 Glycaemic control

Glucose control is monitored on a daily basis by the individual patient using capillary blood samples to measure blood glucose levels. The patient will often adjust their insulin

dose and sugar intake based on these “instant results”. Longer term glucose control exploits the non-enzymatic glycation of haemoglobin to form glycosylated haemoglobin which occurs throughout the life cycle of the erythrocytes. The percentage of haemoglobin which has been glycosylated when measured by High Performance Liquid Chromatography (HPLC), at any one time, gives an indication of the relative glucose control over the preceding eight to twelve week period (Rahbar et al., 1969). Based on findings from the UKPDS and DCCT, patients will be asked to aim for an HbA1c below 7% for optimum control although some authorities recommend that 7.5% be set as a target (Table 1-2). It has been shown that the incidence of diabetic complications is greatly reduced by maintaining strict diabetic control (DCCTRG, 1993). While there are a number of cut-off points used in studies reporting the effect of diabetic control on other disease processes, it is generally accepted that an HbA1c of 7.5% or less represents good glycaemic control although there are benefits from reducing this further. Arbitrary cut off points and classification have been proposed and used in studies however, there is no HbA1c level below which complications cannot occur and no thresholds above which complications are certain to arise (Stratton et al., 2000).

Table 1-2 International guidelines on target HbA1c for adults with Type 1 diabetes mellitus.

Organization	Target HbA1c (%)
National Institute for Clinical Excellence CG15 (2004, 2010)	$\leq 7.5\%$ or $\leq 6.5\%$ in those at risk of arterial disease
Canadian Diabetes Association (2008)	$\leq 7.0\%$, $\leq 6\%$ if clinically safe.
American Diabetes Association (2011)	$\leq 7.0\%$
Scottish Intercollegiate Guidelines Network 116 (2010)	$\leq 7.5\%$
American Academy of Clinical Endocrinologists (2011)	$\leq 6.5\%$
International Diabetes Federation (1998)	$\leq 7.5\%$

1.1.7 Mechanism of diabetic tissue damage

Tissues in the diabetic sufferer are subject to biochemical anomalies which lead, with time, to damage and loss of function. The range of tissues affected is extensive however, worst affected seem to be large and small blood vessels, the kidneys, eyes and nerves (DCCTRG, 1993; Turner et al., 1996). It is thought that the mechanism of damage is similar in most tissues and could be due to a combination of the following factors: increased circulating levels of pro-inflammatory cytokines (Signorelli et al., 2007; Navarro-González et al., 2008) or adipokines (Hajer et al., 2008); alteration in the structure and therefore the antigenicity of structural proteins by the incorporation of non-enzymatically glycated proteins (Hudson et al., 2002; Kim et al., 2005); and the build-up of oxygen free radicals either through metabolic dysregulation in glucose metabolism (Zheng et al., 2009; Muhammad et al., 2009) or through the presence of host derived heat shock proteins (Hooper et al., 2003). One of the difficulties in specifying a single pathway is that the mechanisms outlined above are not necessarily mutually exclusive and may in fact all play a part in the immunopathogenesis of tissue injury.

1.1.7.1 Advanced Glycation End Products (AGE)

Currently the most widely accepted mechanism of tissue damage is that of the non-enzymatic glycation of macromolecules such as structural proteins. When high levels of glucose are present in the blood this reducing sugar is able to form irreversible cross links with proteins through non enzymatic rearrangement with amino acids to form advanced glycation end products (AGEs). Because the cross links are irreversible AGE builds up in the affected tissue. This results in a loss of functionality as well as acting as a stimulus for pro-inflammatory cytokine release through interaction with the Receptor for Advanced Glycation End products (RAGE) found on the surfaces of immune and resident tissue cells (Kim et al., 2005).

This mechanism has been used by researchers to monitor tissue damage in the animal model. Glycation of proteins within the lens of the eye leads directly to visible alterations in the appearance of the lens and subsequent resultant loss of function (Cerami et al., 1979). This pigmentation was originally known as Maillard's browning products although a number of AGEs have now been identified. These include N-(carboxymethyl) lysine

(CML) and pentosidine, the most studied, although others have also been identified (Hudson et al., 2005).

The relationship between the glycation of macromolecules and diabetic complications was established during the United Kingdom Prospective Diabetes Study (UKPDS). It was shown that a 1% rise in glycated haemoglobin was associated with a 37% increase in microvascular disease (Stratton et al., 2000). Accumulation of AGEs in the skin has been shown to be associated with increased levels of diabetic complications (Sell et al., 1991; Beisswenger et al., 1993).

1.1.7.2 Receptor for Advanced Glycation End Products and the inflammatory response

Studies have shown that the interaction between AGEs and AGE binding receptors (RAGE) which bind to, remove and degrade AGE, results in activation of pro-inflammatory pathways (Lin et al., 2009). This chronic, low level inflammatory response to these altered proteins perpetuates the tissue damage through the activation of downstream pro-inflammatory molecules such as cytokines, chemokines and the arachidonic acid metabolites. These in turn have the ability to cause tissue breakdown through up-regulation of molecules such as matrix metalloproteinases (MMPs) (Nah et al., 2007) and osteolytic activators (Hein et al., 2006). RAGE is a signalling receptor and a member of the immunoglobulin superfamily of receptors. While RAGE is expressed in low levels in most tissues, except the lung, many studies have shown that it is up regulated in tissues affected by diabetic tissue damage. These include: vascular smooth muscle (Schmidt et al., 1999); the glomeruli; podocytes and tubular epithelium of the kidney (Bierhaus et al., 1996); the endothelium in peripheral vascular disease (Ritthaler et al., 1995); the retina (Hammes et al., 1999); and neuronal cells (Brett et al., 1993). RAGE is also expressed by cell types of the immune system including macrophages and monocytes, a possible mechanism for interference in the immuno-inflammatory response (Brett et al., 1993). Studies on rat models of diabetic cardiovascular disease have shown that the accumulation of AGE/RAGE is proportional to the increased duration of hyperglycaemia. This is in agreement with human studies which clearly show the relationship between diabetic complications and duration of diabetes (DCCTRG, 1993; Stratton et al., 2000; Pambianco et al., 2006).

1.1.7.3 RAGE and oxidative stress

Activation of RAGE leads to induction of an oxidative process within the affected tissue which could account for a continued increase in tissue damage through the direct production of free radicals. Free radicals cause tissue damage as their oxidative potential is absorbed by the surrounding cells causing damage to DNA and promoting cell death. Oxidative stress and superoxide generation due to hyperglycaemia increase mitochondrial DNA damage and enhance the sensitivity to apoptosis (Suzuki et al., 1999). Myocytes, fibroblasts, endothelial cells and podocytes are all more sensitive to apoptosis in the presence of hyperglycaemia-induced oxidative stress (Frustaci et al., 2000; Susztak et al., 2006; Alikhani et al., 2007). At the molecular level it has been shown that activation of RAGE leads to nuclear translocation of Nuclear Factor Kappa B (NFκB), a transcription factor which is implicated in inflammation within vascular tissues as well as in other parts of the body. Expression of RAGE increases through a positive feedback loop initiated by AGE/RAGE interaction in a mechanism that is dependent on a number of key NFκB sites. Expression of RAGE therefore increases in tissues where there is accumulation of AGE.

According to Hudson et al. (2005) hyperglycaemia can exert a damaging effect on the tissues through at least four pathways. Hyperglycaemia leads to activation of the aldose reductase pathway due to an increase in the amount of glucose being metabolised through the polyol pathway converting glucose to sorbitol. The production of reactive oxygen species, the accumulation of AGEs and activation of the protein kinase C pathway all contribute to possible tissue damage. Activation of cell signalling molecules such as MAP Kinase and NFκB leads to alterations in gene expression and protein function that in turn leads to cellular and tissue dysfunction (Hudson et al., 2005).

Increased production of oxidants in the tissue occurs as a result of the metabolism of excess glucose, lipids and free fatty acids (FFAs) via a number of different pathways. Damaging oxidant production can occur in response to the structural changes in AGEs indirectly. In addition, exposure to certain bacterial challenges can also result in increased superoxide production and tissue damage. It has been concluded that, while oxidative stress may modify and accelerate tissue damage, it may be insufficient in itself to explain the level of microvascular tissue damage seen in diabetes (Hudson et al., 2005).

1.1.8 Conclusion

T1DM is a common chronic inflammatory disease that affects every major system in the human body. The mechanisms by which this damage occurs are being elucidated and investigated with a view to developing strategies to prevent, control and manage the complications of this disease. The oral cavity and its associated structures cannot be immune from the local and systemic effects of this condition. The aim of this thesis is to gain a better understanding of how T1DM affects the oral cavity and to understand the impact that oral health or disease has on patients with diabetes.

1.2 Periodontal disease

1.2.1 Introduction

The periodontal diseases are a heterogenous group of diseases that affect the supporting apparatus of the teeth. For the purposes of this thesis the term periodontal disease will refer to the two most common periodontal conditions, which are initiated by dental plaque, i.e. chronic gingivitis and chronic periodontitis. This introductory section contains an overview of the anatomy of the periodontium, and of the aetiopathogenesis, classification and epidemiology of these conditions in order to form the basis of the work presented in the second, third and fourth chapters of this thesis.

1.2.2 Anatomy

The periodontium, which surrounds the teeth, is made up of the gingivae, cementum, periodontal ligament and alveolar bone. In health, it is resistant to mechanical, microbiological and chemical trauma and is responsible for maintaining the attachment of the teeth.

1.2.2.1 Gingivae

The gingivae are split into the attached gingivae and the unattached gingivae. The gingivae are made up of three types of epithelium and an underlying core of connective tissue. The attached gingiva is covered by keratinised, stratified, squamous, epithelium. The gingival crevice is lined by the sulcular epithelium and the junctional epithelium. The sulcular epithelium is a non-keratinised epithelium which is not attached to the tooth surface. The most apical portion of the sulcular epithelium is adjacent to the junctional

epithelium. The junctional epithelium is a unique structure in the body with two strati basale with cell maturation occurring at both surfaces. The junctional epithelium is around 15-20 cells thick and is attached to the tooth via hemidesmosomes. Underlying the epithelium is a core of connective tissue composed of collagen fibres, fibroblasts, blood vessels and nerves embedded in an amorphous matrix (Lindhe et al, 2008). In clinical health the gingivae contain a mild chronic inflammatory infiltrate and the inflammatory transudate, gingival crevicular fluid, is produced in small amounts (Page and Schroeder, 1976). Clinically the healthy attached gingivae appear pink and firm with stippling present in 30-40% of individuals (Rosenberg et al., 1967, Lindhe et al., 2008). There is a physiological sulcus in health that is normally 1.5-2mm coronal to the amelocemental junction. The depth of the sulcus can be increased either by coronal movement of the gingival margin due to oedema or hyperplasia (false pocketing) or through apical migration of the insertion of the periodontal ligament attachment in disease (true pocketing). Clinical attachment level (CAL) can be calculated using a graduated probe measuring the distance in millimetres from the amelocemental junction to the base of the clinical pocket. As probing depths can be increased in the absence of attachment loss, clinical attachment level measurements are essential to indicate the cumulative attachment loss over time. Clinical probing depths give an indication of current disease and treatment need.

1.2.2.2 Cementum

The cementum is a specialised connective tissue covering the root dentine into which the Sharpey's fibres of the periodontal ligament insert. Cementum consists of two main types namely cellular and acellular cementum. Acellular extrinsic fibre cementum, which is found in the middle and coronal aspects of the root, is an important part of the attachment apparatus, which links the tooth to the alveolar process proper. Cellular mixed fibre stratified cementum contains cementocytes as well as both intrinsic and extrinsic fibres and is found around the apex of the tooth (Lindhe et al., 2008).

1.2.2.3 The periodontal ligament

The periodontal ligament is a soft, richly vascular and cellular connective tissue which is the interface between the roots and the alveolar process. It contains specifically organised fibres which are arranged in a horizontal, oblique and circumferential direction. Sharpey's fibres insert both into the alveolar bone and the cementum of the tooth. The primary collagen type in the periodontal ligament is type 1 collagen. The ground substance within

the periodontal ligament means that the ligament behaves in a viscoelastic fashion distributing the loading applied to the teeth (Lindhe et al., 2008).

1.2.2.4 The alveolar bone

The alveolar bone into which the periodontal ligament inserts is functionally and embryologically linked to the presence of teeth. Patients suffering from hypodontia are deficient in alveolar bone in the areas where no teeth have formed, as the alveolar bone proper is derived from the cells of the developing dental follicle. Functionally, after tooth extraction, the alveolar process associated with the tooth in question will begin to resorb. Alveolar bone comprises an outer cortex of cortical bone, an inner layer of compact bone surrounding the tooth socket and cancellous bone in between. It is this inner layer of compact bone (or bundle bone) into which the fibres of the periodontal ligament insert (Lindhe et al., 2008). The alveolar process follows the course of the amelocemental junction (Birn, 1966).

Alveolar bone is constantly undergoing a process of remodelling due to physiological mesial drift and in response to alterations in loading on the supported teeth. This process is carried out in health by osteoclasts, present in the Howships lacunae of the alveolar bone and by osteoblasts. Osteoblasts are present on the socket walls near the periodontal ligament, the surface of bone trabeculae in cancellous bone and in relation to the outer and inner aspect of the cortical bone (Lindhe et al., 2008). In health the process of remodelling is in equilibrium, also referred to as coupled. However, if there is excessive osteoblastic or reduced osteoclastic activity, uncoupling of this process occurs. This leads to increased net bone deposition or net loss of the cancellous bone in conditions such as osteopetrosis (Hamdan et al., 2006). Similarly excessive action by osteoclasts or reduced osteoblastic activity will give rise to a net loss of bone and resulting loss of alveolar bone support for the tooth (Jeffcoat et al., 2005). Mechanisms which impact on bone turnover will be fully discussed in section 1.2.5.6.

1.2.3 Classification of periodontal diseases

A relatively recent classification has been proposed in 1999 by the International Workshop on Periodontal Diseases and this has largely been accepted as the current classification despite a number of arguments against its application in clinical practice and basis in

pathogenesis (Armitage, 2000). This classification follows many years of confusion in the literature as to the diagnosis and classification of cases of periodontitis which had up until this point made comparisons between studies extremely difficult (Borrel et al., 2005; Page and Eke, 2007). The classification proposed in 1999 reduced the emphasis of the classification on age and was less prescriptive in definitions of levels of disease. Previous classifications and indices of periodontitis measured extent and severity of loss of attachment as detected on clinical examination and prescribed the location and proportion of sites affected. These strict classifications aided in producing phenotypically similar groupings but the lack of agreement resulted in significant heterogeneity in case definitions across research groups and thus these were abandoned in favour of the current classification. This has, however, not ended the on-going controversy over the definitions of cases of periodontitis clinically or in the periodontal literature (Page and Eke, 2007; Demmer and Papapanou, 2010).

1.2.3.1 Gingivitis

Gingivitis is defined as gingival inflammation without any loss of attachment and may be purely plaque related or may be exacerbated by local or systemic factors. Gingivitis may be associated with a slight increase in clinical probing depth as a result of erythema and swelling of the gingivae resulting in false pocketing. There is a loss of the stippling which can characterise healthy gingivae and an increase in the flow of gingival crevicular fluid. The pocket will commonly bleed on gentle probing of the sulcus. This condition is entirely reversible with removal of the aetiological agent (dental plaque) with no permanent loss of periodontal attachment. Chronic gingivitis can persist without the development of progressive periodontal destruction. The causative relationship between dental plaque and gingivitis was convincingly demonstrated by Loe et al. (1965) in a study on healthy young adults, all of whom developed gingivitis within a 21 day period after cessation of normal oral hygiene practices. When mechanical plaque control was reintroduced the swelling and inflammation in the gingivae reduced to baseline levels. These studies definitively proved the hypothesis that gingivitis was caused by plaque and that the inflammation was related to the volume of plaque at the gingival margin.

1.2.3.2 Periodontitis

Periodontitis is defined by Flemmig as “an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss”

(Flemmig, 1999). Periodontitis may present either as clinical probing depths greater than the depth of the physiological sulcus or as gingival recession or a combination of both (Novak et al., 2008). Periodontitis can be classified as either chronic or aggressive. Chronic periodontitis is the most common and is generally considered to be a slowly progressing form of the disease. Chronic periodontitis may be further described as mild moderate or severe (Armitage, 2000). Aggressive periodontitis is a severe and widespread form of periodontal disease which is characterised by rapid attachment loss and bone destruction. The patient will be healthy apart from the periodontitis and there is a propensity to familial aggregation (Armitage, 2000, Michalowicz, 2000). Both chronic and aggressive periodontitis can occur in a localised form affecting a small number of teeth or a generalised form affecting an increased number of sites. While age *per se* has been removed from the disease classification, aggressive periodontitis is commonly diagnosed in teenagers and young adults indicating the rate of disease progression. The effects of chronic periodontitis increase with age and untreated this will progress slowly through life in a pattern of cyclical bursts of disease progression and quiescence resulting in a linear loss of attachment over time (Gilthorp et al., 2003). The distinction between chronic and aggressive forms of disease is related to the age of onset or detection, the rate of progression, the pattern of destruction, the signs of inflammation and the relative amounts of plaque and calculus (Armitage, 2010). The lack of specific guidelines in the definitions compared with previous classifications and the fact that diagnosis is dictated by the past history and future progression of the disease make it difficult to use the current classification in a cross-sectional study without knowledge of either of these factors (Demmer and Papapanou, 2010). As a result of this, groups investigating periodontal disease have been developing criteria for diagnosis of periodontal disease taking account of extent, severity and age (Page and Eke 2007, Demmer and Papapanou, 2010).

The principal headings of the International Workshop on the classification of periodontal diseases are shown in Table 1-3 (Armitage, 2000).

**Table 1-3 Headings from the International Workshop, 1999
(Armitage, 2000)**

Classification of Periodontal conditions

I. Gingival Diseases

A. Dental plaque-induced gingival diseases

1. Gingivitis associated with dental plaque only
 - a. without other local contributing factors
 - b. with local contributing factors
2. Gingival diseases modified by systemic factors
 - a. associated with the endocrine system
 - 1) puberty-associated gingivitis
 - 2) menstrual cycle-associated gingivitis
 - 3) pregnancy-associated
 - a) gingivitis
 - b) pyogenic granuloma
 - 4) diabetes mellitus-associated gingivitis
 - b. associated with blood dyscrasias
 - 1) leukaemia-associated gingivitis
 - 2) other
3. Gingival diseases modified by medications
4. Gingival diseases modified by malnutrition

B. Non-plaque-induced gingival lesions

II. Chronic Periodontitis

- A. Localized
- B. Generalized

III. Aggressive Periodontitis

- A. Localized
- B. Generalized

IV. Periodontitis as a Manifestation of Systemic Diseases

- A. Associated with haematological disorders
- B. Associated with genetic disorders
- C. Not otherwise specified (NOS)

V. Necrotizing Periodontal Diseases

- A. Necrotizing ulcerative gingivitis (NUG)
- B. Necrotizing ulcerative periodontitis (NUP)

VI. Abscesses of the Periodontium

VII. Periodontitis Associated With Endodontic Lesions

A. Combined periodontic-endodontic lesions

VIII. Developmental or Acquired Deformities and Conditions

1.2.4 Aetiology

The primary aetiological factor in periodontal disease is dental plaque (Socransky and Haffajee, 1994). The vast majority of patients who experience an accumulation of plaque will develop non-destructive periodontal inflammation (gingivitis) (Loe, 1978). Chronic periodontitis is a multi-factorial disease which affects a significant proportion of the general population to some degree or another (60%). Severe disease is however much rarer, affecting only 8% of the dentate adult population in Britain (Morris, 2001) (see section 1.2.5 for more detail). There are a number of suggested reasons for an increase in susceptibility to periodontitis. The differences between patients have been suggested to be due to the amount of plaque, the microbial composition of the plaque or the host response to the presence of dental plaque.

1.2.4.1 Microbial aetiology of periodontitis.

Dental plaque is a polymicrobial biofilm containing up to 500 species of bacteria up to half of which are yet to be cultured (Paster et al., 2001). Dental plaque begins to form as soon as the teeth erupt. After prophylaxis the teeth are initially colonised by *Streptococci* and *Actinomyces* species. As the biofilm matures the type of bacteria contained within the plaque changes, from one composed primarily of Gram positive aerobic bacteria to one composed of primarily Gram negative motile rods (Bagg et al., 1999; Kolenbrander et al., 2006). Attempts have been made over the years to identify a specific pathogen or pathogens involved in the aetiology and pathogenesis of periodontitis. While there is a well-documented shift in the microbial flora from health to disease it is difficult to determine whether this microbiological shift is the cause or an effect of the disease. The species most strongly associated with the presence of disease are classified by Socransky and co-workers (1998) as the red complex of bacteria. These are *Tannerella forsythia* (*T. forsythia*), *Porphyromonas gingivalis* (*P. gingivalis*) and *Treponema denticola* (*T. denticola*).

Aggregatibacter actinomycetemcomitans (*A. actinomycetemcomitans*) is also strongly associated with certain forms of periodontal disease, specifically aggressive periodontitis (Berthold et al., 1986; Slots and Ting, 1999; Cortelli et al., 2005). All of these species are commonly present at sites of periodontal destruction at the time of initial diagnosis and their elimination or persistence is associated with prognosis after treatment. Each

bacterium has specific virulence factors which confer biological feasibility on their proposal as putative periodontal pathogens. A further group are classified as the amber species and these are less strongly associated with periodontal destruction. These include *Prevotella intermedia*, *Prevotella nigrescens*, *Peptostreptococcus micros*, *Fusobacterium nuc. vincentii*, *Fusobacterium nuc. nucleatum*, *Fusobacterium nuc. polymorphum* and *Fusobacterium periodonticum*. *Eikenella nodatum*, *Campylobacter gracilis*, *Streptococcus constellatus* and *Capnocytophaga rectus* (Socransky et al., 1998).

The search therefore for a specific pathogen associated with periodontitis has led to the identification of a number of species which may be implicated in disease progression. However, not all diseased sites harbour these periodontal pathogens. A further complication is that putative pathogens can be isolated from patients without periodontitis and from sites not associated with periodontal destruction in mouths affected by periodontitis. The question that remains is why some sites exhibit loss of attachment and others remain resistant in spite of the presence of putative periodontal pathogens within the pocket. It is also worth noting that bacterial succession is occurring throughout the disease process. It may be the early coloniser, initiating the disease process, which is the most important factor, rather than those which subsequently colonise the altered ecological milieu.

Early research focussed on the volume of plaque and calculus present at affected sites. Cross-sectional and longitudinal studies had shown that sites with pathologically deepened pockets occurred most commonly at sites affected by plaque and calculus accumulation (Theilade et al., 1986). The prevailing body of clinical opinion believed that periodontal destruction over time was an inevitable consequence of the presence of plaque. This theory was called the non-specific plaque hypothesis. Support for this view comes from experimental gingivitis studies demonstrating that plaque is the causative agent in the development of gingivitis (Loe et al., 1965).

At this time it was believed that gingivitis inevitably led to periodontitis with loss of clinical attachment and bone loss and that removal of plaque would eliminate the risk of progressing to periodontitis. However studies on the natural history of periodontal disease confounded this principle. Manual labourers from tea estates in Sri Lanka were examined in a prospective study over a period of 18 years. Oral hygiene was universally

poor with generalised plaque, calculus and gingivitis. The importance of dental plaque in disease initiation and progression was demonstrated where attachment loss was evident as young as 16 years of age and mean attachment loss at 30 years was 3mm. Ten millimetres of attachment loss was also not uncommon (Loe et al., 1986). There were, however, three distinct groups who responded differently to what appeared clinically to be the same level of microbiological challenge. The authors found that one group was resistant to disease and had no periodontitis despite poor oral hygiene and chronic gingivitis (11%), a larger group was moderately susceptible to disease (81%) and a third group showed severe and advanced bone loss compared with both other groups (8%). The increased severity in the group experiencing the most severe disease and the lack of periodontitis in the gingivitis only group cannot be accounted for by plaque presence and volume alone. There was no difference in the volume of plaque between the gingivitis only and the periodontitis groups. Subsequent to this study, and other reports from around the world, it is now clear that periodontal destruction is not an inevitable consequence of plaque accumulation (Morris et al., 2001; Reich, 2001; Van der Velden et al., 2006). Other factors, such as differences in the microbial biofilm and the host immuno-inflammatory response, are crucial in determining the development of periodontal destruction.

It is recognised that the bacteria in dental plaque are responsible for the initiation of periodontal disease. This is supported by studies that confirm the association between the accumulation of dental plaque and periodontal disease and the absence of plaque in health (Loe et al., 1986). There is also clear evidence that removal of plaque will result in clinical improvement and that when plaque control is poor the disease will recur (Becker et al., 1984; Axelsson et al. 2004).

Many *in vivo* and *in vitro* studies have confirmed the periodontopathic virulence of many key pathogens (Holt et al., 2005). However, it has been clearly shown that the presence of dental plaque does not always lead to advanced periodontitis and that key periodontal pathogens can be found at sites of relative periodontal health (Wolff et al., 1993; Van der Velden et al., 2006). In addition longitudinal studies have shown that a small group of individuals experience the majority of tooth loss (Hirschfeld and Wasserman; 1978; McFall, 1982). This leads us to conclude that, in addition to the essential nature of the dental biofilm in initiating the inflammatory response, other host factors must also be involved (Kinane et al., 2006). These factors may determine whether the inflammatory

response will be an efficient protective and preventive one or whether an inappropriate or inefficient inflammatory response leads to host induced tissue destruction.

Investigation of the role of the innate and acquired immune response, the process of inflammation and the genetic susceptibility of some individuals to periodontal disease are the subject of ongoing and future research around the world.

1.2.5 Pathogenesis

The pathogenesis of periodontitis has been categorised into four stages through histopathological examination of the development of periodontal inflammation as a result of plaque accumulation. These stages are called the initial, the early, the established and the advanced lesion (Page and Schroeder, 1976). A brief description of each stage in lesion progression follows.

1.2.5.1 Initial lesion

After cessation of normal oral hygiene measures, within 2 - 4 days of plaque accumulation, the early inflammatory response is seen histologically. Vasodilatation, loss of perivascular collagen, margination and active migration of neutrophils and monocytes into the periodontal tissues and junctional epithelium mediated by Intercellular adhesion molecule (ICAM) and Endothelial leukocyte adhesion molecule (ELAM) are observed. The exudation of serum proteins from the dilated capillaries leads to an increase in GCF fluid flow to flush away toxins.

1.2.5.2 Early lesion

The early lesion was shown to be present after 7 - 14 days plaque accumulation. This stage would be clinically detectable as gingivitis with more pronounced vascular changes and an increase in extra-vascular neutrophils.

Histologically the inflammatory infiltrate consists of numerous lymphocytes, predominantly T lymphocytes, immediately below the proliferating basal cells of the junctional epithelium. In order to facilitate the migration of neutrophils and lymphocytes destruction of the gingival connective tissue occurs. This is through apoptosis of fibroblasts and a reduction in the collagen fibre network of the marginal gingivae, via host

and pathogen derived matrix metalloproteinases (Page and Schroeder 1976, Takahashi et al., 1995).

1.2.5.3 Established lesion

The established lesion is an extension of the histological picture seen in the early lesion. However, there is a shift in the cell population in the inflammatory infiltrate with large numbers of plasma cells being the main histological feature in older patients; in younger patients the infiltrate continues to be dominated by lymphocytes (Fransson et al., 1996). The inflammatory infiltrate extends laterally towards the oral epithelium and inflammation becomes more pronounced and clinically noticeable with an increase in swelling, loss of the knife edge margin and the development of false pocketing. T & B lymphocytes and antibodies and complement are present in the inflamed marginal gingivae and gingival sulcus. The junctional epithelium becomes hyperplastic and ulcerated at this stage and bleeding is likely to occur on gentle probing.

1.2.5.4 Advanced lesion

The advanced lesion is unique. At this stage the inflammatory lesion extends into the periodontal ligament and alveolar bone and there is destruction of connective tissue attachment to the tooth (LOA). The junctional epithelium migrates down the root surface to form a true periodontal pocket.

This destruction of the periodontal ligament and the surrounding alveolar bone is thought to be mediated through matrix metalloproteinases and through enhanced osteolytic activity by osteoclasts. While there is some evidence that highly virulent strains of bacteria can invade the tissues this is not normally the case (Vitkov et al., 2005). Some direct tissue damage can occur through direct cytotoxicity of bacterial products such as proteinases, collagenases, epitheliotoxin, cytolethal distending toxin, hemolysin, hydrogen sulphide and ammonia (Haffajee and Socransky, 1994). Dysregulation of host derived factors such as proteinases and proteinase inhibitors, matrix metalloproteinases and tissue inhibitors of metalloproteinases (TIMPs), pro-inflammatory cytokines such as Interleukin-1 α (IL-1 α), Interleukin-1 β (IL-1 β), Tumour Necrosis Factor- α (TNF- α) and others, prostaglandins and the products of polymorphonuclear leukocytes lead to damage to the connective tissue attachment (Kornman, 2008). This is particularly the case where the immunoinflammatory response is unsuccessful in containing the bacterial biofilm and its products.

1.2.5.5 The innate immune response to periodontal microbial infection

The developing dental biofilm consists of initially Gram-positive cocci in health, changing to increased numbers of motile Gram-negative anaerobes in gingivitis and periodontitis (Moore et al, 1994; Socransky and Hafajee, 1997).

Gram-negative bacteria have on their cell wall endotoxin or Lipopolysaccharide (LPS), a potent stimulator of Toll-like receptor 4 (TLR4). The structure of the lipid A moiety and lipopolysaccharide (LPS) have a large bearing on the immunostimulatory capacity and pathogenicity of the organism. LPS is liberated from Gram-negative bacteria and becomes linked to the extracellular acute phase protein LPS-binding protein before binding to CD14. This leads to transfer of LPS to the extracellular domain of the TLR4 receptor and subsequent TLR4 signalling (Akira et al., 2006). TLR4 deficient mice are more susceptible to infection with Gram-negative bacteria. However, Gram-negative bacteria will also activate TLR2 through their cell membrane proteins, TLR5 if they have flagella, TLR9 through recognition of bacterial CpG DNA and Nucleotide-binding oligomerization domain-containing protein 1 and 2 (NOD 1, NOD 2) through peptidoglycan derivatives (Akira et al., 2006, Mogensen, 2009).

Periodontal pathogens have been shown to stimulate TLRs *in vitro*. For example *P. gingivalis* LPS and fimbriae and *Bacteroides fragilis* LPS are all potent TLR2 agonists (Hirschfield et al., 2001, Asai et al., 2001, Erridge et al., 2004). *A. actinomycetemcomitans* and *F. nucleatum* LPS and whole *P. gingivalis* will stimulate TLR4 (Darveau et al., 2004; Yoshimura et al., 2002). Most bacteria can also initiate an immune response via TLR9, which also detects viable bacterial DNA (Bauer et al., 2001). It is therefore clear that the myriad of bacteria that are present in both health and increasing severity of periodontal disease will present a challenge to the innate immune response in the periodontal tissues. Tolerance to the healthy host microflora is achieved through the poorly understood differential recognition of subtle changes in LPS and other Pathogen associated molecular patterns (PAMPs). These changes indicate to the host the immunogenicity and potential pathogenicity of the organisms present.

The junctional epithelium, as described in detail in the section on the anatomy of the periodontium, is the front line in an uneasy peace between the host and the oral microflora. The junctional epithelium is well equipped to recognise invading pathogens, with studies

showing the presence of mRNA encoding TLR2, 3, 4, 5, 6 and 9 in gingival epithelial cells (Kusumoto et al., 2004). The migrating neutrophils that are constantly recruited in small numbers, even where there are no signs of clinical disease, also express the full range of known TLRs (Mogensen, 2009). Neutrophil defects, including defects in margination, adhesion, chemotaxis and inefficient cell killing, lead directly to an increased risk of periodontal destruction. This occurs clearly in diseases with reduced neutrophil numbers and function including Leukocyte Adhesion Deficiency syndrome and Chediak Higashi syndrome (Meyle et al., 1994; Bailleul-Forestier et al., 2008). Conditions resulting in a deficient TLR response in neutrophils could result in an increased susceptibility to periodontal destruction. Tissue dendritic cells and Langerhans cells are present within the gingival epithelium and the underlying connective tissue. These antigen-presenting cells express a wide range of TLRs including TLR1, 2, 3, 4, 5, 6, 8 and 10 (Mahanonda et al., 2007, Mogensen, 2009). These cells will monitor invasion of the bacteria or bacterial products initiating the adaptive immune response.

Gingival and periodontal fibroblasts respond to TLR2, 4 and 9 (Hatakeyama et al., 2003). Cementocytes within the cellular mixed fibre stratified cementum, that may become involved as periodontal destruction progresses to involve and infect the root cementum, have also been shown to express TLR2 and 4 (Nociti et al., 2004).

The alveolar bone is the supporting structure into which the periodontal ligament inserts and is the tissue that is ultimately destroyed by the inflammatory lesion of periodontitis. Osteoblasts and osteoclasts involved in bone turnover also express TLR1, 4, 5, 6 and 9 (Asai 2001) and TLR1, 2, 3, 4, 5, 6, 7, 8 and 9 (Itoh et al., 2003) respectively. It is therefore possible that TLR signalling within the bone can generate an inflammatory response to invading pathogens. Initiation of a cascade of pro inflammatory cytokines within the alveolar bone will tend to lead to pathological resorption of bone through excessive or prolonged production of osteolytic host molecules. These include IL-1, Tumour Necrosis Factor- α (TNF α) and Prostaglandin E2 (PGE₂) which stimulate osteoblast inhibition and osteoclast activation and maturation through the Receptor activator of nuclear factor kappa-B ligand/Osteoprotegerin (RANKL/OPG) axis. Studies have shown enhanced survival of mature osteoclasts in response to TLR4 and TLR2 activation (Suda et al., 2002; Itoh et al., 2003). Conflicting data showing inhibition of osteoclast differentiation in response to TLR stimulation means that we cannot yet define the role of TLRs in bone resorption and remodelling (Takami et al., 2002).

1.2.5.6 Bone remodelling and the RANKL/OPG axis in periodontitis

Periodontal bone loss is the end result of periodontitis. While the destruction of the matrix of the periodontal attachment is through a combination of microbial and host factors, bone destruction is not caused by direct osteolytic damage. It is due to the normal biological mechanism involving physiological bone turnover and repair. The alveolar bone is resorbed by osteoclasts creating a “safety zone”, 0.5-1mm in width, of un-infiltrated connective tissue (Cochran, 2008). This encapsulation of the inflammatory infiltrate is an important part of the host defence mechanism in periodontitis. Osteoclastogenesis is the production of bone resorbing cells which remodel the alveolar bone progressively during bursts of disease progression leading to decreasing bone support and subsequent tooth loss. Pro-inflammatory mediators such as IL-1 β , PGE₂, TNF α , Interleukin 6 (IL-6), Interleukin 11 (IL-11) and Interleukin 17 (IL-17), induced by bacteria and their products through the NF κ B pathway, result in recruitment of osteoclastic precursor cells and their differentiation into active osteoclast cells. Periodontitis leads to an increase in RANKL levels relative to the levels of OPG resulting in an increase in the ratio of RANKL: OPG and subsequent periodontal bone loss (Cochran, 2008). Activation of the osteoclast precursors is controlled by the RANKL/OPG axis (Cochran, 2008).

RANKL is a member of the tumour necrosis factor ligand family (Lerner et al., 2006). RANKL can either be membrane bound or free in the serum. RANKL is expressed on the surface of activated T and B cells as well as other cells activated by the inflammatory process such as periosteal osteoblasts and fibroblasts (Lerner et al., 2006). RANKL bound to the membranes of these activated cells acts on preosteoclasts in the vicinity of the inflammatory lesion inducing maturation into active osteoclasts via receptor activator of nuclear factor Kappa-B (RANK). RANK is expressed on cells such as dendritic cells, B and T cells, fibroblasts and osteoclastic precursors. When RANKL activates RANK, RANK then activates NF κ B via the tumour necrosis factor receptor associated family members (TRAF). Activated osteoclasts are responsible for the controlled destruction of bone in natural remodelling. They also destroy bone in inflammatory diseases affecting bone, as well as during orthodontic tooth movement. The activated osteoclast undergoes structural changes to form a tight junction between the bone surface and basal membrane where it secretes lytic enzymes into a resorption pit to erode underlying bone (Boyle et al., 2003). RANKL also reduces osteoclast apoptosis leading to prolonged osteoclastogenic potential from each cell extending its life cycle and increasing the amount of bone

breakdown carried out. Several pro-inflammatory cytokines for example IL-1, IL-6, IL-11, IL-17, TNF α , Oncostatin M (OSM) and Leukaemia Inhibitory Factor (LIF) are capable of inducing osteoclast activation through this pathway leading to bone resorption. Hormones, which act to increase blood levels of calcium by releasing calcium from the bone, also act via this physiological mechanism. The expression of RANKL on osteoblasts is reduced by anti-inflammatory cytokines such as IL-13, Interferon gamma (IFN- γ), and transforming growth factor beta 1 (TGF- β 1). These anti-inflammatory cytokines suppress the expression of RANKL and/or increase the expression of OPG, its decoy receptor, reducing osteoclastogenesis (Nakashima et al., 2000).

Bone remodelling is a closely controlled system and the effect of RANKL is ameliorated by the presence of the decoy receptor osteoprotegerin. OPG is a member of the tumour necrosis factor (TNF) receptor superfamily also known as osteoclastogenesis inhibitory factor (OCIF). It is a basic glycoprotein comprising 401 amino acid residues arranged into 7 structural domains and it is found as either a 60 kDa monomer or 120 kDa dimer linked by disulfide bonds. By binding RANKL, OPG inhibits NF- κ B a fast acting transcription factor which is a key regulator of innate immunity, inflammation, cell survival and differentiation. Soluble OPG can reduce the amount of RANKL available for interaction with preosteoclasts, reducing cell-cell signalling and osteoclast activation. OPG also induces apoptosis in mature and activated osteoclasts directly (Simonet et al., 1997).

While the absolute amounts of RANKL and OPG present are affected by the presence of inflammation, it is the relative ratio which is more important, maintaining the balance between induction and inhibition of bone destruction. Where there is an excess of OPG this will interact with the available RANKL reducing the osteoclastogenic potential. Where the amount of RANKL significantly exceeds the amount of OPG there will be an increased availability of RANKL thus increasing the potential for osteoclast activation leading to net bone loss. Where both molecules rise or fall together i.e. there is no change in the ratio, there will be a negligible effect on osteoclastogenesis. This effect has been shown in a number of diseases characterised by inflammatory bone loss including multiple myeloma, arthritis, periapical pathology and periodontitis (Dougal and Chaisson, 2006, Menezes et al., 2008; Maruotti et al., 2010).

RANKL and OPG levels can be detected in serum, GCF and saliva by enzyme linked immunosorbent assays (ELISAs). Investigators have also studied tissue samples stained using immunohistochemistry for RANKL and OPG expression while, more recently, reverse transcriptase PCR measurements of mRNA levels in tissue samples have also been studied (Kawai et al., 2006; Cochran, 2008).

The importance of the RANK/RANKL/OPG system on periodontal bone loss has been shown in a number of studies in humans. Studies have shown that in experimentally induced periodontitis, levels of RANKL are relatively higher than OPG leading to an increase in the ratio of RANKL to OPG. These findings have been confirmed by studies in humans comparing health with gingivitis and periodontitis (Nagasawa et al., 2002; Garlet et al., 2004, Bostanci et al., 2007a,2007b; Lu et al., 2006; Liu et al.,2003; Mogi et al., 2004; Vernal et al., 2004; Wara-aswapati et al., 2007; Lappin et al., 2007). The main source of RANKL in the periodontitis lesion is activated T and B cells (Kawai et al., 2006).

1.2.5.7 Animal Studies.

Animal studies demonstrate the importance of pro-inflammatory cytokines such as IL-1 and TNF α in the recruitment and activation of osteoclasts. In a *Macaca fascicularis* primate model, Assuma and co-authors (1998) showed that inflammatory bone destruction in an experimentally induced periodontal lesion could be blocked by intrapapillary injection of soluble receptors to IL1 and TNF α . Blockading the action of these proinflammatory cytokines resulted in an 80% reduction in the recruitment of inflammatory cells surrounding the alveolar bone. Osteoclast recruitment and alveolar bone loss were reduced by 67% and 60% respectively.

Having established that inflammatory cytokines are integral to active bone loss, investigators began investigating downstream molecules activated by these pro-inflammatory proteins. Research has focussed on the interrelationship between the infective agent, the host inflammatory response and the subsequent effect on the underlying bone. This branch of science has become known as osteoimmunology.

Interleukins and other inflammatory proteins do not directly cause osteoclastogenesis by binding to precursor cells; rather pro-inflammatory cytokines act by influencing the

RANK/RANKL/OPG axis leading to osteoclast maturation and activation (Simonet et al., 1997).

In order to identify the source of RANKL in periodontitis lesions the congenitally athymic rat model has been used in a number of studies. Han and co-workers (2006) demonstrated, using congenitally athymic Rowlett rats, that B cells from *A. actinomycetemcomitans*-immunized animals had greater RANKL expression and induced a significantly higher level of osteoclast differentiation from precursors than did those specimens receiving non immune B cells that were not Ag specific. Osteoprotegerin fusion protein eliminated this effect on the level of RANKL expression. RANKL-expressing B cells were recovered from the gingival tissues of recipient rats transfected with *A. actinomycetemcomitans*-binding B cells (ABB). Rats which had received non immune B cells and those which had received B cells which did not bind *A. actinomycetemcomitans* did not demonstrate RANKL expression. Those rats showing RANKL expression showed periodontal and alveolar bone loss (Han et al., 2006).

The interaction between RANKL and its decoy ligand OPG regulates the initiation of osteoclastogenesis. In experimentally (ligature) induced periodontitis lesions in the rat model Jin et al. (2007) showed that administration of intraperitoneal Human OPG-fc reduced alveolar bone loss and histologically suppressed osteoclast surface area at the alveolar crest at weeks three and six.

The influence of the T cell in the development of periodontal bone loss has also been demonstrated in the mouse model. In the non-obese diabetic mouse (NOD) model *A. actinomycetemcomitans*-reactive CD4⁺ T-cells in mice exhibited significantly higher expression of (RANKL) in experimentally induced periodontitis. The antagonistic and indeed protective effect of OPG was confirmed, as there was a significant reversal of alveolar bone loss and reduced RANKL expression in *A. actinomycetemcomitans*-reactive CD4⁺ T-cells after treatment with OPG. The authors of this study hypothesised that OPG may have a potential role to play in prevention or treatment of periodontitis (Mahamed et al., 2005).

1.2.6 Risk Factors

The puzzling issue in periodontal research is the mechanism by which some people can maintain the established lesion for many years without progression to the advanced lesion while others exhibit minimal marginal bone loss and still others experience rapid and extensive loss of periodontal attachment.

Periodontitis is a multi-factorial disease with many differing risk factors playing a part in the clinical progression of the disease. These factors can be categorised as systemic or local risk factors. Systemic risk factors are those which, in combination with local aetiological agents, increase the likelihood of a patient suffering from the disease, or increase the severity of the disease experienced for a given exposure to dental plaque. Large studies of populations have found that periodontal disease is affected by a number of risk factors including increasing age (Albander et al., 1999, 2002), smoking (Bergstrom and Floderus-Myrhed, 1987; Gunsolley et al., 1998, Bergstrom, 2006) genetics (Michalowicz et al., 1994; Michalowicz et al., 2000), race (Albander et al., 1999), gender (Loe and Brown 1991; Albander et al., 1999; Albander et al., 2002; Hyman and Reid 2003), oral hygiene (Morris et al., 2001; Loe, 1986), socio-economic status (Borrell et al., 2006, 2007), obesity (Chaffee and Weston, 2010), psychosocial stress (Dolic et al., 2005), osteopenia and osteoporosis (Tezal et al., 2000) and other medical conditions including type 2 diabetes mellitus (Taylor et al., 2008). The putative risk factors for periodontitis are thoroughly reviewed by Stabholz and co-workers (2010) and Nunn et al. (2003). The evidence from these associations leads us to conclude that periodontitis does not occur simply as a result of plaque accumulation but is also associated with host factors which will modulate the effect of the plaque on a given individual.

1.2.6.1 Genetics

As with many chronic diseases there is a genetic background to the development of periodontitis. The first indication that this might be the case and indeed, some of the evidence enabling us to understand the pathogenesis of periodontal disease, derives from the studies of patients suffering from single gene defects. It has been well established for many years that aggressive forms of periodontitis are often features of other syndromes with a genetic aetiology. An understanding of the effects of single gene defects allows us to recognise the process by which these individuals have lost periodontal attachment.

Examples include leukocyte adhesion deficiency syndrome, a syndrome where a single gene defect results in the inability of the leukocytes to adhere to the endothelium and respond to the chemotactic signalling (Dababneh et al., 2008). Other single gene defects in leukocytes, affecting their ability to respond to chemotactic stimulation or to phagocytose effectively, result in severe periodontal destruction. Periodontitis is associated with the following genetic defects; Down syndrome, Chediak-Higashi Syndrome, Papillon-Lefèvre syndrome, cyclic and severe congenital neutropenia, Cohen Syndrome and the IL-1 pro-inflammatory genotype (Modeer et al., 1990; Parkhill et al., 2000; Kinane et al., 2005; Hart and Atkinson 2007). All have specific defects in their immune response which lead to attachment loss. It is currently estimated that genetic differences account for at least 50% of all periodontitis (Michalowicz, et al., 2000).

1.2.6.2 Smoking and Periodontitis

The effect of smoking on the periodontium is well documented and is thoroughly reviewed by Warnalaksuriya et al. (2010). There is strong evidence in support of a negative impact of smoking on periodontal health. A systematic review appraised 70 cross-sectional studies and 14 case-control studies, all of which indicated an association between smoking and periodontitis (Bergström et al., 2006). A further 21 cohort studies were also reviewed of which 20 indicated that smoking had a negative impact on the periodontium (Bergstrom et al., 2006). Odds ratios reported in the literature range from 1.2-7.1 depending on other factors (Johnson et al., 2004; Do et al., 2008; Thomson et al., 2007).

The mechanisms by which smoking has been proposed to damage the periodontium were thoroughly reviewed by Palmer et al. in a recent article (Palmer et al., 2005). The proposed mechanisms include; alterations in the vasculature of the periodontal tissues in smokers, reduced neutrophil transmigration across the periodontal microvasculature, suppression of neutrophil chemokinesis, chemotaxis and phagocytosis. Altered release of proteases by neutrophils could also be responsible for tissue damage. Defects in cell-mediated immunity and humoral immunity could affect the immune response to the periodontal biofilm in smokers. Cytokine and enzyme levels are also reduced in the GCF of smokers. *In vitro* studies have shown detrimental effects of nicotine and some other tobacco compounds on fibroblast function, including fibroblast proliferation, adhesion to root surfaces and cytotoxicity. It seems likely that the bacteria associated with smoking related periodontitis may be different but there have been contradictory findings in this regard

(Palmer et al., 2005). All current smokers, and those who had recently stopped, were excluded from the studies included in this thesis.

1.2.6.3 Current model of the pathogenesis of periodontitis

From the above discussion around the various risk factors for periodontitis it can be clearly seen that periodontitis is a multi-factorial disease. Kornman (2008) describes the current model of periodontal pathogenesis as including two levels (Figure 1-2). Level A includes the biological mechanisms involved in immuno-inflammatory responses and in bone and connective tissue metabolism. In level B the products of the microbial complexes activate the immuno-inflammatory pathways and subsequently affect the behaviour of the bone and connective tissue metabolism. Genetic and environmental factors will affect the expression of these interactions both in levels of metabolites that can be measured in samples and in clinical signs that can be observed. The current concept is therefore a multilevel model, which recognises that a number of individual elements interact to confer periodontal risk.

Periodontitis occurs when behavioural and environment factors, for example smoking, are combined with the products of microbial complexes (dental plaque). The effectiveness of the host immuno-inflammatory response within the soft tissue and bone will determine whether the disease is contained or leads to inflammatory bone loss which can be measured clinically. For example, alterations in the microbial milieu may subvert or overcome the host response supporting the role of specific bacteria in the pathogenesis of periodontitis (Socransky et al., 1998). Alternatively hyper-inflammatory states seen in the IL-1 genotype or IL-6(-174) could potentially result in an excessive and inappropriate response to a relatively innocuous microbial complex (Kornman et al., 1997; Trevisatto et al., 2003; Raunio et al., 2010) although many studies on the genetic susceptibility to periodontitis are underpowered and a recent review reported that despite speculation there is limited evidence for the IL-1 genotype as a risk factor for chronic periodontitis (Grigoriadou et al., 2011).

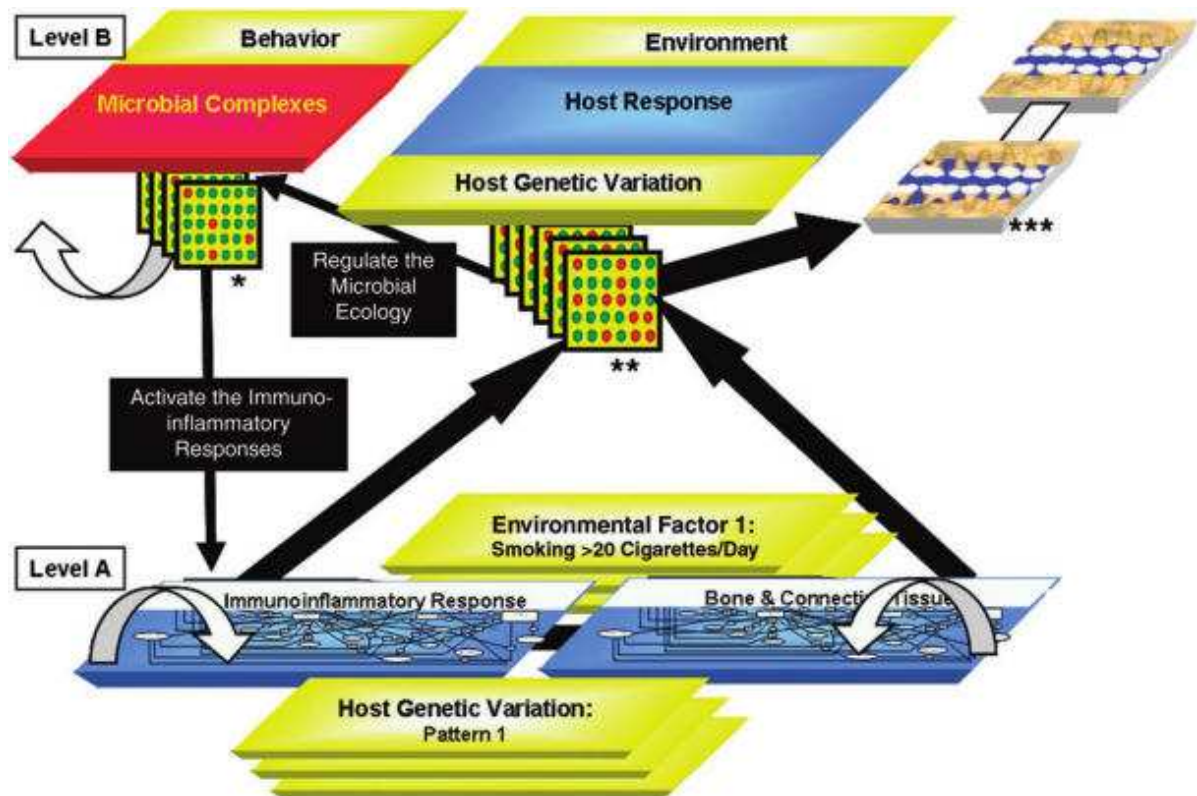


Figure 1-2 Current model of the pathogenesis of periodontal disease (Kornman, 2008)

1.2.7 Epidemiology of periodontal diseases

1.2.7.1 Introduction

Periodontal diseases are among the most common diseases affecting mankind with gingivitis presenting in up to 90% of individuals in some populations. Even in populations with high dental awareness and good access to dental care the prevalence of gingivitis can be as high as 44%, although perhaps as few as 5% of sites are affected in these individuals (Brown et al., 1990). Across the world there are large differences in the reported prevalence of periodontal disease (Papapanou, 1996). While this may indicate a racial predilection it also reflects the prevalence of known risk factors for periodontal disease, the effects of socioeconomic factors and their relationship with oral cleanliness in different geographic locations.

1.2.7.2 Prevalence of periodontal disease

In populations where oral hygiene is poor and access to dental care is limited or non-existent the prevalence of periodontitis is very high. Among the Sri Lankan tea plantation workers attachment loss was evident as young as 16 years of age and the mean attachment loss at thirty years of age was 3mm (Loe et al., 1978). Similarly studies in Kenya found that where oral hygiene was poor, probing depths of ≥ 4 mm were found at 20% of all sites examined (Baelum et al., 1988). 70% of all patients examined in Brazil had at least one site with ≥ 5 mm CAL (Susin et al., 2004). Holtfreter et al. (2009) reported that 51% of subjects had moderate or severe periodontitis in Pomerania. In this study the Centre for Disease Control criteria were used meaning that all patients had at least 2 interproximal sites with CAL ≥ 4 mm or 2 interproximal sites with PD ≥ 5 mm (Page and Eke, 2007). Arguably more relevant to the findings presented in this thesis, studies in developed countries report a much lower prevalence of periodontitis. Periodontal trends in Britain and Europe were the subject of a WHO report in 2001. Reich states in 2001 that only 10% of adults between 35-44 years of age will experience advanced periodontitis (Reich, 2001). There are however, only a small number of countries in Europe for which there are good data on prevalence (Konig et al., 2010). A study in North America reported that moderate disease with probing depths of up to 5mm was found in 20-30% of this age group. Similarly, there was an increasing prevalence with age (65-74years) (Albandar et al., 1999). Studies monitoring periodontal disease in populations over time have shown a reduction in periodontal disease in recent decades (Reich, 2001). A study in Norway, a country generally believed to host a population of highly dentally motivated individuals, was carried out by the same investigators as the famous Sri Lankan tea worker study (Loe et al., 1986). They found, in a young cohort of less than 30 years of age, that attachment loss was less than 1mm at 30 years and there were virtually no deep pockets. This patient group had minimal plaque and calculus deposits (Loe et al., 1986). While this is encouraging and suggests that the initiation of periodontitis can be prevented by good oral hygiene practices, reports from other northern European countries such as Sweden have shown higher levels of disease. In an adult population moderate disease affected 40% of patients, severe disease affected only 13% and the most severely affected accounted for only 3% of the population. This is consistent with the view that periodontal disease as a chronic condition requires a period of years of exposure to initiating factors before signs of

disease are measurable. Encouragingly, the prevalence of periodontitis appeared to be reducing over recent decades (Hugoson et al., 1998, 2008).

A large cross sectional study in the United States involving 15,132 adults reported that gingivitis was present in 44% of patients, but probing pocket depths of 4-6 mm occurred in only around 13% of patients (Brown et al., 1990). These authors reported that the prevalence of clinical attachment loss of ≥ 3 mm was 44% while severe disease, defined as probing depths ≥ 7 mm, was only present in 0.6% of cases. Another North American study reported that the prevalence of pockets ≥ 5 mm and CAL ≥ 5 mm was 8.9% and 19.9% respectively across all subjects. The levels of disease were slightly higher in non-Hispanic blacks (18.4%, 27.9%) and Mexican Americans (14.4%, 28.34%) (Albandar et al., 1999).

1.2.7.3 Periodontal disease in the United Kingdom

Data describing the periodontal condition of the general population of the United Kingdom is scarce although the Adult Dental Health Survey is a useful cross sectional study of oral health that has been conducted on a ten yearly basis since 1968. In addition, data derived from control groups in case control studies examining other risk factors can be used to determine the level of disease in the general population in the United Kingdom.

Tooth extractions

The ultimate outcome of periodontitis is tooth loss and as such a consideration of available data on this outcome is relevant to this overview. Periodontitis is still a significant cause of tooth mortality across the world and in the United Kingdom and is one of the most common reasons for tooth loss in general dental practice. Across the developed world periodontitis accounts for between 18% and 36% of all extractions in national cross-sectional studies (Ainamo et al., 1984; Cahen et al., 1985; Klock and Haugejorden, 1991; Reich and Hiller, 1993; Ong et al., 1996; Angellilo et al., 1996; Murray et al., 1997).

Studies in the UK population have found that dental caries and its complications are still the primary reason for tooth extraction with periodontitis the second most cited cause. In 2001, in a survey of 11,149 teeth extracted in general practice, Agerholm reported that 45% of all extractions were due to caries and 30% were due to periodontitis (Agerholm et

al., 2001). 75% of all extractions due to periodontitis occurred in patients between 41 and 70 years of age. In both 1986 and in 1997 there were no extractions for periodontitis in patients below forty years of age. Another study in 2005 in a survey of 558 extractions reported similar results; 29.1% were attributable to periodontitis (Richards et al., 2005). This finding has been confirmed in two studies over fifteen years in a Scottish population. In 1994 a study of extraction patterns in general dental practice in Scotland reported that of all the extractions carried out in general practice over a week by 139 GDPs (917 teeth) periodontitis accounted for 20% of them (Chestnutt et al., 2000). McCaul and co-workers (2001) ten years later in the same population confirmed that caries and its sequelae were the most common reason for tooth loss; 54.7% of extractions were attributable to caries and 16.3% to periodontitis. Nevertheless the number of extractions attributable to periodontitis increased with age and accounted for a significant proportion of extractions particularly in the lower incisor region where this was the most common reason for extraction (McCaul et al., 2001).

Clinical probing depths and clinical attachment level

While periodontitis can lead to tooth loss, extractions are obviously not a sensitive measure of mild or moderate disease in a population. Data including clinical probing depths and clinical attachment loss describe the prevalence of active and historic disease respectively in the general population.

Robinson et al. (1996), in a survey of adult men in the UK between the ages of 18 and 65 years, reported that clinical attachment loss (CAL) and probing depths of $\geq 4\text{mm}$ affected around a third (28.5% and 31.9% respectively) of all of the group. Bleeding on probing (BOP) was almost universally present with $>90\%$ of subjects exhibiting some gingival bleeding. Similarly, an examination of 100 UK military recruits showed that even in this young group, periodontitis was evident with $\text{CAL} \geq 3\text{mm}$ affecting up to 47% of subjects. The extent of $\text{CAL} \geq 3\text{mm}$ was uncommon (0.9%) (Griffiths et al., 2001). This study also confirmed that the most severe disease affects a small proportion of the population as only a small number of patients had a large number of sites above the threshold for the diagnosis of periodontitis (Griffiths et al., 2001). There is a paucity of data describing the periodontal condition of the population of the United Kingdom and Scotland outwith the national surveys.

1.2.7.4 Adult Dental Health Survey 1998

Until recently, (March 2011) the most comprehensive data concerning the periodontal status of the United Kingdom was derived from the Adult Dental Health Survey 1998 (ADHS). The periodontal findings of this study were reported by Morris et al. (2001). It represents the most comprehensive data available on the level of periodontal disease in the United Kingdom as there were no examinations of Scottish patients in the most recent survey reported in 2011 (The Health and Social Care Information Centre, 2011). Morris reported that the oral hygiene of the UK population is poor with around 70% of patients having visible plaque and calculus present. Even patients who had chosen to brush their teeth immediately prior to examination still had plaque present on one third of their teeth. This is indicative of the ineffectiveness of tooth brushing even when it was employed (Morris et al., 2001).

The ADHS recorded clinical probing depths although a partial mouth protocol was adopted. This is likely to have resulted in under-reporting of both the prevalence and extent of disease (Susin et al., 2005). Mild periodontitis was common and 54% of dentate adults had periodontal pocketing of ≥ 4 mm; however only 5% of patients had pockets deeper than 6mm. Pocketing ≥ 9 mm was seen in only 1% of all dentate responders. Age or increased duration of exposure to risk factors clearly increases the prevalence of periodontitis with deep pockets (≥ 6 mm) seen in only 1% of the 16-24 year old age group but in 15% of over 65 year olds. The number of teeth involved also increased with age. Pocketing of 4mm affected only 5% of teeth in 16-24 year olds but up to 23% of teeth in those over 65 years. Measurement of attachment loss, a better measure of total disease experience, showed that moderate attachment loss of up to 4mm was common (43%), but severe attachment loss of ≥ 6 mm was only found in 8% of adults. There were no cases of CAL ≥ 6 mm in the 16-24 year old group but in those between 55 and 64 years old this figure rose to 17%. 30% of all subjects over 65 had one tooth with ≥ 6 mm CAL. A clear trend is seen with an increasing level of periodontitis with age with attachment loss of ≥ 4 mm affecting 14% of 16-24 year olds but up to 85% of those over the age of 65 years (Morris et al., 2001).

Those patients suffering from severe disease account for 8% of the general population and have on average 2.6 teeth affected, although a further ten teeth on average were affected by moderate disease. When only those suffering from pocketing ≥ 4 mm were analysed, it was

found that there were small differences in socioeconomic factors, dental attendance and oral hygiene between those patients suffering from mild periodontal disease and those with the most severe disease. This large study also found that the oral hygiene and periodontal condition of men was worse than age- matched females and that periodontitis and the abundance of plaque and calculus were slightly lower in patients who attended a dentist regularly and reported regular oral hygiene measures.

Age, gender, previous smoking status and socioeconomic status are potential confounders that should be controlled for in determining the effect of T1DM on the prevalence of periodontal disease in the studies described in this thesis (Morris et al., 2001).

The Adult Dental Health Survey 2009 (The Health and Social Care Information Centre, 2011) recently reported that there has been an over all reduction in the level of moderate periodontal disease with 55% of dentate adults in England and Wales having no periodontal pocketing. 37% of adults had pockets of between 4 and 5.5mm, 7% had pocketing of between 6 and 8.5mm and 1% had pocketing of 9mm or more. Overall the prevalence of severe disease has increased slightly since 1998 although this is likely to be due to increased tooth retention rather than a true increase in disease given that mild to moderate disease has reduced over the same period.

1.2.7.5 Conclusion

In conclusion, mild forms of periodontal disease, including gingivitis, are common conditions affecting a small number of teeth in a large number of patients. Periodontitis with associated moderate loss of attachment affects up to half the population, however this again may be limited to a small number of teeth. Severe attachment loss is uncommon despite poor oral hygiene in the population and only a small number of teeth are likely to be affected by the most advanced attachment loss. The level of periodontitis in the United Kingdom appears to be similar to other developed countries. There is a significant association between increasing age and loss of periodontal attachment. The high prevalence of mild disease in the general population makes it unlikely that differences due to specific risk factors will be detectable. The relative rarity of severe periodontal destruction, in the UK population should mean that any increase in prevalence or severity in high risk groups should be measureable at this level. The extent as well as the

prevalence and severity of periodontitis should be reported in studies investigating differences in periodontal disease levels in different populations.

1.3 Type 1 diabetes mellitus and periodontal disease

1.3.1 Introduction

The association between T1DM and periodontal disease has been investigated repeatedly over the years with the earliest reports beginning in the 1930s (Sheppard, 1936). Early studies can be criticised for their lack of adequate controls and a lack of standardisation in their inclusion and exclusion criteria. A detailed review of the literature will cover the methodological strengths and weaknesses of the studies into T1DM and periodontitis to date.

1.3.2 Terminology and Nomenclature

Diabetes mellitus, like many other complex diseases, has had its share of nomenclature changes over the past decades and this alone makes direct comparisons between studies difficult. T1DM is variously referred to in the literature as juvenile diabetes, insulin dependent diabetes mellitus, insulin deficient diabetes mellitus and early onset diabetes. T1DM has been clearly defined in the current classification and has already been discussed in section 1.1.2 (ADA, 2011). It is important to distinguish clearly between Type 1 and Type 2 diabetes mellitus. Both are defined by hyperglycaemia but they are essentially two separate diseases with different aetiology, pathogenesis, presentation, treatment and complications (Daneman, 2006; Stumvoll et al., 2005). It is the author's view that studies investigating the effect of diabetes on the periodontium should not mix patients with type 1 and type 2 diabetes together (Aspriello et al., 2010). Unfortunately, many of the early studies have mixed diabetic populations making it impossible to separate the two main subtypes of the disease (Glavind et al., 1968; Albrecht et al., 1987; Oliver and Tervonen, 1993; Alpagot, et al., 2001; Persson et al., 2003). This has resulted in a large number of early studies being difficult to interpret in the light of unclear inclusion criteria.

1.3.3 Study Design

1.3.3.1 Demographic characteristics

The recent systematic review by Chávarry and co-workers (2009) reported that the design of studies investigating the periodontal condition of T1DM patients has been inadequate. Factors known to affect the prevalence of periodontitis should be eliminated or adequately controlled for by strict inclusion and exclusion criteria and where possible statistical analysis. Chávarry et al. (2009) concluded that none of the studies into T1DM to date have adequately controlled for confounding factors. The selection of an appropriate control group is central to the validity of the conclusions that can be drawn, but many studies have used convenience samples, limiting the applicability of the conclusions to the wider population (Cianciola et al., 1982; Leeper et al., 1985; Tervonen and Karjalainen, 1997; Sbordone et al., 1995, 1998)

Another weakness within the literature is that many studies on diabetes and periodontitis have focussed on children and adolescents. The prevalence of periodontitis in the juvenile population is relatively low and it is generally accepted that periodontitis normally runs a chronic course throughout life. It is therefore to be expected that even adolescents with diabetes will exhibit little sign of periodontitis at this early stage in life and after a short duration of diabetes. Increases in gingivitis which have been reported may or may not reflect an increase in susceptibility to periodontal destruction over time (Lalla et al., 2006a). Chávarry et al. (2009) called for future studies to be performed in adults with T1DM rather than in children and adolescents.

It has been well documented that there is a genetic component to the pathogenesis of periodontitis as well as to T1DM (Michalowicz et al., 2000; Ounissi-Benkalha and Polychronakos, 2008). It is therefore imperative that the control group does not include siblings of the diabetic patients, since they would have similar environmental and genetic backgrounds. Racial differences in the prevalence of periodontitis are well documented with an increase in periodontitis in Afro-Caribbean, Asian and Hispanic races (Albander et al., 1999). A number of studies have not reported demographic information or have included a number of different races in the study population (Lalla et al., 2006a).

1.3.3.2 Case definition

Well defined criteria for diagnosis of disease should be employed. Full mouth chartings of attachment level and clinical probing depth are the most reliable method of diagnosing periodontitis (Susin et al., 2005). There is also significant heterogeneity in case definitions of periodontitis (Page and Eke, 2007). Most studies only reported differences in the mean CPD and CAL rather than prevalence data and odds ratios for a given level of periodontitis (Khader et al., 2006, Chávarray et al., 2009).

1.3.3.3 Tobacco smoking.

Many studies have not reported the prevalence of smoking in their case and control groups and no study of T1DM has completely eliminated this factor. Since smoking is the most important environmental risk factor for periodontitis it is essential that this information is gathered and allowed for in the statistical analysis. The odds ratio for increased risk of periodontitis due to smoking is affected by age, duration and the cumulative number of cigarettes smoked over time (Warnakulasuriya et al. 2010). Odds ratios reported in the literature range from 1.2-7.1 depending on other factors (Johnson et al., 2004; Do et al., 2008; Thomson et al., 2007). In the studies of T1DM patients which did report smoking data, the prevalence was high (Bridges et al., 1996; Moore et al., 1999). It was therefore difficult to model accurately for the effect of smoking on the periodontium in diabetic patients in these studies and distinguish between the two risk factors. Haber et al. (1993) examined 132 T1DM patients and 88 controls measuring plaque, gingivitis, clinical probing depths and clinical attachment level but did not exclude smokers. On average they found that T1DM smokers had higher levels of periodontitis than healthy controls. However the authors also noted that the smokers had experienced lower levels of periodontal care as, on average, it had been longer since their last prophylaxis. This indicates the possibility that there were differences in the social and/or behavioural risk factors for periodontitis in this group. Interestingly, there was no significant difference between non-smoking patients with T1DM and the healthy controls. The odds ratio for former smokers was 1.8 and for current smokers this was 6.9. The authors concluded that prevalence of periodontitis increased with age, past smoking experience and current smoking exposure. These findings were in agreement with those of Bridges et al. (1996) who examined the effect of smoking and socioeconomic factors in T1DM patients. They found that there was an increase in bleeding index, clinical probing depth and clinical

attachment level in T1DM. However, they also reported that smoking *per se* was related to increased clinical probing depth, clinical attachment level and to missing teeth. Many of the mechanisms by which smoking is related to the development and increased extent and severity of periodontitis are the same as those hypothesised for T1DM. Therefore, it is imperative that smoking data is included and reported in studies examining the impact of diabetes on the periodontium. Moore et al. (1999) reported that levels of periodontitis were similar to national surveys. This study also showed that 35% of all patients with periodontitis were smokers. The odds ratio for having periodontitis if subjects were smokers was 9.73 while the OR for T1DM was 3.36. The three studies described above make it clear that in order to elucidate the role of T1DM in the development of periodontitis current smokers should be excluded from future studies.

1.3.3.4 Sample size

Sample size is of critical importance in all clinical studies both in concluding that an association exists or that no association is present. Small sample sizes are a problem in a number of the studies reported to date. Often studies have been under-powered to detect real differences in disease experience between cases and controls. Conversely, it is also possible that differences reported in small samples are chance findings which would not be seen in a larger population (Manouchehr-pour et al., 1981, Rylander et al., 1987, Pinson et al., 1995, Sbordone et al., 1995, Guthmiller et al., 2001, Yucekal-Tuncer et al., 2003, Aren et al., 2003).

1.3.3.5 Training and calibration

A further weakness was the lack of calibration and training of clinical examiners. Only 4 out of 17 studies on T1DM included in the review by Chávarry and co-workers reported adequate training and calibration of examiners (Hugoson et al., 1989, Harber et al., 1993, Pinson et al., 1995; Guthmiller et al., 2001; Chávarry et al., 2009). In addition, blinding of the examiners to the diabetic status of the subjects was only reported in 1 of the 17 studies (Tervonen and Karjailenen, 1997).

1.3.3.6 Measurement of diabetes

As diabetes is a relatively common disease affecting 4.4% of the Scottish population (SDS, 2009) and 6.4% (3.8-10.2%) of the population worldwide, it is important that the control group are not unknowingly suffering from Type 2 diabetes. A small number of studies

have taken blood tests for blood glucose levels, fructose levels or glycosylated haemoglobin from their control group, however this precaution has not been universal (Firatli et al., 1996, 1997, Salvi et al., 2005). Without this information it is not possible to be sure that there are no undiagnosed diabetic patients in the control group confounding the findings of the study.

1.3.4 Gingivitis and Type 1 Diabetes

Many studies have shown that there is an increase in gingivitis in diabetic patients even at an early age. In a prospective experimental gingivitis study 9 subjects suffering from T1DM were compared with nine healthy controls. It was found that there was an increase in the severity of gingivitis and a reduction in the time taken for the gingivitis to develop despite similar levels of plaque (Salvi et al., 2005). This prospective *in vivo* experimental study of two small but well matched groups provides moderately good evidence that patients with type 1 diabetes are more susceptible to gingivitis. Other cross-sectional studies have also found an increase in gingival redness and swelling in T1DM (Bernick et al., 1975; Ringelberg et al., 1977; Gislen et al., 1980; Faulconbridge et al., 1981; Gusberti et al., 1983; Harrisson and Bowen 1987; Hugoson et al., 1989; Akyuz and Oktay, 1990; De Pommereau et al., 1992; Seppala et al., 1993; Pinson et al., 1995; Pinducciu et al., 1996 and Lalla et al., 2006a). These studies have primarily used indices of gingival inflammation such as the Gingival Index of Loe and Silness (1963) or the Modified Gingival index of Lobene (1986). As gingivitis is caused by dental plaque it is important that a measure of oral hygiene is included in the analysis to ascertain the cause of any increased gingival inflammation. Bridges et al. (1996) reported an increased GI score in T1DM. However when plaque scores were taken into account the association was no longer seen.

A number of studies have shown that in diabetic patients the severity of gingivitis is related to glycaemic control (Kjellman et al., 1970a; Gislen et al., 1980; Gusberti et al., 1983; Harrisson and Bowen 1987; Seppala et al., 1994 and Firalti et al., 1996) and the occurrence of diabetic complications (Rylander et al., 1987). Other investigators have found no increase in clinical signs of gingivitis in patients whose glycaemic control is good (Ervasti et al., 1985). Clinical trials examining the effect of glycaemic control on gingivitis have shown that there can be a small improvement in the gingival condition, seen by a reduction in gingival bleeding or redness, when the glycaemic control is improved (Karjailenen and

Knuuttila, 1996, Sastrojiwoto et al., 1990). While both of these studies showed a slight reduction in gingival bleeding, they did not result in any improvement in the underlying periodontal condition. These studies support the theory that there is a relationship between glycaemic control and gingival inflammation but a clinically significant improvement in gingivitis or periodontitis has not been demonstrated (Karjailinen and Knuuttila, 1996; Sastrowijoto et al., 1990). Gingivitis is found to be increased in differing hormonal states for example pregnancy and puberty (Armitage, 2000). Studies investigating diabetic patients who are pregnant or experiencing puberty have found that they suffer from increased gingivitis at these times compared with non-diabetic subjects (Gusberti et al., 1983, Guthmiller et al., 2001). However, in one study of pregnant patients, most were suffering from gingivitis at the beginning of the study (Albrecht et al., 1987). The study was also conducted on a mixed population of T1DM and type 2 diabetic patients. Another study found an increase in gingivitis in the circumpubertal age range in T1DM in line with the normal adolescent population (Gusberti et al., 1983). When age was taken into account the main contributing factor was average glycaemic control.

In contrast to the above studies there have been a number of reports which have not found a correlation between diabetes and gingivitis (Glavind et al., 1968; Bay et al., 1974, Goteiner et al., 1986; Pinson et al., 1995; Sbordone et al., 1995 and 1998; Bridges et al., 1996; Novaes et al., 1997). These studies have either found no increase in gingivitis or have found that plaque control in the diabetic group is significantly poorer. When plaque scores were taken into account, there was no difference in levels of gingivitis. The studies reporting no differences do not seem to be significantly methodologically superior to those reporting an increase. In light of the available evidence it is not possible to definitively conclude that the prevalence and severity of gingivitis is increased in T1DM. Further studies to elucidate this relationship are required.

1.3.5 Oral Hygiene

As discussed in the section on the aetiology of periodontal disease, dental plaque is the primary aetiological factor in the development of gingivitis and periodontitis. It is therefore important in all measures of periodontal disease that investigators report the level of plaque control in the mouth at the time of diagnosis. Poor oral hygiene in diabetic patients is a possible cause of an increase in periodontal disease (Albrecht et al., 1988; Novaes et al., 1991; Bridges et al., 1996; Firatli et al., 1996; Lalla et al., 2006). A possible

cause of increased plaque accumulation includes increased glucose content of the saliva. This could provide nutrients for the development of the plaque through production of extracellular polysaccharides such as glucans and fructans (Takahashi and Nyvad, 2008). It has also been suggested that lack of compliance with recommended diabetic therapy might in turn reflect on attitudes to maintenance of oral health (Thorstensson et al., 1989a).

Increased plaque accumulation has not been universally reported. A number of studies have found no increase in plaque deposits in T1DM (Bernick et al., 1975; Leeper et al., 1985; Akyuz and Oktay, 1990; De Pommereau et al., 1992). It may be that the differences noted in the various studies are specific to the case and control groups under investigation. Oral hygiene varies according to socioeconomic status, age, sex and other individual factors. Factors affecting oral hygiene must be reported in cross-sectional studies in order to eliminate these as a confounding element in the analysis.

1.3.6 Periodontitis and type 1 diabetes mellitus

1.3.6.1 Cross-sectional studies

By definition periodontitis is the progressive loss of periodontal clinical attachment which can either be manifested as an increase in clinical probing depths and/or recession of the gingivae. Studies reporting on periodontitis should include full mouth clinical probing depths and attachment levels, measured from the amelocemental junction to the base of the probable pocket (Susin et al., 2005). This type of assessment is time consuming for both the patient and the examiner and is expensive to achieve. Therefore many studies have involved partial mouth measurements or have recorded only probing depths. Studies reporting only clinical probing depths in T1DM have reported either no difference in average clinical probing depths or an increase compared with non-diabetic control subjects (Khader et al., 2006, Chávarry et al., 2009). There are no studies to the knowledge of the author that report lower mean clinical probing depths. Studies investigating the prevalence of periodontal disease in adolescents are more numerous than those investigating adult populations. Many of these, while reporting increased levels of gingivitis, do not report any increase in probing depth or loss of attachment.

Glavind et al. (1968) examined 102 subjects (51 diabetic patients and 51 healthy controls) to ascertain the relationship between periodontal status and diabetes duration, insulin

dosage and retinal changes. He found no difference between the groups in oral hygiene or gingival inflammation. The periodontal condition was normal up to 30 years old. However, in the 30-40 year age range there was an increase in mean clinical attachment level (diabetic patients: 4.32mm, NDS: 2.76mm). This study also reported that periodontitis was related to age, duration of diabetes and retinopathy. It contained both T1DM and young type 2 diabetic patients. Cianciola and co-workers (1982) showed that the prevalence of periodontitis was increased in children and adults suffering from T1DM. The authors examined 263 patients with IDDM (T1DM) and 149 non-related controls and 59 non-diabetic siblings. This design could be criticised for the use of siblings who might share a predisposition to diabetes, or to the development of periodontitis. However, the majority of control subjects were not related to the diabetic patients. It is therefore not possible to quantify the effect of using siblings on the analysis. In the sibling control group between 11 and 18 years of age there were no instances of periodontitis. This would suggest that the differences in periodontal status between the diabetic patients and their siblings were due to their diabetic status. Leeper et al. (1985) also used the siblings of T1DM patients as part of his group of age and sex matched controls. The study included only 11 diabetic patients between 11-19 years of age. Plaque index, clinical probing depth, clinical attachment loss and bleeding on probing were all measured. The mean probing depth was increased in T1DM (mean 2.9mm) compared with the control group (mean 2.3mm). The difference between the diabetic patients and their siblings was not significant in this instance, adding weight to the argument against using siblings as controls however the small sample size means that any differences would be unlikely to be statistically significant in any case. The bleeding index was increased but there were no differences in plaque levels indicating that there was an increased prevalence of gingival inflammation in the diabetic population. The size of this study precludes drawing any conclusions from the data. The researchers used the juvenile periodontal index and showed that clinical signs of periodontitis were not seen in patients less than 12 years of age. However, in the 11-18 year old group the prevalence of periodontitis was 9.8% in the T1DM group compared with 1.7% in controls. There were six diabetic patients (4.2%) with severe periodontal destruction with radiographic signs of bone loss and attachment loss of greater than 25% on at least two teeth in the 11-18 year old group compared with only one patient (0.8%) in the control group. As patients aged, there appeared to be an association between duration of diabetes and periodontitis. Both an increase in gingivitis and an increase in radiographic

and clinical signs of bone loss and corresponding attachment loss of up to 39% in patients older than 19 years were evident.

Galea et al. (1986) examined 82 subjects with early onset insulin-dependent diabetes mellitus and reported that there was evidence of increased clinical probing depths and clinical attachment loss in two children 11 and 13 years old respectively. Deep probing depths were first found in patients aged 19 and above, while 60% of 25-29 year olds had established periodontitis. While these figures are well above the reported normal prevalence of periodontitis in the healthy adult population, this study did not have a control group. In addition it is not clear what criteria were used to define “established periodontitis”, and whether these definitions are comparable to those used in epidemiological surveys. Periodontitis was associated with higher blood glucose concentration and diabetic complications (Galea et al., 1986). Another study of 50 diabetic patients (53 controls) showed that there were no differences in the periodontal status between the diabetic patients and the controls (Tervonen and Knuutila, 1986). There was, however, an increase in the number of deep pockets and the number of bleeding sites in the poorly controlled diabetic patients. In this study, the well-controlled diabetic patients had significantly better periodontal health than the healthy controls. There were no differences detected in mean alveolar bone levels. The main difficulty with this study is that it is not possible to determine from the available information the type of diabetes of the cases. In addition, no smoking data were presented. The study was also inadequately powered to allow comparisons between four groups. It may be that the observed reduction in periodontitis in well controlled diabetic patients is representative of health attitudes and behaviours. Good compliance with a diabetic care regime may be linked with a positive attitude to general and oral health (Syrjala et al., 1999, 2002, 2004).

Harrisson et al.(1987) and Pinson et al.(1995) are further examples of studies which have reported no increase in periodontitis in a T1DM population. Both of these studies however examined a paediatric and adolescent population and it may be that the children, in line with the findings of others, had simply not been exposed to dental plaque for long enough to display signs of this chronic disease

Similarly, no increase in full mouth clinical probing depths was noted by Rylander and co-workers (1987) in an examination of 46 T1DM patients and 41 control subjects aged

between 18 and 26 years. However, when only buccal and lingual sites were examined, an increase in gingival redness and prevalence of attachment loss $>2\text{mm}$ was noted. Moore et al.(1999) is one of only a few studies of an adult T1DM population to have found no significant increase in periodontitis. It is interesting that in this study, which included smoking status as a confounding factor, most of the periodontitis could be explained by age and smoking. Rosenthal et al.(1988), in an uncontrolled cross-sectional descriptive study, found that moderate to advanced periodontitis was experienced by 5.8% of the study population of type 1 diabetic patients. In addition to this higher than average prevalence of periodontitis in a population of 11-22 year olds, Rosenthal also reported that those patients who had no periodontitis had a lower mean HbA1c score than those with periodontitis. There was also an association between retinopathy, neuropathy and ketoacidosis and periodontal disease. Similarly, Rylander et al. (1987) also reported that diabetic complications were associated with increases in gingival inflammation. An association between HbA1c and loss of clinical attachment has also been reported by multiple authors (Willerhausen et al., 1991; Safkan-Seppala and Ainamo, 1992; Tervonen et al., 1993, 1997; Patino Marin et al., 2002; Aren et al., 2003; Lalla et al., 2007). These authors reported increased clinical probing depths, clinical attachment loss and radiographic bone loss in poorly controlled subjects but no corresponding increase in well controlled T1DM despite similar plaque levels.

The average HbA1c score is linked to the development of diabetic complications. However, complications are not only found in poorly controlled diabetic patients nor do all patients with high HbA1c levels develop complications at the same rate (DCCTRG, 1993). Thorstensson and co-workers (1996) analysed periodontal data from 39 T1DM patients for associations with other systemic complications of diabetes rather than simply HbA1c. These workers found that periodontitis was associated with proteinuria and renal disease, cardiovascular disease e.g. angina and myocardial infarction, and cerebrovascular disease such as stroke and transient ischaemic attacks. This evidence may suggest that there is a common pathway of pathogenesis in the development of these complications.

Hayden et al. (1989) examined 157 diabetic patients and did not find any relationship between periodontal indices and diabetic status. However, this study only used index teeth to determine periodontal status and did not include the examination of healthy controls. Hugoson et al. (1989) in a study investigating the effect of the duration of diabetes on the

periodontium examined 82 long duration diabetic patients (mean duration 28.9 years), 77 short duration diabetic patients (mean duration 5.2 years) and 77 controls between 20 and 70 years of age. The patients were all insulin-dependent but it appears from further reading that a number of these patients were maturity onset diabetic patients and as such would be classified as having type 2 diabetes mellitus. The authors reported that long-duration type 1 diabetic patients between 40 and 49 years of age had evidence of increased alveolar bone loss. There was an increased prevalence of probing depths of 4 mm and 5 mm in insulin-dependent diabetic patients less than 45 years of age. There was also an increase in the incidence of severe disease (CPD >6mm). This is another example of a study which did not report smoking status.

Sastrowijoto et al. (1989) in a cross-sectional descriptive study showed that there was no relationship between periodontitis and diabetes and therefore no relationship between diabetic control and periodontitis. This study however did not have a control group and consisted of only 22 adults (Sastrowijoto et al., 1989). A similar finding was reported by Akyuz and co-workers (1990) and De Pommereau (1992) who both concluded that there was no increase in periodontitis in T1DM children and adolescents between 5 and 18 years old and 12 and 18 years old respectively. When examining adolescents, Andronikaki-Faldami (1990) also found that there was no increase in periodontitis experience in the 15-25 age group. However, in the same study in a young adult population there was an increase in periodontitis in the 25-36 year age group. This again emphasises the point that periodontitis is a chronic disease which normally takes years for clinical signs to become apparent. Novaes et al. (1991) showed that the prevalence of periodontitis increased with age in a T1DM population and was evident at an earlier age than in healthy controls. The difference in the mean probing depth between these groups was not statistically significant although a trend was reported. Alveolar bone loss was found to be higher in the anterior region in T1DM than in healthy controls. These mixed findings are likely to be due to lack of statistical power in the sample sizes. However, it is interesting that the anterior region appears to be more susceptible than the posterior. This does not follow the normal pattern of disease progression for chronic periodontitis where most commonly the molar teeth are affected in the first instance. There was a positive correlation between periodontal condition and age in diabetic patients but not in healthy controls in this age group.

Pinducciu et al. (1996) examined 131 T1DM but only 20 healthy controls. By using the Community Periodontal Index of Treatment Need (CPITN), these workers reported that 16% of the diabetic patients had a score of 3 or 4. This means that at least one tooth had a clinical probing depth >3.5mm however this does not give the reader any further information about the extent or severity of disease. CPITN scores of 3 or 4 were seen in 44% of diabetic patients over 35 years of age, compared with 12.5% of the controls. Given the small size of the control group it is not surprising that the above findings lacked statistical significance. The authors in turn concluded that there was little difference in periodontitis prevalence between this group of well controlled diabetic patients and healthy controls. If this study had been adequately powered it is possible that statistically significant differences would have been seen; however, it is also possible that this result is entirely spurious due to inadequate sample size.

Recently another study in New York reported that there was an increase in plaque index, gingivitis and clinical attachment loss in a large case controlled study of adolescents with diabetes (94% T1DM) (Lalla et al., 2006a,b). In this study only two quadrants of the mouth were examined. This is not the optimal method of examination and it has been suggested that such partial mouth recordings may significantly underestimate the true prevalence of periodontitis (Susin et al., 2005). This would, however, of course apply equally to both cases and controls in the analysis. This study had a mixture of races represented although these were similarly distributed in the case and control groups. It is impossible to quantify what influence racial predilection for periodontal disease will have had on the final analysis. The level of disease reported in this study was very low and the control subjects were all patients at a paediatric dental clinic. It is possible that selection bias introduced in this way would produce a control group with less periodontal disease than the corresponding local population. A second paper by the same group reported that there was a strong association between mean HbA1c and periodontitis after controlling for age, gender, ethnicity, frequency of prior dental visits and dental plaque (Lalla et al., 2007)

Since the systematic reviews by Khader et al. and Chávarry et al. there have been seven further studies in T1DM. These have been primarily on children and adolescents and therefore do not address the concerns that were raised by these authors about the conclusions that can be drawn from subjects in this age group.

Al-Shammari et al. (2006), in a small study with only 72 T1DM subjects, showed that patients with one or more diabetic complications had a higher risk of periodontal disease. They also reported that smoking was associated with diabetic complications and severe CAL ≥ 7 mm. Luczaj-Cepowicz et al. (2006) examined a group of 50 well controlled diabetic adolescents, with a mean age of 14 years, and 50 matched control subjects. There were no differences in the periodontal disease index between the groups. However there were differences in the PI, the sulcular bleeding index and the periodontal index in the T1DM group. The level of disease was very low in both groups. Dakovic et al. (2008) also investigated a group of T1DM adolescents and showed that diabetes conferred an increased OR of 2.78 for the presence of periodontitis. Plaque scores and gingival inflammation were increased and periodontitis was associated with mean HbA1c, duration of diabetes and bleeding/plaque ratio. Orbak et al. (2008) also showed that children as young as nine years had increased gingival inflammation compared with NDS (n=50 T1DM, 50NDS). Silvestre et al. (2009) examined 180 subjects (T1DM 90, NDS 90) and showed that the T1DM subjects had higher bleeding index, deeper periodontal pockets and more periodontal attachment loss than NDS. Poor glycaemic control and the presence of diabetic complications were associated with higher bleeding index and clinical probing pocket depths. Kaur and co-workers in a study of 145 T1DM patients showed using linear multivariable regression that there was a significant association between T1DM and mean attachment loss. Although the modelling accounted for smoking, 40.7% of T1DM were current smokers and a further 25.5% were previous smokers (Kaur et al.2009). Xavier et al. (2009) looked at predictors of periodontitis in T1DM with no control group. These authors concluded that poorly controlled T1DM patients had higher CPD and CAL than better controlled T1DM. Finally a study published by Saes Busato et al. (2010) reported in another adolescent population that the mean Community Periodontal Index (CPI) was higher in well and poorly controlled T1DM subjects. However, the CPI has limited application in studies reporting the prevalence of periodontitis because it does not record clinical attachment level and does not distinguish between true and false pocketing.

1.3.6.2 Longitudinal studies

Seppala et al. (1993, 1994) carried out a 2 year longitudinal study in T1DM adults. Only 22 of the original 38 diabetic patients examined at baseline were available for assessment at the 24 month follow up. There were higher levels of clinical probing depth, gingivitis scores, clinical attachment level, recession and radiographic signs of approximal bone loss

in the poorly controlled T1DM. These findings were confirmed in a further site by site analysis of the same data. The site by site analysis showed that the greatest difference was found when only the canines were analysed. Overall these differences could not be accounted for by local plaque control as plaque levels showed no differences between the well and poorly controlled groups. It is also worth noting that the mean HbA1c score of the well-controlled T1DM patients was poor. This may mean that those patients who achieve target levels below 7.5% could be at even less risk of periodontal complications than this study suggests. It may also be the case that the cut-off point in HbA1c level for the development of periodontal complications is higher than for other systemic complications. The latter has been established by the large prospective diabetic complications studies (DCCTRG, 1993; Stratton et al. 2000). The conclusions of this study are limited by its small size and the fact that no smoking data were presented.

In another longitudinal study of T1DM subjects between 9 and 17 years old, Sbordone and co-workers (1995) examined 16 T1DM and 16 cohabiting healthy siblings. No differences in periodontitis levels were detected either at baseline or at any of the three follow up time points. A number of criticisms can be levelled at this study namely: the small sample size; the age of the subjects; and the use of cohabiting siblings as a control group. The latter share the same environment and a similar genetic make-up, which may influence their susceptibility to diabetes and periodontitis. It is documented that periodontal pathogens can be transmitted within families conferring a similar microbiological profile and associated risk (Petit et al., 1993a,b).

Firatli et al. (1997) examined the relationship between periodontal status and the duration of IDDM in 44 T1DM children and adolescents in a longitudinal study lasting five years. Clinical attachment loss was associated with duration of diabetes and over a five year period the mean CAL increased from 2.39mm to 3.51mm. These findings are in agreement with those of Novaes et al. (1997) who reported that over a ten year period probing depths increased in diabetic patients.

1.3.6.3 Radiographic analysis

Rutledge et al. (1940) reported a prevalence of periodontitis of 40% in a group of T1DM using radiographic bone levels to determine presence or absence of disease. However no control patients were examined. Sheppard et al. (1936) showed that there was a high

prevalence of periodontal destruction seen radiographically in a population of hospitalised patients with insulin-dependent diabetes mellitus. Interestingly, and in agreement with later studies, he did not detect any signs of radiographic bone loss in patients less than 15 years of age, although the numbers in this category were low. However, in patients older than 20 years of age, mild alveolar bone loss was ubiquitous and moderate to severe bone loss was seen in 56% of subjects. These patients were hospitalised with a variety of medical conditions including tuberculosis and this limits the applicability of the results to the ambulatory population.

Bernick and co-workers (1975) examined 50 diabetic children less than 16 years of age and compared their oral health with 36 healthy controls. They reported that there was an increase in radiographic bone loss in the diabetic patients while plaque levels were similar. The increase in radiographic signs of bone loss is remarkable given the young age of the sample population and the relatively short duration of diabetes experience. These findings are in disagreement with another study which reported an absence of periodontitis in a population of 45 T1DM adolescents aged between 10 and 18 years (Barnett 1984). These conflicting results may be due to differences in the definition of radiographic bone loss or due to real differences between the periodontal experience of the study populations, based on other protective or predisposing factors.

Hugoson et al. (1989) examined 82 long duration, 77 short duration diabetic patients and 77 healthy controls (20-70 years of age) and reported increased radiographic bone loss. This finding was only statistically significant in the 40-49 year age group. This study may have contained a number of patients who would currently be classified as having type 2 diabetes mellitus because they were late onset patients, who had become dependent on insulin. The paper also fails to report the prevalence of smoking in the groups.

Pinducciu et al. (1996) utilised both clinical and radiographic examination to ascertain the prevalence of periodontitis in T1DM by examining 131 T1DM patients and 20 healthy controls. The CPITN, gingival index (GI), mobility score, simplified oral hygiene index (OHI-S) and radiographic examinations were used. An increase in GI, mobility and CPITN score were reported in T1DM. In this study, radiographic signs of alveolar bone loss were not seen in the 5-15 year age group. In the 15-25 year age group, 8.3% of T1DM patients showed radiographic signs of bone loss; for 4.5% of them it was severe (>30% of

root length). In the 26-35 year age group, 5.2% showed signs of radiographic bone loss; for 3% it was severe. In the > 35year age group, 9.1% showed signs of radiographic bone loss and for 5.3% it was severe. The control group showed no signs of radiographic bone loss in subjects less than 35 years old. In patients over 35 years old the prevalence was 12%, for 2% of them it was severe. This study examined both children and adults. The size of the control group in this study was entirely inadequate.

Another study reported that alveolar bone loss in T1DM patients was increased in subjects between 24-36 years of age (average age 29 years). Thirty five T1DM and 10 healthy controls were examined radiographically and alveolar bone loss of >15% of root length was considered the cut off for an increase in bone loss (Tervonen et al., 2000). The authors found no differences between the well-controlled, moderately well-controlled and the non-diabetic subjects. There was a non-significant increase in the radiographic signs of bone loss in the poorly controlled diabetic patients. A larger study of similar design would be required to confirm this inference.

Radiographic analysis as an adjunct to clinical examination in the diagnosis of periodontitis is a useful tool. However, the bone loss must be significant enough to be measured and it is important that well recognised definitions and criteria are used. Radiographs may not be a good tool for measuring incipient bone loss and this may be why some studies utilising radiographs have not detected any differences in the youngest groups whose disease levels are very unlikely to be severe enough to be detected radiographically.

1.3.6.4 Conclusion

Despite anecdotal clinical impressions a review of the available literature does not unequivocally support the widely held belief that T1DM increases a patient's risk of experiencing periodontal destruction. Limitations in sampling; heterogeneity of case and control groups; lack of standardisation in classification both of diabetes and periodontitis; failure to adequately control for confounding factors; and a predilection for studies in adolescents with low levels of periodontitis have meant that firm conclusions cannot be drawn. In the meta-analysis published by Chávarry and co-workers (2009), the authors concluded that there was a need for further studies in adults with T1DM controlling for age, sex, socioeconomic status, glycaemic control, health attitudes and behaviour and smoking in order to confirm this hypothesis.

1.3.7 Mechanisms of periodontal damage in T1DM

In general, the mechanisms of periodontal tissue damage are similar to the mechanisms of microvascular and macrovascular disease in diabetic patients. The periodontium is a complex highly vascularised end organ which is similar to the retina or glomerulus. The current model of the pathogenesis of periodontitis involves the interaction between the microbial challenge, the host immuno-inflammatory response, connective tissue and bone metabolism, and the influence of environmental and genetic risk factors on these processes leading to clinical signs of disease (Kornman, 2008).

It has been postulated that Type 1 diabetes can impact on this process at a number of levels outlined in Figure 1-2.

1.3.7.1 Microbial complexes

It has been postulated that the microbial flora of diabetic periodontitis is significantly different from that of chronic periodontitis. Studies evaluating the microbial flora of periodontal disease are complicated by the diversity of the oral flora and dental plaque in health and disease (Socransky and Haffajee, 1994). While it is reported that the oral cavity harbours hundreds of species of bacteria, many of these are uncultivable, limiting the applicability of conventional culture studies (Mandell et al., 1992; Sbordone et al., 1995, 1998; Thorstensson et al., 1995; Takahashi et al., 2001). Molecular techniques utilising species specific primers to bacterial DNA are improving and can now be used for relative quantification of uncultivable bacteria (Salvi et al., 2005; Lalla et al., 2006b). This technique is, however, limited by the fact that unless messenger RNA (mRNA) is used, non-viable bacteria, which may no longer be involved in the destructive process, are also detected. It is possible to only look for or report the presence of a small number of species of bacteria which have been shown in other studies to be associated with periodontitis. By taking this approach simple answers are generated, but investigators may be asking the wrong questions.

A further limitation to microbial sampling of the subgingival biofilm is that we are sampling the bacteria that are present in established disease states but are not able to show which bacteria were the initiating early colonisers. It is possible that these early colonisers may be responsible for initiating the process of periodontal destruction leading to an

altered ecology and subsequent colonisation of the resultant periodontal pocket with opportunistic pathogens. This issue could of course only be fully documented in a long term prospective study of a healthy cohort with plaque samples taken at every stage in the process over a number of months or years and analysed using molecular techniques.

Bearing in mind the limitations described above there have been a number of studies over the last thirty years that have investigated alterations in the subgingival micro-flora in diabetic periodontitis with conflicting results.

One of the earliest microbial studies on the dental plaque of T1DM patients was by Sanchez-Cordero and co-workers (1979). They utilised conventional culture techniques and showed that increased amounts of staphylococci were found in the subgingival plaque of diabetic periodontitis patients compared with non-diabetic subjects without periodontitis. No further work has been done to either reproduce or develop this finding due perhaps to it being well established that Gram negative anaerobes are largely responsible for chronic periodontitis. Other staphylococcal infections are also more common in diabetic patients with staphylococcal involvement in nasal colonisation, skin infections and delayed wound healing (Tuazon et al., 1975; Graham, 2006). It is possible that staphylococcal infection of the periodontium could lead to periodontal tissue damage leading to loss of attachment. Staphylococci have not been consistently found in either culture or molecular investigations of diabetic periodontal disease.

Mashimo and co-workers (1983) reported that there was an increase in *Capnocytophaga* species and anaerobic vibrios in the subgingival plaque of T1DM patients. They reported that this was significantly different from the microbial flora of patients with localised juvenile periodontitis and chronic periodontitis. This finding was confirmed by Ciantar (2005) who reported an increase in the carriage rate of *Capnoctophaga* species at diseased sites in diabetic patients. Other authors have not been able to reproduce this finding (Sastriwijoto et al., 1989).

Sandholm and co-workers, in an early microbiological study (1989), used only microscopy techniques and concluded that the subgingival microbial flora of juvenile diabetic patients was different. However, the methods used in this study make interpretation of the data very difficult as no bacteria were identified by any other means. It was also reported that

the diabetic patients had more periodontitis than the healthy controls. This being the case, it is not possible to conclude that these differences were as a result of the diabetes when they could result from the different periodontal status of the subjects involved.

Thorstensson and co-workers (1995), in a study of 28 adults with T1DM and 24 non diabetic subjects, reported few microbiological differences apart from an increase in *P. gingivalis* carriage which was higher in diabetic patients. There was no increase in the presence of *P. gingivalis* in deepened pockets of diabetic subjects although this was the case in non-diabetic subjects. This may indicate that *P. gingivalis* was more common in non-diseased sites in diabetic patients as well as the diseased sites. Takahashi et al. (2001) reported that there was an increase in *P. gingivalis* carriage in 12 young people with T1DM and periodontitis. Unfortunately the control group in this study was a periodontally healthy non diabetic control group. No conclusions could therefore be drawn about how periodontal microbiology differs between patients with periodontitis with or without T1DM.

There have been a number of studies that have found no differences in the microbiota of periodontal disease in patients with and without T1DM. Pinduccui et al. (1996) in a study of 131 insulin dependent diabetic patients and 20 controls found no differences in either the aerobic or anaerobic flora. Sastrowijoto and co-workers (1989) showed that there were no differences between the well and poorly controlled diabetic patients. Another study examined the dental plaque of diabetic and non-diabetic siblings over three years and did not detect any differences (Sbordone et al., 1998).

In the most comprehensive study in this area Lalla et al. (2006b) studied 50 adult type 1 diabetic patients and 50 healthy controls that were matched for age, gender and periodontal status. Using a 16s DNA checkerboard technique detecting 12 periodontal pathogens in 8 samples per patient there were differences only in the levels of *Eubacterium nodatum*. The IgG antibody response to these bacteria was similar in both groups.

A review of the preceding literature indicates that there are significant deficiencies in the studies carried out to date. Most studies are limited by inadequate culture techniques, poor matching and small sample size. No single species or group of species has emerged as potential diabetes-specific periodontal pathogens. This would lead us to conclude, on the

basis of the limited available evidence, that there is no significant microbial difference in the dental plaque of T1DM subjects compared with NDS. Further work in adequately powered studies utilising advanced molecular techniques may be required in order to demonstrate whether an altered microbial challenge is responsible for any observed increase in clinical disease.

1.3.7.2 Host response and bone and connective tissue turnover in Type 1 diabetes mellitus

If, as the literature suggests, there are few differences in the subgingival microbiota between diabetic and non-diabetic patients with periodontitis, alterations in the host immuno-inflammatory response to potential pathogens may play a predominant role in the pathogenesis of diabetes associated periodontitis.

Salvi et al. (2005), in a 21 day gingivitis model, demonstrated that there was no difference in the microbiology of the developing lesion, but that diabetic patients showed an earlier and more pronounced inflammatory response to comparable bacterial challenge.

The host-parasite interaction begins at the basic level of the innate immune system. Antimicrobial peptide levels could be altered in the GCF or saliva of T1DM patients leading to evasion of the innate immune system by the periodontal pathogens (Jurevic et al., 2003; Cabras et al., 2010). Modification of TLRs in diabetes could mean that there are alterations in the initial innate response as well as communication between the innate immune system and the adaptive immune system resulting in a prolonged and dysregulated inflammatory response (Devaraj et al., 2008). T1DM has been shown to result in impairment of neutrophil adherence, chemotaxis, and phagocytosis, which may facilitate bacterial persistence in the periodontal pocket and significantly increase periodontal destruction (Manouchehr-pour et al., 1981, McMullen et al., 1981, Gustke et al., 1998). While neutrophils are often hypofunctional in diabetes, especially where glycaemic control is poor, these patients may have a hyper-responsive monocyte/macrophage phenotype, resulting in significantly increased production of pro-inflammatory cytokines and mediators (Salvi et al., 1997a, 1997b). This hyper-inflammatory response could result in elevated levels of pro-inflammatory cytokines in the gingival crevicular fluid. It has been shown recently, in a human 21 day gingivitis study, that the presence of pro-inflammatory cytokines emerged earlier and at higher levels in diabetic subjects compared with controls

(Salvi et al., 2010). Elevated serum levels of inflammatory mediators may be reflected in similarly elevated levels of these mediators in GCF. The level of cytokines in the GCF has been related to the level of glycaemic control in diabetic patients. In one study of diabetic subjects with periodontitis, those with HbA1c levels >8% had gingival crevice fluid levels of interleukin-1 β almost twice as high as subjects whose haemoglobin A1C levels were <8% (Engbretson et al., 2004). The net effect of these host defence alterations in diabetes is an increase in periodontal inflammation. These pro-inflammatory cytokines lead to inflammatory destruction of the periodontal ligament and bone by matrix metalloproteinases in a RANKL dependent pathway leading to attachment loss, and alveolar bone loss (Salvi et al., 2010).

The cause of this increase in pro-inflammatory cytokines may lie in the formation of advanced glycation end-product within the periodontium and their deleterious effects on other organ systems (Schmidt et al., 1996). Interactions between AGEs and RAGE lead to the production of pro-inflammatory cytokines as a result of receptor activation. Increased inflammation may also result from increased levels of reactive oxygen species and the associated tissue damage caused by oxidative stress. It is also possible that circulating levels of pro-inflammatory cytokines could be involved in having a local effect on the periodontium. T1DM patients are in a chronic inflammatory state and serum markers of inflammation are increased such as IL-6, IL-1 β and TNF α (Aribi et al., 2007, Sahakyan et al., 2010a,b). It is hypothesised that the high levels of proinflammatory cytokines in the circulation could exert an effect locally in the periodontium, exaggerating, or enhancing, the immuno-inflammatory response to the subgingival biofilm. This theoretical link is supported by the fact that periodontitis is increased in obese subjects who also exhibit low levels of systemic inflammation and circulating adipokines (Gupta et al., 2010, Hajri et al., 2011). Matrix metalloproteinases are critical components of tissue homeostasis and wound healing, and are produced by all of the major cell types in the periodontium (Ryan et al., 1996). Production of matrix metalloproteinases, such as collagenases, is increased in T1DM patient and is associated with the development of other diabetic complications such as retinopathy and renal disease (Mohammad et al., 2010; Van der Zijl et al., 2010). Alterations in the levels of MMPs result in changes in collagen homeostasis and wound healing within the periodontium. As the evidence for a viral aetiology of both T1DM and periodontitis increases, it is also proposed that susceptibility to viral infection could be a common pathway for the development of periodontitis in T1DM (Jaidane et al., 2010;

Slots, 2010). In addition, there is some evidence from the rat model that reduced capacity for bone repair following inoculation with periodontopathic bacteria could also be a factor in net periodontal bone loss in T1DM (He et al., 2004; Liu et al., 2006). Liu et al.(2006) suggested diabetes enhanced net periodontal bone loss through a combination of increased bone resorption and reduced bone formation. Diabetic patients have been shown to be prone to bone loss throughout the skeleton and bone healing is also reduced in T1DM (Ghandi et al., 2005).

1.3.7.3 Host genetic variation

Candidate gene studies, and genome wide analysis in T1DM, have revealed that the most important of these loci is the HLA class II region on chromosome 6p21 which accounts for 50% of familial aggregation through protective and detrimental effects (Noble et al., 1996, Ounissi-Benkhalha and Polychronakos, 2008). HLA class II antigens are responsible for self versus non-self recognition and antigen presentation to CD4+ T cells. The DR and DQ genes have the greatest influence with certain combinations being of particular importance either conferring susceptibility or resistance. To date, candidate genes have been identified which manifest their effects through defects in T cell maturation, activation and self-tolerance, leading to increased autoimmunity, as well as through defects in antigen presentation and response to viral triggers leading to alterations in self/non self recognition (Morran et al., 2008; Ounissi-Benkhalha and Polychronakos, 2008). Defects in the innate immune system through TLR2 have been reported in type 2 diabetes mellitus and TLR3 polymorphisms have been reported in T1DM (Pirie et al., 2005). In addition, a recent study has shown that the IL-6(-174) genotype is associated with periodontitis and T1DM (Raunio et al., 2010).

It is possible that susceptibility to viral and bacterial infection could affect periodontal risk in T1DM. Genetic defects in the developing immune system, susceptibility to viral and bacterial infection or an altered inflammatory response could all account for an increased genetic susceptibility in T1DM.

1.3.7.4 Behaviour

It is also possible that there is another simple explanation for the increase in periodontitis in poorly controlled T1DM patients. Many studies have shown that oral hygiene is almost universally poorer in poorly controlled diabetic patients compared with well controlled

diabetic subjects and NDS. It is possible that the increase in periodontal disease is due, at least in part, to poor oral hygiene and an increase in the main aetiological agent. This may be due to psycho-social factors affecting motivation and interest in compliance with general as well as oral health advice (Thorstensson et al., 1989a; Syrjala et al., 1999 ; Knecht et al., 2000,2001) or it may be due to alterations in the saliva leading to increased plaque accumulation (Albrecht et al., 1988; Novaes et al., 1991; Bridges et al., 1996; Firatli et al., 1996; Lalla et al., 2006a).

1.3.7.5 Conclusion

There are a number of mechanisms through which T1DM could confer an increased risk of periodontal destruction. These include alterations in the psychological make-up of poorly controlled diabetic patients, alterations in collagen homeostasis and wound healing, alterations in osteoimmunology and increased tissue damage due to an hyper-inflammatory response to a common or subtly different subgingival biofilm.

1.4 Dental caries

1.4.1 Introduction

Dental caries is one of the most common infectious diseases affecting mankind today (Peterson, 2003). Dental caries can be defined as the destruction of dental hard tissues due to acidic and proteolytic attack. The caries process occurs in the biofilm where acidogenic bacteria produce local pH reduction sufficient to cause dissolution of the hydroxyapatite crystals in enamel with resultant demineralisation. The surface zone is a zone of constant flux where remineralisation and demineralisation are occurring constantly but it is only when this finely balanced equilibrium is disturbed that the lesion progresses. If the caries process continues for long enough, and sufficient mineral is lost, progressive porosity and weakening of the tooth structure leads to cavitation. When the lesion extends to the amelodentinal junction the carious lesion will begin to spread laterally, this undermines the enamel and further alterations in the optical properties of the tooth structure occur (Ekstrand et al., 2001). Dental caries may now be detected clinically as either grey/pink/bluish darkening of the dentine. The likelihood of enamel cavitation increases with the increasing size of the dentinal lesion (Fejerskov, 1997). Dental caries is a

complex, multi-factorial disease, known to be influenced by behavioural and dietary factors (Marsh and Percival, 2006).

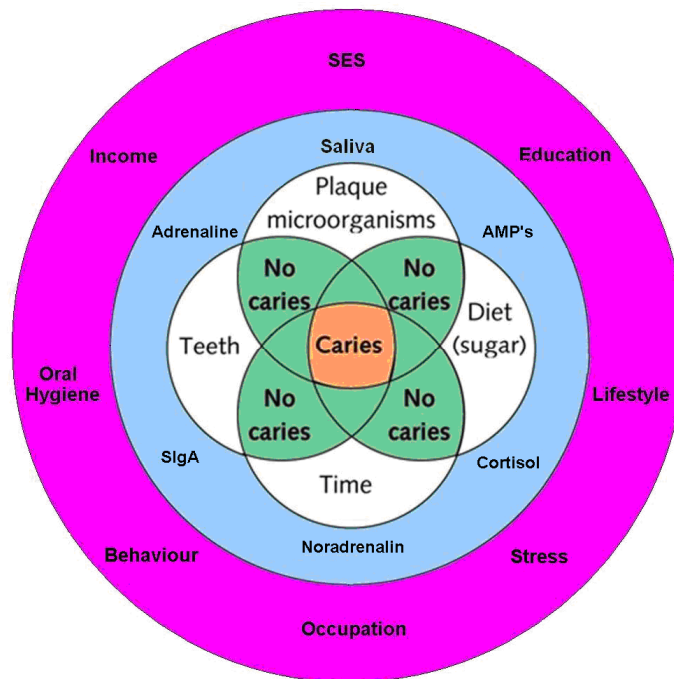


Figure 1-3 Venn diagram showing the multi-factorial nature of caries development (Used with permission Jennifer Malcolm)

1.4.1.1 The dental biofilm

In order for caries to occur a susceptible tooth surface needs to be colonised by a pathogenic biofilm with an adequate source of fermentable carbohydrate for a prolonged period of time. Caries tends to occur in sites of stagnation and is rare on self-cleansing smooth tooth surfaces, except in patients at high risk of caries. The tooth may therefore be susceptible due to its anatomy (normal or disordered formation), iatrogenic factors such as restoration margins and damaged enamel surfaces or due to its local environment such as a reduction in quantity or quality of saliva (Dawes et al., 2008). Caries can be classified according to the site at which it occurs, the tissue which it affects, whether it is primary or recurrent or the activity of the lesion.

Dental plaque is a biofilm which is adherent to tooth structure and its precursor the pellicle. It is made up of a mixture of glycoproteins which begin to form within minutes of toothbrushing. The biofilm is a microenvironment where millions of bacteria live a symbiotic existence. The nature of the biofilm offers protection for the plaque bacteria from the non-specific immune response of the host as well as from chemicals and antibiotics. The early colonisers in dental plaque include *Streptococcus mitis*,

Streptococcus salivarius and *Streptococcus oralis*. These form the initial adherent layer which facilitates the development of the mature plaque, with its more pathogenic bacteria. The main bacterial species thought to be responsible for dental caries is *Streptococcus mutans* although lactobacilli are also involved in the caries process (Takahashi and Nyvad, 2008). Salivary *Streptococcus mutans* and lactobacilli counts are related to caries activity and indicate high caries risk (SIGN, 2000). The characteristics of *Streptococcus mutans* that make it a potent cariogenic bacteria, are that it is aciduric, acidogenic, has the ability to adhere to salivary glycoproteins and produces extracellular polysaccharides (Russell, 2009). It is accepted that in order for caries to develop it is essential for plaque to be present in which are encased the cariogenic bacteria capable of rapid and prolonged acid production.

1.4.1.2 Source of fermentable carbohydrate

Dental caries occurs as a result of fluctuations in the pH of the dental plaque in response to the availability of fermentable carbohydrates as a substrate for the plaque bacteria. Frequent snacking on sugary foods, or even simply eating food with low cariogenic potential, frequently will increase the development of the carious lesion (Faine et al., 1992). Any fermentable carbohydrates can act as a food source for these bacteria. Sucrose is the most potent of the extrinsic sugars, but lactose and fructose can also produce significant reductions in plaque pH. The oral environment is in a constant state of flux with changes in local pH occurring every time food is ingested. Epidemiological evidence for the association of dental caries with consumption of sugars comes from studies in populations with limited access to fermentable carbohydrate. These include the Eskimo population and children raised under strict sugar free diets i.e. the Hope Wood House study in Australia. The caries reduction seen in Europe during the second world war as a result of sugar rationing of the population also provided evidence (Toverud, 1949; Harris, 1963; Costa, 1980). The Vipeholm and Turku studies clearly demonstrated the effect of varying frequency and type of sugar on the development of caries (Gustaffson et al., 1954; Scheinin et al., 1974). A recent systematic review of the role of sugar in the development of dental caries showed that, of the 36 papers that met the authors' inclusion criteria, only two showed a strong relationship between dental caries and dietary sugars, 16 showed a moderate relationship and 18 showed weak or non-existent relationship between the two. The authors concluded that the impact of widespread systemic and topical fluoride had

changed the fundamental relationship between dental caries and sugar (Burt and Pai, 2001).

1.4.2 Caries risk assessment

Guidelines produced by the Scottish Intercollegiate Guidelines Network (SIGN) have identified the following factors as being implicated in the development of caries and should be considered in the management of an individual's risk (SIGN, 2000). The risk of new carious lesions is highest in the young, although caries can also develop at any point throughout life. An individual's risk of caries alters throughout life, as social and dietary habits change. In addition, changes within the salivary glands and exposure of cementum and dentine, through gingival recession and periodontitis, also influence susceptibility to caries. Dental caries is also reduced by removal of the dental biofilm through tooth brushing and the topical application of fluoride in the form of fluoride toothpaste. Caries prevalence is increased by infrequent toothbrushing (Treasure et al., 2001).

As with most diseases, dental caries is most prevalent in deprived groups. Socioeconomic status is a strong predictor for dental decay (Sisson, 2007). Studies in Scotland have shown that patients who are in low socioeconomic groups are at increased risk (Radford et al., 2001). This may be due to low education, poor parental support, poor diet, low dental awareness and irregular dental attendance. Reduced access to dental services may be due to financial restrictions or problems with work or child-care (Hilton et al., 2007; Milsom et al., 2009; Goeterns et al., 2010; Boyce et al., 2010; DiMarco et al., 2010). Patients who have low dental awareness and low dental motivation, due to psychological differences in interacting with and responding to general and oral health advice, are at increased risk of dental decay (Gregory et al., 2007; Syrjala et al., 2004)

Patients who are medically compromised may be at higher risk of developing decay. This may be due to xerostomic medications, lack of self-care, difficulty attending for dental treatment or the use of sugar containing medications. The presence of prosthodontic or orthodontic appliances within the mouth will tend to increase the risk of dental caries, due to plaque stagnation sites, as well as alterations in the oral microflora (Sugihara et al., 2010).

The saliva normally has a protective effect on the oral cavity. Any reduction in salivary flow rate, or buffering capacity, will predispose the individual to caries development. It is also clear that those patients who suffer from reduced salivary gland function, due either to the effect of head and neck irradiation, medication or autoimmune diseases such as

Sjogrens syndrome, are extremely susceptible to caries which can be rapidly destructive (Schwarz et al., 1999; Boutsis et al., 2000; Leung et al., 2004; Flink et al., 2007). As well as its buffering capacity saliva may have a significant role in the prevention of dental caries through the antimicrobial effect of salivary proteins. Specific antibacterial proteins and secretory immunoglobulin A (sIgA) play a role in protection of the teeth from the colonisation and maturation of plaque bacteria (Wallengren et al., 2004).

1.4.3 Caries Diagnosis

It has been difficult, in the past, to record caries at the pre-cavitation level, due to difficulties in standardisation and reproducibility. For this reason caries into dentine, also referred to as the D3 level has been the most frequently used measure of caries in epidemiological studies (Pitts et al., 2004; Schulte et al., 2006; Eitner et al., 2006; Ferro et al., 2007). This involves only scoring cavitated lesions and lesions that are clearly into dentine as shown through spreading underlying staining. While this is clearly not a sensitive measure of all caries experience, it is nonetheless, a standard and valid method of recording dental caries which has been used for decades. New developments, such as quantitative light fluorescence (QLF) or fiberoptic transillumination (FOTI) are available but would be difficult to apply to a large scale cross-sectional study on a full mouth basis (Cortes et al., 2003; Ferreira Zandoná et al., 2010).

1.4.4 Current trends

The prevalence of dental caries has reduced significantly over the last fifty years in the developed world (Reich et al., 2001). Scotland, despite its extremely high prevalence of dental disease, has also benefited from a reduction in decay and tooth loss since national surveys began in 1972. In 1972, the level of edentulousness was 44%, however this figure had dropped to 18% by the most recent survey in 1998. There has also been a significant increase in the number of retained teeth from 21.6 to 23.8 in 1978 and 1998 respectively. Carious, or unsound teeth, reduced from 2.1 per dentate adult in 1978 to 0.9 in 1998 and there was also a corresponding increase in the number of sound and unfilled teeth. New criteria, introduced in 1998 to include visual caries, indicated that 29% of the population had 18 or more sound teeth and 58% of the population had at least one or more decayed or unsound teeth. Overall 24% of dentate adults had visual primary caries, 22% had primary cavitated caries and 8% had recurrent caries. The mean number of missing teeth was 7.5

and the number of teeth that were filled, but otherwise sound, was 8.1. The average number of virgin teeth was 15.7 (Nunn et al., 2001).

The burden of oral disease is unequally distributed throughout the population with an increased prevalence in areas of low socioeconomic status. Living in the north of Great Britain is associated with poorer dental health. Subjects from the north of England were 1.86 times more likely to have some untreated decay. Those living in Scotland had almost two teeth less than those from southern England even after various risk factors were included in a multivariable analysis (Treasure et al., 2001). Caries experience was shown to be associated with brushing less than once a day, lower social class, male gender and irregular dental attendance (Treasure et al., 2001). The survey reports that there is almost 50% less decay in those who attend for regular dental check-ups than those who only go to a dentist when they have trouble with their teeth (Nunn et al., 2001).

1.4.5 Dental caries and Type 1 diabetes mellitus

There have been a number of studies investigating the prevalence and incidence of dental caries in T1DM. This has been a focus of interest because diabetic patients are advised to eat a healthy reduced sugar diet which, taken in isolation, would be expected to result in reduced caries experience. This, however, has not been a universal finding, implicating other factors in the development of caries in this population. Biological plausibility has been hypothesised to explain both the studies finding a reduced and increased caries prevalence. Factors favouring an increased risk include increased glucose concentration in saliva and GCF, a reduced salivary flow rate resulting in reduced buffering and remineralisation and increased frequency of food consumption throughout the day. Studies have also shown a tendency to increased plaque scores in diabetic patients compared with controls (Albrecht et al., 1988; Novaes et al., 1991; Bridges et al., 1996; Firatli et al., 1996; Lalla et al., 2006a). An increase in the volume or nature of plaque could predispose patients with T1DM to the development of dental caries.

1.4.5.1 Study quality

While there have been several studies in this area, there are problems with the validity and reliability of methodology and examiner calibration, and data collected via retrospective examination of dental records. It is also notable that some studies do not have a valid control group and there is a significant risk of selection bias. Some studies have used a convenience sample of T1DM adolescents attending a diabetes summer camp. These

children were closely monitored and had a family history of diabetes. It is possible that this group did not represent a cross section of diabetic adolescents; confounding social factors therefore make interpretation and application of these data difficult. The validity of this kind of sampling of potentially highly motivated diabetic subjects is poor given that they are unlikely to be representative of the general T1DM population (Ziskin, 1944; Goteiner et al., 1986).

Wegner and co-workers in 1971 examined a group of 620 T1DM patients and did not find any reduction in caries prevalence. This study did not have a control group and relied on comparisons with national statistics. Mattson and co-workers (1975) examined only 33 T1DM adolescents and found a reduced number of decayed or filled surfaces (DFS), 13 in the diabetic patients and 20 in the controls. However, this small study did find a delay in premolar eruption in T1DM patients. This could have accounted for a reduced DFS as these teeth would have been exposed to the oral environment for less time than those of the control subjects. Another study reported that the T1DM patients had an increased DMFT; however, the decayed element of this was reduced and the number of filled teeth was increased (Albrecht et al., 1988). This may indicate that patients with diabetes had a similar level of historical decay experience. However, they appear in this study population to have had access to a higher level of care than the control group.

Akyuz and co-workers (1990) also found a reduction in caries level in a T1DM population. However, this paper reported an increase in caries in the poorly controlled patients. This study only included 42 patients with diabetes and 20 control subjects, again limiting the validity of the findings. A further study in Ireland, a country with a high prevalence of caries, reported that there was a reduced prevalence of caries and a higher restorative index in a T1DM adolescent population (Kirk et al., 1991). This was based on comparisons against reported national figures and no control group was examined. The patients with diabetes were also from higher socioeconomic groups which would have affected their initial caries risk. Interestingly, caries was reported more frequently in the group of patients with diabetes who were diagnosed later in life, compared with those diagnosed earlier. Taveres and co-workers (1991) reported in a study of 273 patients, 88 of whom had T1DM, that there was an increase in missing teeth in T1DM. However, there was a reduction in coronal caries, no difference in root caries and an increase in the number of restored root surfaces. It is unknown whether the restorations on the roots were placed for caries management or for other reasons.

Karjalainen et al. (1997) in a study of 80 T1DM patients reported an increase in caries in poorly controlled patients compared with those who were well or moderately controlled. This study also reported that intact dentitions were more common in the non-diabetic control group. An association between the level of metabolic control and caries experience was confirmed in studies by Galea and co-workers (1986), Twetman et al. (1992, 2002, 2005) as well as Syrjala et al. (2003) and Siduikiene et al. (2006) who reported similar findings. Galea and co-workers reported that caries experience varied throughout the mouth with an increase in the incisor region and a reduction in the molar teeth. Caries prevalence was also related to metabolic control (Galea et al., 1986).

Tenovuo and co-workers (1986) compared a T1DM population with an age and sex matched group of healthy controls and found that although there were a lower number of filled surfaces, caries increased in older T1DM patients. Edblad and co-workers (2001) found that there was a significant increase in the caries experience of young adults with T1DM and Moore et al. (2001a) also reported an increase.

There have however been a number of studies that have found no differences in caries levels in T1DM populations (Harrisson and Bowen, 1987; Bacic et al., 1989; Twetman et al., 1989; Swanlung et al., 1991; Pohjamo et al., 1991; Arrieta-Blanco et al., 2003; Chuang Shu-Fen et al., 2005; Lalla et al., 2006a; Do Amaral et al., 2006; Patino-marin et al., 2008, Tagelsir et al., 2010).

1.4.5.2 Caries risk factors in Type 1 diabetes mellitus

Some studies have looked at a number of factors which may have an influence on the rate of caries. Syrjala and co-workers (2003) examined the microbiological parameters of the saliva of T1DM patients. They reported that there were no differences in *Streptococcus mutans* or lactobacillus counts between patients and controls, although there was an association between *S. mutans* counts, high HbA1c and caries. High salivary *S. mutans* counts are associated with active caries and it is likely that the increased *S. mutans* counts were related to increased caries activity rather than the diabetes *per se*. This is in agreement with the findings of Twetman et al. (1992) who also found that *S. mutans* and lactobacilli counts varied in response to caries rate rather than diabetic status. Swanlung and co-workers (1992) reported that *S. mutans* counts were increased in T1DM. Clinically there was no difference in the caries experience of the diabetes patients and the control subjects. In contrast, Tenovuo and co-workers (1986) reported that *S. mutans* counts were

slightly higher and suggested that this was a possible explanation for the higher caries prevalence in T1DM.

Salivary parameters have also been examined by investigators, with most finding differences in flow rates and constituents (Siudikiene et al., 2006; Moreira et al., 2009; Saes Busato et al., 2010). Health behaviour, oral health attitudes and practices have been examined in an attempt to account for possible differences (Syrjala et al., 2004).

1.4.6 Conclusion

While there are weaknesses in the studies to date, it seems impossible to categorise patients with diabetes as a homogenous group with regard to caries risk. Caries is a multifactorial disease and it may be that other factors confer caries risk and account for the heterogeneity of these study findings.

Studies utilising valid selection criteria and an appropriate control group are essential to eliminate confounding factors so that conclusions can be drawn about the effect of diabetes alone on the caries process. Many previous studies have utilised convenience samples for case and control groups thus limiting the applicability of published findings to the general population. The majority of these studies have been on children and adolescents with limited work on adults suffering from T1DM.

In light of the conflicting evidence and heterogeneity in the results reported in the literature it is not possible to reach firm conclusions about whether T1DM alters caries experience or not. It is likely that such is the multi-factorial nature of the disease that diabetes will only contribute to dental caries where other risk factors are present.

1.5 Oral mucosal abnormalities in T1DM

1.5.1 Introduction

Patients with T1DM suffer from a number of systemic complications including microvascular and macrovascular disease as well as an increased susceptibility to infection and poor wound healing (Daneman, 2006). They are also reported to suffer from reduced salivary flow rates, altered salivary constituents and higher salivary glucose concentrations (Banoczy et al., 1987; Cerutti et al., 1988; Borg et al., 1998; Aren et al., 2003). As a result of their general susceptibility to infection, thought to be caused by a number of reported immunological deficiencies, as well as salivary alterations, they are potentially at risk of

greater disruption to the normal oral flora and increased bacterial and fungal colonisation in the oral cavity (Soysa et al., 2005).

The oral flora is incredibly diverse. Traditionally both aerobic and anaerobic culture methods and selective agars were used for identification of species. More recently molecular testing for bacterial DNA or RNA has been used to understand better the presence and effect of uncultivable pathogens and also to identify and type bacteria and fungi phylogenetically. This was originally done using DNA checkerboard techniques but more recently 16S RNA or DNA has been used to identify genetic evidence of bacterial and fungal presence using conventional or real time PCR (Yuan et al. 2001). Refinements to these techniques have meant that amplification of bacterial DNA and identification via international gene banks is now possible. Novel species, hitherto unidentified, are being discovered with regularity.

The oral flora is affected by local and systemic factors most obviously seen with candidal infections, due to either dentures, steroids, HIV-AIDS, smoking or immunosuppressive therapies (Abu-Elteen & Abu-Alteen, 1998; Almstahl & Wikstrom, 1999; Bergmann 1991; Schmidt-Westhausen et al. 1990). Diabetes is linked to compromised immune responses in neutrophils (Wilson et al., 1986; Naghibi et al., 1987). In addition, it is possible that changes to the mucosal immune response and altered cell adhesion, may predispose diabetic patients to bacterial and fungal infection (Darwazeh et al., 1997; Willis et al. 2000a). It has also been reported that alterations in salivary glucose levels may alter the microenvironment to favour the colonisation of the mouth with an opportunistic microflora (Darwazeh et al., 1991). Adhesion to buccal epithelial cells by *Candida* species has also been shown to be increased in cells derived from diabetic patients (Darwazeh et al., 1990; Doroka-Boboskwa et al., 1996). Reduced salivary flow rates reported in diabetic patients (Banoczy et al. 1987; Chavez et al., 2001; Dorko et al., 2005) may limit the eradication of the colonising organisms.

1.5.2 Xerostomia

Xerostomia is the reduction in salivary flow rate and the resultant symptoms of dryness of which a patient complains. Saliva in health has lubricating, remineralising, and antimicrobial properties. Reduction in salivary quality and quantity can have a devastating effect on the dentition and oral mucosa, resulting in rapid carious destruction, mucositis and fungal infections.

Xerostomia is caused by a large number of drugs including, but not limited to, antidepressants, anti-hypertensives, anti-inflammatory agents and chemotherapeutic agents (Scully, 2003). A reduction in salivary flow is also seen in patients who have had previous radiotherapy involving the salivary glands in the field of exposure. Sjogrens syndrome an autoimmune disease affecting the salivary glands is also a significant cause of oral dryness and this can be very difficult to alleviate (MacFarlane & Mason 1974).

Normal salivary flow rates range from 0.3 to 0.5 ml/minute for unstimulated saliva and from 1 to 3ml/minute for stimulated saliva. A patient would normally be classified as having reduced salivary flow rate if the unstimulated rate is below 0.1ml/min and the stimulated rate below 1ml/min (Moore et al., 2001b).

Patients with diabetes are reported to have generally lower salivary flow rates than non diabetic subjects (Ben Aryeh et al., 1988; Thorstensson et al. 1989b; Ben Aryeh et al., 1993; Karjalainen et al., 1996; Ionescu et al., 1998; Chavez et al., 2000; Chavez et al., 2001; Moore et al., 2001b). However there are a number of papers which have not found this association (Belazi et al., 1998; Edblad et al., 2001; Harrison & Bowen, 1987; Swanljung et al., 1992). The reduced salivary flow rates seen were most marked in the poorly controlled type 1 diabetic patients. Theories surrounding aetiology, include specific autoimmune damage to the salivary glands at the onset of the initial disease (Cha et al., 2002; Cinquini et al. 2002; Caldeira et al., 2005), autonomic neuropathy (Ben Aryeh et al., 1996; Lamey et al., 1986) or simply a product of dehydration due to osmotic diuresis (Martin, 1999).

It is possible that T1DM is responsible for a decrease in unstimulated and stimulated salivary flow rates as well as a subjective feeling of dryness (Moore et al. 2001b). This relationship however may be complicated by xerogenic medication, duration of diabetes, glycaemic control and the association with other complications of the diabetes (Conner et al., 1970; Tenovuo et al., 1986; Sreebny et al., 1992; Swanljung et al., 1992). In type 2 diabetes this is thought to be due to concomitant medication (Meurman et al., 1998). However, in T1DM there is evidence that this is not linked to medication, uncommonly prescribed in younger patients, but is rather a true complication of the disease possibly due to either ultrastructural changes in the salivary gland structure or neuropathic autonomic changes (Manfredi et al., 2004).

1.5.3 Oral candidosis

Candida species are part of the normal oral flora and the most commonly identified pathogen is *C. albicans* (Manfredi et al., 2002). There is however an increase in the reporting of previously rare fungi such as *C. glabrata*, *C. dubliniensis*, *C. tropicalis* and *C. krusei*, particularly in medically compromised patients (Willis et al., 2000b; Cislo et al., 2001; Bagg et al., 2003; Tekeli et al., 2004; Aguilar et al., 2005). The oral candidal carriage rate is affected by salivary flow rates and systemic illness. There are studies investigating the elderly (Kuc ey al.,1999), the terminally ill (Sweeney et al.,1998), HIV AIDS sufferers (Kerdpon et al., 2004; Pongsiriwet et al., 2004 ;Schmidt-Westhausen et al., 1990,1991; Tsang & Samaranayake, 2000), children (Pongsiriwet et al., 2004), Sjogren's syndrome sufferers (MacFarlane & Mason 1974; Radfar et al., 2003) and diabetic patients (Tapper-Jones et al., 1981; Aguilar, et al., 2005; Aly et al., 1991; Bai et al., 1995; Belazi et al., 2005; Blackwell et al.,1989; Fisher et al., 1987; Guggenheimer et al., 2000a; Kadir et al., 2002; Kumar et al., 2005;Lamey et al. 1988; Manfredi et al. 2002; Willis et al., 2000a,b) and those suffering from burning mouth syndrome (Samaranayake et al., 1989). Oral carriage rates of *C. albicans* are higher in most of the above groups.

A simplistic explanation for the increased carriage rates in diabetic patients is the increase in salivary glucose levels as a source of nutrients for the fungi. However, it is also possible that changes to the mucosal immune response affect the ability of candida to colonise the oral mucosa. It has been shown that buccal epithelial cells from diabetic patients permit increased adhesion of *C. albicans* (Darwazeh, et al., 1997; Willis et al., 2000a). The mechanism of this binding is unknown but may be due to reduced salivary lysozyme (Pinducciu et al., 1996), production of extracellular proteinases or upregulation of receptors for complement such as inactivated C3b (iC3b) in high glucose concentrations (Manfredi et al., 2006). Alternative theories include increased adhesion of *C. albicans* to epithelial cells when grown in high sugar media due to the development of a fibrofloccular layer on yeast cell surfaces. Accumulation of glycosylation products in epithelial cells may increase numbers of receptors for *C. albicans* (Willis et al. 2000a). In addition it has also been reported that high salivary glucose concentrations may lead to increased resistance to intracellular killing by macrophages (Willis et al., 2000a).

Candidal carriage rates in the healthy population are reported as 2-71% with a mean value of 34% (MacFarlane et al., 1990). This mean value of 34 % seems to be in agreement with other authors (Kadir et al., 2002b; Lamey et al., 1988a; Sedgley & Samaranayake 1994b)

who reported a range from 24% to 31%. Xerostomia and other illnesses can both increase this carriage rate and signs of clinical infection and oral candidal carriage rates among some medically compromised groups can be up to 99% (Schmidt-westehausen et al., 1990; Wahlin et al., 1991, Sweeney et al., 1998).

Diabetic patients were found to harbour *Candida* species in 77% of subjects using an oral rinse technique (Willis et al., 1999). However, there is continued controversy as to whether this figure is accurate. Studies investigating diabetes and oral candidal carriage show a carriage rate of between 23% and 77% (Barlow et al., 1969; Tapper-Jones et al., 1981; Fisher et al., 1987; Lamey et al., 1988; Hill et al., 1989; Darwazeh et al., 1990; Aly et al., 1992; Dorocka-Bobkowska et al., 1996; Guggenheimer et al., 2000a; Willis et al., 2000, Manfredi et al., 2002). Most studies support the position that diabetic patients have higher carriage rates and greater density of colonization than healthy controls (Weinstein et al., 1959; Barlow and Chattawa, 1969; Tapper- Jones et al.,1981; Bhatt et al., 1983; Bartholomew et al., 1987; Fisher et al., 1987; Lamey et al., 1988; Fongsmut et al., 1998; Willis et al., 1999; Kumar et al.,2005). However, some authors did not find a significant association (Darwazeh et al. 1991; Dorocka-Bobkowska et al.,1996; Kadir et al. 2002; Manfredi et al. 2002). It may be that lack of standardisation in sampling and processing methods or inappropriate control groups have contributed to this variance in reporting of candidal carriage. It is also possible that confounding factors such as local risk factors and smoking have not been adequately controlled for in the study design. The rate of oral candidal carriage in tobacco smokers is higher than in non-smokers (Abu-Elteen and Abu-Elteen, 1998; Fongsmut et al., 1998; Willis et al., 1999; Soysa et al., 2005). Aly et al. (1992) found a higher carriage rate in T1DM than in type 2 diabetes, however. Darwazeh and co-workers (1991) found no difference as did other investigators (Dorocka-Bobkowska et al., 1996b). Metabolic control of diabetes has also been suggested as a risk factor for increased candidal carriage, although this is controversial. Studies by Hill and co-workers (1989) and Guggenheimer et al. (2000a) reported that the level of glycaemic control was a contributory factor in candidal carriage though most studies have not found a direct connection (Darwazeh et al., 1991; Dorocka-Bobkowska et al., 1996; Manfredi et al. 2002). Manfredi and co-workers (2002) found that the relationship between oral carriage rates of candida and diabetes was affected more by local factors for example denture wearing than by metabolic factors such as HbA1c or type of diabetes.

It is also worth noting that candidal carriage does not imply candidal infection. Despite a carriage rate of 60% overall, only 3.6% of diabetic patients in one study showed clinical signs of infection (Manfredi et al., 2002).

In summary it is likely that T1DM may affect candidal carriage rate however this has not been shown in a healthy, non-smoking population without dental prostheses. It is possible that glycaemic control could impact on candidal colonisation although there is not a consensus in the literature about this.

1.5.4 *Staphylococcus Species*

Staphylococci are Gram positive facultative aerobic cocci and can be further subdivided into coagulase negative and positive subgroups. Coagulase positive species are known to be responsible for a number of clinical conditions namely angular cheilitis, osteomyelitis, wound infection, mucositis, food poisoning, septic arthritis and septicaemia (Bagg et al. 1995; Bergmann et al., 1989; Smith et al., 2001, 2003). The oral cavity may serve as a reservoir for nosocomial infection elsewhere in the body, e.g. septic arthritis, however this possible source has been under recognised in the literature (Riggio et al., 2010). *Staphylococci* are commonly found in the mouths of healthy subjects (Jackson et al., 1999). The prevalence of *S. aureus* carriage increases with age, with 36% prevalence in the healthy elderly compared with 24% in a healthy adult population in Scotland (Jackson et al., 1999). A study by Sweeney et al. (Sweeney et al., 1998b) identified *S. aureus* carriage rates of 24% in a population of terminally ill patients suffering from xerostomia. Another study also reported increased staphylococcal carriage rates during cytotoxic chemotherapy (1984). A study in patients with leukaemia reported that most patients were carriers of staphylococci (Wahlin & Holm, 1988) while Jobbins et al. (1992), in examining the oral flora of patients with advanced malignant disease, found that 28% carried *S. aureus* in their oral cavities. Patients suffering from hypo-salivation due to salivary gland disorders have been reported to carry *S. aureus* in 21% of cases, although, the sample size in this study was only 28 (Almstahl & Wikstrom 1999). In a study of elderly hospitalised patients the carriage rate of *S. aureus* was only 4% (Wilkieson et al., 1991). These findings are confirmed in a report investigating the oral health of 79 year old Swedish individuals who had a point prevalence of 4% (Ohman et al., 1995). HIV status was not found to be a contributory factor in the carriage of oral staphylococci in a study by Schmidt-Westhausen who found only one isolate of staphylococci in 149 HIV positive patients (Schmidt-Westhausen et al., 1991). Apart from these studies with a low

prevalence of staphylococcal carriage, it is clear that staphylococci are in fact, regular colonizers of the oral mucosa and their isolation is significantly increased by alterations to the patients' systemic condition (Smith et al., 2003). The effect that oral carriage of *S. aureus* will have in the immuno-compromised host is not known although it may serve as a reservoir for future nosocomial infection and cross infection.

1.5.4.1 *Staphylococcus* and T1DM

Massler (1949) first reported that increased levels of *S. aureus* could be found in the mouths of diabetic patients reporting that 45% of 88 diabetic patients carried *S. Aureus*. A subsequent study of dental plaque samples found that staphylococci were present in 10% of healthy patients and 17% of non-diabetic patients suffering from periodontitis harboured the bacteria. However the highest prevalence of 60% was found in patients with diabetes also suffering from periodontitis. The isolation rate of *S. epidermidis*, a coagulase negative staphylococcus, was also noted to be significantly higher in T1DM (Sanchez-Cordero et al., 1979). There are no other published studies reporting the impact of diabetes on oral carriage of *S. aureus* although diabetic patients have been shown to have a higher nasal carriage rate and an increased susceptibility to staphylococcal infection in other body sites (Tuazon et al., 1975, Lipsky et al., 2010).

1.5.4.2 Antibiotic resistance

Methicillin resistant *S. aureus* (MRSA) in the oral cavity is a rare but increasing problem as oropharyngeal eradication is extremely difficult to achieve and this may act as a reservoir for nosocomial infection as well as horizontal spread of infection (Smith et al. 2003). There are no studies examining the relationship between diabetes and oral carriage of MRSA. There are a number of studies documenting the increasing frequency of diabetic foot infections with MRSA (Stanaway et al., 2007; Lipsky et al., 2010; Eleftheriadou et al., 2010). The nose is often cited as the source of the infection although the role of salivary carriage of MRSA in immuno-compromised patients is unknown (Smith et al., 2003).

It can be concluded that there is a paucity of data on the oral carriage of staphylococci in the population generally and specifically relating to T1DM. This is of particular concern regarding *S. aureus* as it is a common cause of wound infections and may further exacerbate this complication of diabetes. The following study will aim to provide a clearer picture of the carriage rates of staphylococci in the oral cavity of patients with T1DM.

1.5.5 Coliforms

Coliforms are facultative anaerobic Gram negative enteric rods which are found throughout the gastrointestinal tract and can be responsible for a number of infections e.g. gastroenteritis or pneumonia due to overgrowth of the commensal organisms or spread to distant sites. They are also found in the mouth in varying numbers, although controversy exists as to whether they are transient colonisers, or a permanent commensal (Samaranayake et al., 1984). The prevalence of oral carriage of coliforms is affected by reduced salivary flow (Sweeney et al., 1998), age (Leung et al., 2003), hospitalisation (Campbell et al., 1983) and immunocompromised states (Jobbins et al., 1992; Tsang & Samaranayake, 2000). A further study reported a significant increase in oral carriage rates in patients hospitalised for renal dialysis (Campbell et al., 1983). Examination of the oral microflora of the staff treating these patients revealed no individuals colonised by coliforms. The authors concluded that the source of the bacteria was not environmental, but rather represented overgrowth arising from the patients altered metabolic and immunological state. Schmidt-Westhausen reported in 1991 that there was a slight difference in the carriage of coliforms between HIV positive patients (20 isolates from 73 patients compared with 7 out of 58 controls) this slight difference however, was in direct contradiction to an earlier retrospective study carried out by the same author in 1990 which reported no increase in HIV positive patients (Schmidt-Westhausen et al., 1990, 1991). Carriage rates in the healthy population were recently reported as 1- 4% from oral rinses and culture techniques (Sedgely et al., 2004) but this is considerably higher (7-10%) if molecular techniques are employed (Sedgely et al., 2005). The variation in the healthy adult population ranges from 0 to 24% with significantly higher rates in Asian countries such as Hong Kong, China, Malaysia and Tibet (Samaranayake et al., 1984; Sedgely and Samaranayake, 1994a,b; Leung et al. 2003). Studies on the oral flora of Scottish hospital staff in 1984 showed a carriage rate of 5% (Samaranayake et al., 1984). Patients with dentures had a higher carriage rate of 12% (Samaranayake et al., 1989). Coliforms have also been associated with Burning Mouth Syndrome, with a 22% carriage rate in patients suffering from burning mouth syndrome compared with only 12% in the control group (Samaranayake et al. 1989b). Interestingly, diabetes can also give rise to the symptoms of Burning Mouth Syndrome (Gibson et al., 1990).

1.5.5.1 Predominant species of oral enterococci

The most common species of coliform isolated from the mouth are *Enterobacter* and *Klebsiella* although there are a large number of other coliforms which are isolated only occasionally (Samaranayake et al. 1989; Schmidt-Westhausen et al. 1990, 1991; Bergmann, 1991; Ohman et al. 1995; Goldberg et al., 1997; Almstahl & Wikstrom 1999; Dennesen et al. 2003; Leung et al., 2003; Hagg et al., 2004). There is evidence that an increase in coliform carriage is often associated with an increase in yeast carriage suggesting a possible symbiotic existence under the right conditions (Schmidt-Westhausen et al., 1990, 1991; Sedgley & Samaranayake 1994).

It is evident from the literature that coliforms form a normal part of the oral flora of a small number of individuals in the western world. This colonisation begins within a short time after birth but the bacteria involved are often transient colonisers and are not reproducibly detected in longitudinal studies (Samaranayake et al., 1984, Makhoul et al., 2002).

The prevalence of coliforms in the oral cavity of T1DM patients has not been studied extensively. However, Schmidt-Westhausen et al. (1990) noted that there was an increase in coliform carriage in patients with diabetes. Therefore it remains a distinct possibility that diabetic patients are similar to other medically compromised groups mentioned above and are likely to have increased levels of coliforms in their oral cavities. Whether this is related to a systemic weakness in their immune system or to local factors such as reduced salivary flow rates remains to be investigated.

1.5.6 Techniques

Researchers have used oral rinses, swabs, imprint sampling, ultrasonic water baths of dental prostheses and endodontic paper points as methods of sampling the oral flora, depending on the types of bacteria being investigated. Culture has been conducted anaerobically and aerobically on selective media (Soysa et al., 2005). The oral rinse method has been shown to be the most reliable method of quantifying the oral flora (Samaranayake et al., 1986). Biochemical and metabolic markers are used to type species at the species and subspecies level according to standard diagnostic protocols. The advances in qualitative PCR and now Real time PCR allow identification of unculturable species and tend to give a more complex picture of the oral flora with a generally higher detection rate than culture methods alone.

1.5.7 Soft tissue lesions

It has been reported that there is an increased prevalence of lichen planus in patients suffering from diabetes mellitus. A paper in 1966, by Grinspan et al. reported that there was a prevalence of oral lichen planus of 40% in patients suffering from diabetes mellitus (Grinspan et al., 1966). Subsequent papers, however, have not confirmed this extremely high prevalence. When studies are reported on patients who have confirmed oral lichen planus, the prevalence of diabetes mellitus is disproportionately high in these groups. One study reported that 40% of all patients with oral lichen planus had diabetes mellitus (Sallay et al. 1989) and a further study in Croatia reported that 20% of patients suffering from oral lichen planus also had diabetes mellitus (Ognjenovic et al., 1998). This was reported to be more than 400 times the national prevalence of diabetes. Apart from the early study by Grinspan et al. (1966), when diabetic patients are examined the prevalence of lichen planus is universally reported to be much lower. Saini et al. (2010) reported that the prevalence of oral lichen planus was only 0.5%; Bordgelly et al. (1993) reported a prevalence of 0.55%. Van Dis and Parks (1995) reported that the prevalence of oral lichen planus was 4% in patients with diabetes but 3% in patients without diabetes mellitus. This paper also reported that there was an association between patients taking non-steroidal anti-inflammatory drugs, anti hypertensive medication and the prevalence of oral lichen planus. Petrou-Amerikanou et al. (1998) reported that the prevalence of oral lichen planus in T1DM was 5.76% however it was three times lower in non-diabetic subjects at only 1.82%. Other studies have not reported any association with oral lichen planus (Guggenheimer et al., 2000a,b). The high prevalence of diabetes in patients suffering from oral lichen planus and the underlying autoimmune risk factors associated with both diseases mean that a connection is biologically feasible. Cross-sectional studies to date have been underpowered to detect a significant difference.

Altered taste, oral dysaesthesia and burning mouth syndrome have all been reported to be more prevalent in patients with diabetes. Burning mouth has been reported to be a presenting symptom of undiagnosed non-insulin dependent diabetes mellitus (Gibson et al., 1990). A study of type 1 diabetic children reported that 11.5% of all children had a burning sensation within the oral cavity (Costa et al., 2004). However, a large study in adults, reported that the prevalence of burning mouth syndrome was only 3.2% in patients with T1DM and 2.1% in patients without diabetes mellitus (Moore et al., 2007).

There are reports that erythema migrans or geographic tongue is increased in patients with T1DM (Saini et al., 2010). Medium rhomboid glossitis, angular cheilitis, depapillation of the tongue, fissured tongue, irritation fibromas, traumatic ulcers and denture stomatitis are all reported to be more prevalent in patients suffering from T1DM (Guggenheiner et al., 2000a,b). It has been suggested there is an increased risk of oral cancer associated diabetes mellitus however this has not yet been clearly established in studies on humans (Ujpal et al., 2002; Ujpal et al., 2004; Dikshit et al., 2006; Saini et al., 2010). The prevalence of oral cancer is extremely high in India and it may be that these differences are not seen in populations that are otherwise at low risk of oral cancer.

In conclusion, there is a significant lack of information about the prevalence or increased risk of soft tissue lesions in patients with T1DM. This is largely due to the fact that a number of studies have combined T1DM and type 2 diabetes mellitus patients in the same group. In addition many studies are underpowered to detect differences in these rare conditions between diabetic and control subjects.

1.6 Measuring oral and general health related quality of life in Type 1 diabetes mellitus

1.6.1 Introduction

Measuring the impact of disease on a patient's quality of life is important, as in many chronic conditions, physiological measurements do not correlate with a patient's experience of ill health. If only physical or biochemical tests are used, then the impact of a disease process on an individual's ability to function and the level of handicap or disablement, both physical and psychological, cannot be measured (Wilson & Cleary, 1995). In addition, it is clear, from clinical practice that individuals respond differently to the same disease process. Patient centred outcomes are increasingly being reported in the literature in addition to the physiological or pathological end point of a specific pharmacological or surgical intervention. The measurement of health related quality of life (HRQOL) has therefore become a focus for research in order to understand and measure the impact of health and disease on quality of life (Guyatt, 1989). The search for valid measures of Health Related Quality of Life (HRQOL) has been extensive but no single method has been shown to be the gold standard in every case. In identifying or developing

a tool for the measurement of health related quality of life it is important that it is valid, specific, sensitive, reliable, responsive and discriminative.

1.6.2 Available tools

Tools described for measuring HRQOL include a single question asking: “how is your quality of life?” This obviously has poor validity, reliability and responsiveness but may give useful information when measuring the health of populations, or for providing information for policy decisions (Torrance et al., 1986). In the other extreme, psychometric measures of HRQOL may include detailed interviews by trained examiners asking many in depth questions. It is more common to use sophisticated tools to measure HRQOL including a number of questions specific to the domain or dimension of quality of life that is being measured. These can be aggregated up to the domain level as well as described as part of the over-all score for the instrument. A domain is an area of experience or behaviour that the tool is trying to measure. The aspect of quality of life that is of interest will determine the domains which the instrument will measure.

Questionnaires can be used to assess overall health related quality of life or may be used in specific disease states to measure more sensitively the impact of that disease process on an individual's function. In general terms, the greater the number of questions the more sensitive the instrument is for detecting changes or differences in HRQOL. This is however limited by resources and can result in missing data if the questionnaire is too long. A balance must be struck between a questionnaire that is invalid because it does not ask enough questions and a questionnaire that becomes invalidated in practice due to the number of missing items recorded (Patrick & Erikson, 1993).

1.6.3 Methods of administration

The method used to administer the measurement of HRQOL can vary. Face to face interviews are the most reliable method of making sure that the response rate is optimal and that there are no missing items and no misunderstandings. Trained assessors are used to conduct these interviews. The interview allows the researcher to probe deeper into other issues and can generate unexpected information useful for hypothesis generation using qualitative as well as quantitative methods. This method is however extremely labour intensive and may also lead to subjects not being honest in their answers or being unwilling to acknowledge that they have a problem. Use of the interview method may restrict the number of subjects that can be evaluated, limiting its applicability and

relevance to larger populations or patient groups. The use of the interview technique may also lead to issues of inter assessor reproducibility and necessitates a training and calibration process to control for this. In addition to this the interviewer method limits the format of questionnaire that can be accommodated (Patrick & Erikson, 1993).

An alternative to the face to face interview technique is the telephone interview conducted by trained examiners. This is less resource intensive and is a useful alternative to the face to face interview while still minimising missed items and allowing opportunity for clarification of any misunderstandings. The use of self-administered questionnaires allows the number of subjects in the study to be larger as it is much less labour intensive. The likelihood of missed items and misunderstandings are generally increased using this method and a reduced response rate is to be expected. This can be offset by the use of validated and reliable questionnaires written in plain language. The relatively larger numbers that are achieved using self-administered questionnaires may offset the aforementioned disadvantages. Proxy or surrogate responders are also used in some conditions although the individual's perception of the quality of life of their close relative may not reflect the patient's own view.

Methods of measuring oral and diabetes related quality of life included focus groups, individual interviews, telephone interviews, and self-administered questionnaires or supported self-administered questionnaires. A search of the literature revealed a number of validated tools for measuring oral health related quality of life (OHRQOL). Consideration of their relative merits included a decision about the validity, reliability, ease of use and acceptance in the scientific literature. Similarly in choosing a tool to measure the diabetes related quality of life the same criteria were used. A combination of the self-administered questionnaire with a trained research assistant available for clarification and explanation was chosen in the study described in this thesis. The research assistant provided the questionnaires and subjects were asked to fill them in on their own. Help was available when required. It was thought that this would reduce the number of missing items and allow clarification to avoid misunderstanding (Patrick & Erikson, 1993).

1.6.4 Diabetes specific quality of life measures

A search of the relevant literature revealed that a number of tools have been used to measure HRQOL in Diabetes. Of these, a recent systematic review concluded that only three were specific to diabetes and could be said to be measuring quality of life (Speight et al., 2008). The Diabetes-Specific Quality of Life Scale (DSQOLS) is a validated tool for

measuring the impact of diabetes on quality of life however this was only available in German. Since simple translation of psychometric tests without further validation and testing is not possible this was not a practical option for this study. There are two other diabetes specific HRQOL tools called the Diabetes Quality of Life (DQOL) and the Audit of Diabetes Dependent Quality of Life (ADDQL).

1.6.4.1 Diabetes Quality of Life

The (DQOL) was developed by Professor Jacobsen of the Joslin Diabetes Centre for use in the diabetes control and complications trial (DCCTRG, 1988) and has been widely used elsewhere. The DQOL consists of 46 items although not all of these are diabetes specific. The subscales measure diabetes worry, social worry, impact and satisfaction. The DQOL is responsive only to the impact of significant interventions but cannot detect subtle changes due for example to changes in the method of insulin delivery. When the DQOL was used in the DCCT as the first diabetes specific measure of QOL no changes in QOL were recorded between intensified or conventional regimes. Using the concept of construct validation, clinicians and researchers would theoretically expect that an intensification of the strict insulin regime used in the DCCT, and the associated impact and limitations as well as the threefold increase in severe hypoglycaemia, would be measured by this QOL tool. As this does not appear to have been the case it is at least possible that the DQOL is not measuring adequately the domain in question (DCCTRG, 1988). The DQOL is also limited by the fact that there is no provision for the patient to indicate, or give weighting to, one aspect of their life over another. A patient may indicate that diabetes has a detrimental impact on their ability to drive a car or use machinery however they may not hold a licence or be required to operate machinery. The impact of this limitation would be given equal weighting alongside domains that have a direct and material impact on the patient's life despite the fact that it is not relevant to the individual concerned.

A recent review of the literature concluded that there were concerns about the acceptability and the face and content validity of the DQOL despite the fact that it is widely used (El-Achhab et al., 2008). They also reported that the DQOL had not been subjected to rigorous psychometric testing such as factor analysis. The number of questions was excessive for the purpose of the study reported in this thesis, due to time constraints, and the fact that the subjects would be experiencing a very intensive clinical examination as well as responding to the questionnaires.

1.6.4.2 Audit of diabetes dependent quality of life

The second diabetes specific quality of life measure is the Audit of Diabetes Dependent Quality of Life. The ADDQOL was developed by Bradley (1999) and contains twenty items. The questionnaire is available for use at no cost with the permission of the copyright holder. The ADDQOL attempts to improve on the reliability and responsiveness of the DQOL by addressing the issue of importance, or relevance, of each domain to the respondent. Subjects are asked to rate the impact of diabetes on different aspects of their life but are then asked to rate how important or applicable that aspect of their life is to them. As described above this is not possible with the DQOL. The ADDQOL contains 18 items that measure the impact of diabetes on different aspects of life. Two further questions measure overall quality of life and diabetes dependent quality of life. There are modifications of the ADDQOL available for the elderly, adolescents and children.

The ADDQOL was developed using past research experience, clinical input and interviews with adults with diabetes making its development both patient and expert driven. For all applicable domains, respondents rate the impact of diabetes on that domain and the importance of the domain for their QOL. The two ratings are multiplied together to provide a weighted impact of diabetes on each domain, which are summed and divided by the number of applicable domains to provide a measure of the 'average weighted impact' (AWI) of diabetes on QOL. The recent review of diabetes specific quality of life concluded that the ADDQOL has been shown to be acceptable and reliable in that internal consistency has been shown, although it has not been subject to assessment of test-retest reliability (El-Achhab et al., 2008). The validity of the ADDQOL has been demonstrated for face and content validity, convergent and divergent states and known and concurrent groups. The tool is responsive and has also been shown to be highly sensitive to subtle changes in diabetes therapy (El-Achhab et al., 2008).

Criticisms include the fact that it includes hypothetical responses on the basis of what it would be like not to have diabetes. A recent recommendation by the Food and Drug Administration recommends that these hypothetical questions are avoided in measures of patient centred outcomes (Food and Drug Agency Center for Drug Evaluation and Research, 2009). It is also possible that the elderly, or those with low literacy levels would find the ADDQOL difficult to complete.

1.6.4.3 Conclusion

In conclusion the ADDQOL is a valid, reliable, responsive and acceptable instrument for the measurement of diabetes specific quality of life and for these reasons it was the tool that was used in the study described in this thesis.

1.6.5 Oral health related quality of life.

Interest in Oral Health Related Quality of Life has been increasing over the last three decades and in dentistry, patient centred outcomes in intervention studies are beginning to be reported. The medical model of disease states indicates that disease is defined by a pathological process that affects the biological and functional integrity of the body (WHO, 1948). Health is however, defined as:

“a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity” (WHO, 1948).

Disease states are observable and quantifiable however, their relationship with the presence or absence of health is not a linear one (Wilson and Cleary, 1995). Conceptually the development of OHRQOL measures has necessitated a change in how we measure health and quality of life in the oral context (Slade et al., 1997).

Oral health related quality of life (OHRQOL) has been defined as:

“the impact of oral disorders on aspects of everyday life that are important to patients and persons, with those impacts, being of sufficient magnitude, whether in terms of severity, frequency or duration, to affect an individual’s perception of their life overall.” (Locker and Allen, 2007)

It has also been defined in the following way:

“a comfortable and functional dentition which allows individuals to continue in their desired social role.” (Dolan, 1993)

This multi-factorial definition of OHRQOL gives researchers great difficulty, as we try to measure something that is related to disease state, but is affected by a multitude of other social factors that will affect the impact on a patient’s ability to function and affect their global quality of life.

The ability of dentists to quantify and assess the impact of oral disease on patients’ quality of life has been greatly aided by the development of a number of tools that have claimed to be doing just this (Slade et al., 1997). Some of the most common tools are the Oral Health Impact Profile questionnaires. These were developed using Locker’s conceptual framework for assessing oral health (Locker, 1988). This model used the World Health

Organisation's International Classification of Impairment, Disability and Handicap (ICIDH) definitions of disease states (WHO, 1980). These may lead to impairment which can result in functional limitation or discomfort and pain which will in turn result in physical, psychological or social disability which may lead to a handicap (Figure 1-4). The idea that the presence of detectable dental disease could lead to symptoms which would lead to impairment and limitations which would in turn lead to disability and subsequent handicap for the subject, ultimately impacting on their quality of life, was the conceptual model on which the OHIP questionnaires were developed. Different questions relate to seven different domains of the Impairment-Disability-Handicap construct.

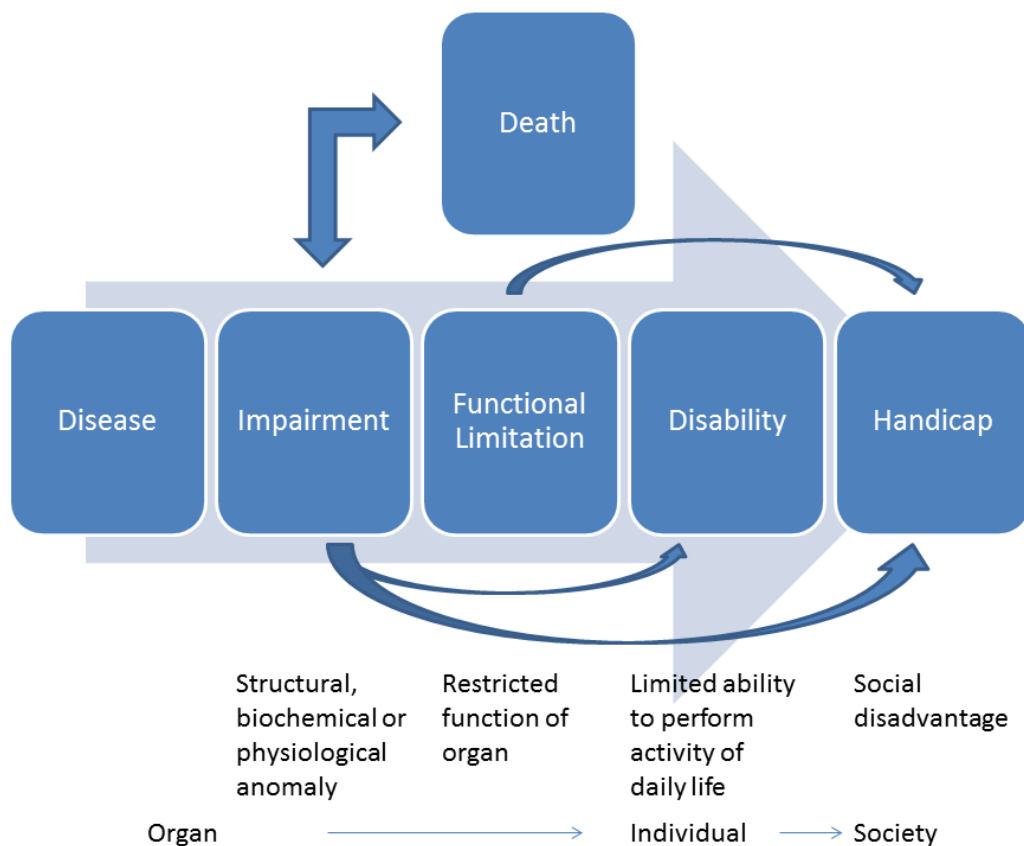


Figure 1-4 Conceptual model of the International Classification of Impairment, Disability and Handicaps (WHO, 1980)

An impairment was defined by the WHO in a report (WHO, 1976) in 1976 which became the basis for the ICIDH, (1980). Impairment was described as:

“any loss of or abnormality of psychological, physiological or anatomical structure or function” (WHO, 1980)

A disability was defined in the same document in the following way:

“any restriction or lack of ability (resulting from an impairment) to perform an activity in the manner or within the range considered normal for a human being” (WHO, 1980)

Disability has more recently been redefined as an umbrella term covering impairments, activity limitations, and participation restrictions. Disability is a complex phenomenon reflecting an interaction between the features of a person’s body and features of the society in which he or she lives. (WHO, 2010)

A handicap was defined as:

“a disadvantage for a given individual, resulting from an impairment or a disability that prevents the fulfilment of a role that is considered normal (depending on age, sex and social and cultural factors) for that individual” (WHO, 1980)

Handicap is no longer a popular term and is no longer used by the world health organisation.

Tools developed for the measurement of quality of life have recently come under some scrutiny in the medical literature in terms of whether they are truly reflecting quality of life issues. However this does not appear to have been the case in dentistry to the same extent. Criteria for assessing QoL tools were proposed by Guyatt and Cook (1994) (Table 1-4) however, it is only in the last few years that the questions about what oral health questionnaires are truly measuring have been addressed (Baker et al., 2006).

Table 1-4 Criteria proposed to assess the validity of QOL measures (Guyatt and Cook, 1994)

Do the authors show that aspects of patients’ lives they have measured are important to the patients?

If not, have previous studies demonstrated their importance?

Do the investigators examine aspects of patients’ lives that clinical experience indicates patients value?

Are there aspects of HRQL that are important to patients that have been omitted?

Were individual patients asked to directly place a value on their lives?

1.6.6 The Oral Health Impact Profile

1.6.6.1 Oral health impact profile 49

The Oral Health Impact Profile 49 (OHIP-49) is used to assess the impact of oral health on an individual's quality of life and this tool was first described and validated in 1994 (Slade and Spencer, 1994). The OHIP-49 was based on the ICIDH model of disease and its consequences (WHO, 1980). It was designed to assess the 'social impact' of oral disorders, that is, the dysfunction, discomfort, disability and handicap caused by these conditions (Locker, 1988). The purpose of the measure is to: assess the priorities of care by documenting social impact among individuals and groups; understand oral health behaviours; evaluate dental treatment and provide information for planning for oral health. It was intended to be a measure of self-perceived oral health.

The OHIP-49 was developed using an initial set of 535 statements obtained from open-ended interviews with 64 dental patients recruited from a range of dental settings. This initial pool was reduced to a set of 46 unique statements based on their form, content and ability to represent one of six domains derived from Locker's conceptual model of oral health. Three additional statements representing the concept of handicap were taken from an existing generic health status measure (Slade & Spencer, 1994).

The qualitative interview based component of the development process suggests that the OHIP is patient-centred and therefore measures aspects of patients' lives that are important to the patients. The item reduction process was carried out by experts, designed to select items according to their fit with a conceptual framework rather than on the basis of their importance to the patients from whom they were derived. It would be expected, although not necessarily proven, that the experts involved in the process would reflect the aspects of quality of life that clinical experience suggests are important to the patients. The process by which the final 46 statements were chosen and the criteria on which others were discarded was not described. Severity weights (from never through to very often) for each of the statements, designed to reflect their relative importance, are also included. The weights for each individual domain have not been widely validated for every population and are therefore not always used. These weightings were based on the judgments of members of community groups, dental practitioners and students in Adelaide, Australia (Slade and Spencer 1994). As a result of this, the weights ascribed may not reflect the severity and/or importance of the events described by the items as perceived by all dental

patients, although one study did show that the weightings were similar in Ontario and Quebec (Jokovic et al, 1997).

The OHIP has been shown to be a valid and reliable test. The internal reliability ranges from 0.7-0.83 for six subscales but only 0.37 for handicap. Test retest reliability was carried out longitudinally and Intra class Correlation Coefficients were 0.42-0.77 for six of the sub scales but only 0.08 for social disability. Two further studies on patients 50+ years in Ontario, Canada and North Carolina showed Cronbach Correlation Coefficients of 0.8-0.9 for all subscales and 0.96 for the overall OHIP score for all groups (Slade et al., 1996; Locker and Slade, 1993).

The OHIP-49 has been shown to detect an association between scale and sub-scale scores and perceived need to visit a dentist (Slade and Spencer, 1994). Further evidence of construct validity of the OHIP as a measure of oral health status has been provided by numerous investigators. Scores distinguish between the dentate and edentulous and show small to moderate correlations in the hypothesised direction with a wide range of traditional clinical indicators and self-perceived oral conditions, such as xerostomia, tooth loss, caries and periodontitis (Slade et al., 1996; Locker and Slade, 1994; Hunt et al., 1995).

Some evidence that the events captured by the OHIP may impact on a patient's general wellbeing has been provided by a study of the institutionalized elderly where there was a relationship between measures of oral health impacts and life satisfaction. Unlike the geriatric oral health assessment index (GOHAI), this association remained clearly significant after controlling for other predictors. The OHIP 49 could be described as an expert-centred measure of subjective oral health that may be capturing events which impact on general well-being and quality of life. However, the items comprising the measure do not necessarily, themselves, demonstrate that this is the case. The OHIP has more recently been subjected to rigorous testing, utilizing structural equation modeling and factor analysis in order to assess the within construct and between construct validity of the OHIP-49 (Baker et al., 2007, 2008). These findings indicate that there is not a simple direct relationship between the separate domains of Locker's conceptual model. The authors conclude that the within and between construct validity of the OHIP, in its current form, is questionable, and that the scale needs to undergo further testing before it can be assumed that it measures the concepts (and model) as originally proposed. The authors, however, concede that they only used a limited number of possible structural equation models and that this secondary analysis was on a group of dental patients who were

dentally fit. They are therefore unable to comment on the applicability of their findings to other dental groups. Nevertheless the OHIP-49 could not be shown to truly reflect the model as described and further investigation and development is warranted.

The authors state that we need to move away from simple descriptive or correlational research in an attempt to understand the complexity of oral health from within a patient-centered perspective. Practically, as the authors acknowledge, the overall OHIP score has been shown repeatedly to correlate with dental disease and to be related to its impact on an individual. Its use as an overall measure can therefore continue to be justified. The lack of direct relationships between domains and indeed the fact that multiple questions may well measure different domains mean that reporting of individual domains should be regarded as having poor validity (Baker et al., 2007, 2008).

Despite the above conceptual limitations the OHIP-49 remains one of the most robust tools for assessing the impact of oral health on an individual's life. A systematic review concluded that the OHIP-49 was valid and reliable. It has been shown to have construct validity and internal validity. Test-retest reproducibility and reliability has been shown by multiple authors. Epidemiological studies have shown that researchers can expect higher OHIP scores in patients with poorer clinical oral status, that is patients with more missing teeth, more retained root fragments, more untreated decay, deeper periodontal pockets and more periodontal recession. OHIP scores are also higher in socially and economically disadvantaged groups and among those who are irregular dental attenders. It has also been shown in longitudinal studies to be responsive to changes in oral status both positively after the provision of new dentures and negatively by further tooth loss (Slade, 1997).

1.6.6.2 The oral health impact profile 14

The greater the number of questions, the greater the validity of the psychometric test (Guyatt, 1994). It has also been demonstrated that the longer a questionnaire the greater the resources in time required for its completion, the greater the likelihood of missing data and the less likely subjects are to complete the questionnaire correctly. Concerns about the length of the questionnaire and a desire for a tool that was more amenable to chairside research led to the development of the short form of the OHIP-49. Distilling the measurement of HRQOL into a few key questions is a goal for clinical investigators. One approach is to develop a long instrument, test it, and use its performance to choose key questions to include in a shorter index. Slade et al. used this approach to create the shorter

oral health impact profile 14 questionnaire based on the more lengthy instrument, the OHIP-49 (Slade et al., 1997).

1.6.6.2.1 Derivation of the OHIP-14

While accepting the desirability of a shorter questionnaire it is essential that the shortened form meet a number of criteria before its use can be recommended and thus the development of a short form of a questionnaire is not a simple task. In deciding whether a short form of a questionnaire is acceptable for discriminative purposes the question is whether both forms of the questionnaire classify people similarly. The extent to which variability in scores, in the full instrument, is predicted by scores of the short version and therefore how well the rating of people's quality of life by the shorter instrument corresponds to ratings by the longer version would indicate whether the use of the short form of the questionnaire would be an acceptable substitute.

In addition, the validity and, if applicable, the responsiveness of the shorter version should then be tested against the full instrument. Correlations of change with independent measures and instrument responsiveness should be comparable. If measurement properties deteriorate, the investigator needs to decide whether trading off the time required to fill in the questionnaire is worth the increases in sample size required for statistical power to detect a difference with a less responsive instrument.

The OHIP-14 questionnaire was developed using a controlled stepwise regression procedure. The authors developed a subset of 14 questions from the full 49 questions using stepwise regression. Individual questions were considered sequentially for their contribution to total r^2 . Items making the greatest contribution to total r^2 were added sequentially, except that no more than two items from each conceptual dimension were permitted to enter the model. This "controlled" regression procedure was also conducted until 14 items, two from each dimension, were selected.

Subsequent comparison between the OHIP-14 and the OHIP-49 showed identification of a subset of 14 questions about the social impact of oral disease that accounted for 94% of variation in total OHIP-49 scores and which had an internal reliability coefficient (α) of 0.88. Summary scores, based on the OHIP-14, displayed the same pattern of variation among socio-demographic groups that was observed using the OHIP-49, and both the OHIP-14 and the OHIP-49 resulted in similar multivariate models relating oral status and socio-demographic variables to social impact. Because of the controlled regression

technique used, the OHIP-14 contains questions that retain the original conceptual dimensions contained in the OHIP-49, and those questions have a good distribution of prevalence. The 14 items ranged in prevalence from 33.1% (Q15) to 1.6% (Q48) while severity ranged from 0.97 to 0.06 (Q48) (Slade et al., 1997). The instrument has now been used in a large number of situations for quantifying levels of impact on wellbeing in settings where only a limited number of questions can be administered.

1.6.6.2.2 Validation

The OHIP-14 has also been subjected to rigorous analysis examining its ability to reflect Locker's conceptual model using structural equation modelling by the same authors who were critical of the OHIP-49. They investigated the validity of the OHIP-14 in the general dental population in the United Kingdom (n=5268), a group of edentulous elders (n=133) and a population suffering from xerostomia (n=85). Structural equation modeling indicated support for the model as applied to each of the samples. All of the direct pathways hypothesized by the model were significant, in addition to several indirect pathways between variables. The authors concluded that the OHIP-14 was applicable at individual, group, and population levels (Baker et al., 2008).

1.6.6.2.3 Responsiveness

Locker measured the responsiveness of the OHIP-14 to a change in oral condition. In order to assess responsiveness, Locker et al. (2004), compared OHIP-14 with global transition judgements on dental changes as a result of the provision of dental treatment in an elderly population. This paper concluded that the OHIP-14 was responsive to changes but that the differences measured were only modest. The authors conclude that this may be because the OHIP-14 was designed to be discriminative rather than responsive to changes. Other findings from this study supported the test-retest reliability of the OHIP-14 which showed excellent reproducibility with an intraclass Correlation Coefficient (ICC) of 0.84. Construct validity was also supported indirectly as patients who were dentally stable did not show any changes in their OHIP-14 score longitudinally, whereas patients who reported improvements in their global transition measures also showed significant reductions in OHIP-14 scores. Unfortunately the minimal important difference was 5 scale points suggesting that studies would require to be very highly powered to detect statistically significant changes.

1.6.6.2.4 Validation in the United Kingdom

It is important that psychometric tests are validated in a range of settings and should ideally have been shown to be valid and reliable in the population under investigation. To date there have been three studies that have utilised the OHIP-14 in the British general population one of which was in a Scottish general dental practice environment.

The 1998 Adult Dental Health Study used the OHIP-14 in a large study of over 5000 patients although no robust validity or reliability testing was published. The ADHS 1998 utilised only one scoring method (the total number of reported problems) stating that the weightings had not been validated in a UK population for the individual sub-scores. There were relationships described between oral health status and OHIP-14 scores and socioeconomic status, lending indirect support for the construct validity of the OHIP-14 in the British general population (Nuttall et al., 2001).

Robinson and co-workers assessed the OHIP-14 in comparison with the Oral Impact on Daily Performance (OIDP) and an interview based method in 179 consecutive patients attending a dental hospital (Robinson et al., 2001). These patients had a mean age of 36 and were from a range of ethnic and socioeconomic backgrounds. 69.1% of all participants were experiencing pain at the time of recruitment and considered their condition a dental emergency. Common diagnoses included irreversible pulpitis (26.6%), periapical periodontitis (13.2%) and periapical abscess (9.9%). All but 8 of the participants had experienced at least one impact and using the “occasional” score, only twenty people reported no impacts. Cronbachs alphas for weighted standardized OHIP (0.91), additive OHIP (0.92) and OIDP (0.88) were excellent. The split half reliability, another measure of reliability, of both OHIP scores was 0.90 and 0.91 respectively. The authors concluded that the face validity of the OIDP was relatively weak due to the complexity of the tool which includes a number of filter and contingency questions, the number of questions and the fact that in their version the questionnaire occupied eight pages. In contrast the OHIP fitted on only one sheet. The OIDP is also limited in content validity by virtue of the fact that, although it is based on Locker’s conceptual model of oral health, it measures only impacts at a functional level unlike the OHIP which records impacts of oral disease at multiple stages of the model. In assessing criterion validity, Robinson et al. (2003), reported significant correlations between both OHIP and OIDP scores with global oral health scores and pain scored on a visual analogue scale. However,

again, the correlation was stronger with OHIP than for OIDP with the global oral health rating. The correlation between weighted standardized OHIP scores and the additive scoring was 1 ($p < 0.001$), lending support for the use of the simpler scoring system. The number of impacts in both OHIP and OIDP were weakly related to the presence of clinical disease. Both methods of scoring the OHIP were significantly related to the presence of oral disease ($p = 0.004$ and 0.005) however, the relationship between OIDP and oral disease was not significant. Completion rates were lower for OIDP and it was also limited by its design, making statistical transformations unsuccessful in order to enable it to be used in regression modeling. The authors concluded that the OHIP-14 (using the summary score) demonstrated superior validity to the OIDP when used in a dental hospital setting. The OHIP-14 was shown to be related to the presence of oral disease conveying construct validity. The actual scores from this study are unlikely to reflect those of the general population, given that many of the patients included were attending for emergency treatment at the time of recruitment. In addition, given the cross-sectional nature of the study, authors did not assess instrument reliability and responsiveness longitudinally (Robinson et al., 2003).

The use of OHIP-14 in Scottish dental patients has been recently validated as part of a study into the impact of impacted wisdom teeth on OHRQOL (Fernandes et al., 2006). 278 patients were recruited from six general dental practices in Tayside, Scotland, and followed for a year, to assess the development of problems related to impacted wisdom teeth. The OHIP-14 was completed at baseline ($n = 278$) and at 1-year follow-up ($n = 169$), and analysed using three different scoring methods: a summary score, a weighted and standardized score and the total number of problems reported. Internal consistency was assessed using Cronbach's α which measures the correlation between questions at baseline and again at follow-up. Cronbach's α coefficients for each of the seven health domains ranged from 0.49 (functional limitation) to 0.74 (physical discomfort) at baseline and 0.30 (functional limitation) to 0.75 (social disability) at 1-year follow-up. Only the functional limitation domain fell below the arbitrary cut off point of acceptability of 0.5, either at baseline or follow-up. Alpha coefficients for all 14 items combined were 0.88 and 0.87 for baseline and follow-up, respectively. Test-retest reliability was also assessed using intraclass correlation coefficients (ICCs). The three methods of scoring, total OHIP-14, weighted and number of problems had ICCs of 0.76, 0.72, 0.77 respectively (Fernandes et al., 2006).

In assessing construct validity the authors demonstrated that, in this group of Scottish dental patients, OHIP scores were related to occurrence of dental pain, the number of missing teeth, no mouthwash used, brushing less than twice a day, educational status, time since last dental appointment, attending only when in pain and infrequent dental attendance. All of these differences were in the hypothesized direction. Stepwise regression confirmed relationships between OHIP-14 scores and condition specific variables both at base line and follow-up. These included presence of dental symptoms, number of teeth, pain scores, reason for last appointment, frequency of seeing a dentist and use of a mouthwash. Although highly significant the r^2 values were only 0.189-0.202. The investigators did carry out periodontal screening and reported bleeding on probing but only as two dichotomous scores. They reported bleeding as either limited to two sextants or present in more than two sextants. They also split the CPI into two groups CPITN 1 and 2 or 3 and 4. Although scores were higher in the group with most bleeding and the higher CPITN scores, these were not significant. It would have been interesting to see whether the patients scoring CPITN 4 compared with the periodontally healthy group had higher OHIP-14 scores, but this comparison was not carried out. Responsiveness was measured using the standardized response mean (SRM). The SRM is equal to the mean change in score divided by the standard deviation of individuals' changes in scores. An SRM of 0.2 indicates a small effect or clinical change, 0.5 moderate, and 0.8 or greater a large effect. The SRMs for patients reporting symptoms within the 1-year study period were 0.55, 0.56 and 0.37 for the summary scores method, the weighted score and the total number of problems respectively. These were only significant in symptomatic patients. The OHIP-14 was moderately responsive to detect the alteration in oral health impact from impacted wisdom teeth.

The authors summarized their assessment of the OHIP-14 by concluding that the OHIP-14 demonstrated good levels of reliability and validity and is responsive to third molar clinical change when used to assess OHRQOL in general dental practice in Scotland. They also concluded that of the three methods studied, the summary scores method performed, as well or better than the other two, and was the simplest to use. Locker and co-workers (2007) also specifically addressed the issue of weighting in both the OHIP 49 and 14 and showed that unweighted and weighted scores discriminated between the groups enrolled in the study. Previous studies had also suggested that psychometric properties are not improved by the use of weights (Allen and Locker, 1997, Allen et al., 2001, Robinson et al., 2003).

1.6.6.3 OHRQOL and periodontal disease.

The majority of studies into the prevalence, progression and treatment of periodontal conditions involve clinical professionally measured outcomes. While these are essential, increasingly researchers are interested in developing patient-centred outcomes that reflect the impact of periodontitis on OHRQOL. There are a number of papers that have specifically investigated the impact of periodontal diseases on OHRQOL. Needleman et al. (2004) reported OHRQOL measures using the OHQOL-UK[©] questionnaire on 205 patients attending a private periodontal practice. He found that the effect of oral health on quality of life was considerable, with many individuals experiencing negative impacts across a broad range of physical, social and psychological aspects of life quality. OHQOL-UK scores were associated with patient's self-reported periodontal health in the past year: experiences of "swollen gums" (p=0.01), "sore gums" (p=0.01), "receding gums" (p=0.01), "loose teeth" (p=0.01), "drifting teeth" (p=0.01), "bad breath" (p=0.01) and "toothache" (p=0.01). The authors also reported that OHQOL-UK scores were correlated with the number of teeth affected by periodontitis (CPD>5mm). Patients who were in a maintenance program had significantly lower scores than new patients. Patients attending a private periodontal practice may not be representative of all patients suffering from periodontal destruction however, this paper was one of the first to begin to address the impact of periodontitis on quality of life.

Another study assessed a number of different OHRQOL measures (OHIP, OHQUOL, GOHAI and a single item self report of oral health) in two different adult patient groups (Jones et al., 2004). All four measures were higher in patients with higher CPITN scores confirming that OHRQOL was related to periodontitis and periodontal treatment need. The OHIP was significantly associated with CPITN score in this study and was a better measure than the GOHAI.

Lawrence and co-workers showed in a birth cohort, at 32 years of age, that the OHIP-14 was related to: DMFS ≥ 12 ; decayed surfaces >0; missing teeth; self reported oral health; attending only when in pain; and also to having sites with greater than or equal to 4mm of clinical attachment loss (Lawrence et al., 2008). Lopez and co-authors (2007) reported in a group of Chilean high school students that high OHIP (Spanish) scores were associated with an increased odds ratio for periodontitis and acute necrotizing gingivitis (OR=2.0,1.6).

Ng and co-workers (2006) investigated the relationship between the OHIP-14 and self-reported symptoms of periodontitis over the preceding 12 months, as well as clinical attachment loss. Swollen gums, sore gums, receding gums, loose teeth, bad breath and toothache were all associated with higher OHIP scores. 5 out of the 7 domains of the OHIP-14 and the OHIP-sum were all significantly associated with high mean CAL and severe attachment loss. Social disability and handicap were not associated with periodontitis in this Chinese population. This differed from the study by Needleman et al. (2004). The Chinese study recruited patients with low dental motivation and it is therefore perhaps not surprising that differences between the study populations were evident and reflect different social attitudes.

Cunha-Cruz and co-workers (2007) found that periodontitis was associated with poorer OHRQOL. In this study, 1497 patients filled in a short OHRQOL questionnaire and gave consent for data to be abstracted from their case notes. Logistic regression models were used to relate both, worse perceived oral health and common oral health-related quality of life problems, to number of teeth with pockets deeper than 5 mm (0–2, 3–4, 5–8, 9–30 teeth), number of teeth with pockets deeper than 8 mm (0, 1–2, 3–19 teeth) and number of missing teeth (0–3, 4–7, 8–11, 12–31 teeth). The adjusted odds ratio for having poorer perceived oral health was 2.78 when patients had more than 9 teeth with pockets deeper than 5mm. The adjusted ratio for having worse perceived oral health was 3.18 in patients with 3 or more teeth with probing depths >8mm. There was also a relationship between the occurrence of common OHRQOL problems and the extent and severity of periodontal destruction as well as the number of missing teeth. Similarly, Aslund et al. (2008) investigated the oral health related quality of life in 251 patients attending the department of periodontology and fixed prosthodontics using the German version of the OHQOL-UK questionnaire. OHQOL-GE scores were significantly associated with patients' self-reported symptoms and problems in the past year: experiences of 'tooth ache' ($p < 0.05$), 'swollen gums' ($p < 0.001$) and 'problems with dental prosthesis' ($p < 0.05$). OHRQOL-GE scores were also directly correlated with the BPE ($r^2 = -0.295$, $p < 0.01$), the number of teeth present ($r^2 = 0.190$, $p < 0.01$) and inversely correlated with age ($r^2 = 0.152$, $p < 0.05$).

Quality of life measures have also been used to report changes on QOL during and after periodontal procedures. Ozelik et al. (2007), in a randomized controlled trial, compared three methods of periodontal treatment using the OHIP-14 and the GOHAI as patient-centred outcomes. They were able to detect differences in OHQOL resulting from

different surgical techniques. There was no control group for comparison at baseline, however, there were differences in the OHIP-14 and GOHAI scores throughout the immediate post-surgical period. Another small study recruited 20 patients with moderate to advanced periodontitis and 16 dentally healthy patients. Patients with periodontitis were treated with root surface debridement within 24 hours. Patients filled in the OHIP-14 at base line and at daily intervals for 7 days. The OHIP-14 was also administered at the final review between 2 and 10 months after initial treatment. This study demonstrated that the median number of impacts was significantly higher among patients with periodontitis compared with the control group at base line, this difference continued throughout the study but patients receiving periodontal treatment saw the mean number of impacts decrease significantly over the study course. The authors concluded that periodontitis is not a silent disease but is one that impacts on an individual's quality of life. They also concluded that periodontal therapy provided in a secondary care setting can result in improvements in a patient's QOL (Jowett et al., 2009).

In conclusion it has been shown that severe periodontitis may not be an asymptomatic silent disease but may in fact lead to significant oral health impacts. These changes are detectable by the measures of OHRQOL available and one would therefore expect that groups of patients with more severe periodontitis would have higher OHIP-14 scores.

1.6.6.4 OHRQOL and Diabetes

There are currently three studies on OHRQOL in diabetic patients. The first of these studies used the Spanish version of the OHIP-14 and examined 159 dentate patients with diabetes mellitus. There was no control group and therefore no conclusions can be drawn about the impact of diabetes per se on OHIP scores. Periodontitis was common in this group with almost 50% of all patients having periodontitis and 21.4% having advanced disease. Gingival bleeding, probing depth, and clinical attachment level ≥ 4 mm were associated with a negative impact on quality of life ($p = 0.013$, $p < 0.001$, and $p = 0.012$ respectively). Diabetic patients with mild-to-moderate and advanced periodontitis had worse OHIP scores than those who were periodontally healthy or had gingivitis (Drummond-Santana et al., 2007). A second study examined the effects of xerostomia on adolescents with T1DM. A total of 52.9% of subjects presented with xerostomia and 40.8% with hyposalivation. OHIP scores were significantly increased by the presence of xerostomia (Busato et al., 2009).

The first study to be carried out in Europe contained a mixture of T1DM and type 2 diabetic patients who were edentulous as well as dentate (Allen et al., 2008). Of the 101 patients recruited only 27 had T1DM and some patients had only been diagnosed with diabetes for one year. Nevertheless, this study is the only one to date that has assessed the impact of diabetes mellitus on OHRQOL in a European population. Only thirty-three per cent of participants were aware of their increased risk for periodontitis (40% of T1DM). The proportion of participants who had attended a dentist within the previous year was only 43%, with 34% not having attended for more than 5 years. Thirty-seven per cent of participants attended for dental treatment once a year, with 63% attending only when they had a problem. Subjects filled in the OHIP-20 and 66% reported that they had frequent problems with food catching around teeth and under dentures and 43% reported that their diet was unsatisfactory because of their teeth. Unfortunately, this pilot study did not include a control group and so no conclusions can be drawn directly about the impact of oral disease on non-diabetic subjects from the same area. The authors reported that the OHIP-20 scores were actually lower than in a similar study carried out in the Dental Hospital in Cork. This may be due to the reasons for patients attending the Dental Hospital. The authors suggest that the impact and complications of diabetes mellitus may lessen the relative importance placed on the oral cavity by this group (Allen et al., 2008). While there is no direct evidence for this, it is one feasible explanation that merits further investigation.

1.7 Recommendations from the FDI and IDF

The World Dental Federation (FDI) and the International Diabetes Federation (IDF) jointly organized a symposium on Oral Health and Diabetes in 2007 during the FDI Annual World Dental Congress in Dubai, United Arab Emirates. The presenting experts and all participants welcomed the initiative of the two convening organizations to emphasize the interrelationship between diabetes and oral health. Experts noted the rising burden of diabetes worldwide and stated that there was an urgent need to inform professionals, people with diabetes, government policy makers as well as the general public about the relationship between diabetes and oral health.

Participants and experts agreed that collaboration between dentists, physicians and policy makers was key to oral health promotion and the prevention of oral disease in patients with

diabetes. The recommendations from this symposium to maintain oral health and minimize its impact on diabetes management are listed below.

- To include prevention of oral disease and promotion of oral health as an essential component of diabetes management.
- To establish periodontal disease formally as a routine complication of diabetes in order to increase awareness amongst health professionals, people with diabetes and policy makers.
- To initiate and support research leading to evidence-based treatment strategies to improve health and oral health of people with diabetes.
- To include routine oral screening of people with diabetes.
- To introduce screening for diabetes in the dental office among high-risk populations.
- To improve knowledge about the reciprocal link between diabetes and oral health among all stakeholders, health professionals, people with diabetes, the public and policy makers.
- To increase the focus of dental education for undergraduate and post graduate students on the association between diabetes and oral health.

The participants recommended that the World Dental Federation and the International Diabetes Federation work more closely in future, to develop joint policy statements and practice guidelines, to advocate for increased awareness among all stakeholders and to implement tangible actions in this context (IDF, 2007).

It is in this global context that the work contained within this thesis was carried out. With an ever increasing number of patients affected by diabetes mellitus we need more information about how best to prevent and manage the oral complications of T1DM. This can only be achieved by having reliable evidence of the prevalence, severity and extent of these complications. It is hoped that the data gathered in the studies described in the following chapters will inform oral and general health policy for patients suffering from T1DM in Scotland and further afield.

2 Type 1 diabetes mellitus and periodontal disease: A cross sectional study

2.1 Introduction

The relationship between diabetes mellitus and periodontitis is one that has received significant attention over the last 50 years. The association between the various types of diabetes mellitus and periodontitis has been the focus of two recent systematic reviews with meta-analysis (Khader et al., 2006; Chávarry et al., 2009). There is now a considerable body of literature including large cross-sectional and prospective studies that demonstrates clearly that there is a link between type 2 diabetes mellitus and periodontal destruction (Khader et al., 2006; Chávarry et al., 2009; Taylor, 2009). However, Chávarry and co-workers (2009) concluded that a causal relationship between T1DM and periodontitis was not established by the available literature.

The reason that this relationship has not yet been clearly established is largely due to the quality of the studies that have been carried out to date. Criticisms include: the studies were under powered; they did not distinguish clearly between the types of diabetes; and they failed to control for confounding factors such as smoking, social class and other risk factors. In addition, there was a failure to confirm the status of non-diabetic controls. There are a number of studies reported in children and adolescents with T1DM. However, these groups are unlikely to have significant periodontal disease because of their young age. The authors of the systematic review called for high quality studies, in adults with T1DM, designed to overcome the shortcomings of the published literature (Chávarry et al., 2009).

Type 1 diabetes is a disease which is increasing in prevalence (International Diabetes Federation, 2009). The burden on society and on individuals suffering from the disease is significant. The frequency of complications has been shown to be reduced by good glycaemic control and management of complications has improved as patients are made aware of the risks and are screened appropriately (DCCTRG, 1996; Stratton et al., 2000). Screening for complications such as peripheral neuropathy and retinopathy often means that they are identified and treated at an early stage when they are more easily managed. Prevention and early diagnosis of diabetes associated periodontitis will only be possible if

there is evidence available to inform, and educate, diabetes physicians, general medical practitioners, diabetes support staff and patients. While evidence of a link is equivocal, we cannot move forward in developing our understanding of the disease process. In addition, we cannot make progress in implementing public health measures to tackle the oral complications of T1DM.

The aim of this chapter is to provide definitive evidence of the relationship, if any exists, between T1DM and periodontal disease. The research questions were:

- Is the prevalence of periodontal disease increased in non-smoking T1DM adults compared with healthy non diabetic control subjects (NDS)?
- Is the prevalence of periodontal disease increased in non-smoking PCD compared with non-smoking well controlled diabetic subjects (WCD) and NDS?

2.2 Materials and Methods

2.2.1 Training and calibration

A single blinded trained examiner recorded all the clinical examinations (DR). Preparation involved clinical training in measurement of the position of the gingival margin, and clinical probing depth and in the use of the Modified Gingival Index (Lobene, 1986) and the Plaque Index (Silness and Loe, 1966). Training was conducted by external, and internal, experienced clinical periodontal researchers. This was in order to improve reproducibility and to provide a second trained and calibrated examiner in the event that the primary examiner was unable to perform the examinations. This was never required during the duration of the study.

Training included a consensus session where the standard was set between the experienced researchers and the examiner using clinical photographs of various levels of gingival inflammation and plaque accumulation. The position of the gingival margin in relation to the cemento-enamel junction and periodontal probing technique were also discussed.

Calibration included intra and inter-examiner reproducibility. As there was to be only one examiner, intra-examiner reproducibility was the most important factor in training and calibration. There was one calibration session prior to the commencement of the study and one shortly after the clinical examinations had started.

During the study, over 10% of all patients had at least one quadrant of their periodontal charting repeated in order to ensure that recordings were reproducible. It was not practical for patients to return on a separate day for repeat measurements due to difficulties with recruitment and attendance at appointments in this population. In order to avoid the examiner recalling measurements, all repeat examinations were carried out later in the morning after all the charting had been completed and patients had taken a break. The decision about which quadrant to repeat was taken randomly by the dental research nurse.

Reproducibility was assessed by calculating Kappa scores for all periodontal indices. The percentage within 1mm or 1 point on the scale and 2mm or 2 points on the scale was also calculated. The mean bias and measurement error were calculated for all indices. Kappa scores were rated against the criteria proposed by Altman for the level of agreement (1991) (Table 2-1).

2.2.2 Study Design

The study protocol was approved by the Glasgow Royal Infirmary local research ethics committee. The nature and purpose of the study was explained to potential recruits and those willing to participate signed a consent form.

2.2.2.1 Subjects

Patients with diabetes, 20-55 years of age, were recruited from out patient departments of Glasgow Royal Infirmary, the Southern General Hospital, the Victoria Infirmary and Stobhill Hospital in Glasgow, and the Royal Alexandra Hospital in Paisley. All hospitals were in the West of Scotland and were within the Greater Glasgow and Clyde Health Board. Healthy control subjects (20–55 years old) were recruited from physiotherapy outpatient clinics, through the buddy system and via an advertisement in a national newspaper. The buddy system involves each diabetic subject asking a friend of the same gender and similar age to consent to joining the study as a healthy control. Consenting buddies were then contacted by the dental research nurse and an appointment for the examination was arranged.

2.2.2.2 Exclusion criteria

- Subjects were excluded from taking part in the study if they had a history of smoking within the past five years. It would have been preferable to have included only subjects with no exposure to tobacco smoking. However, the prevalence of

cigarette smoking in the West of Scotland is such that it was felt that this would impact on recruitment and final sample size (Scottish household survey, 2009).

- Subjects were excluded if they were pregnant at the time of recruitment. It is well recognized that the hormonal changes during pregnancy have a profound impact on the gingival inflammatory response.
- Patients who were immunosuppressed either due to medication or concurrent illness were excluded due to the possible impact of this on the periodontal tissues.
- Any patient who was on medication with side effects shown to affect the periodontal tissues (other than metformin which can show taste disturbance) or those who had been on antibiotics or anti-inflammatory drugs within 6 weeks of the examination were excluded.
- In order to minimize the possible complication of racially related genetic differences affecting the prevalence of periodontitis, only subjects of North European Caucasian origin were recruited.
- Subjects with less than 20 teeth were excluded. This was to ensure that patients had sufficient teeth on which to measure any periodontal disease present.
- Subjects who were unable to consent were excluded from participation.

2.2.2.3 Descriptive data

Data describing age, gender, social class, oral health behaviour, and body mass index were collected prior to the clinical examination.

Area based social deprivation was assessed by using the Deprivation category (DEPCAT) and Scottish Index of Multiple Deprivation (SIMD) of each participant based on their home post code, available in table form from the Scottish Government (Scottish executive, 2006). Both DEPCAT and SIMD are area-based measures of deprivation. DEPCAT is based on the Carstairs index developed for Scotland as an alternative to the Townsend Index of deprivation (England and Wales) (Townsend, 1988; Carstairs and Morris, 1990). The Carstairs index is based on four census indicators: low socioeconomic status, lack of car ownership, overcrowding and male unemployment (Carstairs and Morris 1990). The SIMD 2006 combines 37 indicators across 7 domains, namely: current income, education,

housing, health, skills and training, employment, geographic access and crime. These domains are used to give an aggregate deprivation score for a post code area. SIMD is the preferred index as it uses more information to arrive at the score and in addition is available for smaller postcode areas. Some DEPCAT scores are only available for the first number of the second part of the postcode whereas SIMD is available at the level of the last two digits and therefore refers to a more precise area. DEPCAT has seven scores while SIMD has only five. We recorded and reported DEPCAT as well as SIMD because it was the area based measure of deprivation that was used in the most recent available data from the Adult Dental Health Survey (Kelly et al., 1998). Data on car ownership, educational level and ability to pay for dental treatment were also recorded.

Body mass index (BMI) is defined as the individual's body weight divided by the square of his or her height. The formula which produces a unit of measurement of kg/m² was first described by Adolphe Quetelet in the 19th century and was validated by Keys (Keys, 1972). The patients' weight and height were measured prior to the clinical examination by the dental research nurse. Weight was recorded to the nearest half kilogram; height was recorded to the nearest centimetre. The BMI was calculated using the freely available National Institute for Health (NIH) BMI calculator (www.nhlbisupport.com/bmi/).

$$\text{BMI} = \frac{\text{mass (kg)}}{(\text{height(m)})^2}$$

Pack years were calculated for those who had smoked in the past and are a quantification of past smoking experience over a period of time. Pack years are calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked and dividing the total by 20. Patients were identified as never smokers and former smokers based on their response to individual questioning by the dental research nurse. Never smokers were defined as those individuals who had a pack year score of less than 0.25 during their lifetime.

Patients were also asked about their tooth brushing frequency, use of adjunctive interdental cleaning aids, mouthwash use, registration with a dentist, frequency of dental attendance, reason for attendance, dental anxiety and dentally related symptoms.

The glycaemic control for the T1DM patients was calculated using the average of all available HbA1c measurements over the last 2 years. A two year period was chosen in order to give a representative picture of the patients' glycaemic control over a prolonged

time period likely to affect the periodontium. Patients were separated into well and poorly controlled based on an average HbA1c of $\leq 7.5\%$ as defined by the Scottish Diabetes Survey (SDS, 2009).

2.2.3 Clinical examination

Participants responded to questions concerning their social and dental history using a customised oral health questionnaire.

All clinical examinations took place in Glasgow Dental Hospital and School. To avoid diurnal variation in salivary flow, examinations were mostly scheduled in the morning. Clinical examination normally took around 120mins.

The clinical examination took place in the following order:

1. The patient entered the dental clinic and was met by the dental research nurse who explained the nature of the examinations. The patient was encouraged to ask any questions if they felt that they still had unanswered queries.
2. The dental research nurse ensured that all of the relevant paperwork had been completed and that there had been no change to the patient's medical history since recruitment. The need for secrecy about the diabetic status of the subject was re-emphasized to the individual at this time.
3. Saliva sampling and the oral rinse were collected first. Details of this process are described fully in chapter 4.
4. Examination of the 4 key sites one in each quadrant of the mouth (normally the mesiobuccal aspect of the 1st or 2nd molar tooth) was carried out. The Modified Gingival Index (MGI) (Lobene, 1985) was recorded first followed by the Plaque Index (PI) (Silness and Loe, 1964), gingival crevicular fluid flow, clinical probing depth and bleeding on probing. This is further described in chapter 3.
5. A full mouth dichotomous plaque score was recorded at six sites around every tooth. Where plaque was visible or detectable by running a probe across the surface it was recorded simply as present.

6. A full mouth dental chart was recorded as per the ADH survey 1998 (Nunn et al., 2001). Recording of missing teeth, existing carious lesions, and dental restorations and trauma was based on clinical observation using a dental mirror and explorer.
7. At this point there was a break to allow the patient to have a snack. All patients had a break to ensure that the examiner remained blind to diabetic status.
8. Full mouth periodontal chartings were recorded at six sites around every tooth (mesiobuccal, distobuccal, mesiolingual, distolingual, midlingual and midbuccal) using a manual probe (University of North Carolina PCP 15). The distance between the cemento-enamel junction and the gingival margin was recorded to the nearest whole millimetre. If the gingival margin was coronal to the cemento-enamel junction a negative value was recorded. Gentle probing, approximately 25g/cm², was performed by inserting the probe into the gingival crevice along the long axis of the tooth until resistance was felt. Clinical probing depth (CPD) was defined as the distance between the gingival margin and the bottom of the probeable crevice or pocket rounded down to the nearest whole millimetre. These two parameters were used to compute clinical attachment level (CAL). Bleeding from the base of the pocket was recorded 30 seconds after probing. Third molar teeth were excluded from all recordings unless they were fully erupted and were replacing a missing molar tooth in the same quadrant.
9. The study protocol dictated that the non-diabetic status of the control group was confirmed by HbA1c testing of venous blood. Blood was taken from the antecubital fossa of all subjects by the dental research nurse, who was a trained phlebotomist, in accordance with the requirements of the local ethics committee. Two samples of anti-coagulated blood were collected in K2 EDTA and one in Sodium Heparin. One sample of coagulated blood was collected in a plain tube (vacutainerTM). The blood samples that were not required for HbA1c testing were separated by centrifugation (1048 xg for ten minutes), aliquotted and stored at -80°C. HbA1c testing was carried out on EDTA anticoagulated blood at the diagnostic haematology laboratory in Glasgow Royal Infirmary. High Performance Liquid Chromatography (HPLC) was conducted using the

Menarini HA-8160 analyzer (Menarini Diagnostics Firenze, Italy) according to existing diagnostic protocols. An HbA1c of <6.0% was required to confirm the non-diabetic status of control subjects (ADA, 2009).

2.2.4 Data handling

All data were handled confidentially and were stored on a password protected computer. Hard copies of forms were stored in a fireproof locked safe in a secure office. Clinically relevant findings were recorded in the patients' notes. Where the patient provided the General Dental Practitioner's contact details, a letter was sent to the dentist informing him/her that the patient had participated in the study and of any disease noted. The oral health questionnaire was completed on paper forms and later transcribed into a custom made Microsoft AccessTM database. The caries, key sites and plaque charts were loaded directly into Microsoft Access forms at the chairside. The periodontal charting was loaded into a custom made charting programme. These programmes did not allow automatic or easy analysis and it was necessary to design Microsoft VisualbasicTM programmes to extract the data. These were designed by Mr Colin Cairnie in conjunction with the author. Repeated testing of the programmes confirmed their reliability. It was possible to generate summary data for any caries or periodontal parameters by modifying the underlying programme. All data were backed up on a daily basis and stored on a DVD separately from the dedicated laptop. The patient was allocated a unique identifier which was used to link data for future analysis.

2.2.5 Statistical analysis

2.2.5.1 Power calculation

It was estimated that the level of severe periodontitis (\geq one tooth with ≥ 6 mm CAL) in the general population in Glasgow, for the age group under investigation, would be around 5% (Morris et al., 2001). Emrich and co-workers (1999) showed that type 2 diabetes mellitus conferred a three times increased risk of periodontitis in poorly controlled type 2 diabetic patients. Assuming that the increase in risk would be similar we conservatively estimated an increase in prevalence of two and a half times. Sample size was estimated on a prevalence of severe periodontitis in poorly controlled diabetic subjects of two and a half times that in non-diabetic subjects (i.e. 12.5% and 5% respectively). Using a simple matched pairs design (PCD, NDS), if the proportion of disagreements is 0.115 it was

estimated that a sample size of 136 pairs would have 80% power to detect a difference in proportions of 0.075 using a two-sided McNemar's test at the 5% significance level (calculations performed using StatXact). If the data are considered as sets of matched triplets, then, for a ratio of 2:1 (2 T1DM patients (1PCD, 1WCD):1 NDS) the approximate sample size for the control group is (3x sample size for 1:1 matching) divided by four (i.e. approximately $\frac{3}{4}$ of 136 = 102) (Schlesselman and Stolley, 1982). Correspondingly the size of the T1DM group (including PCD and WCD) would be 2x the new size of the control group (i.e. $102 \times 2 = 204$). If we therefore aim to examine 136 WCD and 136 PCD it will be possible to compare the matched T1DM patients with the controls looking for similar differences with greater than 80% power, as the actual sample size required is less than that for comparing only PCD with NDS. Therefore, a recruitment target of 272 type 1 diabetic patients and 136 non-diabetic subjects was set.

All data analysis was undertaken using Stata (Intercooled v10.0; StataCorp LP) or PASW 18 (PASW 18, SPSS Inc)

2.2.5.2 Descriptive data

Demographic and other characteristics of the study sample according to the primary outcome variable (severe periodontitis) and exposure/predictor variable (diabetes status) were described using the five number summary (median, inter-quartile range, and range) for skewed data and means (95% confidence intervals) for symmetrically distributed data.

2.2.5.3 Univariable analysis

In the univariable analysis the prevalence of periodontitis in T1DM (WCD + PCD) and only PCD was compared to NDS using Pearson's Chi-squared test or Fisher's exact test (when expected cell size < 5).

For the secondary outcomes that were symmetrically distributed, linear regression models compared T1DM vs NDS and PCD vs NDS whilst controlling for age and gender. Where the secondary outcomes were skewed, the comparison was carried out using Mann-Whitney rank sums tests to compare underlying distributions.

2.2.5.4 Multivariable analysis

For the multivariable analysis binary logistic regression models were used to estimate OR [95% CI] for severe periodontitis in T1DM versus NDS and PCD versus NDS.

Adjustments were made in the first instance for age and gender and then additionally for: SIMD, level of education, BMI (weight/height²), smoking status (never smoked, previously smoked) and attendance at a dentist.

For all models, each potential confounder was included in the model separately. Due to potential for collinearity between SIMD and education level, each variable was offered to the final model separately and then simultaneously. A final model was then produced consisting of all potential confounders. The final models were rerun restricting the analysis to only those subjects who had *never* smoked. Due to the large number of comparisons, a significance level of 1% was used throughout.

2.3 Results

2.3.1 Recruitment

Two hundred and ninety-six patients with diabetes and one hundred and sixty-five control subjects were recruited to the study. Figure 2-1 describes the flow chart of the study protocol and records the number of patients assessed, enrolled, examined and finally those available for analysis. In total 209 T1DM patients and 115 NDS were examined. Three diabetic patients were subsequently excluded because they were later shown to have Type 2 diabetes. Three did not have complete periodontal chartings but were included in the secondary analysis. Three NDS subjects were excluded: 1 was a former very heavy smoker (82 packyears), 1 was borderline Type 2 diabetic and 1 was excluded due to the medical history. An unmatched cross sectional analysis was carried out due to the fact that there were insufficient patients in the WCD group to achieve statistical power. In addition multivariable analysis was used to allow for correction of confounding factors. The cross-sectional analysis of the primary outcome for the whole diabetic group included 112 NDS and 203 T1DM. In model 2 which examined the differences between PCD and the NDS, there were 112 NDS and 169 PCD. The numbers examined for the secondary outcomes included 112 NDS and 205 T1DM patients for oral mucosal lesions and conditions and 110 NDS and 204 T1DM patients for hard tissue.

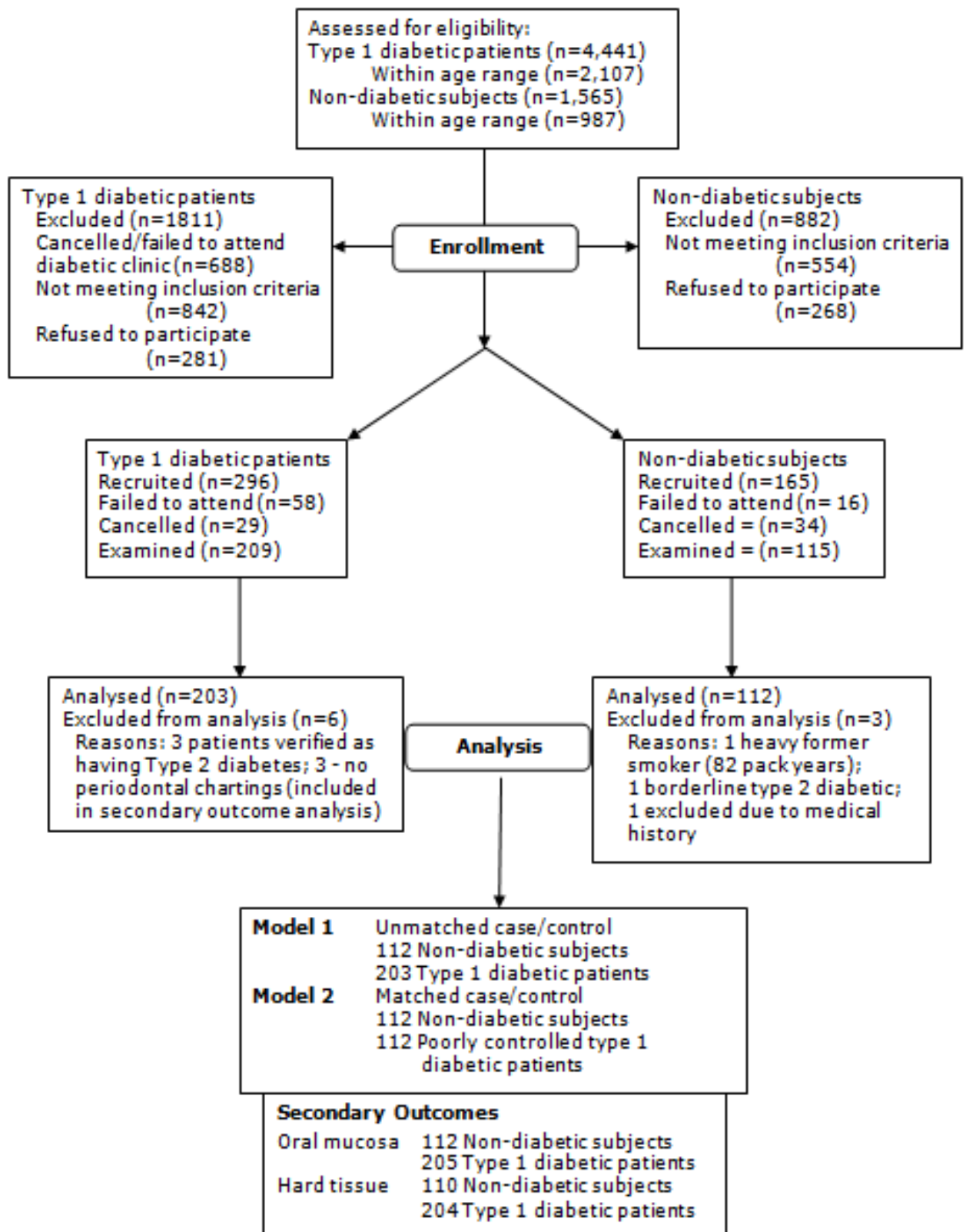


Figure 2-1 Flow chart of recruitment process

2.3.2 Calibration

The results of the intra-examiner calibration confirmed that there was an acceptable level of reproducibility by the single examining dentist. During the first calibration exercise all scores were fair or moderate apart from bleeding on probing which was poor throughout (Table 2-2). The initial inter examiner Kappa scores were lower although these still showed moderate agreement for CPD and CAL ($\kappa = 0.45, 0.41$) and good agreement for the position of the gingival margin ($\kappa = 0.67$) (Table 2-2).

The calibration exercise was repeated following further training shortly after the beginning of the clinical examinations and the scores were improved (Table 2-2). These data were considered acceptable and the clinical examinations continued.

The results of the repeat examinations carried out by the author throughout the study showed very good, good or moderate agreement (Gingival margin position $\kappa=0.82$, CPD $\kappa =0.64$, CAL $\kappa = 0.58$ and BOP $\kappa = 0.48$). The percentage within 1mm was greater than 98.9% for CPD and CAL (Table 2-3).

Table 2-1 Interpretation of Kappa scores (Altman, 1991)

Value of K	Strength of agreement
< 0.20	Poor
0.21 - 0.40	Fair
0.41 - 0.60	Moderate
0.61 - 0.80	Good
0.81 - 1.00	Very good

Table 2-2 Summary of calibration exercise

		CPD	Rec	CAL	BOP
Intra-examiner calibration	K	0.63	0.77	0.54	0.277
	Perfect agreement	69.6%	84.3%	61.3%	74.2%
	Within 1	97.5%	98.0%	94.1	N/A
	Within 2	100.0%	100.0%	99.0%	N/A
	Mean bias	0.025mm	0.00mm	0.03mm	0.092
	Measurement error	0.62mm	0.47mm	0.78mm	0.502
Inter-examiner calibration	K	0.45	0.67	0.41	0.15
	Perfect agreement	66.7%	86.9%	60.1%	62.4%
	Within 1	97.0%	98.2%	93.5%	N/A
	Within 2	99.4%	100.0%	99.4%	N/A
	Mean bias	0.60 mm	0.30 mm	0.89 mm	0.13
	Measurement error	0.67 mm	0.43 mm	0.79 mm	0.60

CPD = Clinical probing depth

CAL = Clinical attachment loss

Rec = gingival margin position/recession

BOP = Bleeding from the base of the pocket

Within 1: agreement between replicates to within 1 unit on measurement scale.

Within 2: agreement between replicates to within 2 units on measurement scale.

Mean bias: mean of differences between replicates.

Measurement error: standard deviation of differences between replicates

Table 2-3 Summary of ongoing calibration

		CPD	Rec	CAL	BOP
Intra-examiner calibration	K	0.64	0.82	0.58	0.48
	Perfect agreement %	78.1	89.4	70.9	74.6
	Within 1	99.8	99.9	98.9	N/A
	Within 2	100	99.9	99.9	N/A
	Mean bias	0.00mm	0.00mm	0.01mm	0.0011
	Measurement error	0.48mm	0.34mm	0.58mm	0.5

CPD = Clinical probing depth

CAL=Clinical attachment loss

Rec= gingival margin position/recession

BOP=Bleeding from the base of the pocket

Within 1: agreement between replicates to within 1 unit on measurement scale.

Within 2: agreement between replicates to within 2 units on measurement scale.

Mean bias: mean of differences between replicates.

Measurement error: sd of differences between replicates

2.3.3 Demographic data of non-participants

Table 2-4 describes the descriptive statistics for those individuals who initially agreed to join the study but subsequently did not attend and those who were included in the analysis. Data were available for 117 non-participants and 318 participants for age, gender, diabetic status and socio-economic status, using both DEPCAT and SIMD. Lifestyles variables including alcohol consumption, smoking status and body mass index were also included. There were statistically significant differences between those who participated and those who did not for smoking status and socio-economic status. 78.6% of non-participants were never smokers compared with 87.7% of participants ($p = 0.02$). There were differences between participants and non-participants by DEPCAT but not by SIMD. For DEPCAT there was no clear pattern of non-attendance by deprivation. There were more patients in DEPCAT 1 and 2 (the least deprived) and 7 (the most deprived) who did not attend their scheduled appointment. There were no other statistically or clinically relevant differences between the study participants and those who did not attend.

Table 2-4 Descriptive statistics for participants and non-participants

	Non-participants	N	Participants	N	p value ^a
N	117		318		
Age median, (IQR), [range]	39 (29, 45), [19-55]	117	37.8 (27, 44), [20-56]	318	0.5
Gender % (n)		117		318	0.6
Female	52 (61)		55 (175)		
Male	48 (56)		45 (143)		
Diabetic status % (n)		117		318	0.84
Diabetic	65 (76)		64.8 (206)		
Non-diabetic	35 (41)		35.2 (112)		
Socio-economic					
DEPCAT % (n)		115		318	0.001
1	8.7 (10)		9.1 (29)		
2	22.6 (26)		10.1 (32)		
3	10.4 (12)		12.3 (39)		
4	22.6 (26)		21.7 (69)		
5	7.0 (8)		16.7 (53)		
6	6.1 (7)		13.8 (44)		
7	22.6 (26)		16.4 (52)		
SIMD % (n)		111		307	0.42 ^a
1	30.6 (34)		24.1 (74)		
2	16.2 (18)		12.4 (38)		
3	16.2 (18)		19.5 (60)		
4	14.4 (16)		18.9 (58)		
5	22.5 (25)		25.1 (77)		
Missing	5.1 (6)		3.5 (11)		
Lifestyle variables					
Alcohol consumption-units/week median, (IQR), [range]	6 (1, 12), [0-40]	115	5 (1, 10), [0-40]	318	0.52 ^a
Missing % (n)	1.7 (2)				
Smoking status % (n)		117		318	0.02
Never smoked	78.6 (92)		87.7 (279)		
Smoked in past	21.4 (25)		12.3 (39)		
Pack years for previous smokers median, (IQR), [range]	5 (1, 14.9), [0-28]	25	5 (1, 12) [0-46]	39	0.85
Body Mass Index median, (IQR), [range]	26.6 (23.8, 29.3), [20.7-40.8]	111	26.1 (23.4, 29.9), [18.4-49.2]	318	0.46 ^a
Missing % (n)					

^a p values were calculated without missing data

2.3.4 Demographic data for patients with periodontitis

Table 2-5 describes the demographic characteristics of those patients with severe periodontitis (SP) as defined by one site of clinical attachment loss greater than or equal to 6mm on at least one tooth. Patients who were diagnosed with SP had a higher median age of 44 compared with 35 in the non-periodontitis group ($p \leq 0.0001$). Patients in the periodontitis group were also more likely to be male compared with the non-periodontitis group ($p = 0.02$) and had a higher BMI ($p = 0.009$). Patients with SP were more likely to attend the dentist once a year and to use inter-dental brushes ($p=0.03$ and $p=0.009$ respectively). Those who were registered with a GDP and attended a dentist regularly were however, no less likely to suffer from severe periodontitis than those who did not. Patients who did not have SP were more likely to have proceeded to higher education than those who did (80.8% compared with 69% ($p = 0.05$) respectively). They were also less likely to have been smokers in the past ($p = 0.002$).

Table 2-5 Descriptive statistics for subjects with and without severe periodontitis

	Clinical attachment level of < 6mm	N	Clinical attachment level of \geq 6mm on at least one tooth	N	p value
N	243		72		
Demographics					
Age median, (IQR), [range]	35 (27, 42), [20-56]	243	44 (38, 49), [20-55]	72	<0.0001
Gender % (n)					0.02
Female	58.4 (142)	243	43.1 (31)	72	
Male	41.6 (101)		56.9 (41)		
Most recent % HbA1c median, (IQR), [range]	7.5 (5.3, 8.7), [4.3-13.7]	235	8.0 (5.7, 9.0), [4.7-11.9]	70	0.07 ^a
Missing % (n)	3.3 (8)		2.8 (2)		
Body Mass Index median, (IQR), [range]	25.6 (23, 29.8), [18.4-49.2]	2243	26.7 (24.8, 31.1), [21-45.8]	72	0.009
No. units alcohol/week median, (IQR), [range]	5 (1.2, 10), [0-40]	243	4.5 (1, 11.8), [0-40]	72	0.63

^a p values were calculated without missing data

Table 2-5 cont.

	Clinical attachment level of <6mm	N	Clinical attachment level of ≥6mm on at least	N	p value
N	243		72		
Socio-economic status					
DEPCAT % (n)		243		72	0.96
1	8.6 (21)		11.1 (8)		
2	11.1 (27)		6.9 (5)		
3	11.9 (29)		12.5 (9)		
4	21.4 (52)		23.6 (17)		
5	16.5 (40)		16.7 (12)		
6	14.0 (34)		12.5 (9)		
7	16.5 (40)		16.7 (12)		
SIMD % (n)		235		70	0.98 ^a
1	24.7 (58)		22.9 (16)		
2	12.3 (29)		12.9 (9)		
3	18.7 (44)		21.4 (15)		
4	19.6 (46)		17.1 (12)		
5	24.7 (58)		25.7 (18)		
Missing	3.3 (8)		2.8 (2)		
Level of Education%(n)					0.05 ^a
Secondary school	19.2 (46)		31.0 (22)		
College/University	80.8 (193)		69.0 (49)		
Missing	1.6 (4)		1.4 (1)		
Car ownership % (n)					
Car ownership % (n)	86.6 (207)	239	84.5 (60)	71	0.70 ^a
Missing	1.6 (4)		1.3 (1)		
Lifestyle variables					
Smoking status %					
Smoking status %		243		72	0.002
Never smoked	90.9 (221)		76.4 (55)		
Smoked in past	9.1 (22)		23.6 (17)		
Pack years for previous smokers median, (IQR), [range]					
Pack years for previous smokers median, (IQR), [range]	2.3 (1, 7.6), [0-46]	22	12 (2.8, 15.4), [0-41]	17	0.02

^a p values were calculated without missing data

Table 2-5 Cont.

	Clinical attachment level of <6mm	N	Clinical attachment level of ≥6mm on at least one tooth	N	p value
N	243		72		
Registered with dentist % (n)	78.7 (188)	239	84.5 (60)	71	0.32 ^a
Missing	1.6 (4)		1.4 (1)		
Attendance at dentist % (n)		239		71	0.03 ^a
At least once a year	62.8 (150)		71.8 (51)		
Occasionally	10.0 (24)		5.6 (4)		
Only when in	27.2 (65)		22.6 (16)		
Missing % (n)	1.6 (4)		1.4 (1)		
Oral hygiene habits% (n)					
Tooth brushing		239		71	0.20 ^a
Never or less than 1 per day	2.5 (6)		4.2 (3)		
Once per day	15.5 (37)		25.4 (18)		
Twice per day	69.9 (167)		60.6 (43)		
More than twice	12.1 (29)		9.9 (7)		
Missing % (n)	1.6 (4)		1.4 (1)		
Use of floss	50.2 (120)	239	43.7 (31)	71	0.35 ^a
Missing % (n)	1.6 (4)		1.4 (1)		
Use of interdental brushes	6.7 (16)	239	18.3 (13)	71	0.009 ^a
Missing % (n)	1.6 (4)		1.4 (1)		
Toothpick use	7.9 (19)	239	18.3 (13)	71	0.024 ^a
Missing % (n)	1.6 (4)		1.4 (1)		

^a p values were calculated without missing data

2.3.5 Demographic data according to diabetic status

Table 2-6 describes the demographic characteristics of the 3 groups: NDS, WCD (HbA1c \leq 7.5) and PCD (HbA1c $>$ 7.5) (SDS, 2009). Demographic characteristics were analysed for differences across the 3 groups. There were no differences in age, gender, duration of diabetes, socio-economic status, car ownership, alcohol consumption, smoking status, registration with a dentist, attendance at a dentist or oral hygiene habits.

Differences approaching significance were noted in BMI where PCD had slightly higher BMI than either of the other 2 groups. The median was 26.7 compared with 25.2 in both other groups ($p = 0.02$). In addition, the level of education was lower in the PCD 29.1% having received only secondary education compared with 14.7% in the WCD group and 11.6% in the NDS.

Table 2-6 Descriptive statistics for non-diabetic subjects and diabetic patients

	Non-diabetic subjects	N	Well controlled diabetic patients	N	Poorly controlled diabetic patients	N	p value
N	112		34		172		
Demographic characteristics							
Age median, (IQR), [Range]	38 (29, 44), [20-56]	112	37 (28,42), [22-53]	34	38 (27, 44), [20-55]	172	0.59
Gender % (n)		112		34		172	0.07
Female	63.4 (71)		55.9 (19)		49.4 (85)		
Male	36.6 (41)		44.1 (15)		50.6 (87)		
Average % HbA1c median, (IQR), [Range]	NA		7.2 (6.8, 7.3), [5.6-7.5]	34	8.8 (8.3, 9.7), [7.6-13.9]	172	NA

^a p values were calculated without missing data

Table 2-6 cont.

	Non-diabetic subjects	N	Well controlled diabetic patients	N	Poorly controlled diabetic patients	N	p value
Most recent % HbA1c median, (IQR), [Range]	5.2 (4.9,5.4), [4.3-5.8]	102	7.1 (6.8, 7.4), [5.1-7.7]	34	8.7 (8.1, 9.6), [6.1, 13.7]	172	
Missing % (n)	8.9 (10)		0		0		
Duration (years)Median, (IQR), [Range]	NA		21 (10.9, 26), [6-39]	34	19 (11.6,25.8), [4-41]	172	0.63
Socioeconomic status							
DEPCAT % (n)		112		34		172	0.3
1	11.6 (13)		8.8 (3)		7.6 (13)		
2	9.8 (11)		8.8 (3)		10.5 (18)		
3	8.0 (9)		23.5 (8)		12.8 (22)		
4	22.3 (25)		11.8 (4)		23.3 (40)		
5	14.3 (16)		14.7 (5)		18.6 (32)		
6	19.6 (22)		8.8 (3)		11.0 (19)		
7	14.3 (16)		23.5 (8)		16.3 (28)		
SIMD % (n)		109		34		165	0.76 ^a
1	25.0 (28)		23.5 (8)		22.1 (38)		
2	9.8 (11)		17.6 (6)		12.2 (21)		
3	18.8 (21)		11.8 (4)		20.3 (35)		
4	22.3 (25)		14.7 (5)		16.3 (28)		
5	21.4 (24)		29.4 (10)		25.0 (43)		
Missing	2.7 (3)		2.9 (1)		4.1 (7)		
Level of Education							
% (n)		110		34		168	0.001 ^a
Secondary school	11.6 (13)		14.7 (5)		29.1 (50)		
College/University	86.6 (97)		85.3 (29)		68.6 (118)		
Missing	1.8 (2)		0		2.3 (4)		
Car ownership %							
(n)	82.7 (91)	110	85.3 (29)	34	88.1 (148)	168	0.45 ^a
Missing	1.8 (2)		0		2.3 (4)		

^ap values were calculated without missing data

Table 2-6 Cont. Lifestyle variables

	Non-diabetic subjects	N	Well controlled diabetic	N	Poorly controlled diabetic	N	p value
Lifestyle variables							
Alcohol consumption-units/week median, (IQR), [Range]	5.3 (1, 11), [0-40]	112	4.5 (1, 9), [0-27]	34	5 (1, 10), [0-40]	172	0.77
Smoking status % (n)		112		34		172	0.72
Never smoked	85.7 (96)		88.2 (30)		89 (153)		
Smoked in past	14.3 (16)		11.8 (4)		11 (19)		
Pack years for previous smokers median, (IQR), [Range]	5.5 (1.4, 12) [0-46]	16	3.3 (0.6, 7.4) [1-8]	4	4.8 (1, 14) [0-41]	19	0.64
Body Mass Index median, (IQR), [Range]	25.2 (22.6, 28.9) [18.4-49.2]	112	25.2 (22.9, 28.5), [19.1-36.7]	34	26.7 (24.3, 31.0), [19.6-43.2]	172	0.02

^a p values were calculated without missing data

Table 2-6 cont. Dental variables

	Non-diabetic subjects	N	Well controlled diabetic patients	N	Poorly controlled diabetic patients	N	p value
N	112		34		172		
Dental Variables							
Registered with dentist							
% (n)	74.1 (83)	110	70.6 (24)	34	82.6 (142)	168	0.07 ^a
Missing	1.8 (2)		0		2.3 (4)		
Attendance at dentist % (n)							
		110		34		168	0.15 ^a
At least once a year							
	62.5 (70)		55.9 (19)		65.7 (113)		
Occasionally							
	6.3 (7)		8.8 (3)		10.5 (18)		
Only when in pain							
	29.4 (33)		35.3 (12)		21.5 (37)		
Missing	1.8 (2)		0		2.3 (4)		
Oral hygiene habits % (n)							
Tooth							
		110		34		168	0.1 ^a
Never or less than 1 per							
	1.8 (2)		2.9 (1)		3.5 (6)		
Once per day							
	10.7 (12)		11.8 (4)		22.7 (39)		
Twice per							
	71.4 (80)		70.6 (24)		62.8 (108)		
More than twice per day							
	14.3 (16)		14.7 (5)		8.7 (15)		
Missing	1.8 (2)		0		2.3 (4)		
Use of floss % (n)							
	51.8 (58)	110	47.1 (16)	34	45.9 (79)	168	0.63 ^a
Missing	1.8 (2)		0		2.3 (4)		
Use of interdental brushes %							
	10.7 (12)	110	8.8 (3)	34	8.1 (14)	168	0.75 ^a
Missing	1.8 (2)		0		2.3 (4)		
Use of toothpicks % (n)							
	8.9 (10)	110	17.6 (6)	34	9.3 (16)	168	0.31 ^a
Missing	1.8 (2)		0		2.3 (4)		

^a p values were calculated without missing data

2.3.6 Prevalence, severity and extent of periodontitis in Type 1 diabetic subjects and poorly controlled Type 1 diabetic subjects

2.3.6.1 Prevalence of Periodontitis: Clinical Attachment Loss

Table 2-7 presents the prevalence of severe periodontitis and moderate combined with severe periodontitis for NDS, T1DM and the PCD.

Figure 2-2 shows the prevalence of SP as defined by one site with $CAL \geq 6mm$ for the three groups (NDS 20.5%, T1DM 24.1%, PCD 27.2, $p = 0.49, 0.26$.) Clinical attachment level greater than or equal to 6mm at at least 4 sites, was higher in both the T1DM (10.8%) group and the PCD (11.8%) group compared with the NDS (5.4%). The difference was not statistically significant ($p = 0.14, p = 0.12$) due to the small number of cases detected in this study (Figure 2-3).

Figure 2-4 shows the prevalence of moderate combined with severe periodontitis was higher in the T1DM group (79.8%) and PCD (79.2%) compared with the NDS (61.6%) ($p=0.001, p=0.002$ respectively).

Table 2-7 Prevalence and severity of periodontitis in non-diabetic subjects compared with type 1 diabetic and poorly controlled patients

	NDS	All T1DM patients	PCD	p value^a	p value^b
N	112	203	169		
Missing teeth median, (IQR), [range]	4 (3, 6) [0-17]	4 (3, 6) [0-16]	4 (3, 6) [0-16]	0.47	0.56
Severe periodontitis					
Clinical attachment level \geq 6mm on at least 1 tooth % (n) (95% CI)					
	20.5 (23) (13.5, 29.2)	24.1 (49) (18.4, 30.6)	27.2 (46) (18.1, 35.0)	0.49	0.26
Clinical attachment level \geq 6mm at at least 4 sites % (n) (95% CI)					
	5.4 (6) (1.9, 11.3)	10.8 (22) (6.9, 15.9)	11.8 (20) (7.7, 21.1)	0.14	0.12
Clinical probing depth \geq 6mm on at least 1 tooth % (n) (95% CI)					
	21.4 (24) (14.2, 30.2)	25.6 (52) (19.8, 32.2)	27.8 (47) (18.9, 35.9)	0.49	0.26
Clinical probing depth \geq 6mm at at least 4 sites % (n) (95% CI)					
	3.6 (4) (0.9, 8.9)	10.8 (22) (6.9, 15.9)	11.2 (19) (6.3, 19.0)	0.1	0.1
Moderate and severe periodontitis Clinical attachment level \geq 4mm on at least 1 tooth % (n) (95% CI)					
	61.6 (69) (51.9, 70.6)	79.8 (162) (73.6, 85.1)	79.3 (134) (69.8, 85.8)	0.001	0.002
Clinical probing depth \geq 4mm on at least 1 tooth % (n) (95% CI)					
	84.8 (95) (76.8, 90.9)	90.1 (183) (85.1, 93.9)	91.1 (154) (83.1, 95.0)	0.2	0.13

^a NDS vs T1DM ^b NDS vs PCD

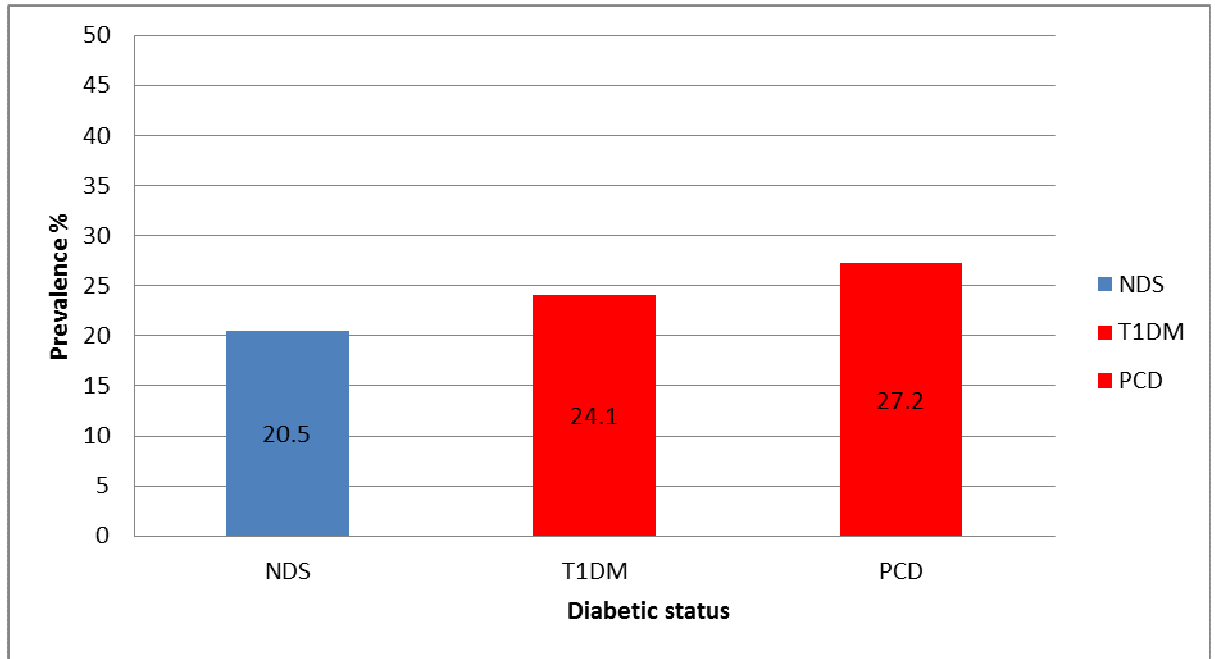


Figure 2-2 Prevalence of severe periodontitis (1 tooth \geq 6mm CAL) for NDS, T1DM and PCD

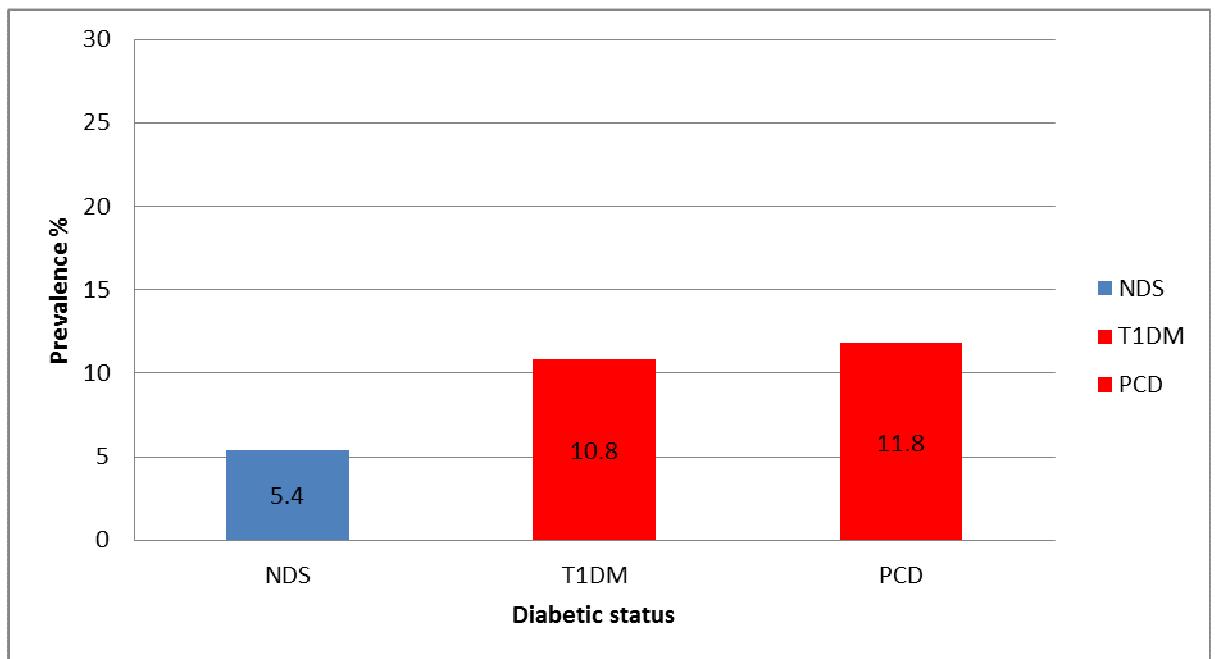


Figure 2-3 Prevalence of severe periodontitis (4 sites \geq 6mm CAL) between NDS, T1DM and PCD

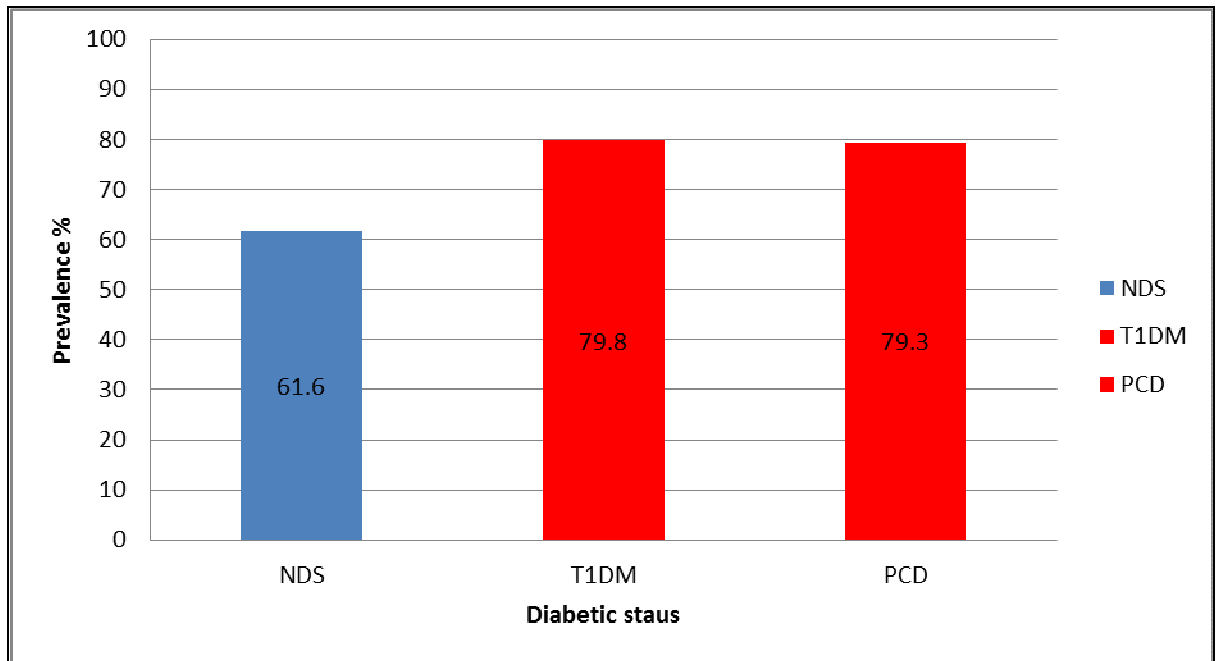


Figure 2-4 Prevalence of both moderate and severe periodontitis combined (minimum 1 site \geq 4 mm CAL) between NDS, T1DM and PCD

2.3.6.2 Prevalence of Periodontitis: Clinical Probing Depth

There were no significant differences in the prevalence of severe periodontitis or moderate combined with severe periodontitis as detected by clinical probing depths, between any of the 3 groups (Table 2-7). The prevalence of severe periodontal disease as defined by 4 sites \geq 6mm CPD was three times higher in the PCD compared with the NDS (NDS 3.6%, T1DM 10.8%, PCD 11.2% $p = 0.1$, $p = 0.1$) (Figure 2-6).

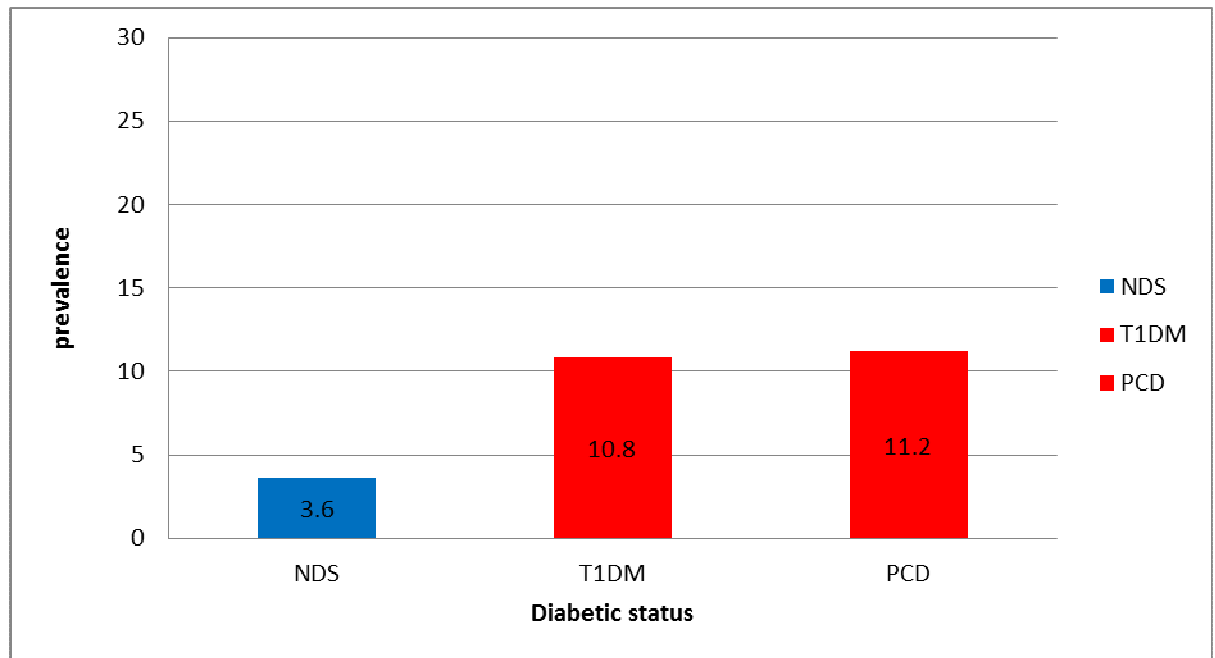


Figure 2-5 Prevalence of Severe Periodontitis (4 sites \geq 6mm CPD) between NDS, T1DM and PCD

2.3.6.3 Severity and Extent of Periodontal Disease

The extent of periodontitis was calculated using the median number of sites affected by severe periodontitis and moderate periodontitis combined with severe periodontitis. There were no differences in the extent of severe periodontitis as defined by the number of sites with ≥ 6 mm CAL due to the small number of sites affected by this level of disease (median number of sites [range] CAL ≥ 6 NDS = 0 [0-29], T1DM = 0 [0-69], PCD = 0 [0-69], $p = 0.39$, $p = 0.163$). There were also no differences in the extent of severe periodontitis as defined by the number of sites with CPD ≥ 6 mm (median number of sites [range] CPD ≥ 6 NDS = 0 [0-14], T1DM = 0 [0-37], PCD = 0 [0-37], $p = 0.24$, $p = 0.124$) (Table 2-8).

The extent of moderate combined with severe periodontal disease (median number of sites with clinical attachment loss ≥ 4 mm) was more widespread among T1DM and PCD compared with NDS (median number of affected sites NDS = 1, T1DM = 3, PCD = 4 ($p = 0.0003$, $p = 0.0004$)). The extent of current moderate and severe periodontitis as seen in the number of sites affected by CPD ≥ 4 mm was significantly higher for PCD and T1DM compared with NDS (median number of sites ≥ 4 mm CPD NDS = 5, T1DM = 7, PCD = 8 ($p = 0.005$, $p = 0.001$)) (Table 2-8) (Figures 2-6 & 2-7).

Gingivitis measured using full mouth bleeding on probing scores was significantly higher in the PCD group compared with T1DM and the NDS. The median percentage bleeding

on probing for NDS was 36% compared with 46% for T1DM and 54% for PCD ($p \leq 0.001$). Plaque scores were also significantly higher in both the PCD and the T1DM groups (NDS 63%, T1DM 73%, PCD 75% $p \leq 0.001$) (Table 2-8).

Combined measures of severity and extent and severity of periodontitis are represented by mean full mouth CAL and CPD (Fig 2-8 & 2-9). Figure 2-8 shows that NDS had lower mean full mouth clinical attachment level (1.53mm) compared with T1DM (1.85mm) and PCD (1.86mm) ($p \leq 0.001$, $p \leq 0.001$) respectively. Figure 2-9 shows that the mean clinical probing depths were all significantly greater in the T1DM and PCD groups. 2.17mm compared with 2.29mm in the T1DM and 2.31mm in the PCD group ($p = 0.01$, $p \leq 0.005$) (Table 2-8).

2.3.7 Multivariable analysis

The results of the multivariable analysis are shown in Table 2-9. Odds ratios were calculated for comparisons between the NDS and the whole T1DM group. The unadjusted odds ratio was 1.26 (0.69, 2.33). The fully adjusted model, including BMI, attending a dentist, SIMD, previous smoking experience, education, age and gender gave an overall odds ratio of 1.19 [0.62, 2.27] ($p = 0.6$). Odds ratios were also calculated comparing NDS with the PCD. The unadjusted model gave an odds ratio of 1.5 [0.72, 3.11] ($p = 0.28$). The fully adjusted model including BMI, attending a dentist, SIMD, previous smoking experience, age and gender gave an odds ratio for the poorly controlled group of 1.43 [0.74 to 2.75] ($p = 0.29$). Restricting the analysis to those who had never smoked increased the fully adjusted OR [95% CI] comparing T1DM and PCD with NDS to 1.35 [0.66 to 2.8] ($p = 0.41$) and 1.58 [0.75 to 3.33] ($p = 0.23$) respectively.

Table 2-8 Extent and severity of periodontitis in non-diabetic subjects compared with T1DM and poorly controlled diabetic subjects

	NDS	T1DM	PCD	<i>p value</i>	<i>p value</i>
N	112	203	169		
Number of sites clinical attachment level \geq 4mm median, (IQR), [range]	1(0, 6), [0-76]	3(1, 11), [0-91]	4(1, 11.5), [0-91]	0.0003	0.0004
Number of sites clinical attachment level \geq 6mm median, (IQR), [range]	0(0, 0), [0-29]	0(0, 0), [0-69]	0(0, 1), [0-69]	0.39	0.163
Number of sites clinical probing depth \geq 4mm median, (IQR), [range]	5(2, 10.5), [0-45]	7(3, 15), [0-84]	8(1, 11.5), [0-91]	0.005	0.001
Number of sites clinical probing depth \geq 6mm median (IQR), [range]	0, (0, 0), [0-14]	0 (0, 1), [0-37]	0 (0, 1), [0-37]	0.24	0.124
Mean clinical attachment level (mm), (95% CI)	1.53	1.85	1.86	<0.001	<0.001
Mean clinical probing depth (mm), (95% CI)	2.17, (2.11, 2.23)	2.29, (2.23, 2.35)	2.31 (2.24, 2.38)	0.01	<0.005
Bleeding on probing (%) median, (IQR), [range]	36 (26, 52), [4-80]	46 (33, 61) [7-100]	49 (36, 64) [7-100]	<0.001	<0.001
Plaque score (%) median, (IQR), [range]	63(52, 75) [14-99]	73 (63, 84) [7-99]	75 (65, 85) [7-99]	<0.001	<0.001

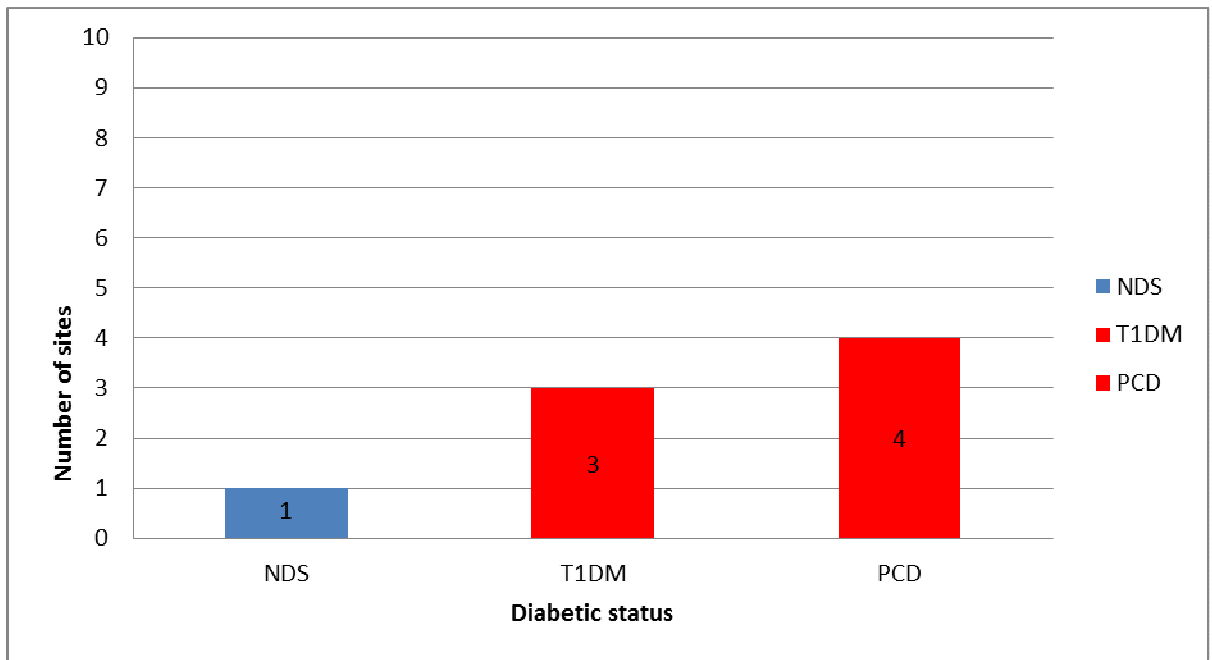


Figure 2-6 Extent of moderate periodontitis between NDS, T1DM and PCD (Median number of sites affected ≥ 4 mm CAL)

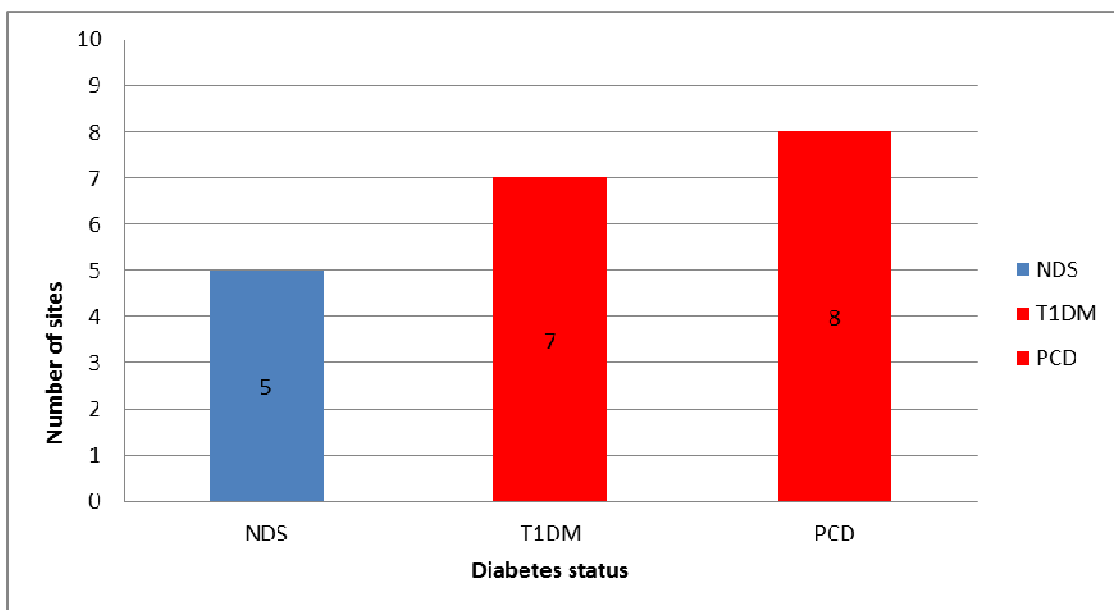


Figure 2-7 Extent of moderate periodontitis between NDS, T1DM and PCD (Median number of sites affected ≥ 4 mm CPD)

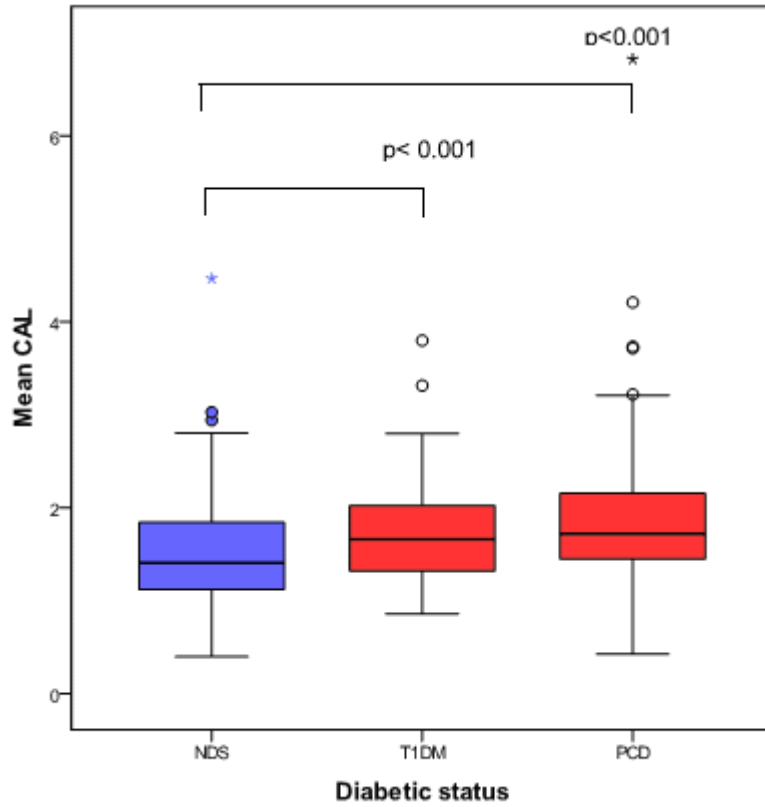


Figure 2-8 Mean full mouth CAL by diabetic status

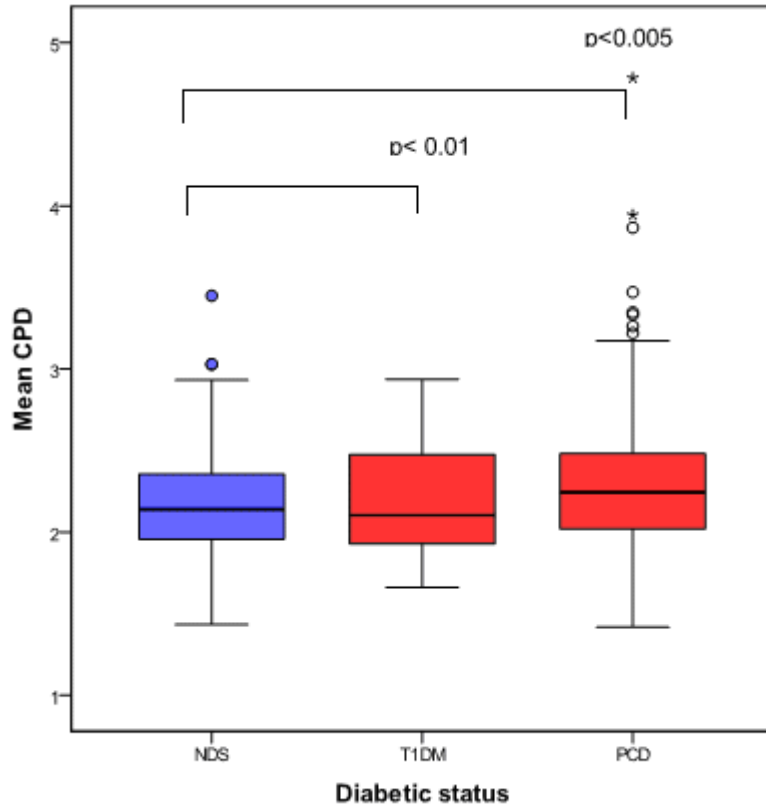


Figure 2-9 Mean clinical probing depths by diabetic status

Table 2-9 Prevalence, unadjusted and adjusted Odds Ratios and 95% confidence intervals for severe periodontitis (A minimum of one tooth with CAL \geq 6mm) in type 1 diabetic patients, and poorly controlled patients versus non-diabetic subjects

	Severe periodontitis % (n)	Unadjusted OR [95% CI]*	Adjusted OR [95% CI] with education**	Adjusted OR [95% CI] with SIMD**	Adjusted OR [95% CI]***	Adjusted OR [95% CI]***	Adjusted OR [95% CI]***
						Type 1 diabetic Never smokers only (n=263)	Poorly controlled Never smokers (n=234)
Non-diabetic subjects	20.5 (23/112)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Type 1 diabetic patients	24.1 (49/203)	1.26 [0.69, 2.33]	1.21 [0.64, 2.29]	1.23 [0.65, 2.32]	1.19 [0.62, 2.27]	1.35 [0.66 to 2.8]	
p-value		0.45	0.57	0.53	0.6	0.41	
Non-diabetic subjects	20.5 (23/112)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)		1.00 (ref)
Poorly controlled patients	27.2 (46/169)	1.50 [0.72, 3.11]	1.61 [0.78, 3.34]	1.46 [0.72, 2.99]	1.43 [0.74 to 2.75]		1.58 [0.75 to 3.33]
p-value		0.28	0.20	0.29	0.29		0.23

* Patient age and gender in unadjusted models. ** BMI (weight/height squared), attending a dentist (y/n), previous smoking experience (y/n), age and gender.*** Fully Adjusted for BMI, attending a dentist, SIMD, previous smoking experience, education, age and gender.

2.4 Discussion

2.4.1 Calibration

Accurate recording of periodontal indices is essential to ensure the reliability of the clinical data. Training and calibration of examiners was carried out according to standard protocols with external training by an independent periodontal researcher as described in the Materials and Methods section. The results of the training and calibration are presented within this thesis. Overall we can be confident in the reproducibility of the clinical examiner and the reliability of the clinical data for the gingival margin position, CPD, CAL and BOP with all kappa scores showing moderate to very good agreement and with 98.9% of all CPD and CAL measurements being within 1mm.

Inter-examiner agreement for BOP was initially unacceptably low ($\kappa = 0.15$) although there was perfect agreement in 62.4% of cases (Table 2-2). The Kappa score is low, in part, because there are only two options, bleeding or no bleeding. The probability of getting agreement by chance is therefore 50%. High kappa scores therefore require that agreement is very high relative to the probability of agreement by chance, that is, well in excess of 50%. Clinically the relatively low level of agreement in bleeding on probing could be due to the possibility that trauma caused by the first examiner may not cause initial bleeding but may make bleeding more likely when the gingival or periodontal pocket is traumatised for a second time. In addition, variation in the position and force used by examiners could lead to differences in the tendency of the pocket to bleed. Since, logistically, there is no other way for calibration to be carried out this was considered an acceptable explanation. The kappa score for BOP for the single examiner showed a moderate level of agreement throughout the study ($\kappa = 0.48$).

The steps necessary to improve the intra-examiner scores further are likely to make the data less valid; for example only recording visible and accessible sites or similar strategies. Some studies have, for example, used only buccal sites in order to improve reproducibility. This can hardly be said to be representative of the clinical situation where periodontal destruction can occur anywhere in the mouth and is, in fact, more common on the lingual and palatal aspects of teeth (Kingman et al., 2002).

2.4.2 Comparison with the relevant literature

The conclusion of most narrative reviews published over the last few decades has been that there is an association between T1DM and periodontal disease. More recent systematic evaluation of the literature has called this assumption into question with one review in 2006 reporting that the extent of periodontal disease was similar in T1DM and NDS (Khader et al., 2006). A meta-analysis by Chávarry et al. (2009) concluded that there was no statistical difference in the severity of periodontitis in type 1 diabetic patients compared with controlled subjects and called for further research into this area.

The review by Chávarry and co-workers (2009) specifically criticised previous studies for a number of methodological inadequacies including: lack of a power calculation; not confirming the diabetic status of diabetic patients and controls; inadequate training and calibration of examiners; not reporting and adjusting for confounders such as smoking; inappropriate statistical analysis; the source of the control group; not blinding the examiners; and poor response rates. Chávarry reported that of the 17 papers investigating a link between T1DM the majority had significant omissions in their study design and reporting.

The aim of the study reported in this thesis was to focus on the effect of T1DM on the oral tissues in a population of white European non-smoking subjects who were of similar age and from the same geographic area, thereby reducing demographic, socio-economic and genetic variation. This study confirmed that the prevalence, severity and extent of periodontal disease is increased in T1DM. This was the first study in type 1 diabetic adults to confirm this association in the absence of recent or current smoking.

Data synthesis and meta-analysis on the studies reporting clinical probing depths between T1DM compared with NDS showed that the average difference in CPD was 0.11mm ($p=0.137$) (Chávarry et al., 2009). The magnitude of difference was similar in the study reported in this thesis with a mean difference of 0.12mm between NDS and T1DM and a mean difference of 0.17mm between NDS and PCD. These differences were not significant in the meta-analysis but were highly statistically significant in our study ($p = 0.008$, $p < 0.001$ respectively) (Chávarry et al., 2009). The difference between the study presented here and the meta-analysis could be due

to differences in the spread of the data and heterogeneity in the studies included in the meta-analysis.

Studies reporting clinical attachment loss showed a mean difference in CAL of 0.26 ($p = 0.054$). In this study the mean difference was 0.32mm for NDS compared with T1DM ($p < 0.001$) and 0.38mm for NDS compared with PCD ($p < 0.001$). Mean CPD or CAL levels give a good indicator of overall damage to the periodontium and can be used in populations where levels of disease are very low such as adolescent populations (Lalla et al., 2006, 2007). They do not however give any information about the prevalence of current or historical periodontal disease at a patient level or indicate the need for treatment. Many sites of 4mm CAL or CPD would lead to a higher mean figure than where only one or two sites are severely affected by periodontitis in an otherwise healthy mouth. In addition, it is not possible to draw conclusions about the extent of periodontal disease between the two groups. In the meta-analysis by Khader et al., (2006) it was concluded that there was an increase in severity but that there was no difference in extent of periodontal disease. We have shown that there is an increase in the level of periodontitis in T1DM and PCD compared with NDS using the following parameters: prevalence of moderate combined with severe periodontitis, measured using CAL; severity measured by mean CAL and CPD; and extent measured by the median number of sites with CAL and CPD of ≥ 4 mm.

Many studies have presented their results as mean CAL or CPD (Guthmiller et al., 2001; Alpagot et al., 2001; Aren et al., 2003; Yucekal-Tuncer et al., 2003). A few studies report the extent of disease as the percentage of sites with specified levels of disease but this is not common. Khader and co-workers found only three studies reporting this data in their systematic review (Khader et al., 2006). Some studies have presented the prevalence of different levels of periodontal disease (Rylander et al., 1986; Sandholm et al., 1989; Hugoson et al., 1989; Thorstensson et al., 1993; 1995); however, odds ratios are not commonly used (Haber et al., 1993). This thesis presents a full picture of the prevalence, extent and severity of periodontal disease in T1DM.

2.4.3 Recruitment

Recruitment was much more difficult than expected and required that participation was sought from a number of hospitals spread throughout the city. Extending

recruitment to other sites increased the time commitments for the dental research nurse and required the Principal Investigator and another part time research nurse to become involved in recruitment. The identification of clinicians at the different sites able to become involved, the burden of bureaucracy and the time taken for amendments and extensions to the ethical approval meant that this process took months rather than weeks. Other measures taken to increase recruitment were to increase the age limit to 55 years; using the buddy method to recruit controls and placing an advertisement for the study in the Metro newspaper.

It is possible that using a number of methods of recruitment of controls increased the heterogeneity of the control group reducing the effect of participation bias from any one of the various methods employed. As a result of the reduced rate of recruitment the project was extended by six months. This has implications for the cost of running studies involving human recruitment.

A pilot study was conducted prior to the start of recruitment but this only assessed the number of patients eligible for recruitment and not the number who would agree to participate. We were recruiting in deprived areas of the West of Scotland and found the drop-out rate, between recruitment and participation, by DEPCAT, was different across the whole social spectrum. The higher drop-out rate among more affluent participants could have been due to work commitments. The slightly higher number of subjects who dropped out from the lowest socio-economic group fits with the common pattern where study participation is generally lower in more deprived groups (Conway et al., 2010). There was no difference across the socio-economic spectrum using the preferred current measure of deprivation (SIMD) between those who participated and those who did not. Therefore it is unlikely that the differences detected using DEPCAT would have changed the results.

2.4.4 Sample size

The target sample size was predetermined by a power calculation. Unfortunately the target was not reached despite the efforts described in the following section. Due to relatively poor glycaemic control among the diabetic patients recruited we had more PCD than required (see section 2.4.6). The lack of WCD reflects the average metabolic control of the population from which the subjects were drawn (HbA1c around 8.7 for the age-group of interest; (SDS, 2009). The fact that statistically

significant differences in extent and severity of periodontitis were found, without reaching the sample size predicted by our power calculation, indicates that a real difference exists. It was disappointing not to achieve our initial target. The results of the multivariable analysis indicate that there may be an increased risk of severe periodontitis in non-smoking type 1 diabetic patients and this is supported by other secondary measures of periodontal disease. However we cannot reach firm conclusions about the odds ratios of severe periodontitis based on the available data. Given the high level of disease in both groups it is unlikely that the differences would have been statistically significant even if we had achieved our recruitment target.

2.4.4.1 Control group

A number of previous studies used inappropriate control groups such as siblings or patients attending a specialist paediatric dentistry department (Cianciola et al., 1982; Leeper et al., 1985). We used three sources of control subjects as described in sections 2.2.2.1 and 2.4.3. Some researchers have criticised using hospital based controls because it has been suggested that they may not be true healthy controls. Nonetheless the use of hospital based controls is encouraged in order to ensure that the factors other than the diseases under investigation are similar in both study groups. We recruited from the drop-in physiotherapy clinics in the same hospitals in an attempt to recruit patients from the same geographic and socioeconomic background. Subjects recruited were attending these clinics with minor physical injuries and were otherwise fit and well. No patients with significant impairment issues or chronic arthritic conditions were recruited. The use of the buddy system was intended to identify a group of controls of the same gender and similar age as the T1DM patients who recommended them. Finally the use of the advertisement in the national newspaper was only moderately successful. The criticism of advertising is that there is the potential for bias introduced by self-selection. The Strengthening the reporting of observational studies in epidemiology guidelines (STROBE) suggest that controls with other diseases may have advantages over population-based controls for hospital-based cases (Von Elm et al., 2007). The guidelines state that they better reflect the catchment population of a hospital and have greater comparability of recall and ease of recruitment. It is however also acknowledged that they can present problems if the exposure of interest affects the risk of developing or being hospitalized for the control condition(s). To remedy this problem the authors suggest that a mixture of the best

defensible control populations is used (Von Elm et al., 2007). In using different sources for our controls we have hopefully avoided the potential bias introduced by any one of the three methods used. It could be argued that the generalizability has been improved by the inclusion of different types of control subject although it is not possible to quantify this effect. In our experience the most successful method was the hospital out-patient recruitment, confirming the recommendations of the STROBE guidelines.

2.4.4.2 Confirmation of non-diabetic status of control subjects

Studies that did not confirm the non-diabetic status of the control group could have inadvertently recruited subjects with undiagnosed diabetes into the “healthy” control group. Diabetes mellitus affects 4.4% of the population and it is estimated that there are another 1.1 million patients who are currently undiagnosed in the United Kingdom representing around 2% of the adult population (Diabetes UK, 2010). In a study aiming to recruit 136 healthy adult controls it is theoretically possible that 2 or 3 subjects could be suffering from undiagnosed type 2 diabetes. If these few control subjects had severe periodontitis as a result of having undiagnosed type 2 diabetes then this could affect the results significantly. We screened our control subjects using HbA1c as a marker and excluded one middle aged female with severe periodontitis because she was borderline for type 2 diabetes.

2.4.4.3 Controlling for other confounders

In our study we attempted to control for age, gender, race, systemic health and smoking by careful phenotyping, and our exclusion criteria. The mean ages for the three groups were similar. There is a near linear relationship between periodontal disease and age and so it is critical that there are no significant differences in age between T1DM and the NDS (Morris et al., 2001). Most studies have shown that there is a higher prevalence of periodontal disease in men compared with women. There were more males in the PCD but this was adjusted for in the careful statistical modelling. Patients with medical conditions or taking drugs that are known to affect oral and periodontal health were excluded from participation. In the multivariable analysis age, gender, pack years, BMI, attendance at a dentist, deprivation and education were adjusted for in the analysis. It is most interesting to note the effect of a multivariable analysis whereby similar prevalence rates (NDS 20.5%, T1DM

24.1%, PCD 27.2%) can mask differences that are only revealed by multivariable analysis (OR 1.58 $p = 0.23$). The aim of both the inclusion/exclusion criteria and the multivariable analysis was to focus on the effect of diabetes on the periodontium once all other confounders had been accounted for.

2.4.4.4 Comparison between periodontal data from the UK and the control group

The prevalence of chronic periodontitis in the study group was significantly higher than was expected based on available population data (Morris et al., 2001). While we did see a high prevalence of periodontal disease in our T1DM we also saw a correspondingly high prevalence in the NDS. In the multi-variable model the odds ratio in never smoking poorly controlled diabetic adults for having 1 site with ≥ 6 mm of CAL was 1.58. Because we did not meet our recruitment target and because the level of disease in the control group was higher than the estimate used for the power calculation this was not statistically significant ($p = 0.22$)

Robinson (1996), in a survey of adult men between the ages of 18 and 65 years including smokers reported that CAL and CPD of ≥ 4 mm affected around a third (28.5%: 31.9%) of all of the group. The prevalence of moderate combined with severe periodontitis (CAL and CPD ≥ 4 mm) in the NDS in our study was 44% higher for CAL and more than double for CPD (41.1%, 63.4%). The study by Robinson was carried out in the general population and would have included smokers. It was therefore surprising to see such high levels of moderate disease in our control group.

The high level of moderate periodontitis in the control population reflects the response to local aetiological factors i.e. plaque exposure where oral hygiene is universally poor. The additional effect of T1DM where oral hygiene is poor is harder to calculate in this population. This effect was seen in a recent study in Pomerania where the extent of periodontal disease was high in both groups 24.3% of sites in the diabetic patients had CAL ≥ 4 mm while the control group had 19% of all sites affected. No significant difference in extent of disease was found between the groups (Kaur et al., 2009).

2.4.4.5 Adult dental health Survey 1998

In the Scottish population of the Adult Dental Health Survey (ADH) (Morris, 2001), the level of SP in the general population was 8% across the whole age group from 16 years upwards. This study population included current and former smokers. If anything we would have expected the NDS would have less disease than that recorded in the ADH survey. The prevalence of SP in the NDS in the current study was 20.5%. There are a number of reasons why this level of disease may well be representative of the local population.

The ADH survey did not stipulate a minimum number of teeth for inclusion and as a result included both fully dentate and partially dentate in the periodontal examinations. The mean number of teeth was lower in the ADH study compared with our control group (23.8 versus 27.2) and therefore there were fewer teeth on which to measure any disease present. In addition to this the level of deprivation appears to have been higher in our sample compared with the ADH survey. There was an almost fourfold increase in the proportion of subjects from the most deprived areas included in the current study (DEPCAT 6 and 7: 30.2% versus 8.0% in ADH study). This over representation of DEPCATs 6 and 7 is more representative of Glasgow and Paisley where the study took place. It has been shown that low socioeconomic status and low educational attainment are associated with poor glycaemic control as well as higher levels of oral disease (Mühlhauser et al., 1998; Larsson et al., 1999; Ross et al., 2001; Bower et al., 2007).

The ADH study used a partial mouth recording method and this may account for part of the difference noted. It has been shown that partial mouth recording while acceptable and indeed more practical in large population studies does lead to underreporting of periodontal disease levels (Susin et al., 2005; Beck et al., 2006; Kingman et al., 2008). Susin (2005) showed that the level of under reporting increased as the CAL increased. With $CAL \geq 6\text{mm}$ a number of different partial mouth recording methods were evaluated. Some methods of partial mouth recording could underestimate the true prevalence by up to 76% (12-76%) (Table 2-10). The largest effect was seen where some index teeth methods resulted in an underestimation when only mesial and buccal sites were examined. The study by Beck (2006) showed that partial mouth recording had a marked impact on

underreporting of $CAL \geq 4\text{mm}$ and $CAL \geq 6\text{mm}$ (6-56% and 11.3-70.8% respectively).

The ADH periodontal examination involved none of the methods described above. It involved full mouth recordings at only two sites per tooth using the buccal sites in the upper jaw and the lingual sites in the lower jaw; a total of 56 sites were examined. Two methods reported by Beck examined 42 randomly selected sites and underestimated disease in 17.5% ($CAL \geq 4\text{mm}$) and 28.2% ($CAL \geq 6\text{mm}$) of cases. It is reasonable to assume that an underestimation of prevalence of in the region of 20% took place in the ADH survey because of the partial mouth recording that took place.

Severity as measured by mean CAL and mean CPD is not seriously affected by partial mouth recording techniques (Beck et al., 2006). In another study, partial mouth recording resulted in an underestimation of the prevalence of periodontitis as defined as $CAL \geq 2\text{ mm}$ and $CAL \geq 3\text{ mm}$ by 22% and 36% respectively. The extent of CAL was less affected by partial mouth recording, in that the percentage of sites with no sign of early attachment loss was underestimated by up to only 11%. However, the percentage of sites with $CAL \geq 1\text{ mm}$ and $\geq 2\text{ mm}$ were overestimated by 11% and, 7% respectively (Eaton, 2001).

Table 2-10 Underestimation of true prevalence using partial mouth recording techniques. Redrawn from Susin (2005)

	Full mouth				Random half mouth			
CAL mm $\geq 6\text{mm}$	6 Sites*	MB-B	MB-B-DB	MB-B-DL	6 Sites	MB-B	MB-B-DB	MB-B-DL
	43.6	32.5	36.6	38.9	36.2	24.7	28.3	31.4
% Underestimation	-	34%	19%	12%	20%	76%	54%	39%

The level of untreated periodontal disease in the control population may also reflect the system of remuneration for periodontal treatment that exists under the National Health Service in Scotland where many general dental practitioners do not feel that such treatment is adequately funded. Subjects not registered with a dentist were no

more likely to have CAL of ≥ 6 mm than those who were (24.2% (n=15/62) versus 24.2% (n=60/248) respectively; p =1.0) (Table 2-5).

A concern may be that there was the potential for participation bias in the NDS sample. It is possible that patients who were worried about their teeth and/or had bleeding gums may have been more likely to have agreed to attend the Dental School for a thorough examination as part of a research study. It is possible that those study participants who responded to the advert had concerns about their oral health. Although no incentives were given, participants were informed that there was the possibility of remedial dental treatment if disease was found during the examination. Given that about a quarter of all non-diabetic subjects reported that they had difficulty paying for dental treatment this is another feasible explanation. However, we will show in chapter 6 of this thesis that the impact of dental disease (OHIP-14) was no worse in the NDS than in the ADH survey for the general population.

2.4.5 Glycaemic control.

During recruitment no account was taken of HbA1c and all willing diabetic participants were recruited regardless of diabetic control. The glycaemic control of the study participants therefore reflects the level of control among diabetic patients in the respective diabetic units. The diabetic subjects were split into WCD and PCD groups based on the target suggested by the Scottish Diabetes Survey of 7.5% (SDS, 2009). This is a conservative cut off for good glycaemic control that is also recommended by the current National Institute for Health and Clinical Excellence (NICE) guidelines (NICE CG15, 2004, 2010). NICE states that blood glucose control should be optimised towards attaining DCCT-harmonised HbA1c targets for prevention of microvascular disease (less than 7.5%). In those at increased risk of arterial disease an HbA1c of less than or equal to 6.5% is recommended (NICE, 2004).

The risk of serious hypoglycaemia is increased as the target HbA1c is lowered and this is one reason why patients rarely achieve such low levels. There is currently significant debate about the target that should be set for HbA1c. The American Diabetes Association (ADA) guidance suggests an HbA1c goal of <7%, while other diabetes organizations, such as the American Association of Clinical Endocrinologists (AACE) and the International Diabetes Federation (IDF) have set their HbA1c

targets at <6.5% (AACE Diabetes Mellitus Clinical Practice Guidelines Task Force, 2007; ADA, 2011). If we had used the cut-off of 6.5% for adequate glycaemic control then we would have only included seven patients in the WCD. Diabetic patients with an HbA1c of 6.6% would have been classified as poorly controlled. This figure is not only unrealistically low but would have diluted any effect of increased HbA1c on the prevalence of oral complications. The evidence suggests that a target HbA1c of 7.5% or even slightly higher is sufficient to reduce the risk of microvascular complications (DCCTRG, 1993). Little discernible benefit is derived in terms of diabetic microvascular disease from attempting to reduce HbA1c below this level. The main advantage is gained in the reduction of macrovascular disease as HbA1c is reduced toward normal levels. However, this target has recently been brought into question in type 2 diabetic patients due to an increase in all causes of mortality in the intensive glucose control arm of a large trial (ACCORD Study Group, 2008). The proposed mechanism of periodontal destruction is thought to be similar to the pathogenesis of microvascular disease and so a target of 7.5% seems reasonable for pathological as well as practical reasons (SIGN, 2010).

The majority of study patients fell into the PCD group (83%) (Table 2-6). It has been reported that complications are associated with high levels of deprivation in T1DM (Bachmann et al., 2003). However, the Scottish Diabetes Survey (2009) showed that T1DM patients generally show poor metabolic control (mean HbA1c over the previous 15 months in patients >20 years in Greater Glasgow and Clyde was similar to that recorded in this diabetic group at around 8.7%). The proportion of patients achieving an HbA1c of 7.5% or less in the greater Glasgow and Clyde area was only 22.1%. These figures are similar to the level of glycaemic control in England where less than 28.6% of patients met the same target (National Diabetes Audit 2008-2009).

2.4.5.1 Glycaemic control and periodontal disease

Clinical studies have shown that an HbA1c of 7.5% or less is required to reduce the incidence of microvascular diabetic complications (DCCTRG, 1996; Stratton, 2000). As this target is not being routinely met by T1DM patients in the West of Scotland then the prevalence of diabetic complications, oral and general, will be higher within this population. If poor glycaemic control is independently related to periodontal disease then we can hypothesise that the burden of dental care will be high among

T1DM subjects in the West of Scotland. A number of studies have shown that in diabetic patients the severity of gingivitis is related to glycaemic control (Gislen et al., 1980; Kjellman et al., 1970a,b; Gusberti et al., 1983;Harrisson et al., 1987, Seppala et al., 1993, 1994; Firalti et al., 1996). Seppala (1993, 1994) in a longitudinal study in T1DM adults reported that patients with poorly controlled diabetes mellitus had higher clinical probing depths, gingivitis scores, clinical attachment loss, recession and radiographic signs of approximal bone loss than controls. Willerhausen (1991) in a European study of 42 T1DM compared with 118 healthy controls reported that there was a significant correlation between HbA1c and loss of clinical attachment as well as between insulin dose and the gingival condition.

Discussion with diabetic colleagues revealed that they were unsurprised by the proportion of patients failing to meet the targets set and that this is an ongoing problem for diabetologists. While other organisations are recommending still lower target levels for HbA1c it appears that moderate and poor HbA1C levels remain high in the United Kingdom. The extent and severity of periodontitis in type 1 diabetes reported in this study indicates that a large proportion of diabetic adults in the United Kingdom is at risk of developing periodontitis as a result of having poor glycaemic control.

2.4.6 Smoking

Because of the effect of smoking on periodontal health all participants were never smokers or had not smoked in the last five years. The hospitals were primarily located in deprived areas of Glasgow. In 2008 it was reported that in the most deprived groups in Scotland smoking prevalence was 45% compared with only 11% in the least deprived (Scottish Household Survey, 2009). The SDS 2009 reported that 24.6% of all T1DM in Scotland are current smokers and a further 22% are ex-smokers. In light of the general trend by deprivation this is likely to have been higher in deprived subjects with diabetes. It is possible that this inequality could have affected recruitment to the study.

We chose a cut-off of five years for former smokers. The odds ratio for periodontitis starts to reduce as soon as an individual stops smoking and reduces from 3.97 to 3.15 within 0-2 years of stopping smoking. This continues to reduce with time and reduces to 1.15 eleven years after cessation of smoking (Tomar et al., 2000). It has been

shown that ex-smokers have a reduced risk of periodontitis compared with smokers although their risk is not as low as those who have never smoked (Warnakulasuriya et al., 2010). The effect of smoking on periodontitis is dose dependent and this can also be taken into account when analysing the data, using pack years as an estimate of smoking exposure over time. We used a pack year score of less than 0.25 and not having smoked for five years to classify subjects as never smokers. The five subjects who had between 0 and 0.25 pack years had smoked socially in their early twenties for a short time. There is clear evidence that low levels of smoking over short periods of time have a negligible effect on the periodontium (Tomson et al., 2007).

National statistics suggest that 75% of the population would have been eligible to participate. However, the impact of our definition of non-smokers, and the deprivation levels in the areas in which we were recruiting, resulted in the proportion of the population that was eligible to be recruited being much lower (SDS, 2009). If we had insisted that study participation be limited to only those who had never smoked then the number of potentially eligible participants would have been reduced even further. There were 39 subjects in the study population who were former smokers (having stopped a minimum of five years previously). Significantly higher proportions of the SP group were former smokers and had a higher pack year score (SP group 12 pack years; no SP group 2.3 pack years ($p=0.02$)) (Table 2-5). This confirms the importance of attempting to restrict studies of the impact of diabetes on periodontal health to never smokers to avoid confounding. This indicates that while our exclusion criteria had been designed to eliminate current smoking as a confounding factor past history of smoking was still a significant risk factor for periodontitis. One subject that was excluded from the analysis after examination had a history of 82 pack years and suffered from severe generalized periodontitis. The individual in question had smoked heavily for a long time although he had stopped more than five years before the examination.

2.4.7 Obesity

A potential confounder was the increased levels of obesity in the study population. A recent systematic review and meta-analysis concluded that there is evidence that obesity is itself a risk factor for chronic periodontitis (OR 1.35) (Chaffee, 2010). Differences approaching significance were noted in BMI between the groups where

PCD had slightly higher BMI than either the whole T1DM or NDS. The median was 26.7 compared with 25.2 in both other groups. ($p= 0.02$). Comparison with the information from the Scottish Diabetes Survey 2009 showed that BMI levels in our diabetic population were similar to levels among diabetic patients in Scotland generally. The percentage of T1DM patients who were overweight or obese was 61.5% in Scotland and 64.5% in our study group (SDS, 2009). 25.1% of all T1DM patients in Scotland are obese (SDS, 2009). T1DM is not as closely related to obesity as type 2 diabetes mellitus. Nonetheless it is possible that the increased levels of obesity may reflect general health attitudes and may in addition add to the inflammatory burden leading to increased risk of periodontal disease (Pischon et al., 2007; King et al., 2009). This was confirmed in the present study by the significantly higher BMI in the SP group compared with those without SP (Table 2-5). However, the independent impact of adipokines and the chronic inflammatory burden of this on the periodontium in diabetes is unknown.

2.4.8 Oral hygiene

One variable that was not included in the multivariable analysis was dental plaque score. Although plaque is required for the initiation of gingival inflammation it has been shown that microbial differences account for only around 20% of variance in disease expression (Page et al., 1997). Other factors may play a more important aetiological role in periodontitis including variations in genotype and tobacco smoking (Michalowicz et al., 1991; Grossi et al., 1994, 2000). The level of interest in general and oral health may be low in the study population. Oral hygiene was generally poor (Table 2-8) despite the fact that in the NDS 87.2% reported brushing twice daily or more. 52.7% claimed to be using floss and 50.9% reported that they used mouthwash. Oral hygiene as described by the percentage of sites with plaque was significantly higher in PCD compared with NDS and WCD. It is obvious from the plaque scores that the oral hygiene measures, if applied as claimed, are not being performed adequately. This is consistent with the data from the ADH study where patients who had just brushed their teeth prior to examination still had plaque present at one third of all sites (Morris, 2001). As plaque is on the causal pathway, it was not included as a confounding factor in the multivariable analysis. It could be argued that PCD have worse periodontal disease because they have higher plaque scores. The counter argument is that whether PCD have higher levels of periodontal disease

because of higher plaque scores, RAGE deposition in the gingivae or hyper-responsive T cells is irrelevant. The key question is whether they have a higher level of disease than NDS regardless of the aetiology. If it is because of a hyper-responsive cell type then it will be necessary to identify ways of modifying this. If, on the other hand, it is because of greater plaque accumulation then we need to understand this process and modify it accordingly.

Plaque accumulation can relate either to an increased rate of deposition and maturation of the oral biofilm or a reduced frequency and efficiency of self-performed plaque control. Of the poorly controlled diabetic subjects 26.5% reported that they brushed their teeth once per day or less while only 12.7% of the NDS and 14.7% on the WCD reported brushing so infrequently. Oral hygiene habits, dental attendance patterns, smoking, socio-economic status, psychosocial considerations, gender, salivary flow rate and glucose levels in saliva can all affect the development of dental plaque (Kelly et al., 1998; Heitz-Mayfield et al., 2005; Marton et al., 2008). The inclusion criteria for this study and the adjustment for confounding should mean that the groups were well matched socio-economically as well as demographically and in lifestyle variables. The only significant difference was in the proportion of subjects who had a university/college education which was lower in the PCD. It is to be expected that patients who are unable to achieve good glycaemic control are from less well educated and less affluent backgrounds (National Diabetes Audit, 2009). This was taken into account in the statistical models. T1DM and particularly PCD were shown to have significantly lower median salivary flow rates than NDS (Table 5-4 chapter 5 (p=0.003 and 0.01 respectively)) and this together with increased glucose concentrations in saliva and a negative response to health care advice in the PCD group may have contributed to the higher plaque scores in the former group (Hanson et al., 1987; Paulander et al., 2004).

2.4.9 Clinical implications for practice

The study described above showed that severity, extent and the prevalence of moderate periodontitis was higher in T1DM. These findings will serve as a spring board from which laboratory based studies can be funded in order to elucidate the aetiopathogenesis of diabetes related periodontitis in T1DM patients. This will hopefully enable us to identify optimum methods of preventing and treating

periodontitis in these patients. In addition this information can be used to make a case for an increased dental input into the management of T1DM. We found there was a lack of awareness of oral complications of diabetes amongst specialist diabetes workers and diabetic patients. This has been confirmed by other researchers (Kunzel et al., 2007; Quijano et al., 2010). In addition study participants had often never been informed that their diabetic condition made them at higher risk of periodontal disease. Patient information, staff training and improved periodontal screening either in primary or secondary care are required in order to reduce the impact of this preventable disease which leads to significant morbidity and ultimately loss of oral function. Given that we have excluded smokers from our study and still found a high level of disease in type 1 diabetic patients, we can only speculate that the level of periodontitis in the population of smoking diabetic patients (24.6%) would be much higher. It has also been suggested that there is a bidirectional relationship between T1DM and periodontitis (Iacopino et al., 2001). This theory proposes that the systemic inflammatory burden induced by the presence of periodontal inflammation will lead to an increase in insulin resistance in the tissues resulting in higher blood glucose levels and higher glycated haemoglobin levels. It is therefore possible that prevention or treatment of periodontal disease could have a beneficial impact on diabetic management (Simpson et al., 2010; Teeuw et al., 2010). Conversely good glycaemic control in conjunction with good oral hygiene could be beneficial to periodontal status and prevent the development of diabetes related periodontitis. The impact of glycaemic control on the prevalence and severity of periodontal disease will be the subject of chapter 3

2.5 Conclusions

Type 1 diabetes is a risk factor for the development of periodontal disease. The prevalence of moderate combined with severe periodontitis is increased in non-smoking adult T1DM patients. The severity of periodontal disease as measured by mean CAL and CPD levels was higher in T1DM patients compared with NDS. The extent of moderate combined with severe periodontitis was also increased in PCD. Poor oral hygiene in PCD patients may play a part in the increased levels of periodontal disease. Levels of periodontal disease were higher than expected in both T1DM patients and NDS due in part to full mouth periodontal charting and the large

number of subjects from deprived backgrounds. Further research is required to establish the pathogenesis and the methods of prevention and treatment of diabetes associated periodontal disease.

3 Type 1 diabetes and periodontitis: relationship between periodontitis, age, glycaemic control and duration

3.1 Introduction

In the previous chapter of this thesis it was shown that there is an increase in the prevalence, severity and extent of periodontitis in T1DM patients. This relationship exists in the absence of smoking, the main environmental risk factor. This chapter will investigate the relationship between age, glycaemic control and duration of diabetes, and the prevalence of periodontal disease.

Micro-vascular complications have been shown to increase significantly when the HbA1c increases beyond 7.5%. Risk of macro-vascular disease is increased at all HbA1c values above normal levels (DCCTRG, 1993). The effect of glycaemic control on the periodontium in non-smoking patients with T1DM is not known. It would therefore be useful to identify whether there is a similar cut-off point below which the risk of periodontal disease is not increased or beyond which periodontal disease is inevitable.

There is some evidence that periodontitis and gingivitis are increased in PCD patients as compared with WCD patients. Studies have however not been consistent in defining poor control. Adequate glycaemic control is defined by some organisations as $\text{HbA1c} \leq 6.5\%$ while studies into the periodontal health of T1DM patients have used a median HbA1c of 8%, 8.5% or even greater than 10% to 13% to define poor control (Seppala et al., 1993; Karjalainen et al., 1996; Tervonen and Karjalainen, 1997; Syrjala et al., 2003; Lappin et al., 2009; Saes Busato et al., 2010). By treating HbA1c as a continuous scale, as well as dividing the T1DM patients into quintiles of equal sized groups, the relationship between HbA1c and periodontal disease will be investigated.

Rosenthal et al. (1988) reported that T1DM patients who had no periodontal disease had a lower mean HbA1c than those with periodontal disease. In addition, these authors found an association between micro vascular diabetic complications including retinopathy, neuropathy and ketoacidosis, and periodontal disease. Periodontal

disease has also been associated with the diabetic complications of long term poor glycaemic control such as proteinuria, renal disease, cardiovascular disease e.g. angina and myocardial infarction, and cerebrovascular disease such as stroke and transient ischaemic attacks (Thorstensson et al., 1996).

There is agreement within the published literature that there is an association between HbA1c and loss of clinical attachment (Willerhausen et al., 1991; Safkan-Seppala and Ainamo, 1992; Ternoven et al., 1997, 2000; Patino Marin et al., 2002, Aren et al., 2003 and Lalla et al., 2007). These studies all showed the effect of metabolic control on periodontal disease, reporting that clinical probing depths, clinical attachment level and radiographic bone loss were increased in PCD patients. There was no corresponding increase in WCD patients despite similar plaque levels. It is therefore believed that poor glycaemic control has a detrimental effect on periodontal health. However, the nature of this relationship has not been fully elucidated when controlling for other risk factors. In addition, a number of studies have reported no relationship between glycaemic control and certain periodontal parameters (Sastrowijoto et al., 1989, Akyuz and Oktay, 1990, De Pommereau et al., 1992).

There is continued controversy about the effect of diabetes on gingivitis with many studies reporting an increase in gingival inflammation (Kjellman et al., 1970b; Gislen et al., 1980; Gusberti et al., 1983; Harrisson and Bowen 1987; Seppala et al., 1992 and Firalti et al., 1996). However oral hygiene has also been repeatedly shown to be poorer in PCD patients (Albrecht et al., 1988; Novaes et al., 1991; Bridges et al., 1996; Firatli et al., 1996; Lalla et al., 2006a). Where this is taken into account, some studies have shown that the association persists. Others have concluded that the observed increase in gingivitis can be explained by oral hygiene alone (Hove et al., 1970). In this chapter, we will present the ratio of the percentage bleeding score for the whole mouth to the full mouth plaque score, known as the periodontal susceptibility index. We will also examine in detail the relationship between plaque accumulation and gingival inflammation at index sites.

The research questions for this chapter are:

- Do T1DM patients have a different age of onset of periodontal disease compared with NDS?
- Does glycaemic control have an effect on periodontal disease levels independent of other risk factors?
- Are T1DM subjects more susceptible to plaque related periodontal inflammation than NDS?
- Does duration of diabetes have an effect on periodontal disease levels independent of other risk factors?

3.2 Methods

The patients were examined as described in chapter 1. We carried out subgroup analysis to determine whether there was any relationship between age, HbA1c and duration of T1DM, and periodontal disease. The information recorded from the index sites was also used to determine the relationship between the PI, MGI and CPD in patients with and without T1DM.

Training included a consensus session where the standard was set between the external trainer, the principal investigator and the examiner using clinical photographs of various levels of gingival inflammation and plaque accumulation. Calibration included intra and inter-examiner reproducibility. There were two calibration sessions and MGI and PI charts were repeated throughout the study.

3.2.1 Predictors of periodontal disease in type 1 diabetic patients

In order to investigate the effect of age, glycaemic control and duration on periodontal disease prevalence, the patients were first grouped according to diabetic status (NDS; T1DM) then by age in 10 year increments. Subjects were also divided into five quintiles based on their average HbA1c over the last two years. Duration of diabetes was analyzed as a continuous variable. Initially, the data was cross-tabulated according to age and HbA1c quintile, in order to assess whether there was any obvious relationship between these variables and periodontal disease. Age and HbA1c quintile were cross tabulated with the presence of two measures of severe

periodontitis (CAL \geq 6mm and CPD \geq 6mm) and moderate plus severe periodontitis (CAL \geq 4mm).

3.2.2 Periodontal susceptibility

The periodontal susceptibility index was calculated by dividing the number of sites with bleeding on probing by the number of sites harboring visible plaque. A high ratio of percentage bleeding sites to the percentage of sites with plaque indicates a high susceptibility and a low ratio indicates resistance to inflammation (Trombelli et al., 2004, 2006). The mean periodontal susceptibility index was calculated for the three groups WCD, PCD and NDS.

3.2.3 Index sites

3.2.3.1 Sampling and indices

Examination of the 4 key sites, one in each quadrant of the mouth (normally the mesiobuccal aspect of the 1st or 2nd molar) was carried out. The MGI was completed first, followed by the PI, then gingival crevicular fluid (GCF) was collected.. Clinical probing depth and bleeding on probing were recorded after GCF had been collected.

A) Plaque Index: each site was given a score from 0 to 3, as described by Silness and L e (1964):

- 0: no plaque.
- 1: plaque only detected by running a probe over the surface of the tooth.
- 2: moderate accumulation of soft plaque that can be seen with the naked eye.
- 3: the presence of abundant deposits of soft plaque.

B) Modified Gingival Index: each site was given a score from 0 to 4, according to Lobene (1986).

- 0: absence of inflammation.

- 1: mild inflammation — slight change in colour, little change in texture, of any portion of but not the entire papillary gingival unit.
- 2: mild inflammation — criteria as above but involving the entire papillary gingival unit.
- 3: moderate inflammation — glazing, redness, oedema and/or hypertrophy of the papillary gingival unit.
- 4: severe inflammation — marked redness, oedema and/or hypertrophy of the papillary gingival unit, spontaneous bleeding, congestion or ulceration.

C) Probing depth, defined as the distance between the gingival margin and the bottom of the probeable pocket rounded down to the nearest whole millimeter.

3.2.3.2 Summary of index sites

Mean MGI

The MGI for each site was summed and divided by the number of sites giving a mean MGI for each subject. This average was used to indicate the amount of gingival inflammation present at the index sites.

$$\text{MeanMGI} = \frac{\text{MGI1} + \text{MGI2} + \text{MGI3} + \text{MGI4}}{4}$$

Mean PI

The plaque index was determined as above and the mean PI for the four sites was calculated by summing the PI scores and dividing by the number of sites.

$$\text{MeanPI} = \frac{\text{PI1} + \text{PI2} + \text{PI3} + \text{PI4}}{4}$$

Mean Ratio MGI: PI

The ratio of MGI: PI is a marker of susceptibility to inflammation. Abundant plaque and a low inflammation score (in the absence of smoking) may indicate that the subject has a reduced inflammatory response to the irritating effect of dental plaque. In contrast, subjects with a low plaque score and severe gingival inflammation may indicate that an individual has a heightened inflammatory response to plaque. The mean ratio of MGI: PI was first calculated at a site level and then the mean proportion across the four sites was calculated.

$$\text{MeanratioMGI : PI} = \frac{\text{MGI1} / \text{PI1} + \text{MGI2} / \text{PI2} + \text{MGI3} / \text{PI3} + \text{MGI4} / \text{PI4}}{4}$$

3.2.4 Statistical analysis

To investigate the effect of potential risk factors for severe periodontitis (1 site with CAL \geq 6mm) (SP) logistic regression analysis was used to determine the relationship between SP and age, HbA1c and duration of diabetes. Logistic regression analysis for HbA1c controlled for age, gender, SIMD, smoking status, dental attendance, education and BMI. Odds ratios, 95% confidence intervals and p value were calculated. The spearman correlation coefficient was calculated between HbA1c and bleeding on probing. The chi squared test was used to test for differences in proportions in the cross tabulations. Differences between the periodontal susceptibility index, PI, MGI, gingival crevicular flow were compared between NDS; WCD and PCD using ANOVA where the distributions were shown to be normally distributed. The cross-tabulation, bivariate correlations and logistic regression was carried out using the statistical package SPSS 15 (SPSS Inc.) and the ANOVA tests were carried out using Minitab (Minitab 16, Minitab Inc. Philadelphia).

3.3 Results

3.3.1 Calibration

The initial calibration was carried out prior to the beginning of the study and the results were mixed (Table 3-1). Kappa scores showed fair agreement for the plaque index ($\kappa = 0.34$) between the two examiners but the MGI was unacceptably low at 0.19. The intra-examiner agreement for the primary examiner was good for MGI ($\kappa = 0.66$) and fair for the PI ($K = 0.36$). A further training exercise was carried out and further calibration was performed. The intra-examiner reproducibility for the primary examiner was good for the MGI ($\kappa = 0.61$) and moderate for the PI ($\kappa = 0.43$). The mean bias was low and the percentage within one unit was 100% for the MGI and 98.1% for the PI. For the inter-examiner calibration the mean bias was significantly lower and 94.7% of all MGI scores were within one unit on the measurement scale. For the PI 98.8% of all repeats were within one unit of measurement. The inter-examiner scores were improved with both scales showing moderate agreement, MGI ($K = 0.41$) and PI ($K = 0.52$).

The primary investigator repeated MGI and PI charts throughout the study to test for reproducibility. The intra-examiner reproducibility for the primary examiner throughout the study was good for the MGI ($\kappa = 0.77$) and for the PI ($\kappa = 0.64$) according to the criteria proposed by Altman (1999). The mean bias was low and the percentage within one unit was 99.1% for the MGI and 100% for the PI. The reproducibility of the recording of bleeding on probing was moderate with 74.6% perfect agreement and a kappa score of 0.48 (Table 3-2).

Table 3-1 Summary of 1st and 2nd calibration exercises

		MGI	PI	MGI	PI
		Time 2	Time 2	Time 1	Time 1
Intra-examiner Calibration	K	0.61	0.43	0.66	0.36
	Within 1	100.0%	98.1%	95.2%	100.0%
	Mean bias	-0.02	0.03	0.01	0.08
	Measurement error	0.52	0.65	0.63	0.63
Intra-examiner Calibration	K	0.66	0.53	0.45	0.54
	Within 1	98.1%	98.8%	82.1%	100.0%
	Mean bias	-0.09	-0.08	-0.50	-0.07
	Measurement error	0.54	0.58	0.90	0.46
Inter-examiner Calibration	K	0.41	0.52	0.19	0.34
	Within 1	94.7%	98.8%	76.2%	95.2%
	Mean bias	0.12	-0.04	0.23	0.30
	Measurement error	0.75	0.58	1.13	0.65

Within 1: agreement between replicates to within 1 unit on measurement scale

Mean bias: mean of differences between replicates

Measurement error: SD of differences between replicates

Table 3-2 Summary of ongoing calibration

		MGI	PI
Ongoing Intra-examiner calibration	K	0.77	0.64
	Perfect agreement	86.4	80.7
	Within 1	99.1	100
	Within 2	100	100
	Mean bias	0.09	0.04
	Measurement error	0.39	0.43

Within 1: agreement between replicates to within 1 unit on measurement scale

Within 2: agreement between replicates to within 1 unit on measurement scale

Mean bias: mean of differences between replicates

Measurement error: SD of differences between replicates

3.3.2 Prevalence of severe and moderate and severe periodontitis by age

Periodontitis was very common in both groups with severe periodontitis (CAL \geq 6 mm on a minimum of one tooth) affecting 40% of the NDS >50 years of age and 64% of the diabetic subjects in the same age group. The prevalence of periodontitis increased with age in both groups (Fig 3-1). The relationship with age was almost linear with $r^2 = 0.9602$ (T1DM) and $r^2 = 0.9567$ (NDS) respectively. The prevalence of severe periodontitis in T1DM subjects under thirty years of age was 3.25 times as high in T1DM compared with NDS (NDS 2.8%, T1DM 9.1%).

Moderate combined with severe periodontitis was also consistently more common among T1DM compared with NDS. In every age group the prevalence of periodontitis was higher among T1DM (Fig 3-2). The differences were most marked in the under thirty year group and in those over fifty years of age. CAL of \geq 4mm was twice as common in the under thirties and the over fifties. There was no age group in which there were no instances of severe periodontitis in either T1DM or NDS groups. Logistic regression was carried out to determine whether there was a different relationship between age and severe periodontitis in T1DM and NDS. The risk of severe periodontitis increased more than 17 fold [CI 2.1-139.1] between the youngest and oldest NDS however the same odds ratio was only 7.6 [CI 3.0-19.3] in the T1DM group. The level of disease was much higher in the youngest T1DM group (Table 3-3).

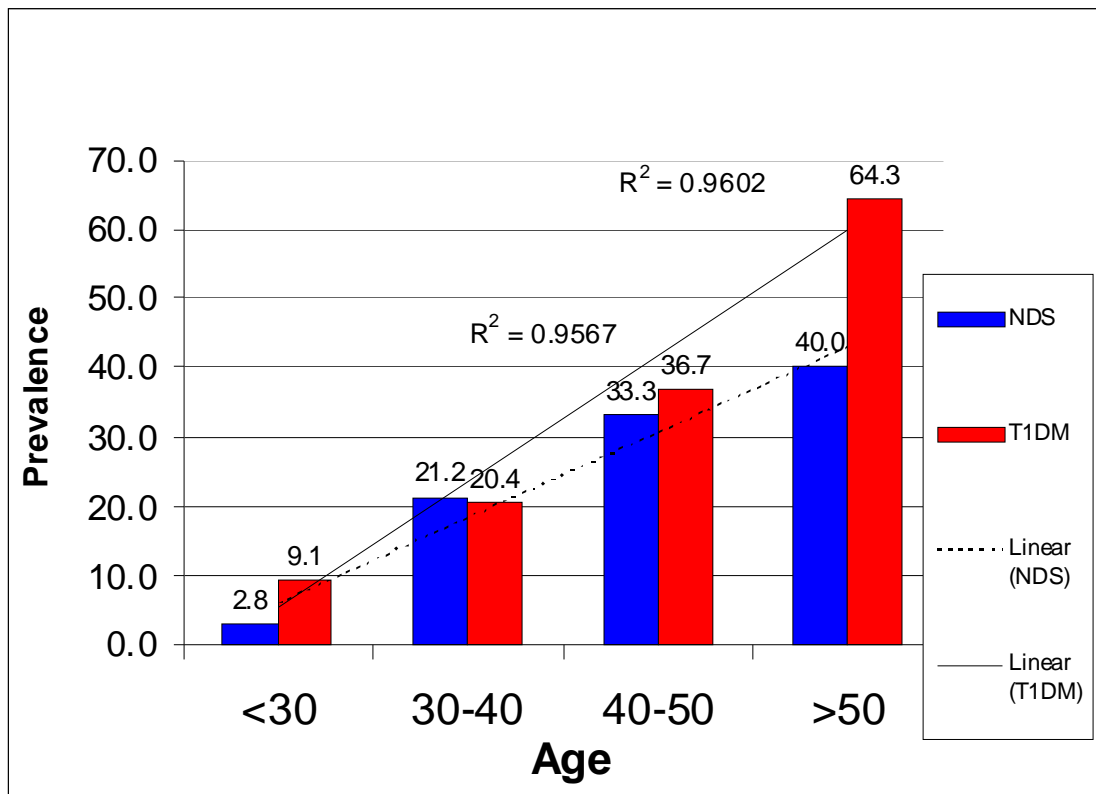


Figure 3-1 Severe periodontitis CAL ≥ 6mm by age and prevalence

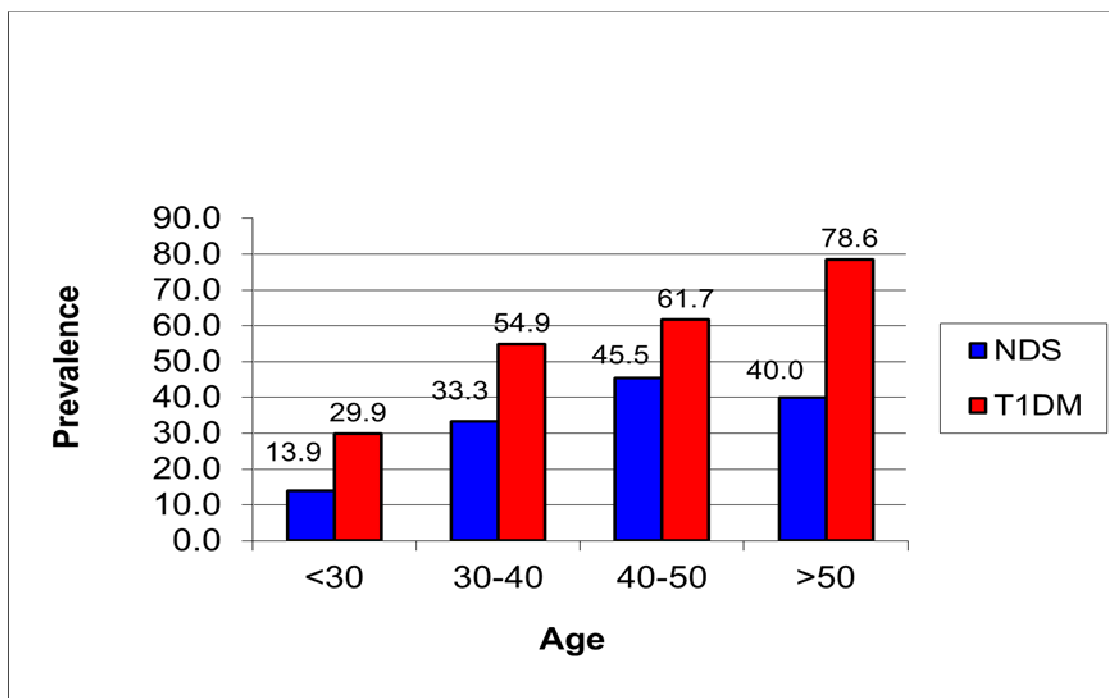


Figure 3-2 Moderate combined with severe periodontitis CAL ≥ 4 mm by age and prevalence

Table 3-3 Table showing the increasing relative risk of CAL \geq 6mm with age across three equally sized age groups

		Percentage with Severe Periodontitis	Severe Periodontitis (OR)	95% Confidence intervals	P value
NDS	Age group 1	3.1%	1(ref)		-
	Age group 2	18.4%	7	[0.81-60.3]	0.077
	Age group 3	35.7%	17.22	[2.1-139.1]	0.008*
T1DM	Age group 1	9.5%	1(Ref)		-
	Age group 2	21.2%	2.58	[0.97-6.85]	0.058
	Age group 3	44.4%	7.66	[3.04-19.3]	0.000*

* statistically significant

3.3.3 Periodontal treatment need by age.

The prevalence of severe periodontitis as defined by treatment need (≥ 6 mm CPD on a minimum of one tooth) was also examined by age. The prevalence of CPD ≥ 6 mm was higher in the younger age groups but there was no difference in the older age groups. Among patients less than 30 years old the prevalence was 3.8 times higher among the T1DM patients (10.4%) compared with NDS (2.8%) ($p= 0.018$). The prevalence of 6mm CPD was also higher among the 30-40 year age group with NDS 6.1% and T1DM 12.2% ($p >0.05$). There were no differences between T1DM patients and NDS in the 40 to 50 or the over 50 year age groups (Figure 3-3).

Figure 3-4 shows that the prevalence of moderate periodontal treatment need (clinical probing depths ≥ 4 mm) was seven fold higher in patients less than 25 years old suffering from T1DM compared with NDS. 41.5% of all diabetic patients under 25 had at least one tooth with CPD ≥ 4 mm compared with only 5.9% of the NDS ($p=0.018$). When all patients under thirty were analysed there was a 2.3 times increase in prevalence (NDS 16.7% T1DM 39.5%, $p=0.011$). There were no significant differences in any other age groups.

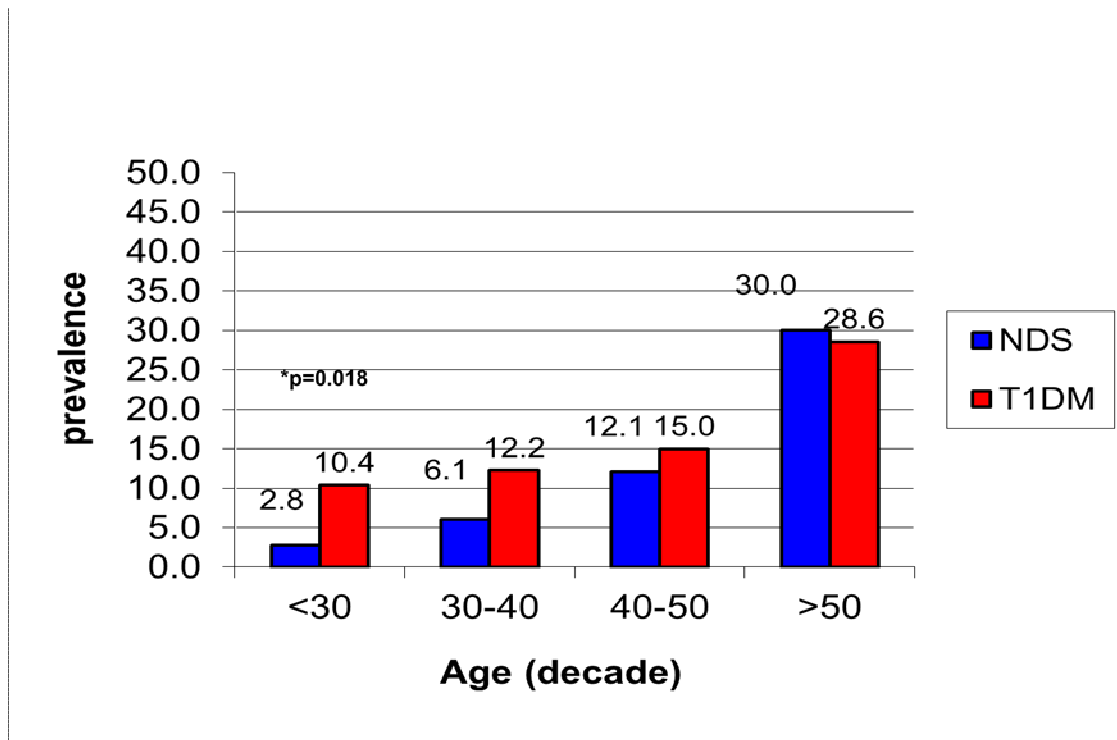


Figure 3-3 Clinical probing depth ≥ 6 mm on a minimum of one tooth by age (decade). (*p value calculated using chi squared test)

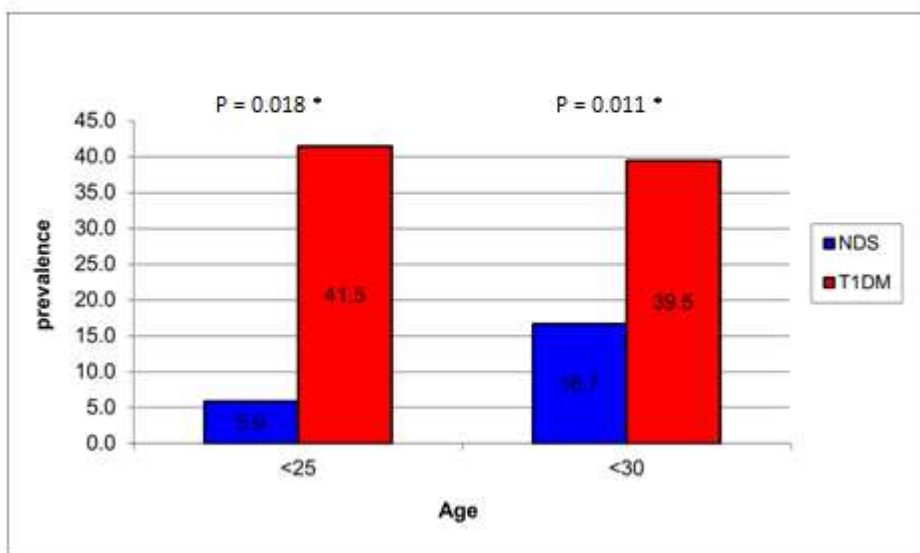


Figure 3-4 Prevalence of moderate combined with severe periodontal treatment need in patients less than 25 years and less than 30 years of age (* p value calculated using chi squared test)

3.3.4 Glycaemic control and periodontal disease

3.3.4.1 Bleeding on probing

Bivariable analysis showed that there is a positive correlation between average HbA1c and bleeding on probing (Figure 3-5). A Spearman correlation showed that this relationship was statistically significant (correlation coefficient 0.31 $p = 0.000$).

3.3.4.2 Periodontitis

The influence of HbA1c on the prevalence and extent of periodontitis is less clear. The patients with the lowest HbA1c had the lowest prevalence of SP. However the group with the highest prevalence, and the most widespread disease, was the moderately controlled group (Figures 3-6 and 3-7).

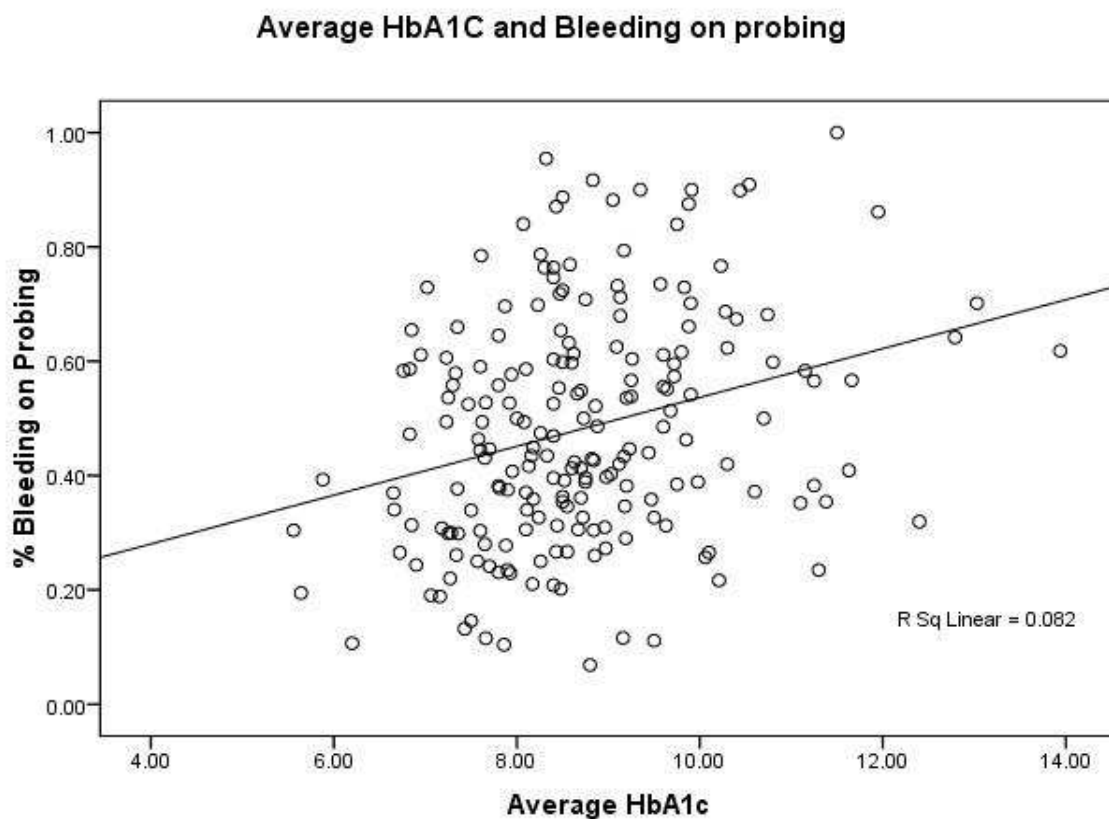


Figure 3-5 Scatterplot of Average HbA1c against the percentage of sites with bleeding on probing

Figure 3-6 shows which HbA1c quintile the patients with severe periodontitis were in. There was a pattern of increasing proportion of periodontal patients in the groups as the HbA1c increases until the last group. Figure 3-7 shows that there was an increase

in the extent of periodontitis as defined by the number of sites ≥ 4 mm CAL with increasing HbA1c although this pattern was also reversed in the most poorly controlled group. Figure 3-8 shows that of those patients who were in the most poorly controlled group 41.5% were under twenty five and 62.6% were under 30 years of age. There was an inverse relationship between age and HbA1c. Since this was the age group with the lowest prevalence of periodontal disease it is not surprising that the prevalence of severe periodontitis was low in this group where young diabetic patients were over represented. A negative relationship between HbA1c and age, and a positive association between periodontal disease and age, meant that in order to see whether the relationship between periodontitis and HbA1c required a multivariable logistic regression model.

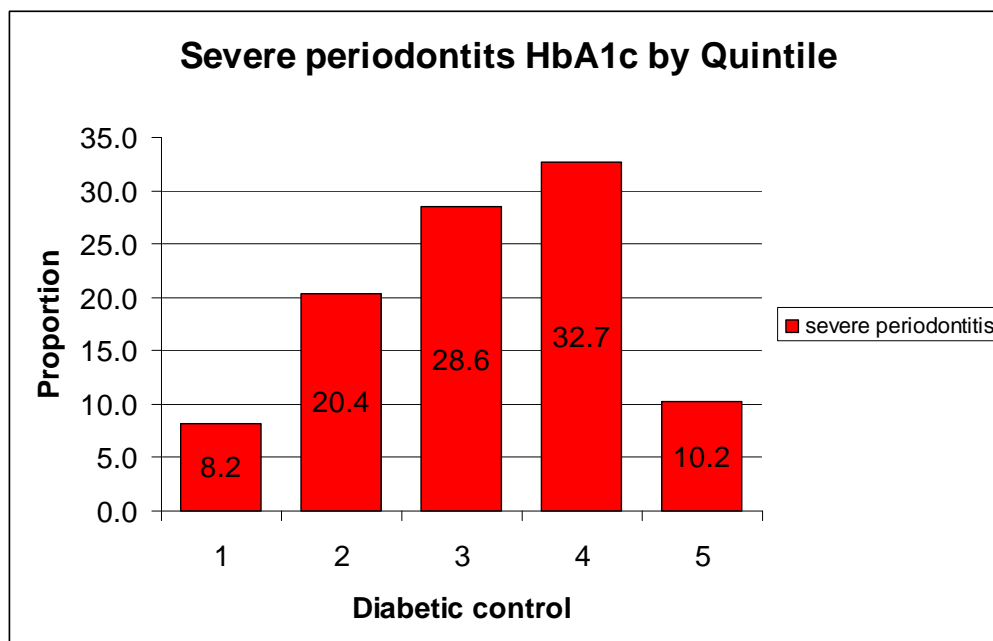


Figure 3-6 Bar chart of the proportion of patients with severe periodontitis who were in each quintile of HbA1c

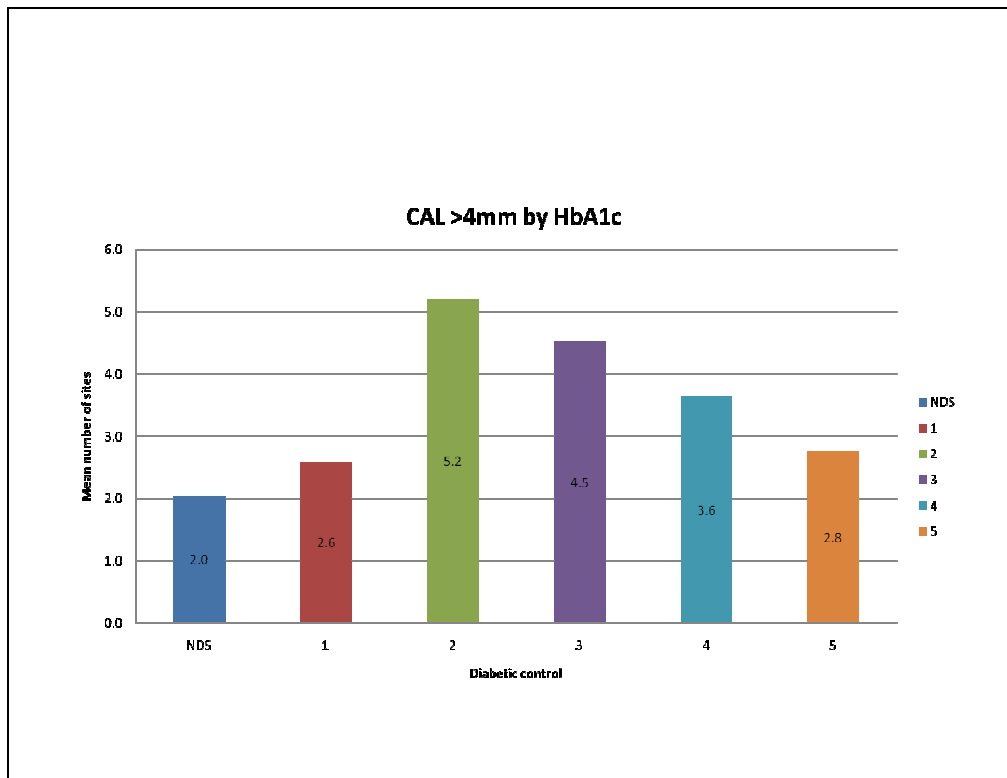


Figure 3-7 Mean number of sites \geq 4mm by quintile of HbA1c

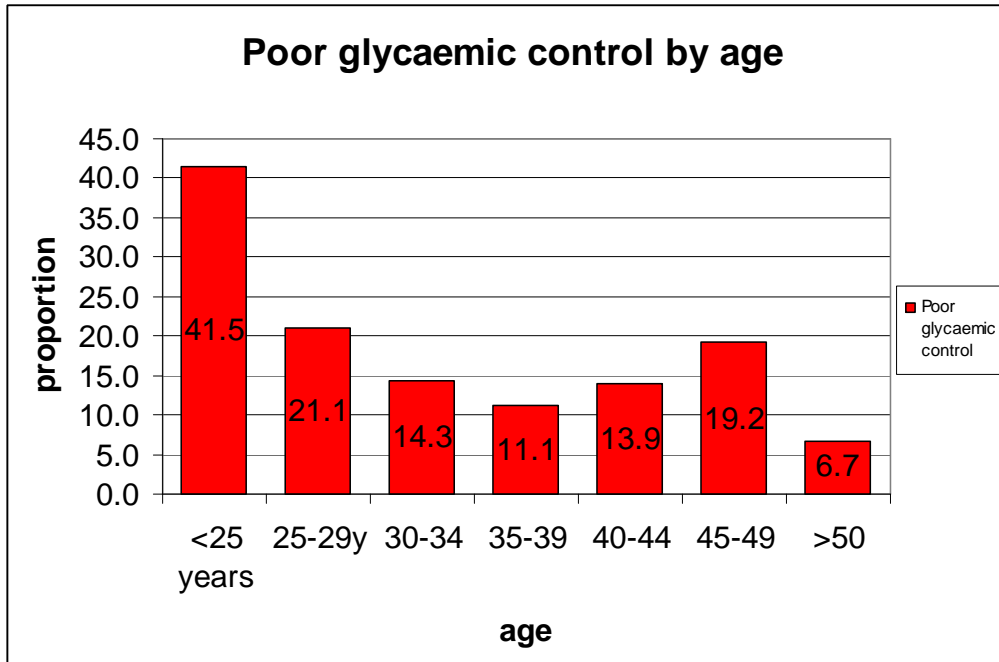


Figure 3-8 Bar chart showing the age distribution of the group with the highest HbA1c

Multivariable logistic regression analysis was used to calculate odds ratio and 95% confidence intervals for severe periodontitis ($CAL \geq 6mm$) against the HbA1c groups controlling for age, gender, BMI, previous smoking status dental attendance, SIMD and education. The adjusted Odds Ratio for all quintiles of HbA1c was higher than the lowest reference group with the fourth quintile rather than the fifth having the highest risk. The adjusted Odds Ratios for quintiles 2-5 were 2.78 [CI 0.92-8.36], ($p = 0.07$), 2.68 [CI 0.87-8.20] ($p = 0.085$), 3.996 [CI 1.334-11.97] ($p = 0.013$) and 2.806 [CI 0.901-8.739] ($p = 0.075$) (Figure 3-9 and Table 3-4).

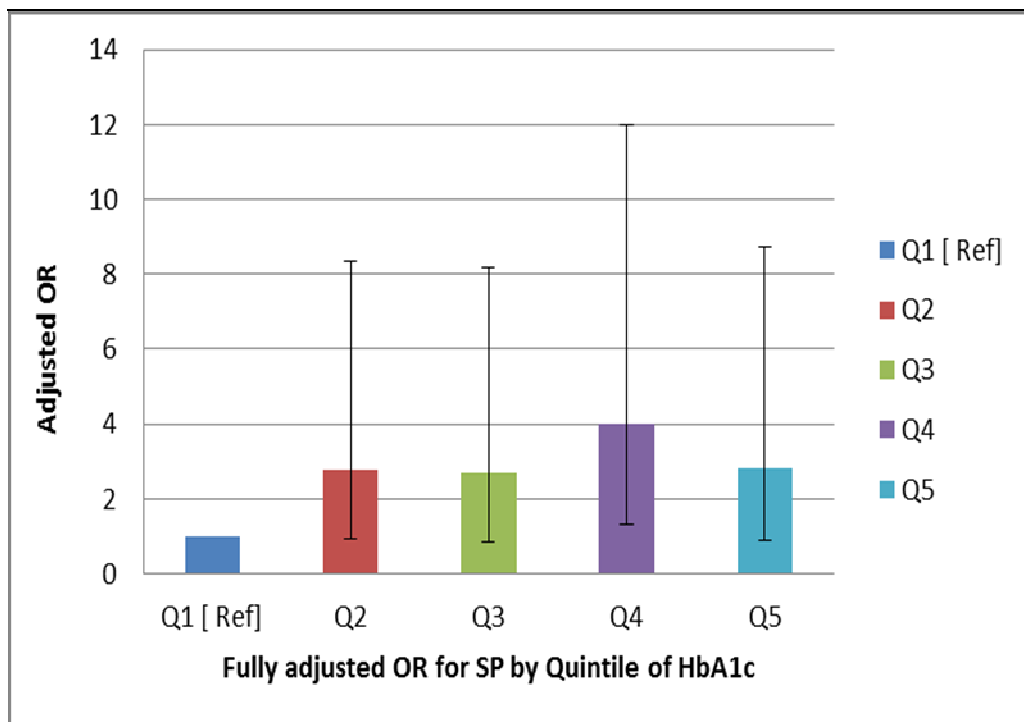


Figure 3-9 Fully adjusted odds ratios (error bars 95% confidence intervals) for severe periodontitis by quintile of HbA1c. Adjusted for age, gender, smoking status, BMI, attendance, education and SIMD.

Table 3-4 Fully adjusted odds ratio for severe periodontitis

Quintiles of HbA1c	Severe Periodontitis (OR)	95% Confidence intervals	P value
1	1 (ref)		-
2	2.775	[0.92-8.36]	0.07
3	2.675	[0.87-8.195]	0.085
4	3.996	[1.33-11.97]	0.013
5	2.806	[0.90-8.74]	0.075

a Variable(s) entered on step 1: HbA1c, age, gender, smoking status, BMI, attendance, education, SIMD

The level of disease was much higher in the youngest T1DM group. An interaction test to test whether HbA1c impacted on the relationship between SP and age showed that this apparent difference in the age distribution was not statistically significant ($p = 0.692$). The relationship between HbA1c and SP was not a linear one. Correcting for apparent differences in age did not remove this effect and despite multivariable logistic regression no clear pattern emerged.

3.3.5 Duration of diabetes and risk of SP

The relationship between the duration of diabetes and severe periodontitis was investigated initially using a bivariate logistic regression analysis. In the initial analysis the relationship appeared to be significant with an increase in risk of 5.1% with every year of duration (1.051 CI [1.014-1.09] $p = 0.007$). A series of models were constructed where duration was adjusted for age. This attenuated the relationship and there was no longer a significant association between duration and SP (1.003 [0.962-1.045] $p = 0.891$). There was a strong correlation between age and duration ($p = <0.00$) (Fig 3-10).

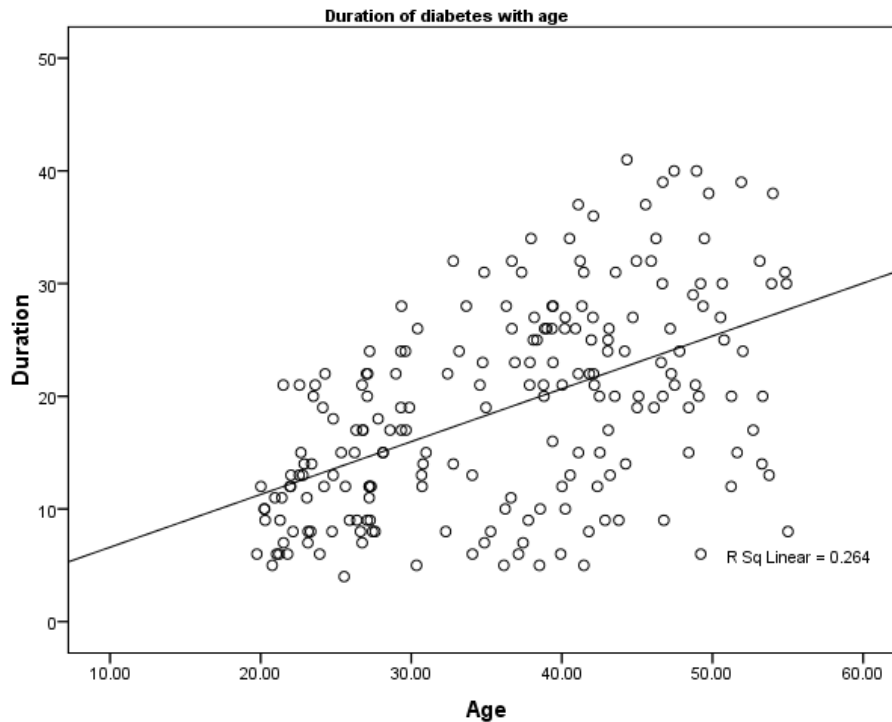


Figure 3-10 Scatterplot of age against duration of diabetes

3.3.6 Index sites

When the index sites were examined, there were no differences in the number of bleeding sites affecting the first or second molars and the average probing depth across the three groups was not significantly different. The mean MGI across the 4 teeth was significantly higher in the PCD as compared with both the NDS ($p = 0.000$) and WCD ($p = 0.001$) (Figure 3-11, Tables 3-5).

The likelihood of all 4 sites having an MGI of zero, that is to say, no visible signs of inflammation, were significantly higher in the NDS and WCD; 25.2% and 24.2% of the NDS and WCD had no visible signs of inflammation, however only 11.2% of the PCD had an MGI of zero ($p < 0.0001$). The mean plaque index was, however, higher for the PCD compared with both NDS and the WCD ($p < 0.001$, $p = 0.006$) (Figure 3-12, Tables 3-6).

There were no differences in the probing depths of the sites (Fig 3-13, Table 3-7) (NDS 2.68mm, WCD 2.51mm, PCD 2.74mm $p = 0.262$).

The periodontal susceptibility index, which is the ratio of the percentage bleeding sites to the percentage of sites where there is visible plaque, was calculated and is shown in the box plot (Figure 3-14). This showed that there was a slight increase in the ratio of the number of bleeding sites to the number of sites with plaque in PCD

compared with NDS (NDS = 0.63 [CI 0.57-0.68], PCD 0.695 [CI 0.64-0.69] p = 0.13). This may mean that there is an increased susceptibility to gingival inflammation for a given level of plaque. However, the difference detected was not statistically significant and therefore no conclusions can be drawn at the whole mouth level.

In order to account for the presence of plaque at the site level the ratio of the modified gingival index to the plaque index was calculated. There were no statistically significant differences between the three groups in the ratio of MGI to PI (Figure 3-15, Tables 3-8).

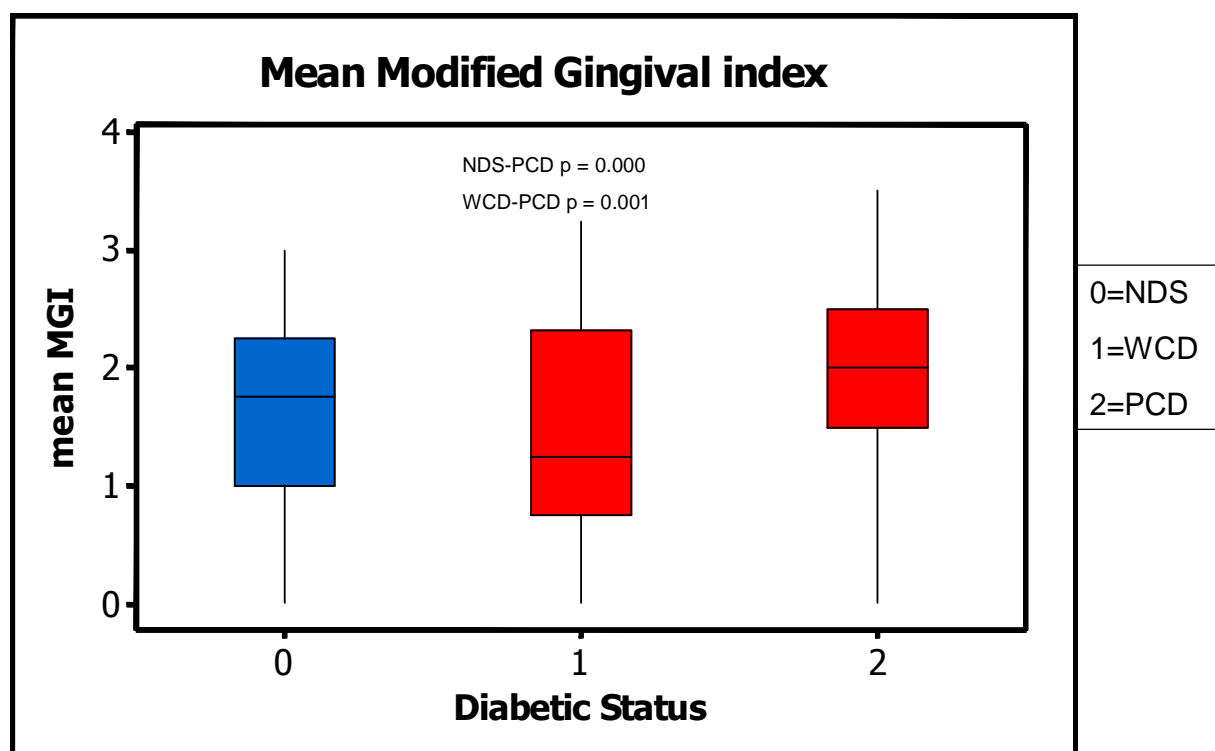


Figure 3-11 Box plots comparing the distribution of the Modified Gingival Index in NDS, WCD and PCD subjects. Median, interquartile range and extremes.

Table 3-5 Mean MGI by Diabetic status

	N	Mean	Std. Deviation	Min	Max	Std. Error	95% Confidence Interval for Mean		NDS v WCD P	NDS v PCD P
							Lower Bound	Upper Bound		
NDS	110	1.56	0.804	0	3	0.0767	1.407	1.711	0.848	0.000
WCD	34	1.48	0.877	0	3.25	0.1505	1.172	1.784		
PCD	170	1.99	0.695	0	3.5	0.0533	1.882	2.09		

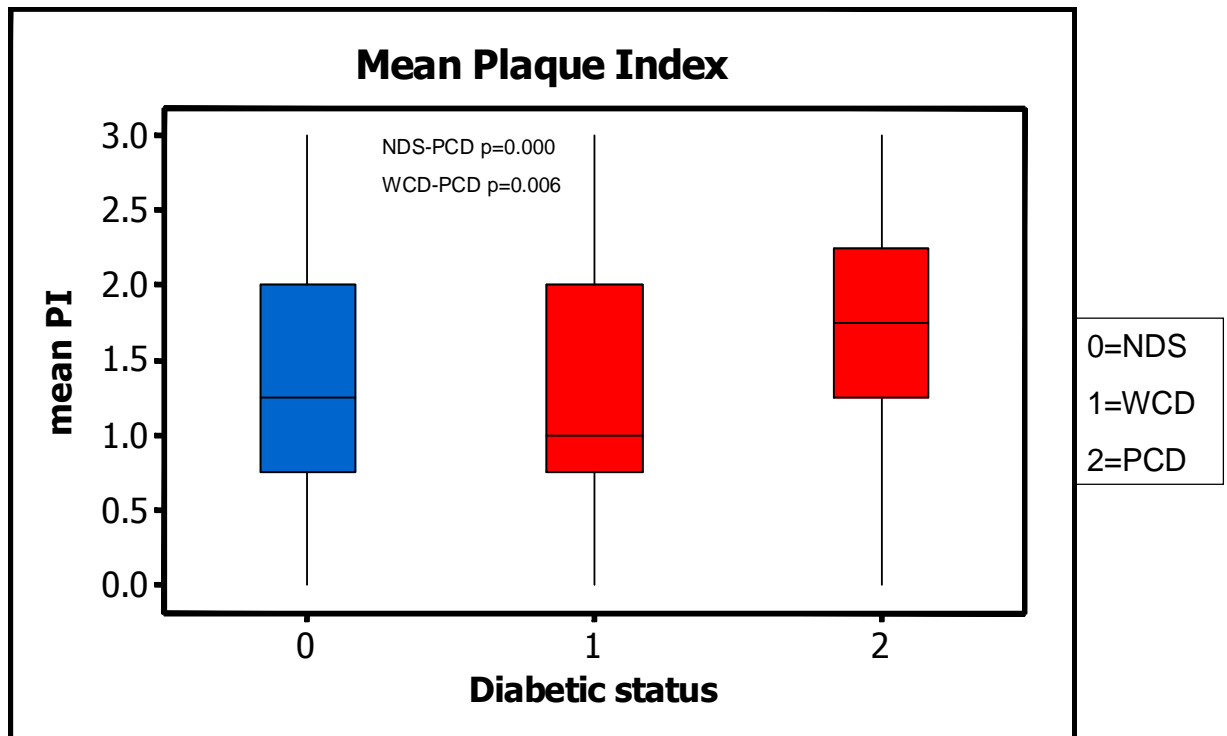


Figure 3-12 Box plots comparing the distribution of the Plaque Index in NDS, WCD and PCD subjects. (Median, interquartile range and extremes)

Table 3-6 Mean Plaque Index by Diabetic status

	<i>N</i>	<i>Mean</i>	<i>Std. Deviation</i>	<i>Min</i>	<i>Max</i>	<i>Std. Error</i>	<i>95% Confidence Interval for Mean</i>		<i>NDS v WCD</i>	<i>NDS v PCD</i>
							Lower Bound	Upper Bound	P	p
NDS	110	1.3	0.786	0	3	0.075	1.16	1.45	0.985	0.000
WCD	34	1.28	0.804	0	3	0.138	1	1.56		
PCD	170	1.73	0.754	0	3	0.058	1.62	1.84		

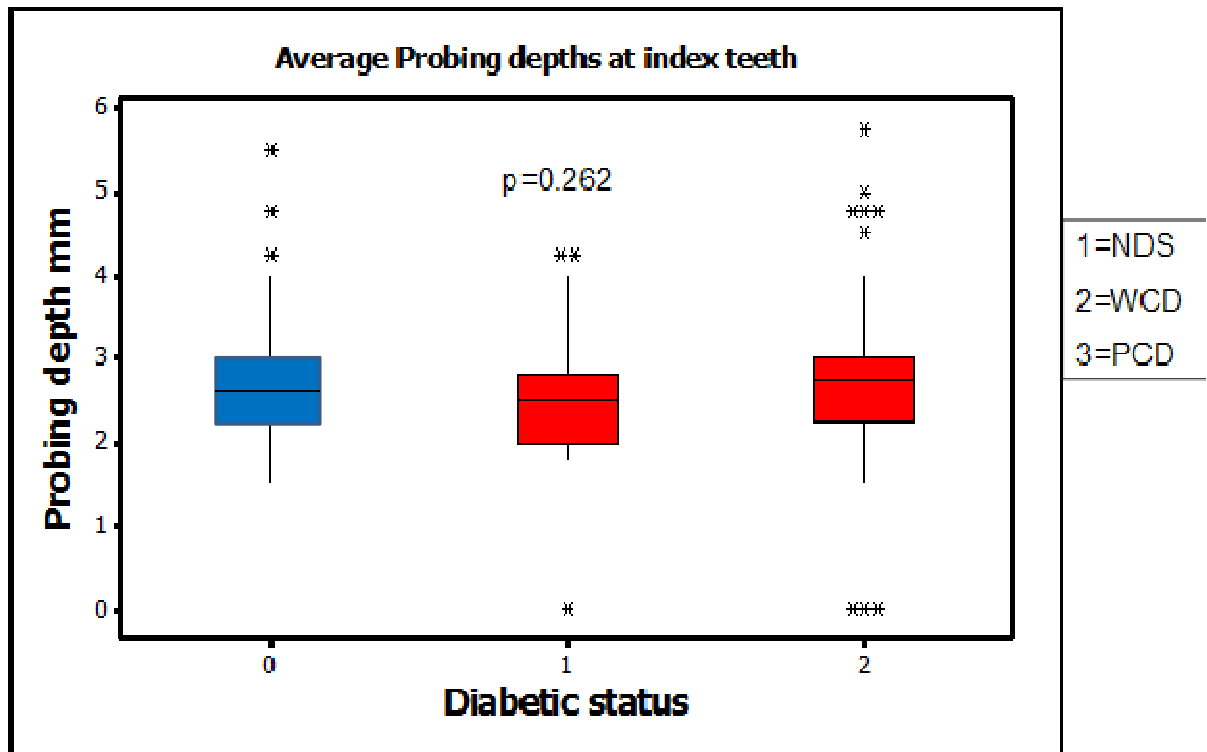


Figure 3-13 Box plots comparing the distribution of the clinical probing depths in NDS, WCD and PCD subjects. (Median, interquartile range and extremes)

Table 3-7 Mean clinical probing depths (mm) comparing NDS v WCD and NDS v PCD

	N	Mean	Std. Deviation	Min	Max	Std. Error	95% Confidence Interval for Mean		NDS v WCD	NDS v PCD
							Lower Bound	Upper Bound	p	P
NDS	110	2.68	0.686	2	6	0.065	2.55	2.81	0.462	0.821
WCD	34	2.51	0.815	0	4	0.14	2.22	2.79		
PCD	170	2.74	0.774	0	6	0.059	2.62	2.85		

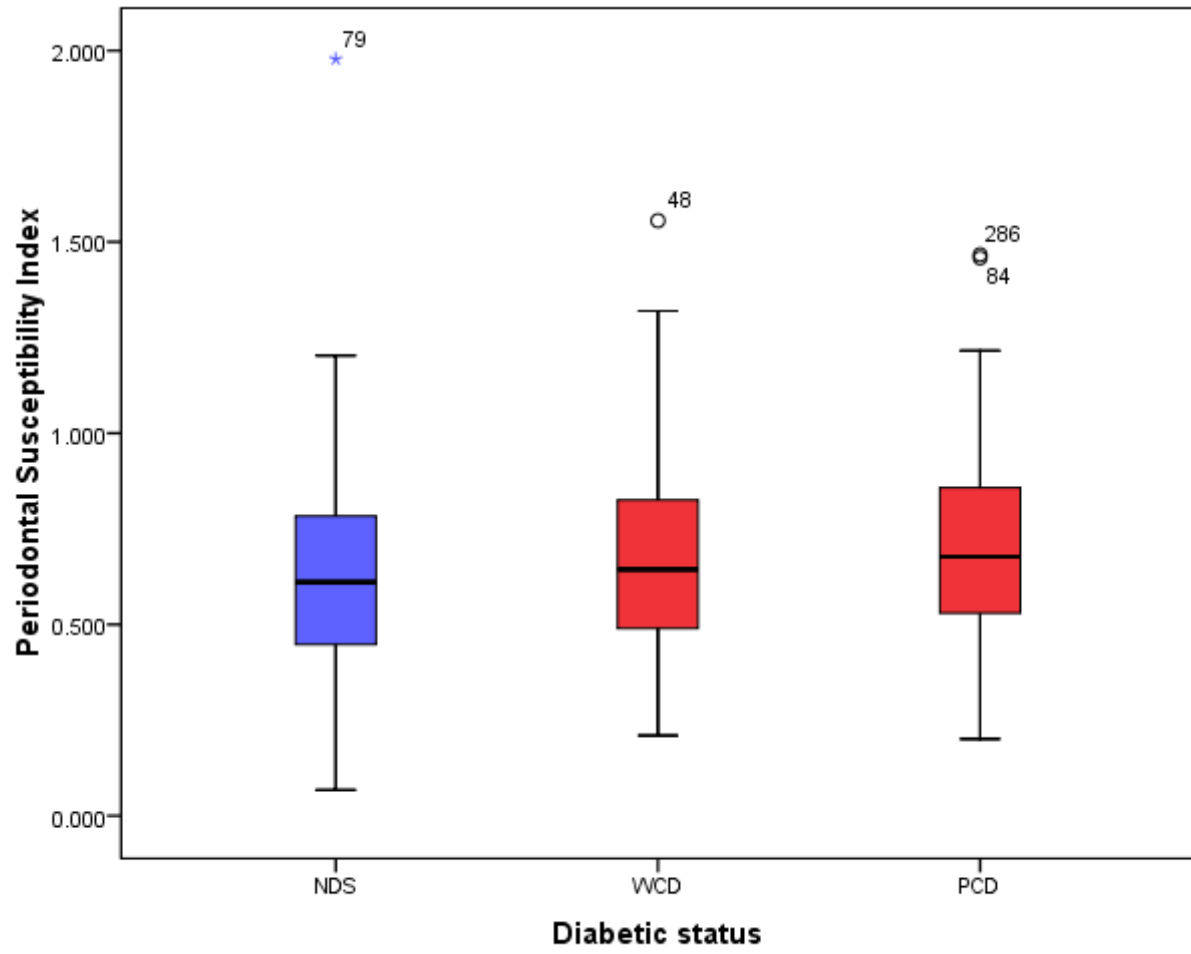


Figure 3-14 Box plots showing the distribution of the periodontal susceptibility index (ratio of bleeding/plaque) in NDS, WCD and PCD subjects

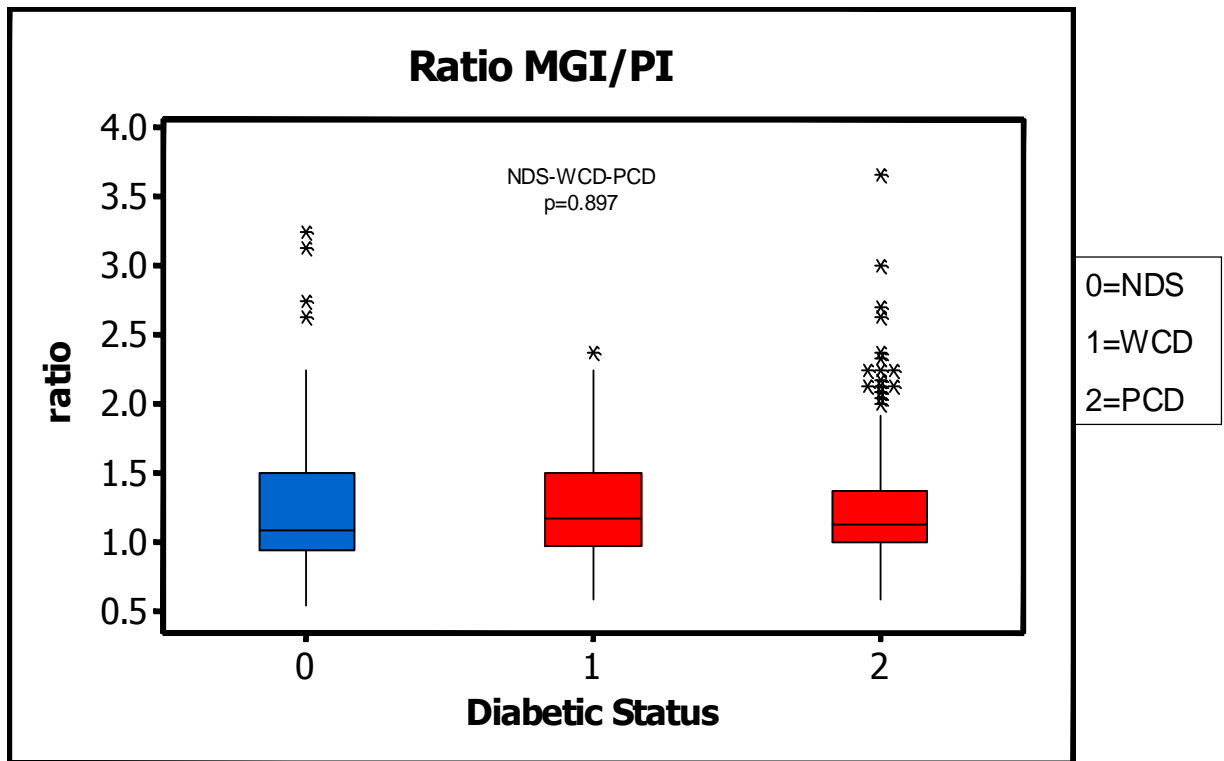


Figure 3-15 Box plots comparing the distribution of the ratio Modified Gingival Index/Plaque Index in NDS, WCD and PCD subjects. Median, interquartile range and extremes.

Table 3-8 Ratio of MGI: PI by diabetic status

	N	Mean	Std. Deviation	Min	Max	Std. Error	95% Confidence Interval for Mean		NDS V WCD	NDS V PCD
							Lower Bound	Upper Bound	P	P
NDS	110	1.28	0.512	0.542	3.25	0.049	1.179	1.37	0.898	0.95
WCD	34	1.23	0.415	0.583	2.38	0.071	1.089	1.38		
PCD	170	1.27	0.476	0.583	3.67	0.036	1.186	1.33		

3.4 Discussion

3.4.1 Age

The prevalence of SP increases linearly with age in diabetes patients as it does in healthy subjects. There is no lower age limit where moderate or severe periodontal disease does not occur in either group, although the prevalence of periodontal disease seems to be much higher in younger type 1 diabetic subjects. This is in agreement with studies in adolescents which showed that low levels of periodontitis are present even in younger age groups (Lalla et al., 2006a). The differences in CAL between T1DM and NDS continue through life but the differences in CPD decrease with age. The differences in change in probing depth with age are interesting, as they seem to show that there is an accumulation of disease experience and therefore periodontal tissue loss over time. However, the probing depths do not seem to get interminably deeper and are maintained at depths similar to those of middle-aged patients with chronic periodontitis. In the ADH survey 1998 the level of CPD was always lower than CAL. Between the 35-44 year age group and the 45-54 years age group there was only a 1% increase in the prevalence of CPD >5.5mm while there was an increase from 3% up to 10% in the same groups for CAL >5.5mm. This shows that while CAL is cumulative CPD will not necessarily increase in the same way with increasing age (Morris et al., 2001). Of real interest was the fact that the prevalence of severe periodontitis in T1DM subjects under thirty years of age was 3.25 times as high in T1DM compared with NDS age-matched controls (NDS 2.8%, PCD 9.1% $p > 0.05$). This suggests that the onset of periodontitis occurs earlier than in non-diabetic subjects where an increased duration of exposure to local aetiological factors such as plaque and inflammation is required to cause a comparable level of periodontal destruction. This increase in such young adults has not been specifically reported before although Andronikaki-Faldami and co-authors did report an increase in periodontal disease in their 25-36 year age group despite not seeing any difference in the subjects less than 25 years of age (1990). A number of studies in children and adolescents have reported increased severity of disease in young diabetic patients compared with age matched controls. In these studies, the prevalence of significant periodontal disease was very low in both groups (Lalla et al., 2006; Luczaj-Cepowicz

et al., 2006; Dakovic et al., 2008; Silvetre et al., 2009). This is also similar to the finding of retinopathy in young diabetic patients. Retinopathy affects diabetic patients at a younger age than non-diabetic patients although the disease itself is little different (Morello et al., 2007). It is possible that the pathology of periodontitis is similar in T1DM to NDS however the natural history is accelerated in the former.

3.4.2 Glycaemic control

3.4.2.1 Gingival inflammation

Bivariate analysis showed that there is a positive correlation between average HbA1c and bleeding on probing (correlation coefficient 0.31 $p < 0.000$). Poor glycaemic control is therefore related to an increase in periodontal inflammation as demonstrated by bleeding from the base of the pocket. This analysis did not however take account of other confounding factors specifically including the presence of plaque. It is not possible to distinguish between gingivitis and periodontitis with this index.

3.4.2.2 Prevalence and extent of periodontitis

The influence of HbA1c on the prevalence and extent of periodontitis is less clear. Although the diabetic patients with the lowest HbA1c had the lowest prevalence of SP, the group with the highest prevalence and the most widespread disease was the moderately controlled group. It may be that differences in the age ranges across the five groups can account for this difference although the multivariable analysis did include age as a potential confounder. The age group with the highest levels of periodontal disease were however, older than the worst controlled diabetic patients (Fig 3-8).

There was an increase in the extent of periodontal disease as measured by the number of pathologically deepened pockets (≥ 4 mm) with increasing HbA1c although this pattern was also reversed in the most poorly controlled group. This is unlikely to be because increasing HbA1c is protective for the periodontium but rather it may reflect other differences between the groups. While it was suspected that age may have been confounding this relationship a multivariable regression analysis model of the prevalence of SP against HbA1c was not modified significantly with the inclusion of age in the model. It may be that with the small numbers involved the data was not powerful enough to detect this relationship and to account adequately for the

overwhelming effect of age on periodontal disease experience. Alternatively, there may be other unknown factors that account for this confounding effect as well as differences in age with HbA1c. The mean age of those subjects in the most poorly controlled group was lower than that of those in the moderately controlled groups. More than 60% of the group with the highest quintile of HbA1c were under thirty years of age and as such we would expect this group to have a correspondingly lower prevalence of SP. In this circumstance the increased age and duration of exposure to local aetiological agents may be more important than the HbA1c alone.

3.4.2.3 Comparison with the literature

A number of studies have shown a relationship between periodontal disease and glycaemic control (Gislen et al., 1980; Kjellman et al., 1970a; Gusberti et al., 1983; Harrison et al., 1987; Seppala et al., 1993, 1994; Firalti et al., 1996). Seppala and co-workers (1993, 1994) in a longitudinal study in T1DM adults reported that patients with poorly controlled diabetes mellitus had higher clinical probing depths and clinical attachment loss than controls. Willerhausen and co-workers (1991) in a European study of 42 T1DM compared with 118 healthy controls reported that there was a significant correlation between HbA1c and loss of clinical attachment.

Patients with poorer glycaemic control had more bleeding on probing and a higher prevalence, extent and severity of periodontal disease, although, due to confounding with age, this relationship was not linear although there was a positive correlation. It is not possible using our data to predict a safe level of glycaemic control below which periodontal disease will not occur. In the well-controlled groups, there were patients who suffered from periodontal disease. For other diabetic complications, it has been possible to identify safe levels of glycaemic control due to the relative rarity of the condition in the general population (DCCTRG, 1993). Retinopathy is rare in young non-diabetic patients and it was therefore possible to identify a safe level of glycaemic control below which this did not occur. Due to the multi-factorial nature of periodontal disease and its high prevalence in our control group it is not possible to distinguish between periodontitis caused by local risk factors, for example poor oral hygiene, and that caused by diabetes alone. Diabetic patients with good control had levels of periodontal disease that were less than the healthy controls. This could reflect the background risk of periodontal disease in the Scottish population. It is possible that if we had used a lower cut off point for HbA1c we would have been able

to demonstrate even more of a difference between the WCD and the PCD. This would however have been impossible within our study population given the rarity of patients achieving HbA1c levels of <6.5% or even <7%.

The possible mechanisms by which periodontal disease is exacerbated in diabetes include the deposition of advanced glycation end products (AGE) and their associated inflammatory effects, an altered systemic inflammatory response due to adipokines and circulating inflammatory cytokines, an immune deficiency or alterations in osteoimmunology or simply poor oral hygiene and dental compliance (Mealey et al., 2007; King, 2008; Chavarry et al., 2009). Most if not all of these would normally be worse in PCD.

The deposition of AGE in the tissues happens at a rate dependent on the concentration of glucose in the serum over time. This damage is however accumulative over time and so, while it is true to say that a high HbA1c will lead to an increase in AGE/RAGE related tissue damage, this will still take a finite amount of time to occur (DCCTRG, 1993; Stratton et al., 2000; Pambianco et al., 2006). This would explain why the younger patients with the worst HbA1c results do not have the highest levels of periodontal disease due to the fact that the natural history of the disease still requires a time factor to cause the disease.

Similarly, the functionality of leukocytes in terms of chemotaxis and phagocytosis has also been shown to be dependent on HbA1c and is therefore less of an issue in well controlled patients (Marhoffer et al., 1992; Gallacher et al., 1995). There are some immune deficiencies in diabetic patients that have been demonstrated to be present regardless of glycaemic control. These are inherent deficiencies of specific cell lines in T1DM regardless of external factors specifically affecting HLA type and TLR responsiveness (Pirie et al., 2005; Morran et al., 2008). T regulatory cells (T reg) from T1DM subjects display decreased suppressive characteristics, which suggest a defect in T reg function in people suffering from T1DM leading to excessive tissue damage in the pancreas causing beta cell destruction (Brusko et al., 2005; Lindley et al., 2005). The genetic and immunoregulatory factors that predisposed the patient to developing diabetes may be the same as the ones that predispose the patient to periodontal disease (Kopitar et al., 2006). If this were to be the case then it would not be the diabetes per se or even glycaemic control that would be the cause of the periodontitis but rather they would be two separate diseases with a common genetic risk profile.

Dyslipidaemia is increasingly thought to be important in the development of both macrovascular and microvascular disease (Tseng et al., 2019; Grauslund et al., 2010). This may be related to the pro-inflammatory effect of adipokines. The majority of patients in this study were overweight and this is a factor that we have not investigated. It is possible that the lipid profile and levels of adipokines could be an important factor in diabetes associated periodontitis (Karthikeyan et al., 2007; Fentoğlu et al., 2011). As with cardiovascular risk, the lipid profile or circulating levels of adipokines are important mediators of periodontal destruction. The relationship between glycaemic control and dyslipidaemia is not linear. It is possible that there was no significant relationship found between HbA1c and periodontal disease because the latter is in fact related to the lipid profile and adipokines in the circulation. However, Lim et al. (2007) recently showed that despite the fact that there was a trend to increased dyslipidaemia in a mixed population of type 1 and type 2 diabetic subjects the most significant correlation was between HbA1c and the percentage of sites with probing depths greater than or equal to 5mm. We have no available information on dyslipidaemia due to limitations on the availability of past laboratory data for our subjects, however it would have been interesting to investigate the effect of this on periodontal status. Interestingly a recent study has shown statistically significant reductions in low density lipoprotein cholesterol and total cholesterol in response to periodontal therapy. The role of periodontal treatment as an adjunct to reducing hyperlipidaemia as well as hyperglycaemia is an intriguing one that may merit further investigation (Kiran et al., 2005).

3.4.2.4 Non immuno-inflammatory hypotheses

It is possible that PCD patients have more disease simply because they do not look after their mouths as well as WCD patients. Patients who are compliant with diabetic control measures such that they are capable of maintaining glycaemic control in the target range over a two-year period are patients who are generally highly motivated individuals (Skinner and Cameron, 2010). This study showed some evidence that the PCD patients were slightly less well educated. In addition social factors do play a role in the epidemiology of periodontal diseases (chapter 2 Table 2-6). There is however also evidence from the psychological literature that patients react differently to disease states and chronic conditions. It has been shown that patients who have positive health behaviour toward their diabetes also have positive oral health

behaviour (Knecht et al., 1999a,b, 2000, 2001, Syrjala et al., 1999). This may relate to self-efficacy and the health locus of control. High self-esteem was also found to relate to good adherence with prescribed exercise regimens and adjustment of insulin doses. In the dental sphere, logistic regression analysis showed an association between self-esteem and frequency of tooth brushing (Knecht et al., 2001). Put simply, it may be that the “type of patient” who can cope with their diabetes by maintaining rigid control of every aspect of their diet and lifestyle is psychologically the same “type of person” who takes good care of their teeth. There are a series of papers showing this phenomenon. The group which conducted this research concludes that the connection between these psychological factors may be one of the explanatory variables that make up the difference between NDS, WCD and PCD (Knecht et al., 1999a,b, 2001; Syrjala et al., 1999). The applicability of these studies to the Scottish diabetic population has never been shown. However, further study on these possible links in a Scottish population could illuminate this explanation further.

3.4.3 Susceptibility to periodontal inflammation

In order to account for the presence of plaque, the ratio of the percentage of bleeding sites to the percentage of sites with plaque was calculated (Van der Velden et al., 1985). The difference in the susceptibility index was not significant although it was slightly higher in the PCD. There were no group differences in the MGI when the PI was corrected for. The periodontal susceptibility index has been shown to be higher in patients with a history of severe periodontitis compared with older patients with copious plaque but little periodontal attachment loss (Van der Velden et al., 1985). There have been three studies in diabetic subjects that specifically reported the ratio of bleeding sites to plaque. Paolantonio et al. (1989) reported that there was a higher ratio in patients with diabetes and periodontitis compared with healthy controls. Sastrowijoto et al.(1990) was unable to show that patients with a family history of diabetes and impaired glucose tolerance had any change in this ratio although the subjects in this study did not have any periodontal breakdown at the outset and it was concluded that the short duration of periods of hyperglycaemia were not sufficient to cause changes in the gingival condition. Dakovic and co-workers did however show that adolescents with T1DM had a higher bleeding/plaque ratio (OR 1.25; 95% CI 1.06-1.48). It is possible that the hormonal susceptibility to gingival inflammation in this age group exacerbates this increase. The same study also showed that there was a

weak positive relationship between the number of teeth affected by periodontal destruction and the bleeding/plaque ratio (regression coefficient 0.17; $p = 0.021$).

As the periodontal susceptibility index is a full mouth index it is a less accurate reflection of what is happening on a site basis. We have no way of knowing, from the susceptibility index, whether the sites that bled were also the ones where plaque was present, although this is likely. The findings at the index sites indicated that there was an increase in gingival inflammation in T1DM. However, this can be explained by the increase in plaque accumulation independent of diabetic status.

Based on this study we can conclude that there is insufficient evidence to support the hypothesis that adult patients with T1DM are more susceptible to plaque induced inflammation than non-diabetic subjects. This is in agreement with a number of studies that have found no correlation between diabetes and gingivitis (Glavind et al., 1968; Bay et al., 1974; Goteiner et al., 1986; Pinson et al., 1995; Sbordone et al., 1995, 1998; Bridges et al., 1996; Noaves et al., 1997). These studies have either not found an increase in gingivitis at all or have found that the plaque control in the diabetic group is significantly poorer. When this is taken into account there is no difference in gingivitis other than that resulting from the increase in plaque scores.

A number of studies have however shown that in diabetic patients the severity of gingivitis is related to glycaemic control (Kjellman et al., 1970a; Gislen et al., 1980; Gusberti et al., 1983; Harrisson et al., 1987; Seppala et al., 1992; Firatli et al., 1996). Differences in the age of the subjects may have played a part as some studies that found differences were in adolescents. These hormonal effects are less relevant in an adult population. However, Salvi et al. (2005, 2007, 2010) showed that the rate of development and severity of gingivitis is higher in diabetic patients. There were also higher levels of IL-1 β and MMP 9 in the T1DM patients at certain time points throughout the studies. The sample size was extremely small (controls $n=9$, T1DM $n = 9$) but the prospective nature of this study means that it provides more reliable evidence than the cross-sectional data presented in this chapter.

We cannot determine with certainty the temporal relationship between poorly controlled diabetes and periodontal disease or well-controlled diabetes and the absence of periodontal disease. It could be argued by those unfamiliar with the natural history of periodontal disease, that periodontal disease is a risk factor for the development of diabetes rather than diabetes mellitus being a risk factor for periodontal disease. This is not likely, given that periodontal disease is relatively rare

in youth while this is the time at which T1DM is diagnosed. What is perhaps more interesting is whether having a healthy periodontium leads to good glycaemic control or indeed whether having a diseased periodontium leads to an increased risk of poor glycaemic control. This two way or “bi-directional” relationship is one that was proposed by Iacopino et al. (2001) and has been the subject of two recent systematic reviews (Simpson et al., 2010, Teeuw et al., 2010). We have shown that well controlled diabetic subjects have less periodontitis than poorly controlled subjects. However we cannot establish whether the good control has prevented the development of periodontitis or whether the poorly controlled diabetes mellitus has led to periodontal damage. A prospective longitudinal study would be required to elucidate this relationship. This would require periodontal healthy recently diagnosed T1DM subjects to be followed through life with detailed periodontal charting at regular intervals.

3.4.4 Duration of diabetes

There was no relationship between diabetes duration and periodontal disease. While it may be expected that the length of time for which a subject has had diabetes would be an important factor in the development of periodontal disease this does not appear to be independent of age or glycaemic control. Those patients who have had diabetes for a long time are also older. The increasing age was more important than the duration of diabetes. Glavind et al. (1968) reported that periodontitis was related to duration of diabetes and Leeper and co-workers also reported that periodontitis increased with duration (1985). The authors did not use multi-variable analysis and therefore they could have been reporting the relationship with age rather than duration. Firatli et al. (1997) examined the relationship between periodontal status and the duration of IDDM in 44 T1DM children and adolescents in a longitudinal study lasting five years. Clinical attachment loss was associated with duration of diabetes. Over a five year period the mean CAL increased from 2.39mm to 3.51mm. Silvestre et al. (2009) reported that there was an increase in gingival bleeding and periodontitis in patients who had had diabetes for more than ten years compared with those of short duration of diabetes. This was a bivariate comparison and no age data was presented for either group. It has been shown that the accumulation of AGE in the tissue increases with increasing duration of diabetes and exposure of diabetes and thus human studies clearly show the relationship between diabetic complications and

duration of diabetes (DCCTRG, 1993; Stratton et al., 2000; Pambianco et al., 2006). There have been a number of studies that have concluded that duration of diabetes is not an independent risk factor for periodontitis (Arrietto-Blanco et al., 2003; Lalla et al., 2006, 2007). It is possible that the effect of duration can be seen in adolescents because diabetes exposure is the main risk factor at this age. However this effect is attenuated with age as increased age confers a greater risk of periodontitis than duration of diabetes alone.

3.5 Conclusions

This second results chapter has attempted to elucidate the effect of age, glycaemic control and duration of diabetes on the periodontal findings. We have shown that periodontitis is evident in young adults although it is not necessarily particularly severe in this age group. The level of disease noted in young diabetic patients would necessitate periodontal treatment and this has service and cost implications as well as implications for the patients themselves. According to the British Society for Periodontology a BPE of 3 indicates a moderate periodontal need that can normally be managed in general practice. A BPE of 4 is categorised as complexity 2 and may require specialist intervention, particularly if there is a systemic modifying factor such as diabetes mellitus (Dowell and Chapple, 2002). Those patients who have diabetes mellitus and a BPE of 4 in one sextant represent a group of patients who may well require treatment in secondary care. The cost and service implication of treating the periodontal disease of this group will be significant and given that this appears to be starting in patients as young as 25 could represent a significant lifetime cost in periodontal treatment and subsequent maintenance.

Poorly controlled patients had higher full mouth plaque scores and higher mean PI scores at the index sites. This accounted for most of the increase in MGI and bleeding scores. This indicates that either their oral hygiene is simply inadequate or that they have higher plaque levels for some organic reason. Biofilm formation may progress quicker in a glucose rich environment and this may lead to more rapid regrowth of plaque. Reduced salivary flow rates may lead to an increased accumulation of dental plaque and there is also evidence that *C albicans* can interact with early bacterial colonisers and increase biofilm formation (Silverman et al., 2010). Regardless of the reason for the higher plaque levels, better local measures to control plaque are

essential if these patients are to avoid developing periodontal disease and its sequelae. Patient education and oral hygiene instruction may be an effective way of reducing the risk of developing periodontal disease in diabetic patients in addition to improving glycaemic control.

Duration of exposure to diabetes was not an independent risk factor for periodontal disease progression. This is not a modifiable risk factor as the patient cannot control the date of their diagnosis. Within the limits of this cross-sectional study there does not appear to be an inevitable progression in periodontal destruction with increasing duration of exposure to diabetes.

Poor glycaemic control was also shown to be damaging to the periodontium with higher levels of disease in patients with poorer levels of glycaemic control. In the multivariable regression analysis there did not appear to be a linear relationship between the prevalence of severe periodontitis and increasing HbA1c quintiles. We were not able to show a threshold effect and therefore we cannot advise a certain target HbA1c to avoid periodontal disease. The main difference was seen at levels of HbA1c above the physiological range. This conferred at least a twofold increased risk of severe periodontitis. The small number of cases in some groups and the strong association of periodontitis with age may have reduced the power to detect the full extent of the impact of HbA1c on periodontitis. The fully adjusted model did not elucidate the relationship further. Given the similarities between periodontitis and other microvascular complications a holistic approach to treatment should be adopted. Patients should be advised that poor glycaemic control is a risk factor for periodontal disease and should be encouraged to maintain HbA1c levels as near the physiologically normal range as possible.

4 Markers of bone turnover in Type 1 diabetes mellitus and periodontitis

4.1 Introduction

The mechanism through which diabetes mellitus confers increased risk of periodontitis in T1DM is unclear, although a number of mechanisms have been proposed (Graves, 2006). It is unlikely that differences in subgingival microflora are to blame as a number of studies both culture based and using 16s molecular microbiology have shown no significant differences (Sastrowijoto et al., 1989, Lalla et al., 2006b). It is therefore more likely to be the host response to the common subgingival microflora that leads to the destruction of the periodontal ligament and alveolar bone. There is some evidence that diabetes associated periodontitis may be due to cytokine dysregulation due to excessive TNF α expression (Salvi et al., 1997a,b, 2010). It is hypothesised that this hyper-inflammatory process leads to production of cytokines, such as IL-1 and IL-6, that influence osteoclast maturation and activation via a receptor activator of nuclear factor-kB ligand (RANKL) dependent pathway. The production of RANKL and the subsequent activation of bone destruction could lead to excessive alveolar bone loss. Bone resorption and bone formation are processes that are “coupled” but can take place independently (Corral et al., 1998). The function of bone resorbing osteoclasts is regulated by interaction with fibroblasts in the ligament between the tooth and the bone (Kanzaki et al., 2002). These periodontal ligament fibroblasts are involved in both stimulatory and inhibitory processes. RANKL interacts with receptors on the surface of osteoclasts to stimulate bone resorption. Osteoprotegerin (OPG) also produced by periodontal ligament fibroblasts (Kanzaki et al., 2002) blocks the activity of RANKL by binding to RANK. High levels of RANKL are expressed during root resorption of deciduous teeth and, raised levels of OPG are expressed, where no root resorption is normally taking place, i.e. adjacent to permanent teeth (Fukushima et al., 2003). RANKL levels are much higher in periodontitis patients than in those who have never had periodontitis (Bostanci et al., 2007).

The expression of OPG in periodontal tissues is decreased in both moderate periodontitis and advanced periodontitis, while RANKL mRNA is increased in

periodontitis at the advanced stage (Liu et al., 2002). Higher circulating levels of OPG are found in T1DM patients than in healthy individuals (Kim et al., 2005). Plasma OPG concentrations have been shown to be implicated in the regulation of vascular calcification, atherogenesis, coronary artery disease (Dhore et al., 2001; Jono et al., 2002) and associated with progression of coronary artery calcification in type 2 diabetes mellitus (Anand et al., 2007).

An increased ratio of RANKL to OPG in periodontitis may determine local bone resorption. This ratio can be measured either in GCF, serum or at the mRNA level within tissues (Cochran, 2008). C-terminal telopeptide of type 1 collagen (ICTP) is the carboxyterminal telopeptide of type 1 collagen, the only collagen type found in mineralized bone (Risteli & Risteli, 1993). ICTP is released into the circulation during bone resorption. Increased amounts of this product in serum or plasma indicates an increase in osteoclast activity which has been shown in multiple myeloma and osteoporosis, as well as other diseases characterised by bone resorption (Elomaa et al., 1992, Hassager et al., 1994, Abildgaard et al., 1997). ICTP has also been shown to be related to bone resorption associated with chronic periodontitis. Oringer et al. (2002) showed that ICTP and IL-1 were increased in periodontally diseased sites. Sarwent also showed that levels of ICTP changed during healing after periodontal surgery and were increased when recombinant platelet derived growth factor was used as an adjunct to surgery. Another study showed that ICTP is highly correlated with clinical features of periodontal disease, that levels decreased in response to treatment and that ICTP had been shown to possess predictive properties for possible future disease activity (Kinney et al., 2007). In a study investigating the possibility of using saliva as a source of ICTP, ICTP was only detected in the saliva of patients with periodontitis (Frodge et al., 2008). Gurlek et al. (2009) demonstrated that salivary ICTP levels positively correlated with the number of bleeding sites but also demonstrated that there was no difference in ICTP levels in smokers and non-smokers where the levels of periodontal disease were similar. Rheinhardt et al. (2010) showed that elevated baseline ICTP levels during periodontal maintenance was also associated with increased risk of 1- and 2-year loss of alveolar bone density.

Osteocalcin is a major non-collagenous protein of the bone matrix that is produced by osteoblasts. Serum osteocalcin levels have been used as biomarkers for bone formation because this protein is released into the circulation during this process

(Bullon et al., 2005). A number of studies have measured osteocalcin levels in relation to periodontitis and in diabetic patients (Shi et al., 1996; Alexopoulou et al., 2006; Gurlek et al., 2009) but there are no studies examining diabetic patients with periodontitis. Shi et al. (1996) showed that patients with periodontitis had lower levels of serum osteocalcin than healthy controls. Rheinhardt and co-workers also showed that the ratio of osteocalcin to ICTP was highest while bone was being laid down during healing of a surgically induced wound in the alveolar bone (Rheinhardt et al., 2005). Higher serum levels of osteocalcin were measured in rats, with experimentally induced periodontitis, treated with doxycycline and alendronate indicating higher bone formation due to the inhibition of osteoclast activity by the alendronic acid (Buduneli et al., 2005). Liu and co-workers (2009) reported that plasma levels of osteocalcin were higher in aggressive periodontitis than in healthy controls although this study did not include any data on the relative ICTP levels.

Bone loss seen in periodontitis is associated with a perturbation in the balance of RANKL and OPG and may be reflected in alterations in osteocalcin and/or ICTP levels. Our hypothesis is that higher levels of OPG reported for T1DM patients modify the level of destruction seen in those patients also suffering from periodontitis.

The aim of this study was to measure the circulating levels of the markers of bone metabolism in T1DM patients with and without periodontitis. The research question was:

- Does the presence of periodontitis have an influence on the plasma concentrations of markers of bone metabolism in diabetic patients and systemically healthy individuals?

4.2 Methods

4.2.1 Subjects

The ethical approval, recruitment, consent and inclusion and exclusion criteria have been described in chapter 2. The age range of the subjects included in this sub group was 22-55 years and included both male and female Caucasians, who were generally healthy and had at least 20 teeth. To be included in the periodontitis groups patients had to have a minimum of two sites with clinical probing depth and clinical

attachment loss of ≥ 5 mm. Non-periodontitis control subjects had no sites with clinical attachment loss or clinical probing depths of > 4 mm.

Full mouth six point clinical attachment level and clinical probing depth chartings were carried out on all subjects as described in chapter 2. The most recent HbA1c available from the relevant diabetic clinic was used to categorise the T1DM subjects. Sixty of the patients recruited had T1DM and 38 were NDS. 10 of the NDS were recruited from the periodontal department of Glasgow Dental Hospital and School.

4.2.2 Measuring RANKL, OPG, ICTP and osteocalcin

The plasma was separated from the blood by centrifugation (1048 xg for ten minutes), stored in aliquots at -70°C and thawed immediately prior to assay. The human soluble RANKL (hsRANKL) Enzyme Linked Immunosorbent Assay (ELISA) development kit (Peprotech EC, United Kingdom), the human OPG (hOPG) ELISA development kit (R & D systems, United Kingdom) the UniQ ICTP Enzyme immunoassay (EIA) kit (Orion Diagnostica, Finland) and the osteocalcin immunoassay kit (BioSource Europe, Belgium) were used. ICTP assays were performed using a 1/3 dilution of plasma and osteocalcin assays were performed using a 1/5 dilution of plasma. The kits contained all the necessary solutions for performing the assays and method used for each assay followed the manufacturers' guidelines. All samples were run in duplicate.

4.2.2.1 ELISA solutions

Phosphate buffered solution (PBS) - 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 , 5 mM KH_2PO_4 , pH 7.2 - 7.4, 0.2 μm filtered.

Wash buffer - 0.05% Tween 20 in PBS, pH 7.2 - 7.4

Reagent diluent - 0.1% Bovine serum albumin (BSA) in PBS with 1% heat inactivated [56°C for 30 mins] goat serum, pH 7.2 - 7.4, 0.2 μm filtered

Streptavidin-HRP - Streptavidin conjugated to horseradish peroxidase

Substrate solution - Tetramethylbenzidine (TMB) (Vector Laboratories, Burlingame USA). Four drops of buffer stock solution, six drops of TMB stock solution and 4 drops of hydrogen peroxide were sequentially added to 10ml distilled water.

Stop solution - 0.12 M HCl

4.2.2.2 Osteoprotegerin and RANKL ELISA Protocol

1. The capture antibody provided (biotinylated goat anti-human OPG or RANKL) was diluted to the working concentration of 2 μ g/ml in PBS without carrier protein.
2. The 96-well immunolon 4 microplates (Thermolaboratories, United Kingdom) were immediately coated with 100 μ l per well of the diluted capture antibody.
3. The plates were sealed and incubated overnight at room temperature.
4. Each well was aspirated and washed using a multi-channel plate washer (Nunc immunowash 12 , Immunomed, Denmark) at least three times with 400 μ l wash buffer ensuring that all liquid was removed at each step.
5. After the last wash, any remaining wash buffer was removed by inverting the plate and blotting it against clean paper towels.
6. The plates were blocked by adding 300 μ L of reagent diluent to each well.
7. The plates were incubated at room temperature for a minimum of 1 hour and washed again as described in step 4.
8. 100 μ L of standards or samples (plasma diluted 1:5 for OPG and 1:10 for RANKL) in diluent, were added to each well and the wells were covered and incubated for 2 hours at room temperature.
9. The aspiration/wash steps were repeated as in step 4.
10. 100 μ L of the detection antibody, diluted in reagent diluent was added to each well, covered with a new adhesive strip and incubated for two hours at room temperature.
11. The aspiration/wash steps were repeated as in step 4.
12. 100 μ L of the working dilution of Streptavidin-HRP (streptavidin conjugated to horseradish-peroxidase) was added to each well. The plates were covered and incubated for 20 minutes at room temperature.
13. The aspiration/wash steps were repeated as in step 4.
14. 100 μ L of tetramethylebenzidine (TMB) chromagen substrate solution was added to each well and incubated for 20 minutes at room temperature.
15. 50 μ L of stop solution was added to each well and the plates were gently tapped to ensure thorough mixing.

16. The optical density of each well was determined using a photometric microplate reader set to 450 nm with a reference filter at 630nm (Dynex MRX II, Thermal Fisher, UK). The results were calculated from the standard curve.

4.2.2.3 ICTP EIA protocol

1. All the reagents, controls and patient samples were brought to room temperature at least 30 minutes before use.
2. The excess strips were removed from the plate frame and returned to the pouch and closed tightly.
3. 50 μ L of ready to use calibrators (0 μ L, 1.0 μ L, 2.5 μ L, 5 μ L, 10 μ L, 25 μ L, 50 μ L), control (lyophilised in human serum reconstituted in 0.5ml of distilled water) and patient sample in duplicate was pipetted into the 96 well microtitre plate provided. Two wells were reserved for the substrate blank.
4. 50 μ L of ICTP enzyme conjugate (ready to use peroxide labelled ICTP) was pipetted into all wells except the blanks.
5. 50 μ L of ICTP antiserum (ready to use rabbit antiserum) was pipetted into all wells except blanks. The antiserum was applied to all wells within 3 minutes.
6. The plates were incubated on a plate shaker at 18-25°C for 2 hours. Use a shaking speed of 600-1000 rpm.
7. The strips were washed 4 times with the wash solution (80 ml EIA wash concentrate diluted to 1000ml) on a plate washer. Using 300-500 μ L of the wash solution per well. Any remaining moisture was removed by tapping the strips firmly against absorbent paper.
8. 100 μ L of ICTP substrate (ready to use Tetramethylbenzidine in aqueous buffer) was pipetted into all wells and the plates were incubated on a plate shaker at 18-25 °C for 30 minutes.
9. The enzyme reaction was stopped by adding 100 μ L of stopping solution (ready to use 0.5M H₂SO₄) into all wells.
10. The plates were shaken for 15-30 seconds to mix the reagents.
11. The absorbances of all wells was read at 450 nm on a photometric microplate reader with a reference filter at 630nm (Dynex MRX II, Thermal Fisher, UK) within 10minutes. The results were calculated from the standard curve.

4.2.2.4 Osteocalcin ELISA protocol

1. All the reagents, controls and patient samples were brought to room temperature at least 30 minutes before use.
2. The lyophilized standards and controls were reconstituted to the volume specified on the vial label with distilled water and were mixed with gentle inversion.
3. The wash solution was prepared by diluting 2ml of the concentrate wash solution in 400ml of distilled water and stirring with a magnetic stirrer.
4. 25 μ l of each calibrator, control and sample was pipetted into the 96 well microtitre plate precoated with anti-human osteocalcin provided.
5. 100 μ l of working anti-OST-HRP conjugate was pipetted into all the wells and the plates were incubated for 2 hours at room temperature on a horizontal shaker set at 700 plus or minus 100..
6. The liquid was aspirated from each well and the plates were washed three times by dispensing 500 μ L of wash solution into the well and aspirating the liquid.
7. 100 μ l of the chromogenic solution (ready to use TMB) was pipetted into each well within 15 minutes following the washing step.
8. The microtiterplate was incubated for 30 minutes at room temperature on a horizontal shaker set at 700 plus or minus 100 rpm avoiding direct sunlight.
9. 200 μ l of the provided Stop solution was pipetted into each well.
10. The absorbancies were read at 450 nm (reference filter 630 nm) within 1 hour using the Dynex MRX II (Thermal Fisher, UK) photometric plate reader and the results were calculated from the standard curve.

The method used for each assay followed the manufacturers' guidelines. The concentration of sRANKL and OPG in each of the samples was then determined by comparing the average sample optical density readings with the concentrations from the assay standard curve. The relevant assays for these proteins are capable of detecting both the free and RANKL-OPG complex (Lappin et al., 2007). The relative plasma concentrations of OPG and sRANKL and osteocalcin and ICTP (nanograms per millilitre) were calculated.

4.2.3 Statistical analyses of the ELISA/EIA data

A pilot experiment, where OPG levels were measured and a two-fold difference obtained, was utilized in statistical power calculations. With a power of 80% and an $\alpha = 0.0125$ the minimum number of patients required for the comparisons was 68. Non-parametric distribution-free statistical tests were employed to analyse the data. The postulated null hypothesis was “no statistically significant difference” and the maximum number of comparisons was four.

The relationship between the percentage of glycated haemoglobin in the blood (HbA1c%) and [RANKL], [OPG], [osteocalcin] and [ICTP] was determined using the Spearman correlation coefficient, correcting for multiple testing by applying a Bonferroni correction.

4.3 Results

4.3.1 Demographic and clinical parameters

A full mouth six point periodontal charting was successfully carried out for 96 of the 98 patients, recording the following: gingival recession, clinical probing depth (CPD) and clinical attachment level (CAL) measured from the amelocemental junction. Only 94 of the 98 patients had complete records of bleeding on gentle probing.

Once the study population was divided according to the periodontal criteria, 14 had to be excluded from the dataset because they did not fit into either the periodontitis or the non-periodontitis groups (two because the clinical data was not complete and 12 because they did not meet the criteria for either gingivitis alone or periodontitis for example a patient may have had 1 site with >4 mm CAL and therefore could not be included in the healthy group). Two non-diabetic patients who did not have full mouth BOP recorded were retained since full mouth records of CPD and CAL were available.

The non-diabetic group consisted of 16 male and 22 female subjects and the diabetic group consisted of 28 male and 32 female patients. The T1DM patients were divided into two groups according to the median HbA1c for the whole group. The low HbA1c ($<8.5\%$) group consisted of 12 male and 18 female patients and the high HbA1c ($>8.5\%$) group of 16 male and 14 female patients. The median ages of the patients in the non-diabetic and the diabetic groups (Low HbA1c% and High

HbA1c% groups) were very similar; non-diabetic patients 40 (range 21 – 55) years, diabetic patients with Low HbA1c% 34 (20 – 50) years and diabetic patients with High HbA1c% 40 (20 – 54) years. Clinical data are presented in Table 4-1. The number of sites and teeth with clinical probing depths and clinical attachment loss greater than 4mm and the number of sites bleeding on probing are indicated. The NDS group appeared to have lower average levels of bleeding on probing than the T1DM group. The diabetic patients with low HbA1c% appeared to have lower numbers of teeth and sites with CAL >4mm and CPD >4mm than the diabetic patients with high HbA1c, but there was no statistically significant difference in these parameters between the groups. The Kruskal-Wallis test results comparing the three groups were as follows: CPD >4mm, $p= 0.31$; Number of teeth with CPD >4mm, $p=0.26$; CAL >4mm, $p= 0.48$ and Number of teeth with CPD >4mm, $p= 0.36$.

Although, the number of teeth and sites with CPD >4mm was similar in both T1DM and NDS periodontitis groups there were more sites and a greater number of teeth with CAL >4mm in the T1DM periodontitis group. None of these differences were statistically significant.

Table 4-1 Number of sites and teeth with clinical probing depths > 4mm, sites and teeth with clinical attachment loss > 4mm and proportion of sites bleeding on probing (mean ± standard deviation)

Diabetic status (n)	Number of sites CPD ^{*1} >4mm	Number of teeth CPD ^{*1} >4mm	Number of sites CAL ^{*2} >4mm	Number of teeth CAL ^{*2} >4mm	Proportion of sites BOP ^{*3}
Non-diabetic (38) ‡ (36)	4.13 ±5.83	2.16 ±2.49	2.82 ±5.99	1.39 ±2.57	0.34 ± 0.17
Diabetic (60)	3.77 ±7.6	2.14 ±3.85	2.14 ±3.85	3.22 ±5.79	0.48 ±0.18
Periodontal status (n) †	Number of sites CPD >4mm	Number of teeth CPD >4mm	Number of sites CAL >4mm	Number of teeth CAL >4mm	Proportion of sites BOP
Non-diabetic control (17)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.35 ± 0.14
Non-diabetic periodontitis (17) † (15)	8.71 ± 6.09	4.47 ± 1.91	6.18 ± 7.84	3.00 ± 3.2	0.32 ± 0.21
Diabetic (26)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.16
Diabetic periodontitis (24)	8.86 ± 10.02	4.87 ± 4.82	7.5 ± 7.10	4.38 ± 3.49	0.55 ± 0.16

*1 CPD = Clinical Probing Depth

*2 CAL = Clinical Attachment Loss

*3 BOP = Bleeding on Probing

‡ 2 patients do not have full CAL or BOP data

† 2 of the Non-diabetic (periodontitis) patients do not have full mouth BOP data and one diabetic has this information missing, but full charting of CAL and CPD are available.

‡ 2 non-diabetic patients and 10 diabetic patients were omitted from the periodontal status analysis as they failed to meet the inclusion criteria.

4.3.2 Plasma concentrations of RANKL, OPG, ICTP and osteocalcin

The assay sensitivities and range of the standard curves are shown in table 4-2 below.

Table 4-2 Assay Sensitivity and range of hsRANKL, osteoprotegerin, ICTP and osteocalcin assays

	hsRANKL	OPG	ICTP	Osteocalcin
Sensitivity	7.8pg/ml	3.9pg/ml	0.5ng/ml	0.4ng/ml
Standard curve range	13.6-1000pg/ml	7.8-500pg/ml	1-50ng/ml	1-90ng/ml

Diabetic patients had a higher concentration of OPG, a lower concentration of osteocalcin and a lower RANKL: OPG ratio than non-diabetic patients. The Mann-Whitney U Test indicated that both findings were statistically significantly different (OPG: $p < 0.001$; osteocalcin: $p = 0.047$, RANKL: OPG: $p < 0.001$). The differences between the groups in RANKL and ICTP levels were not statistically significant.

4.3.3 Influence of glycated haemoglobin on plasma RANKL, OPG, ICTP & osteocalcin

Diabetic patients were divided into two groups High and Low depending on their most recent HbA1c. The median HbA1c (8.60%) was taken as the cut-off point and the two groups were compared with each other and the non-diabetic group (Table 4-3). For consistency within this thesis the groups were also analysed using the cut-off point of $\leq 7.5\%$ and both are reported here. The results obtained for OPG, indicated a statistically significant difference between some of the groups according to the Kruskal-Wallis test (Figure 4-2). When the three groups were compared and analysed post hoc with the Mann-Whitney U Test and a Bonferroni correction for multiple comparisons, statistically significant differences were found for the following comparisons: non-diabetic group and WCD (HbA1c $\leq 7.5\%$) group ($p < 0.016$) and the non-diabetic group and PCD (HbA1c $> 7.5\%$) group ($p < 0.001$). PCD showed the lowest concentrations of osteocalcin (Figure 4-1 median= 5.19 ng/ml). The results

showed a statistically significant difference between the groups according to the Kruskal-Wallis Test ($p = 0.04$). When the three groups were analysed by the Mann-Whitney U Test with Bonferroni correction, a statistically significant difference in plasma osteocalcin concentrations was observed when comparing the NDS and the PCD group ($p = 0.016$) before correction (0.06 after correction), but not between the WCD and PCD groups ($p = 0.07$). There were no statistically significant differences in RANKL or ICTP concentrations between any of the groups (Kruskal wallis $p = 0.649$, $p = 0.092$ respectively) (Table 4-3) (Figure 4-1).

There was a statistically significant difference in the RANKL: OPG ratio (Table 4-2) between the NDS and the T1DM patient groups according to the Kruskal-Wallis test ($p = 0.0023$). In the post hoc analysis: both PCD and WCD groups were statistically significantly different from the NDS ($p = 0.024$ & $p = 0.001$, respectively), but not from each other ($p = 0.080$).

Table 4-3 Influence of glycated haemoglobin on plasma RANKL, OPG, ICTP & osteocalcin

Diabetic status (n)	RANKL (ng/ml)	OPG (ng/ml)	RANKL:OPG (Mol:Mol)	ICTP (ng/ml)	Osteocalcin (ng/ml)
Non-diabetic (38)	0.83 (0.36-1.26)	1.11 (0.65-1.55)	1.45 (0.57-2.83)	4.78 (3.20-6.24)	7.18 (4.62-14.60)
Low HbA1c ($\leq 8.5\%$) (30)	0.66 (0.17-2.37)	2.2 (1.51- 3.13)	0.65 (0.24-1.14)	3.34 (1.8 5.81)	5.4 (2.98-10.82)
High HbA1c ($> 8.5\%$) (30)	0.62 (0.22-1.8)	1.88 (1.31-3.11)	0.86 (0.24-1.44)	3.46 (2.49-5.85)	5.31 (2.79-8.19)
Diabetic status (n)	RANKL (ng/ml)	OPG (ng/ml)	RANKL:OPG (Mol:Mol)	ICTP (ng/ml)	Osteocalcin (ng/ml)
Non-diabetic (38)	0.83 (0.36-1.26)	1.11 (0.65 -1.55)	1.45 (0.57-2.83)	4.78 (3.20-6.24)	7.18 (4.62-14.60)
WCD ($\leq 7.5\%$) (11)	0.54 (0.15-1.69)	1.76 (1.19-2.74)	0.39 (0.24-1.14)	2.94 (1,17-7.81)	9.32 (3.52-20.17)
PCD ($> 7.5\%$) (49)	0.66 (0.19-1.99)	2.23 (1.38-3.17)	0.76 (0.22-1.44)	3.79 (2.43-5.76)	5.19 (2.64-8.29)

Indicates statistically significant differences between groups

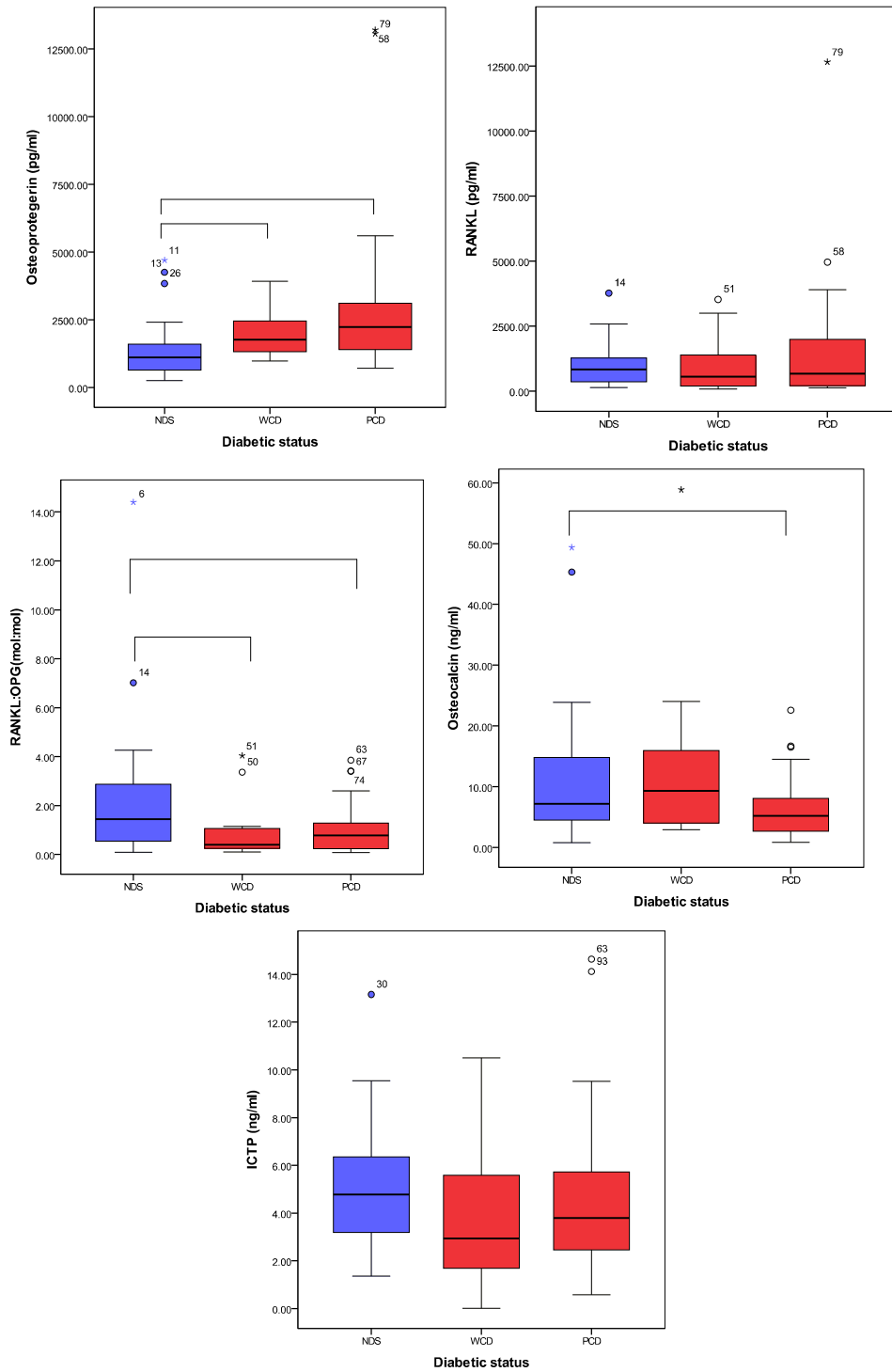


Figure 4-1 RANKL, OPG, Osteocalcin and ICTP by diabetic control.

The box plot with quartiles, showing outliers circles and extreme values(asterisks), represents the plasma concentration of Osteoprotegerin (OPG), RANKL, RANKL:OPG ratio, ICTP and Osteocalcin and in 38 non-diabetic patients and 63 patients with diabetes split into two groups based on their HbA1c%: 11well controlled diabetic patients% (HbA1c ≤7.5%) and 49 poorly controlled diabetic patients (HbA1c >7.5%). ICTP, C-terminal telopeptide of type 1collagen; RANKL, receptor activator of nuclear factor-κB ligand.

4.3.4 Influence of periodontitis on plasma RANKL, OPG, ICTP and osteocalcin

There was no difference in the extent of periodontitis between the non-diabetic and the diabetic patients. Fourteen patients (10 diabetic patients; 4 non-diabetic patients) were excluded from the rest of the study because of difficulty in placing them in either the periodontitis group or the non-periodontitis control group. Therefore the groups consisted of: T1DM non-periodontitis $n = 26$; T1DM with periodontitis $n = 24$; NDS non-periodontitis $n = 17$; and NDS with periodontitis $n = 17$.

When the patients were allocated according to their disease status, T1DM with periodontitis had the highest plasma concentration of OPG (Table 4-4, Figure 4-2). The Kruskal-Wallis Test indicated that the differences were statistically significant ($p < 0.001$). The Mann-Whitney U Test with a Bonferroni correction indicated statistically significant differences in plasma OPG (Figure 4-2) in the following comparisons: non-diabetic patients and T1DM without periodontitis ($p = 0.009$); NDS with periodontitis and T1DM with periodontitis ($p < 0.0001$) and; NDS without periodontitis and NDS patients with periodontitis ($p = 0.004$).

The highest osteocalcin levels were in the non-diabetic patients without periodontitis and the lowest in the non-diabetic patients with periodontitis. The levels in the non-diabetic patients with periodontitis were similar to those in both diabetic patient groups, i.e. the diabetic patients with and without periodontitis. Post hoc tests showed statistically significant differences between the non-diabetic patients without periodontitis and the non-diabetic patients with periodontitis ($p = 0.003$); the NDS without periodontitis and T1DM without periodontitis ($p = 0.009$) (Table 4-4, Figure 4-2). There were no statistically significant differences in RANKL or ICTP concentrations (Table 4-4, Figures 4-2). Statistically significant differences in the ratio of RANKL: OPG (Table 4-4, Figure 4-2) were observed between the following patient groups: the non-diabetic patients without periodontitis and the non-diabetic patients with periodontitis ($p = 0.01$) and the diabetic patients with periodontitis and the non-diabetic patients with periodontitis ($p < 0.0001$).

Table 4-4 Comparison of median (Quartile 1–3) plasma RANKL, OPG, osteocalcin, ICTP levels and RANKL:OPG ratios in the patient groups

Periodontal status (n) †	RANKL (ng/ml)	OPG (ng/ml)	RANKL:OPG (Mol:Mol)	ICTP (ng/ml)	Osteocalcin (ng/ml)
Non-diabetic (17)	0.65 (0.31 – 1.24)	1.29 (1.06 - 1.70)	0.94 (0.43 - 1.70)	4.80 (3.02 – 6.81)	13.26 (6.7-19.9)
Non-diabetic Periodontitis (17)	0.953 (0.69 – 1.25)	0.646 (0.60- 1.39)	2.22 (1.65 - 3.06)	5.22 (4.21 - 6.62)	5.00 (3.5-7.2)
Diabetic (26)	0.83 (0.15 – 2.34)	1.938 (1.39 – 3.13)	0.88 (0.20 - 1.98)	3.24 (2.07 – 6.07)	6.23 (4.38 – 10.54)
Diabetic Periodontitis (24)	0.63 (0.25 – 1.68)	2.30 (1.41 – 3.07)	0.85 (0.28 - 1.14)	3.71 (2.26 - 5.57)	5.31 (3.63 – 10.81)

} Indicates statistically significant differences between groups (Mann Whitney U test. $p < 0.05$)

4.3.5 Correlations

The plasma concentrations of RANKL did not show any statistically significant correlation with HbA1c%; Spearman's correlation coefficient (ρ) = -0.070 $p = 0.494$. The plasma concentration of OPG indicated a statistically significant positive correlation ($\rho = 0.391$ $p < 0.000$) with the HbA1c% in blood. The plasma concentration of osteocalcin indicated a statistically significant negative correlation ($\rho = -0.226$; $p = 0.025$) with HbA1c% and with age ($\rho = 0.32$ $p = 0.001$). These relationships remained statistically significant when the data were controlled for the presence of periodontitis (HbA1c% and OPG, $p = 0.008$ and HbA1c% and osteocalcin, $p = 0.009$) the gender ($p = 0.008$ and $p = 0.009$) or the age ($p = 0.006$ and $p = 0.004$) of the patients. Osteocalcin concentrations also negatively correlated with the number of teeth with CAL > 5mm ($\rho = -0.335$, $p = 0.001$). Plasma ICTP concentrations did not correlate with HbA1c% ($\rho = -0.141$; $p = 0.167$), OPG levels or the number of teeth affected by periodontal disease, but there was a positive correlation between ICTP and RANKL ($\rho = 0.29$; $p = 0.004$ and the RANKL:OPG ratio ($\rho = 0.310$; $p = 0.002$). Bleeding on probing correlated with the number of deep sites ($\rho = 0.406$ $p < 0.001$) and with the HbA1c% ($\rho = 0.464$; $p < 0.001$).

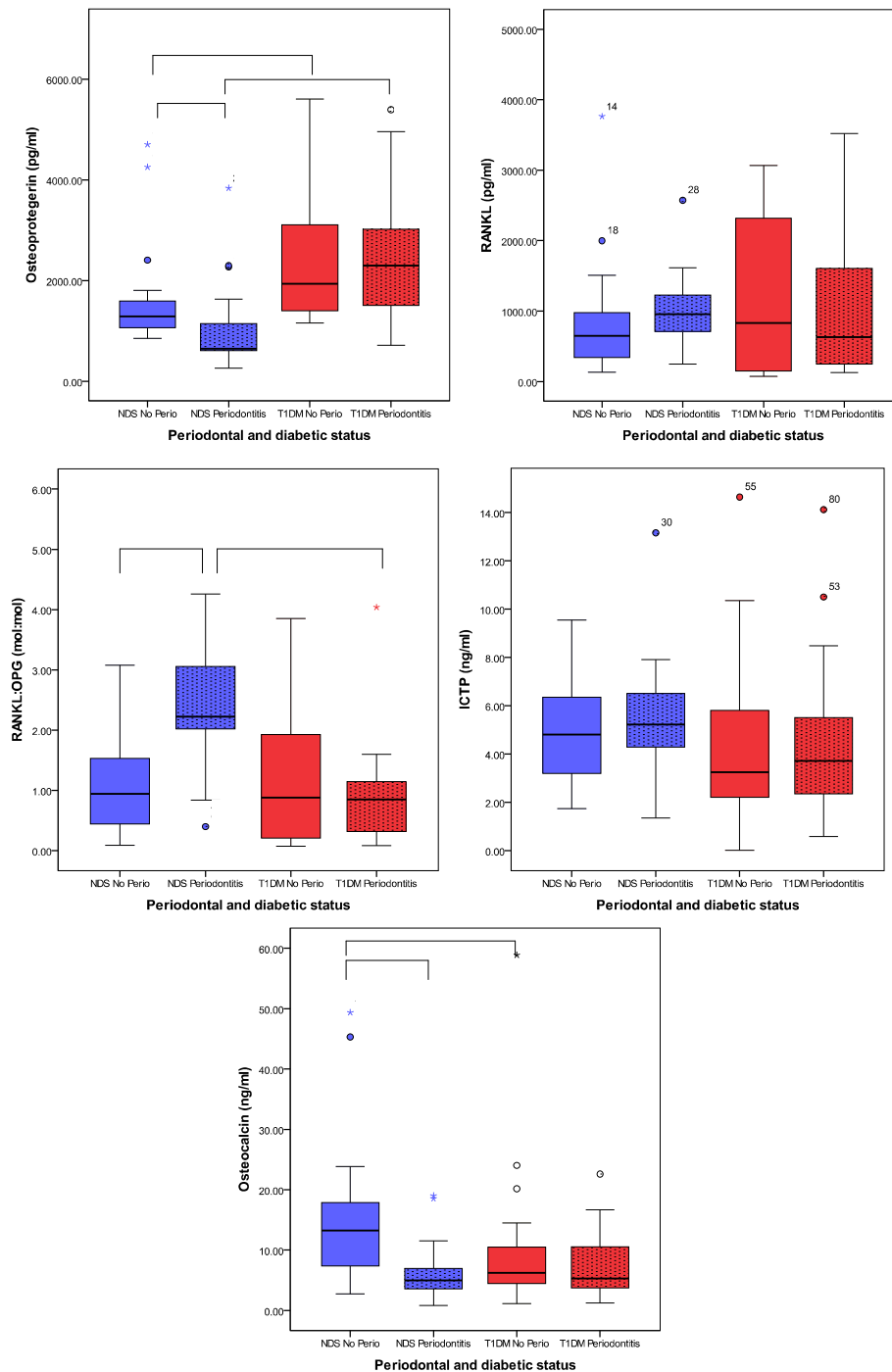


Figure 4-2 RANKL, OPG, Osteocalcin and ICTP in Type 1 diabetes mellitus and periodontitis

Box plot with quartiles, showing outliers (circles) and extreme values (asterisks), represents the plasma concentration of osteoprotegerin (OPG), RANKL, RANKL:OPG ratio, ICTP and osteocalcin in 34 patients without type 1 diabetes mellitus (non-diabetic) and 50 patients with diabetes each split into two groups based on the presence of periodontitis. Seventeen of the non-diabetics had periodontitis and 17 did not and 26 diabetics had periodontitis and 24 did not. ICTP, C terminal telopeptide of type 1 collagen; RANKL, receptor activator of nuclear factor- κ B ligand

4.4 Discussion

There are several mechanisms that might explain the greater incidence of periodontitis in diabetes: a greater susceptibility to infection as a result of diminished neutrophil function; the formation of advanced glycation end products which increase oxidative stress in the tissues; and the binding of advanced glycation end products to cell surface receptors which stimulates the increased production of inflammatory cytokines and causes delayed wound healing. These mechanisms would induce periodontal destruction through osteoclastogenesis. When this study was begun it was thought that, based on a hyper-inflammatory model of periodontal destruction in T1DM, there would be higher levels of RANKL leading to increased bone destruction and a relative reduction in OPG the decoy receptor. It has been shown by Salvi and co-workers that human GCF from type 1 diabetic patients with periodontal disease has higher levels of both PGE2 and IL-1 as compared with GCF from matched non-diabetic patients (Salvi et al., 1997b). Furthermore, monocytes isolated from periodontal patients with type 1 diabetes produce significantly greater amounts of TNF- α , IL-1 β , and PGE2 in response to lipopolysaccharide (LPS) compared with non-diabetic patients (Salvi et al., 1997a,b). It was therefore hypothesised that dysregulation of pro-inflammatory cytokines such as IL-1, IL-6 and TNF α due to prolonged TNF α expression would lead to an increased propensity to inflammatory bone loss (Naguib et al., 2004).

4.4.1 RANKL and Osteoprotegerin

The results of the first studies into RANKL and OPG and their relative ratios were unexpected. Activation of the transcription factor nuclear factor-kB following ligation of RANKL to the cell surface receptor RANK is an event that promotes osteoclast formation (Hofbauer et al., 2000) and also induces the production of a cysteine proteinase and cathepsin K, in osteoclasts. Cathepsin K is involved in bone matrix solubilisation (Crotti et al., 2003). OPG is a member of the TNF receptor family that is expressed by osteoblasts and other cell types, notably insulin secreting β cells of the pancreas (Schrader et al., 2007). OPG inhibits osteoclast formation by high affinity binding to RANKL and prevents RANKL from coupling with the RANK receptor (Hofbauer et al., 2000). This tends to result in reduced bone resorption. In periodontitis, increased concentrations of RANKL are found in diseased tissues, and

the disruption to the balance between RANKL and OPG concentrations is associated with disease severity (Liu et al., 2003; Crotti et al., 2003; Garlet et al., 2006). Numerous studies have shown lower serum and salivary concentrations of OPG and lower expression of OPG in periodontitis and peri-implantitis patients compared with healthy individuals (Belibasakis et al., 2007; Bostanci et al., 2007; Lappin et al., 2007; Budunelli et al., 2008; Akiran et al., 2011). The results of the present study confirmed that higher OPG concentrations are detected in diabetic patients, and indicated that there is a positive correlation between OPG and HbA1c%. The extremely high levels of OPG in the serum meant that this decoy receptor would tend to protect the periodontium by reducing bone loss. An examination of the literature revealed that high levels of OPG had already been reported in patients with type 1 diabetes in the context of other diseases (Kim et al., 2005). High plasma levels of OPG are also linked to endothelial cell dysfunction (Secchiero et al., 2006) and the early onset of diabetes mellitus and have implications in the higher susceptibility of diabetic patients to coronary artery disease (Anand et al., 2007; Ishiyama et al., 2009) and myocardial infarction (Avignon et al., 2005, 2007). Hyperglycaemia does not appear to be directly responsible for elevated plasma OPG in diabetic patients (Knudsen et al., 2007). However, our results suggest a strong relationship between HbA1c% and increased OPG concentrations, acting independently of other factors such as age, gender or periodontitis. A question that has yet to be resolved is whether high levels of OPG are directly involved in the pathogenic process or are indicative of the process taking place. The pathogenesis of type 1 diabetes mellitus is caused by immune mediated pancreatic β cell destruction. Schrader et al. (2007) indicated that cytokine induced OPG production may protect pancreatic b cells from further damage. This may be partially mediated through inhibition of p38 mitogen-activated protein (MAP) kinase phosphorylation. The high levels of OPG that we detected were therefore not a novel finding in themselves although no one had reported RANKL, OPG and RANKL: OPG ratios in diabetic patients with and without periodontitis. The fact that the group with the highest levels of OPG, an osteoprotective molecule, was found in the worst controlled group with the most periodontal disease was counter-intuitive.

4.4.2 ICTP and Osteocalcin

We decided to investigate the bone turnover in diabetic patients using two different molecules. Rather than assessing this just at the level of the regulator, we examined

the effect of the smoking gun, ICTP, a molecule only released during bone destruction and of osteocalcin, a molecule that is associated with bone remodelling in general and new bone formation in particular. By examining bone turnover both at the level of upstream activation and maturation of osteoclasts and osteoblasts, as well as the effect of this in real terms, we were able to see whether there was a relationship between the RANKL:OPG ratio and the apparent increase in periodontal bone loss in the group that should theoretically be protected.

The first question to answer was whether ICTP levels were reduced by OPG as per the current theory. ICTP, a specific biochemical marker of bone resorption, was measured (Bacovsky et al., 2002). It was shown that there was a decrease in ICTP when the RANKL: OPG ratio was reduced. This confirmed that the high levels of OPG are having dampening down the bone destructive process. However, this did not explain the increase in periodontitis levels in this group. Although lower levels of ICTP were observed in the plasma of the diabetic patients there was no statistically significant difference in ICTP levels between non-diabetic and diabetic patients. Increased levels of this bone marker have been observed in the GCF of periodontitis patients (Giannobile et al., 1999; Giannobile et al., 2003). Our results would tend to agree with these findings, since higher median plasma concentrations of bone markers were observed in the groups with periodontitis, but the differences failed to reach statistical significance. This failure to see a statistically significant difference may be due to measuring ICTP in blood rather than GCF or to the lack of sufficient statistical power for determining differences in this particular marker. The lower levels of ICTP that were observed possibly reflect the influence of OPG at reducing bone resorption. If the problem was not excessive bone loss it could be a reduction in new bone formation or a reduced regenerative capacity.

Serum osteocalcin is presently considered to be a valid marker of bone turnover when resorption and formation are coupled, and a specific marker of bone formation when formation and resorption are uncoupled (Giannobile et al., 2003). It has been shown that osteocalcin increases during exercise as the bone remodels to cope with the additional strain as well as during periods of bone growth and healing after fracture (Ivaska et al., 2007; Banfi et al., 2010). Although diabetes has been established as a risk factor for periodontitis and the osteocalcin levels correlate to periodontal status in non-diabetic patients (Bullon et al., 2005), the osteocalcin concentrations in diabetic patients and their relationship to periodontitis have not been established. In this study

both diabetic and periodontitis patients have lower levels of osteocalcin than non-diabetic patients without periodontitis, suggesting lower bone formation. Reduced osteocalcin levels in the presence of periodontitis have been reported before by other researchers (Bullon et al., 2005; Buduneli et al., 2005, 2007) and the present data show a statistically significant inverse relationship between the extent of periodontitis and the osteocalcin levels. The data also suggest that the failure to adequately control diabetes leads to a reduction in plasma osteocalcin and that the elevated blood glucose acts independently of the presence of other factors such as, age, gender or periodontitis to influence the expression of osteocalcin. Interestingly, in a prospective study in post-menopausal women with periodontitis, low levels of osteocalcin were correlated with improved outcomes of periodontal treatment (Bullon et al., 2007). In addition to this it has also been shown that the cyclooxygenase-2 inhibitor, celecoxib, can increase osteocalcin levels in experimentally induced periodontitis and thereby theoretically reduce alveolar bone loss. This finding would tend to indicate that there may be an uncoupling of the remodelling process and a reduction in the capacity of bone to regenerate during and after periods of inflammatory bone loss. This finding was confirmed in a number of studies that have shown a reduced bone density and capacity for bone formation in T1DM (Khazai et al., 2009; Masse et al., 2010).

4.4.3 Reduced bone regeneration

The mechanism of action could be due to alterations in the number, structure or function of osteoblasts in T1DM. A number of studies in the rat model have concluded that a reduced capacity for bone formation is a contributory factor in the development of periodontitis. Following inoculation of bacteria, diabetes was shown to suppress the amount of reparative bone formation that occurs after *P. gingivalis*-induced bone resorption (Graves, 2006). In a mouse calvarial model, treatment of diabetic mice with a caspase-inhibitor, that blocked apoptosis, significantly improved the formation of new bone in diabetic mice following inoculation of *P. gingivalis* (Al-Mashat et al., 2006). The same principle has also been shown in a periodontal model of alveolar bone loss. Alveolar bone loss was induced by placement of ligatures in the rat, and a cycle of bone loss and subsequent bone formation was examined. Osseous repair following induction of periodontal bone resorption was significantly limited in diabetic mice being less than half of that of the non-diabetic mice (Liu et al., 2006). Bone repair in diabetes is characterized by decreased expression of genes

that induce osteoblast differentiation and result in healing (Bouillon et al., 1991; Kawaguchi et al., 1994; He et al., 2004). Periodontal fibroblasts derived from diabetic patients have altered alkaline phosphatase activity and a reduced capacity to form mineralised tissue (Hobbs et al., 1999). It was also shown by Santana et al. (2003) that AGEs could have a detrimental effect on osseous healing in diabetic patients. An increase in apoptosis of bone lining cells, the precursors of osteoblasts, has been suggested to be the mechanism. Apoptosis has been implicated in the pathogenesis of a number of diabetic complications including neuropathy, cardiovascular disease and nephropathy; (Frustaci et al., 2000; Srinivasan et al., 2000; Susztak et al., 2006).

There is evidence that diabetes suppresses osteoblastogenesis after bacterial challenge and the net bone loss could be due to reduced bone formation because of apoptosis of bone lining cells (He et al., 2004). There is also evidence that AGE products are able to induce apoptosis of osteoblasts in diabetic patients via the MAP kinase and cytosolic apoptotic pathway (Alikhani et al., 2007a,b). The reduced plasma osteocalcin concentrations in the diabetic patients in the present study could be explained by a reduction in the number of osteocalcin secreting osteoblasts. Apoptosis of matrix-producing cells may be a critical factor in the repair of soft and hard connective tissue and may represent an important mechanism through which diabetes has a negative effect on the periodontium (He et al., 2004; Liu et al., 2006; Graves et al., 2006). The findings of our study add weight to the theory that diabetes associated periodontal destruction may not be solely a destructive process, but may also be related to a reduced capacity for healing subsequent to bacterially induced bone loss, as the process is uncoupled due to apoptosis of bone lining cells. This the first study to measure these four markers of bone turnover in diabetic patients with periodontitis.

4.4.4 Periodontal parameters

In this study in order to avoid possible confounding between the periodontitis and the non-periodontitis groups we defined the level of disease before the analysis, i.e. a diagnosis of periodontitis was confirmed by a minimum of two sites with ≥ 5 mm CAL and CPD. Therefore 14 patients with only one site of ≥ 5 mm CAL and/or CPD were excluded. Statistically significant differences were not observed in RANKL concentrations, but in contrast, statistically significant differences in OPG and osteocalcin and RANKL:OPG ratios were observed.

The finding that the level of BOP was greater in the diabetic patients could also be partly explained by a dysfunction of the endothelial cells in the gingival blood vessel walls. Further studies are required to confirm this. We expected to see a significantly higher level of BOP in the periodontitis groups compared with those without periodontitis but in the non-diabetic patients this was not the case. This may be due to the poor level of oral hygiene seen in the general population in the United Kingdom (Morris et al., 2001). Although the number of teeth and sites with ≥ 5 mm CPD were similar between the diabetic and non-diabetic periodontitis groups the diabetic periodontitis group had more teeth and sites affected by $CAL \geq 5$ mm. These differences were accounted for by a greater prevalence of gingival recession in the diabetic patients with periodontitis.

4.5 Conclusion

In conclusion, diabetic patients do not appear to be more susceptible to bone destruction, but may have a defect in bone formation. The reduced osteocalcin levels in type 1 diabetic patients suggests that these patients have a reduction in their intrinsic ability to replace bone, such as has been destroyed during “acute bursts” of periodontitis. This may make them more susceptible to progression of this disease. In support of this, other researchers have not indicated a significant change in bone density in diabetic patients but have indicated that bone formation may be lower in diabetic patients (Bridges et al., 2005, Oz et al., 2006). Future studies in type 1 diabetic patients should investigate markers of bone metabolism in combination with bone density measurements and pancreatic β cell destruction.

5 Caries and oral mucosal abnormalities in Type 1 diabetes mellitus

5.1 Introduction

It has been suggested that diabetes has a wide range of effects on oral health beyond that of periodontal disease. There are reports indicating that patients suffering from T1DM could be more prone to dental infections and dental caries, have higher carriage rates of fungal and bacterial pathogens, a higher prevalence of fungal infections and lower salivary flow rates (Ship, 2003). It has also been reported that T1DM patients suffer from burning mouth syndrome (BMS) (Moore et al., 2007) and alterations in the oral mucosa due to autoimmune conditions such as lichen planus and other mucosal abnormalities such as leukoplakias. Reactive fibrous growths have also been reported to be more common in diabetes mellitus patients (Albrecht et al., 1992, Petrou-Amerikanou et al., 1998; Moore et al., 2000; Ujpal et al., 2004). It has also been reported that poorly controlled diabetes and smoking can lead to an increased risk of oral leukoplakias and other precancerous lesions. (Dikshit et al., 2006; Meisel et al., 2010).

The key factors involved in these disease processes are the patients' increased susceptibility to infection due to defects in the innate and adaptive immune system, a common autoimmune pathogenesis, poor healing capacity, ultrastructural changes due to AGE deposition in the mucosa and salivary glands or neuropathy affecting the autonomic nervous system and peripheral nerves.

There have been a number of studies investigating the prevalence and incidence of dental caries in T1DM with varying results (Akjuz et al., 1990; Taveres et al., 1991; Karjalainen et al., 1997; Twetman et al., 2005). Diabetic patients are advised to eat a reduced sugar diet which, taken in isolation, would be expected to result in a reduced caries risk. This however has not been a universal finding, implicating other factors in the development of caries in this population. Biological plausibility has been hypothesised to explain both the studies finding a reduced and increased caries prevalence. Factors supporting the possibility of a reduced caries risk are mainly related to the dietary restrictions previously prescribed; that is a diet essentially free of

processed sugars. However, more recently such prescriptive and restrictive regimes have fallen from favour to be replaced with advice on a healthy balanced diet. Factors favouring an increased caries risk include: increased glucose concentration in saliva and GCF, providing substrate for the plaque bacteria, a reduced salivary flow rate, resulting in reduced buffering and remineralisation; and increased frequency of food consumption throughout the day. Studies have also shown a tendency to increased plaque scores in diabetic patients compared with controls, which is also a risk factor for the development of dental caries (Albrecht et al., 1988; Novaes et al., 1991; Bridges et al., 1996; Firatli et al., 1996; Lalla et al., 2006a; Kaur et al., 2009).

The aim of this part of the study is to answer the following questions:

1. Is the prevalence and extent of dental caries increased in T1DM and with worsening glycaemic control?
2. Is the prevalence of oral mucosal abnormalities increased in T1DM and with worsening glycaemic control?
3. Are there differences in the oral mucosal microflora between NDS and T1DM?

5.2 Materials and methods

5.2.1 Examination of the dental hard tissues

Training and calibration of the single blinded examiner was carried out prior to the examinations by an experienced trained caries examiner from the Community Dental Services.

This was a cross-sectional study of 204 adults aged 20-55 years with T1DM and 110 NDS. Details of recruitment and the inclusion and exclusion criteria were described in chapter 2.

Caries was assessed by visual inspection of the teeth in a dental chair under a dental light. A CPITN probe was used only to remove debris and confirm cavitation. The teeth were examined wet and obvious caries was recorded for each surface. The tooth was split into 6 sections namely mesial, occlusal, distal, buccal, lingual and root surfaces and a score was recorded for each. Caries was assessed at the level of the dentine. Frank cavitation was recorded as caries and un-cavitated lesions were only

recorded where the examiner was sure that dentine caries was present as shown by clear shadowing in the dentine and decalcification of the overlying enamel. Where there was any doubt the principle was to score the tooth surface as non-carious. The DMFT and DMFS were calculated. Within this index; F was used for the filled teeth or surfaces; D denoted the number of untreated carious teeth or surfaces without regard to whether the lesion was in enamel or involved the root; and all teeth that were not present for any reason were treated as M for missing. The care index (more correctly called the restorative index) was calculated by dividing the number of filled teeth by the total DMFT. This gives an indication of the amount of dental care that the individual has received. The total number of individuals who had more than 18 sound and untreated teeth was also calculated as described in the ADH survey (Nunn et al., 2001).

5.2.2 Examination of the oral mucosa

Prior to the study commencing, the examiner was trained in the diagnosis of oral mucosal disorders by a consultant at Glasgow Dental Hospital and School. Patients were asked whether they had experienced any altered taste in the last twelve months and if they had, whether they had experienced a metallic taste or an unpleasant taste. Patients were also asked whether they had experienced any pain in the preceding twelve months. Where patients answered “yes” to this question and described the pain as burning or tingling these patients were tentatively categorised as having oral dysaesthesia or burning mouth syndrome. Any mucosal abnormalities were recorded and where there was doubt as to the diagnosis, or where further treatment was required, the patient was referred to a consultant in oral medicine.

5.2.3 Subjective assessment of xerostomia

Patients were asked a series of questions relating to oral dryness known as the Fox’s xerostomia index (Fox, 1987):

1. Does your mouth feel dry when eating a meal?
2. Do you find it difficult to swallow dry foods?
3. Do you sip liquids to help you swallow?
4. Do you wake during the night with a dry mouth?
5. Do you have too much saliva, too little or are you not aware of saliva.

5.2.4 Saliva collection

Saliva was collected according to a modification of the method described by Navazesh (1982). The method is described below.

The patient was instructed to refrain from intake of any food or beverage (water exempted) one hour before the collection period. Smoking, chewing gum and intake of coffee were prohibited during this hour.

The patient was then informed that the purpose of the investigation was to measure the salivary flow at rest. The patient was instructed to minimise all facial movements particularly movements of the mouth.

1. To begin saliva collection the patient was asked to void the mouth of saliva by swallowing.
2. The patient was asked to lean slightly forward over the tube and funnel. This was demonstrated to the patient.
3. The patient was instructed to keep his/her mouth slightly open and to allow saliva to drain into the funnel. The patient was also instructed to keep his/her eyes open.
4. At the end of the 5 minute collection period, the patient was asked to collect any remaining saliva in his/her mouth and expectorate into the funnel and test tube.
5. The saliva volume was measured using micropipettes (Finnipipette, Lab systems).

All samples were collected between 8:30 and 11:30 to avoid diurnal variation.

5.2.5 Microbiological sampling

The oral microflora was assessed using the oral rinse method (Samaranayake et al., 1986, Smith et al., 2003). The patient was asked to rinse their mouth for 30 seconds with 9ml of sterile phosphate buffered solution (PBS) pH 7.2; 0.1M. The clinical pharmacy department at the Western Infirmary took responsibility for the preparation and quality control of the sterile oral rinse solution (Western Infirmary, NHS Greater Glasgow and Clyde).

Samples were transferred to the oral microbiology laboratory (Diagnostic services, Oral microbiology department, Glasgow Dental Hospital and School, NHS Greater Glasgow and Clyde) for immediate processing.

5.2.6 Sample preparation and culture technique

Samples were treated according to the laboratories standard operating procedures. Briefly the samples were processed in the following way.

The oral rinses were centrifuged at 1717 xg for 10 minutes and the supernatant was discarded. The pellet was reconstituted with 1ml sterile PBS and diluted to 10^{-2} and 10^{-3} . 50 μ L of the dilutions was spiral plated (Spiral systems inc. Cincinnati, U.S.A.) onto Columbia blood agar (Oxoid, Basingstoke, U.K.). The neat reconstituted solution was plated onto the following selective agars: Mannitol salt agar, Sabouraud dextrose agar (Oxoid, Basingstoke, U.K.) and Pagano-Levin agar. The Pagano-Levin was prepared in house according to the following standard operating procedure: 10g neutral bacteriological peptone (Unipath Ltd. Basingstoke, U.K.), 1g yeast extract (Unipath Ltd. Basingstoke, U.K.), 40g dextrose (BDH, Poole U.K.), 15g technical grade agar (Unipath Ltd. Basingstoke, U.K.) was dissolved in distilled water with frequent mixing and autoclaved for 15mins at 115 $^{\circ}$ C. The solution was cooled to 50 $^{\circ}$ C and 10ml Triphenyltetrazoliumchloride (TTC) and 5ml of gentamycin was added (1ml and 0.5ml respectively/100ml).

The plates were incubated at 37 $^{\circ}$ C and read at 48 hours.

5.2.6.1 Strain Identification and sensitivity testing: Staphylococci:

1. Colonies that were suspected to be *Staphylococci* species, based on colony colour and morphology, were sub-cultured, purified and Gram stained.
2. An agglutination test was performed on all staphylococcal species using the Staphaurex kit (Remel Europe Ltd, Kent, U.K.) and a selective chromogenic agar (SAID bioMe´rieux SA, Marcy l’Etoile, France). Staphaurex and SAID positive strains were identified as *S. aureus* and antibiotic sensitivity testing was performed.
3. *Staphylococcus aureus* strains were tested for resistance using the Clinical Laboratory Standards Institute (C.L.S.I.) disc diffusion method for the

following antibiotics: clarithromycin, clindamycin, fucidin, gentamycin, oxacillin, mupiricin (high and low level resistance), rifampicin, tetracycline, trimethoprim, vancomycin and neomycin (Oxoid, Basingstoke, U.K.). Zone diameters were used to determine cut off points for sensitivity using standard laboratory controls (CLSI M100-S19 Kirby Bauer Interpretational Zone Diameters (mm)).

4. Those strains that were coagulase negative were further identified using API 32 Staph (bioMérieux SA, Marcy l'Etoile, France).
5. All results were reported as cfu/ml.

5.2.6.2 Strain Identification and sensitivity testing: Fungi

1. Positive fungal growth was firstly subjected to the germ tube test. 5ml of dilute horse serum was placed in a bijoux and a small amount of one yeast colony was emulsified into the serum and incubated for 4 hours at 37⁰C. One drop was placed on a slide and examined microscopically for germ tube production at x10 and x40 objective. Germ tube positive strains were tentatively identified as *C albicans*. Identity was confirmed using the API 20C (bioMérieux SA, Marcy l'Etoile, France).
2. Antifungal sensitivity testing was carried out using the C.L.S.I. disc diffusion method for nystatin and miconazole (Oxoid, Basingstoke, U.K.). E tests were used to determine the sensitivity pattern to amphotericin, fluconazole and itraconazole (bioMérieux SA, Marcy l'Etoile, France). A 2 MacFarland turbidity specimen was prepared in sterile saline and the entire surface of the Casitone agar was swabbed evenly with the suspension. The surface was allowed to dry for 10 to 15 minutes before the E-test strip was placed onto the surface with forceps. The plates were incubated for 24-48 hours at 35⁰C depending on the candidal species being tested. When the growth became distinctly visible the MIC value at the point of the intersection between the zone edge and the E-test strip was reported as the minimum inhibitory concentration.
3. Results were reported as either sensitive or resistant. Strains were sensitive to fluconazole if the MIC was $\leq 8\mu\text{g/ml}$, sensitive dependent on the dosing regime

(S-DD) 16-32 μ g/ml and resistant above 64 μ g/ml. Strains were sensitive to itraconazole if the MIC was \leq 0.125 μ g/ml, S-DD 0.25-0.5 μ g/ml and resistant above 1 μ g/ml. Where the level of susceptibility was intermediate the minimum inhibitory concentrations were expressed in μ g/ml. Nystatin and miconazole were reported based on the diameter of a zone of clearing according to laboratory standards (CLSI M44-A, Kirby Bauer Interpretational Zone Diameters (mm)).

5.2.6.3 Strain Identification and sensitivity testing: Coliforms

1. Cultures that were suspected to be coliforms, based on colony morphology and appearance, were sub-cultured overnight, purified and Gram stained. In addition an oxidase test was performed using Pyo-Test@MW990 (Medical Wire & Equipment Co (Bath) Ltd, Wiltshire, England).
2. Oxidase positive gram negative rods were further identified using the API20E (bioMe´rieux SA, Marcy l’Etoile, France).
3. Oxidase negative gram negative rods were further identified using the API20NE (bioMe´rieux SA, Marcy l’Etoile, France).
4. Sensitivity testing was carried out for all coliforms using the CLSI disc diffusion method for the following antibiotics: cefuroxime, cefpodoxime, ciprofloxacin, ceftriaxone, gentamycin, tazosin and trimethoprim (Oxoid, Basingstoke, U.K.). Zone diameters were used to determine cut points for sensitivity using standard laboratory controls (CLSI M100-S19 Kirby Bauer Interpretational Zone Diameters (mm)).
5. Stains were also tested for the presence of extended spectrum beta-lactamase (ESBL).

All cultures were reported as cfu/ml using the oral rinse plate method. All reports were checked and signed off by a consultant in oral microbiology.

5.2.7 Data handling

Please see chapter 2 for details of data handling.

5.2.8 Statistical analysis

The differences between the distributions of the caries outcomes, the salivary flow rate and the density of colonisation across the three groups were calculated using the

Kruskal Wallis test, followed by the Mann Whitney U test with a correction for multiple comparisons. The data were not normally distributed. Therefore the data were summarised using medians and interquartile ranges. The categorical data including the proportion of cases: with specific oral mucosal abnormalities; complaining of xerostomia, carrying *C albicans*, *Staphylococci* or *Coliforms*; or having reduced salivary flow were tested using the chi squared or Fisher's exact test (< 5 cases). The significance level was set at 0.01 due to the number of comparisons. All statistical tests were carried out using SPSS18 (SPSS inc).

5.3 Results

5.3.1 Dental caries

The calibration exercises performed prior to the study revealed that the inter-examiner reproducibility was very good to excellent (Table 5-1). The kappa scores for inter-examiner calibration for caries, restorations and missing teeth were 0.83, 1.0 and 0.91. The ongoing calibration throughout the study confirmed that intra-examiner calibration was excellent with kappa scores of 0.98, 0.98 and 1.0 for caries, restorations and missing teeth.

Table 5-1 Results of calibration exercise.

		Caries scale¹	Caries free/caries present²	Filled³	Missing⁴
Inter-examiner Calibration	K	0.75	0.83	1	0.91
	Perfect agreement	91.8%	93.9%	100%	99.2%
	Within 1 unit	97.4%	-	-	-
	Mean bias	0.13	0.006	0.000	0.007
Intra-examiner Calibration	Measurement error	0.54	0.247	0.000	0.085
	K	0.96	0.98	0.98	1.0
	Perfect agreement	99.7%	98.9%	99.5%	100%
	Within 1	99.8%	-	-	-
	Mean bias	0.00	0.00	0.00	0.00

Perfect agreement

Within 1: agreement between replicates to within 1 unit on measurement scale

Mean bias: mean of differences between replicates

Measurement error: SD of differences between replicates

1 Agreement across the scale of caries rating 0-4

2 Agreement in dichotomous score caries free/carious surface

3 Agreement between filled and non-filled surfaces

4 Agreement between missing and non-missing teeth

The results are presented in Table 5-2 and in the following figures. There were no differences in median (interquartile range) DMFT between NDS, WCD and PCD (NDS, 16 (16-20.5), WCD 15 (10-20), PCD, 15 (10-21) $p= 0.9$) (Figure 5-1). There was also no difference in the DMFS scores between the three groups (NDS 54 (34.5-73), WCD 49.5 (35-72.5), PCD 52 (33-80) $p= 0.967$) (Figure 5-2). All three groups had the same median number of teeth missing (4 $p =0.655$) (Figure 5-3). The number of filled teeth and filled surfaces was slightly higher in the NDS. The median number of filled surfaces for the PCD group was 2.5 higher than the WCD (filled surfaces NDS 21(10-38), WCD 17.5 (8.5-34.5), PCD 20 (7-37)). The PCD group had the lowest number of filled teeth with 8 restored teeth compared with 8.5 (WCD) and 9 (NDS) ($p = 0.763$, $p = 0.178$) (Figure 5-4).

The number of decayed surfaces was highest in the PCD group (median, NDS 2, WCD 2, PCD 3 $p= 0.088$). The number of decayed teeth was also slightly higher in PCD compared with NDS (Median NDS 1, WCD 1.5, PCD 2 ($p=0.075$)) (Figure 5-5). The care index was lowest in the PCD patients and highest in the NDS (median NDS 0.59 (0.39-0.71), WCD 0.52(0.36-0.62), PCD 0.52(0.31-0.64) $p=0.03$) (Figure 5-6).

The number of patients with one or more decayed teeth was very high in all three groups and was slightly higher in the PCD group compared with WCD and NDS (NDS 68.8%, WCD 70.6%, PCD 78.8%) (Figure 5-7). There were no statistically significant differences in any caries related parameters between the three patient groups. The number of subjects with more than 18 sound and unfilled teeth was similar across the three groups (WCD 45%, WCD 47.1%, PCD 47.6% $p= 0.909$) (Figure 5-8).

Table 5-2 Dental caries and diabetic status.

Diabetic status	NDS (110)		WCD (34)		PCD (170)		P value
	Median (IQR)	min-max	Median (IQR)	min-max	Median (IQR)	min-max	
DMFT	16 (10-20.5)	1-32	15 (10-20)	2-23	15 (10-21)	0-32	0.9
DMFS	54 (34.5-73)	2-167	49.5 (35-72.5)	4-151	52 (33-80)	0-150	0.967
No. of decayed teeth	1 (0-3)	0-15	1.5 (0- 3.5)	0-7	2 (1-4)	0-14	0.075
Missing teeth	4(3-6)	0-17	4(3-6)	0-16	4(3-6)	0-16	0.655
No. of filled teeth	9 (6-14)	0-22	8.5 (4-12)	0-19	8 (4-12)	0-21	0.178
No. of decayed surfaces	2 (0-5)	0-33	2 (0-7.25)	0-21	3 (1-6)	0-30	0.088
No. of Filled surfaces	21 (10-38)	0-97	17.5 (8.5-34.5)	0-88	20 (7-37)	0-93	0.763
Care index	0.59 (0.39-0.71)	0-1	0.52 (0.36-0.62)	0-1	0.52 (0.31-0.64)	0-1	0.03*
Untreated decay N,(%)	75 (68.8%)		24 (70.6%)		131 (78.8%)		0.211
>18 virgin teeth N,(%)	49 (45%)		16 (47.1%)		80 (47.6%)		0.909

Kruskal Wallis test * Statistically significant p<0.05

Chi squared test †

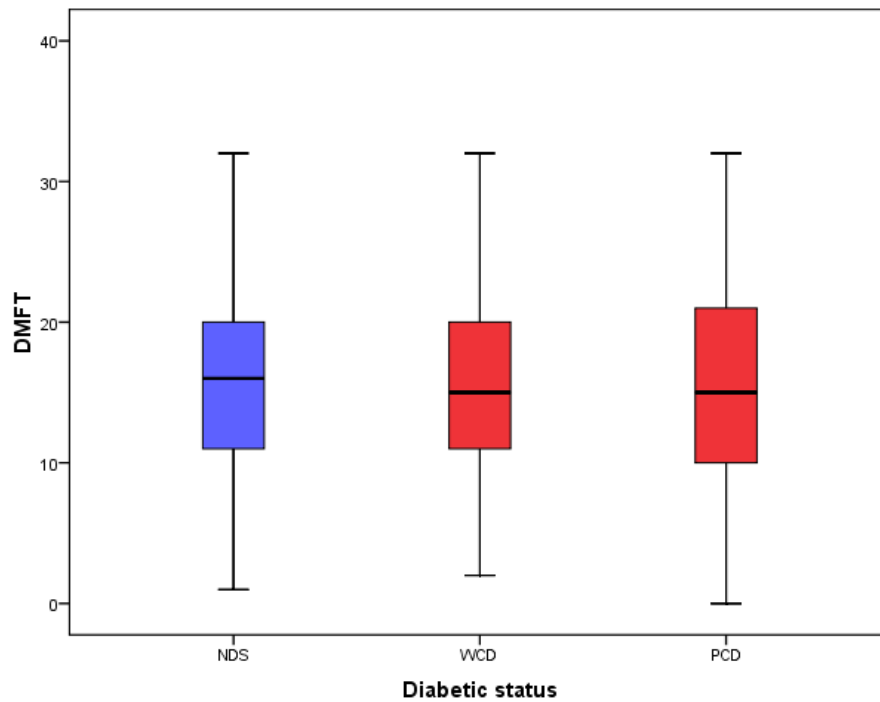


Figure 5-1 Box plot showing median and interquartile range of decayed missing and filled teeth by Diabetic status, WCD HbA1c \leq 7.5%, PCD HbA1c >7.5%

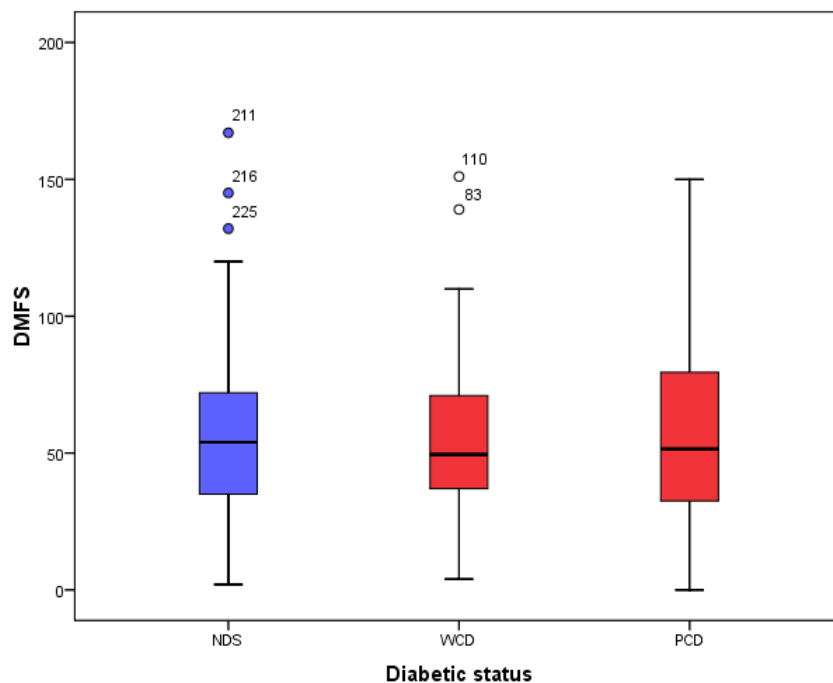


Figure 5-2 Box plot showing median and interquartile range of decayed missing or filled surfaces by Diabetic status, WCD HbA1c \leq 7.5%, PCD HbA1c >7.5%

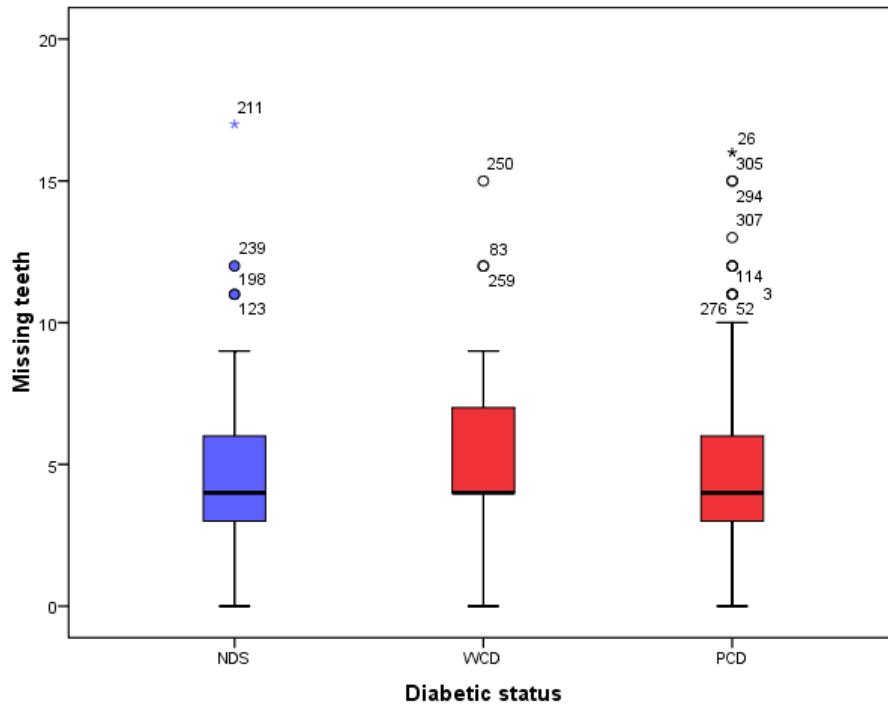


Figure 5-3 Box plot showing median and interquartile range for the number of missing teeth by diabetic status, WCD HbA1c ≤ 7.5%, PCD HbA1c > 7.5%

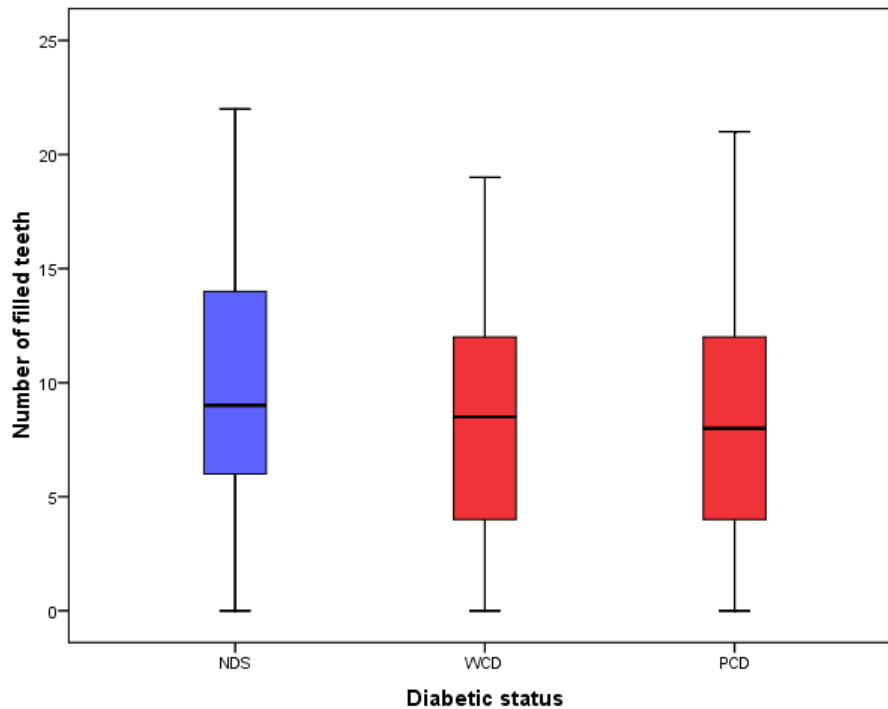


Figure 5-4 Box plot showing median and interquartile range for the number of filled teeth by diabetic status, WCD HbA1c ≤ 7.5%, PCD HbA1c > 7.5%

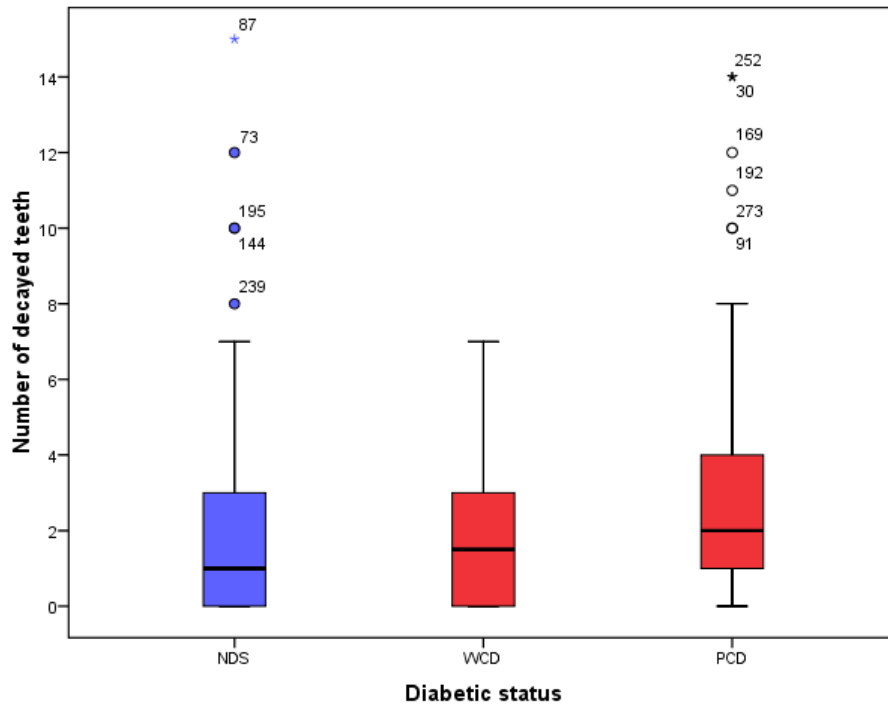


Figure 5-5 Box plot showing median and interquartile range for the number of decayed teeth by diabetic status, WCD HbA1c ≤ 7.5%, PCD HbA1c > 7.5%

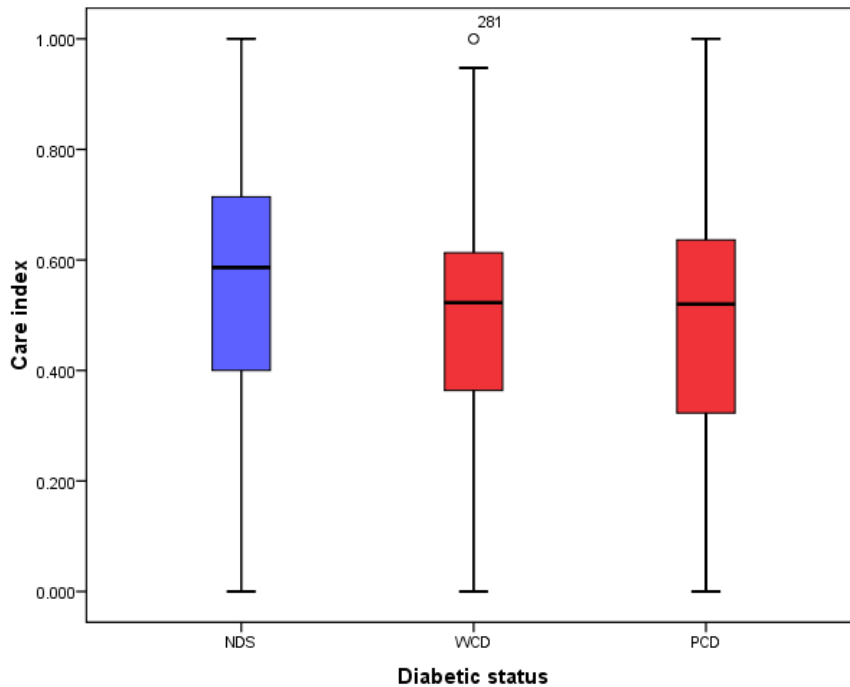


Figure 5-6 Box plot showing median and interquartile range for the number of care index by diabetic status, WCD HbA1c ≤ 7.5%, PCD HbA1c > 7.5%

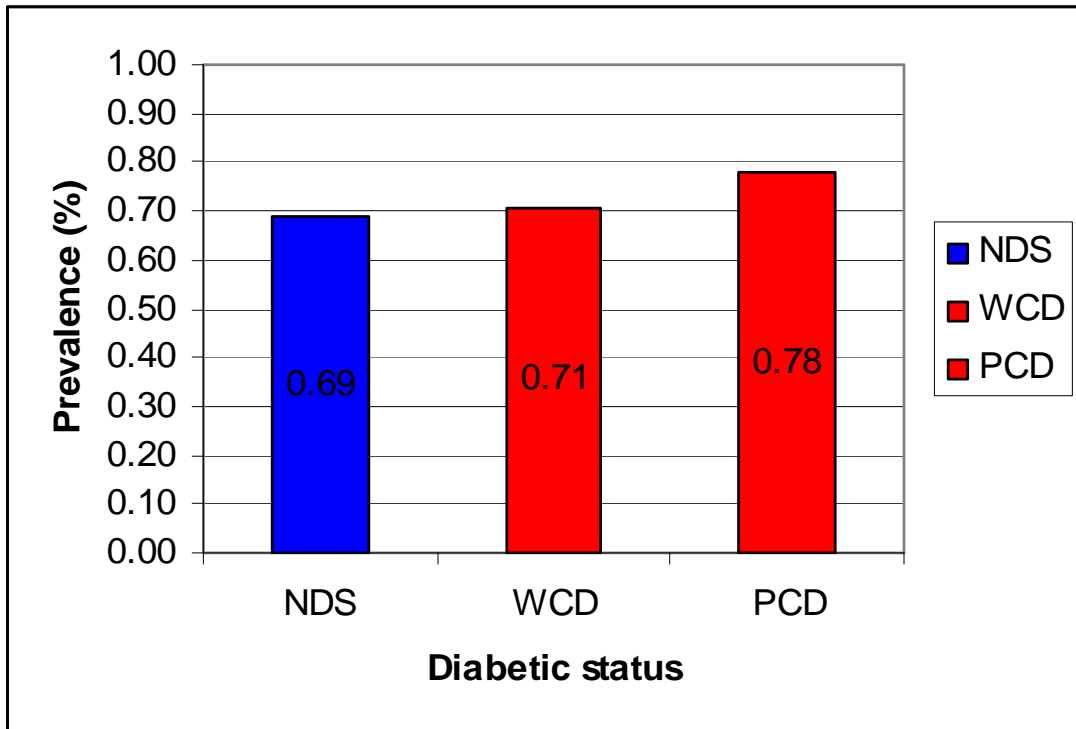


Figure 5-7 Bar chart showing the percentage of subjects in each group who had at least one decayed tooth by diabetic status, WCD HbA1c ≤ 7.5%, PCD HbA1c > 7.5%

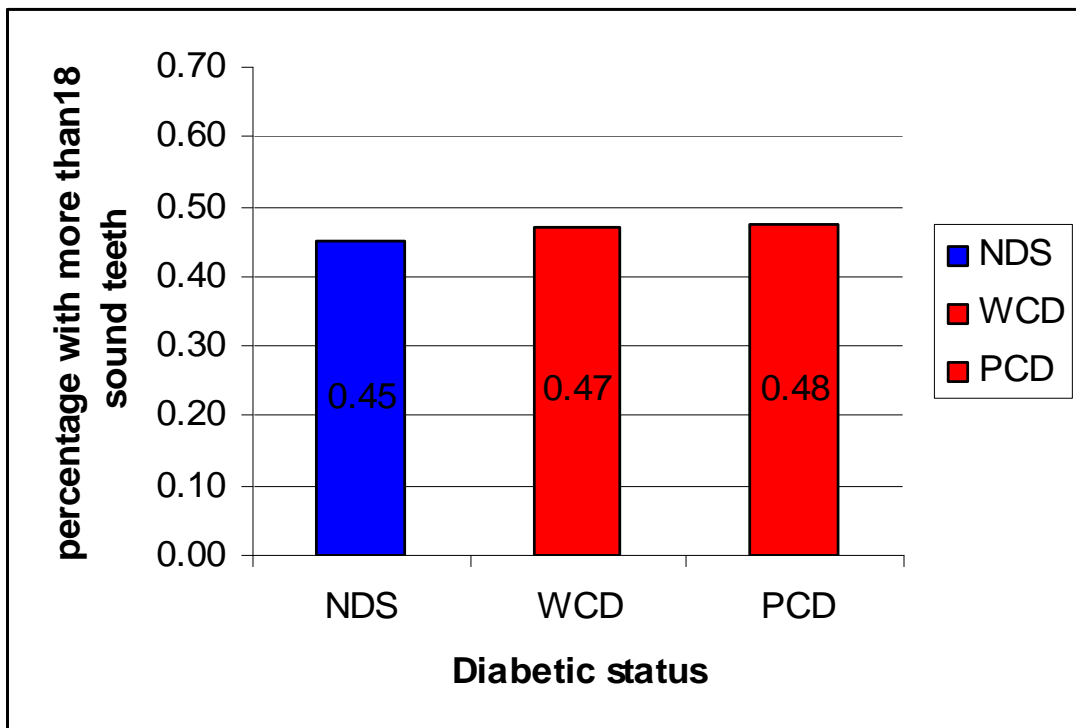


Figure 5-8 Bar chart showing the percentage of patients in each group who had more than 18 sound and untreated teeth

5.3.2 Oral mucosal abnormalities

All lesions are shown in Table 5-3 and in figures 5-9 and 5-10. The lesions most commonly noted were frictional keratosis and lesions of the tongue including fissuring, glossitis and depapillation but there were no differences between the T1DM and NDS groups. There were also no differences in the prevalence of oral candidal lesions, including median rhomboid glossitis, angular cheilitis, and denture stomatitis. The number of patients with oral mucosal lichen planus or lichenoid reaction was three times higher in the diabetic group (NDS 0.8%, T1DM 3% $p=0.168$). There were no other differences between the groups.

Table 5-4 shows the median salivary flow rates, the presence of all oral mucosal abnormalities, the presence of all candidal lesions and the microbiological data for non-diabetic subjects and the diabetic groups. Both T1DM and PCD had lower salivary flow levels over the 5 minute sampling period than NDS (Section 5.3.4). There was no difference in the overall prevalence of oral mucosal lesions across the 3 groups when all lesions were considered (NDS 28.6%, T1DM 31.2%, PCD 28.7%). There were no differences in the prevalence of dry mouth, altered taste, metallic taste or unpleasant taste across any of the groups. There was also no difference in the prevalence of altered sensation such as burning-mouth or tingling across the groups.

Table 5-3 Summary of all mucosal abnormalities detected in non-diabetic subjects and type 1 diabetes mellitus patients

Soft tissue lesions	Diabetic n=205	Non-Diabetic n=112
Frictional keratosis	20	8
Tongue lesions	15	8
fissured tongue	5	3
geographic tongue	3	2
glossitis	3	1
coated tongue	2	1
depapillation of tongue	2	
median rhomboid glossitis		1
Candidal lesions	7	4
denture stomatitis	5	1
angular cheilitis	1	2
median rhomboid glossitis		1
papillary hyperplasia	1	
Abscess	7	4
Lichenoid/lichen planus	6	1
Apthous ulcer	5	3
Desquamative gingivitis	1	1
Petichiae	2	
Florid gingivitis	1	
Gingival hyperplasia	1	1
Herpes labialis	1	
Lymphadenopathy	1	2
Mucocoele	1	
Pigmentation	1	
White/red patch	1	
Epulis		1
Ibroma		1
Pericoronitis		1

Table 5-4 Salivary flow rate, mucosal abnormalities and oral microflora in NDS, T1DM and PCD

	Non-diabetic subjects	N	All type 1 diabetic patients	N	Poorly controlled diabetic patients	N	p value^a	p value^b
N	112		205		172			
Salivary flow mls/five minute sample, median, (IQR), [range]	0.8 (0.4 to 1.5) [0 to 4.2]	110	0.5 (0.25 to 1.0) [0 to 6.5]	202	0.5 (0.25 to 1.0) [0 to 6.5]	169	0.003	0.01
Missing % (n)	1.8 (2)		1.5 (3)		1.8 (2)			
Dry mouth % (n)	7.3 (8)	110	10.4 (21)	201	10.7 (18)	168	0.42	0.45
Missing % (n)	1.8 (2)		2.0 (4)		0.6 (1)			
Altered taste	10.7(12)		8.7 (18)		8.7 (15)		0.68	0.554
Unpleasant taste	9.0(10)		6.9(13)		7.5 (12)		0.49	0.66
Metallic taste	10.9(12)		8.5(16)		9.4 (15)		0.54	0.68
Burning mouth	7.1(8)		3.4 (7)		3.5 (6)		0.21	0.26
Missing % (n)	1.8(2)		9.3 (19)		7.5 (13)			
All soft tissue lesions % (n)	28.6 (32)	112	31.2 (64)	205	28.7 (49)	171	0.7	0.55
<i>Candidal</i> lesions % (n)	2.7 (3)	112	3.4 (7)	205	2.3 (4)	171	0.75	0.99
<i>Candida albicans</i> carriage % (n)	48.5 (54)	112	59.5 (122)	205	58.5 (100)	171	0.11	0.145
Colony forming units median, (IQR), [range]	230 (60 to 725) [20 to 48,000]	54	520 (160 to 1,500) [5 to 24,000]	107	600 (165 to 1,500) [5 to 24,000]	88	0.008	0.006
Missing % (n)	0		9.2 (11)		10.2 (10)			
<i>Staphylococcus aureus</i> carriage % (n)	27.7 (31)	112	33.2 (68)	205	33.3 (57)	171	0.31	0.36
Colony forming units median, (IQR), [range]	640 (120 to 4,200) [16 to 100,000]	31	500 (160 to 1800) [3 to 150,000]	65	660 (200 to 1,975) [20 to 150,000]	54	0.64	0.92
Missing % (n)	0		4.3 (3)		7.1 (3)			

^a type 1 diabetic patients vs non-diabetic subjects ^b poorly controlled patients vs non-diabetic subjects

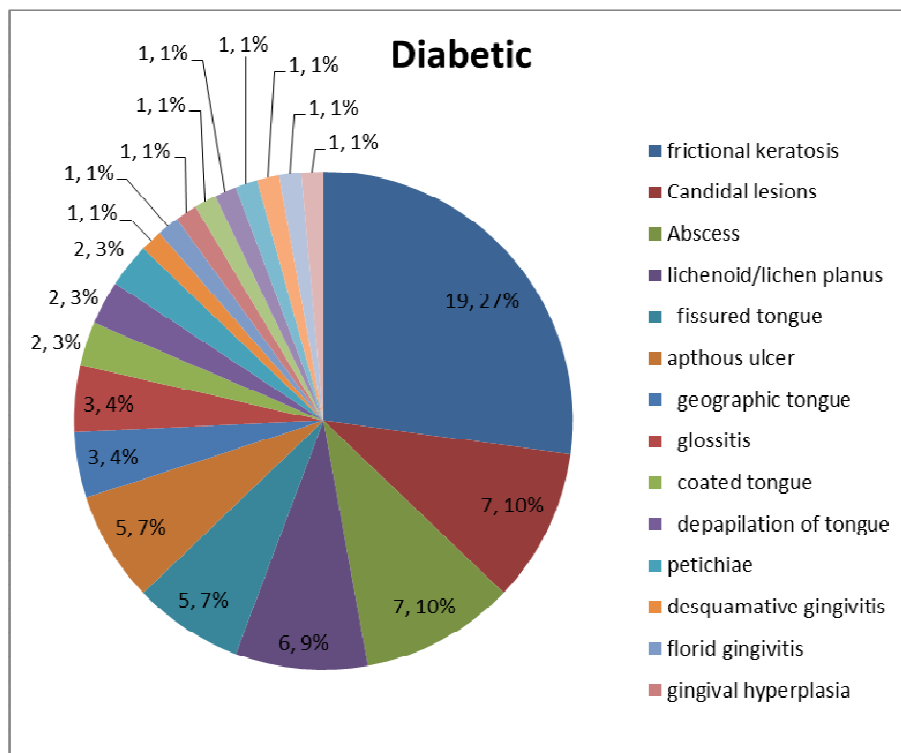


Figure 5-9 Pie chart showing all oral mucosal lesions in patients with T1DM number of lesions, % of lesions

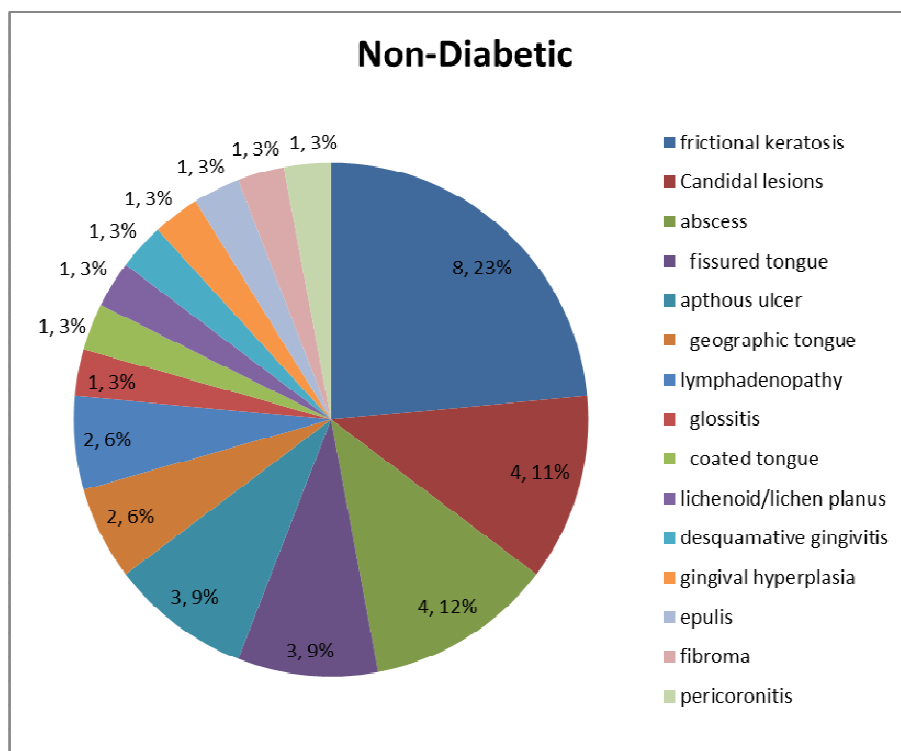


Figure 5-10 Pie chart showing all oral mucosal lesions in the non diabetic subjects number of lesions, %of lesions

5.3.3 Microbiological sampling

There was no difference in the prevalence of *C albicans* carriage across the groups. (NDS: 48.6%, T1DM: 59.5%, PCD: 58.5% p= 0.346, p= 0.142). Where candidal colonisation was detected the growth was heavier in T1DM compared with non-diabetic patients

(NDS:T1DM $p = 0.008$, NDS:PCD $p = 0.006$). There was no difference in the prevalence of *S. aureus* carriage between the groups (NDS: 27.7%, T1DM: 33.2%, WCD: 35%, PCD: 33.3%). There was also no difference in the density of colonisation where carriage was detected (Table 5-4).

All bacteria and fungi recovered and identified are listed in Table 5-5. There was no obvious difference in the species detected between the NDS, T1DM and PCD. Between 4 and 6% of the specimens were positive for coliforms however, there was no increase in the carriage of coliforms in T1DM compared with the NDS group. Antibiotic and anti-fungal resistance was rare. There was one patient who was culture positive for methicillin resistant *S. aureus* (M.R.S.A). There were 3 clinical isolates of *S. aureus* that were reported as resistant to clarithromycin, 2 strains that were resistant to clindamycin, 8 strains that were resistant to fucidin, 4 were resistant to oxacillin, 3 were resistant to tetracycline and one was resistant to neomycin. There was no pattern of staphylococcal antibiotic resistance in T1DM compared with NDS.

Anti-fungal resistance was also rare and there was only one *Candida* clinical isolate that was resistant to amphotericin, three that were resistant to fluconazole and 12 that were resistant to itraconazole. Again the numbers were low and preclude comparisons between the groups.

Table 5-5 All species recovered and identified, in NDS, WCD and NDS.

Species recovered	NDS (111)	T1DM (205)	PCD (172)
<i>C. albicans</i>	48.6 (54)	59.5 (122)	58.5 (100)
<i>C. dubliniensis</i>	1.8 (2)	2 (4)	2.3 (4)
<i>C. guilliermondii</i>	0.9 (1)	2 (4)	2.3 (4)
<i>C. glabrata</i>	1.8 (2)	0.5 (1)	0 (0)
<i>C. tropicalis</i>	0 (0)	0.5 (1)	0 (0)
<i>Saccharomyces cerevisiae</i>	0.9 (1)	0.5 (1)	
<i>S. aureus</i>	28 (31)	33 (69)	33 (57)
<i>Coagulase negative staphylococci</i>	9 (10)	3 (6)	4 (6)
Coliforms	3.6(4)	3.4(7)	3.5 (5)
<i>Enterobacter sakazakii</i>		0.5 (1)	0.58 (1)
<i>Enterobacter cloacae</i>		1 (2)	
<i>Chryseobacterium indologenes</i>		0.5 (1)	0.58 (1)
<i>Citrobacter freundii</i>		0.5 (1)	0.58 (1)
<i>Klebsiella oxytoca</i>	1.7(2)	0.5 (1)	0.58 (1)
<i>Klebsiella terrigena</i>		0.5 (1)	0.58 (1)
<i>Serratia spp.</i>	0.9 (1)		
<i>Serratia liquefaciens</i>	0.9 (1)		
<i>Stenotrophomonas maltophilia</i>		0.5 (1)	0.58 (1)
Streptococcus			
<i>Streptococcus. constellatus</i>		0.5 (1)	0.58 (1)

5.3.4 Salivary flow and the Fox's xerostomia index

The median salivary flow measured in millilitres per 5 minute sample was 0.8ml for the non-diabetic subjects (interquartile range (IQR), 0.4-1.5), and 0.5ml (IQR 0.25-1.0) for the T1DM and PCD groups (NDS:T1DM $p = 0.003$ NDS: PCD $p=0.01$) (Table 5-4). When the groups were divided into those with normal salivary flow (>0.1 ml/minute), reduced salivary flow ($>0.1<0.2$ ml/minute) and very low salivary flow (>0.1 ml/minute) there were more PCD patients in the very low salivary flow group (41%) compared with either the NDS or the WCD (29%, 27% $p=0.043$)(Figure 5-11). There were also slightly more NDS with normal salivary flow than PCD (NDS: 43% vs PCD: 32% $p=0.07$) (Figure 5-11).

When the T1DM patients were divided into quintiles, the number of patients reporting that they had problems with a dry mouth was relatively low across all five groups and there was no difference between the groups (NDS, 7.3% T1DM; 10.1%, Q1, 7.3%, Q2, 12.8%, Q3, 2.4%, Q4, 13.2, Q5, 15% ($p = 0.31$)(Figure 5-12). The number of patients reporting symptoms of dry mouth was twice as high in the group with the highest HbA1c compared with the group with the lowest HbA1c and the NDS group. The second question related to those subjects having problems swallowing dry food and again, this was relatively rare (NDS, 10% T1DM; 13.6%, Q1, 14.6%, Q2, 7.7%, Q3, 7.3%, Q4, 10.5%, Q5, 27.5% ($p = 0.47$) (Figure 5-13). Again, there was a striking difference between the NDS and the better controlled T1DM groups. Quintile 5 was almost three times more likely to complain of difficulty swallowing dry food compared with NDS. There were slight differences between the groups for those who required liquids for swallowing dry foods with (NDS, 33.6% T1DM; 40.7%, Q1, 46.3%, Q2, 25.6%, Q3, 31.7%, Q4, 42.1%, Q5, 57.5% ($p = 0.34$) Figure 5-14). The biggest difference was between quintile 2 and quintile 5 which had the highest prevalence again.

Q1 had more symptoms of xerostomia than Q2, Q3 and Q4 for difficulty swallowing dry foods, requiring liquids for swallowing and for the overall Fox's positive index. The number of patients who were woken by a dry mouth was higher in the NDS group compared with the other 2 groups. Specifically 19.1% of the NDS were affected, while only 7.3% of the Q1 and 14.1% of the T1DM group reported being woken with a dry mouth. 22.5% of the worst controlled T1DM group reported waking with dryness (Figure 5-15). The difference in those patients who felt that they had too little saliva was also significantly different between the best and worst-controlled diabetic subjects with 7.5% of the best controlled diabetic patients (Q1) reporting that they felt they had too little saliva, compared with 15% of the worst-controlled diabetic patients (Q5). There were no

differences in the overall number of patients across the three groups who responded positively to any of the Fox's Index Questions (NDS: 44.5%, T1DM:46.7%, Q1:46%, Q2:37%, Q3:36.6%, Q4:52.6%, Q5:60%) (Figure 5-16). Across all of the Fox's questions there was a trend towards an increase in the number of individuals reporting symptoms of xerostomia with increasing HbA1c. Patients were asked whether they were aware of their salivary function. There were no differences across the groups regarding those patients that reported that they felt they had too little saliva, (NDS: 10%, T1DM:12.6%, Q1:7.5%,Q2: 15.4, Q3:7.3%,Q4:13.2%, Q5:15%) (Figure 5-17).

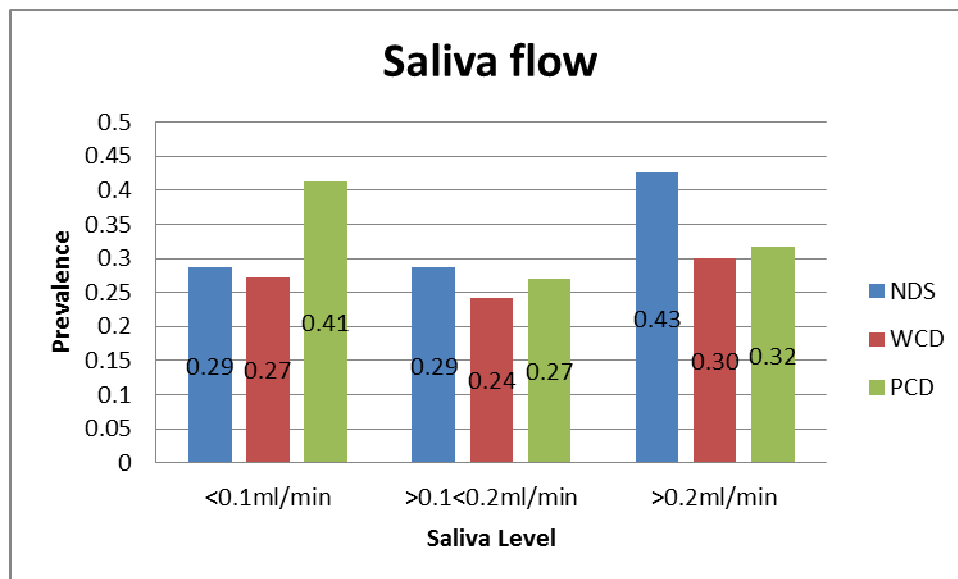


Figure 5-11 Bar chart showing the proportion of individuals who had normal >0.2ml/min, reduced>0.1<0.2ml/min and severely reduced <0.1ml/min across NDS, WCD, PCD

Table 5-6 Self reported symptoms of xerostomia comparing non diabetic subjects with type 1 diabetic patients

	NDS (110)	T1DM (199)	Q1 (41)	Q2 (39)	Q3 (41)	Q4 (38)	Q5 (40)	P
dry mouth (%)	7.3	10.1	7.3	12.8	2.4	13.2	15	0.31
Problem swallowing (%)	10.0	13.6	14.6	7.7	7.3	10.5	27.5	0.047*
liquid for swallowing (%)	33.6	40.7	46.3	25.6	31.7	42.1	57.5	0.034*
Woken with dry mouth (%)	19.1	14.1	7.3	20.5	12.2	7.9	22.5	0.20
Fox's positive (%)	44.5	46.7	46.0	37.0	36.6	52.6	60.0	0.26
too little saliva (%)	10.0	12.6	7.5	15.4	7.3	13.2	15	0.26

Chi squared test* p<0.05

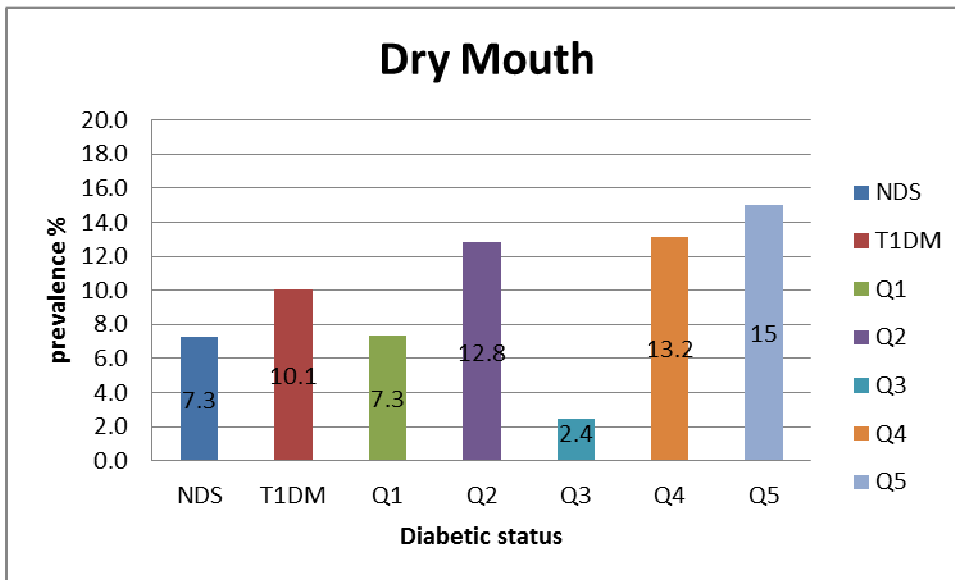


Figure 5-12 Bar chart showing the number of patients in the T1DM, Quintile 1-5 by HbA1c and NDS who reported that they felt that they had a dry mouth

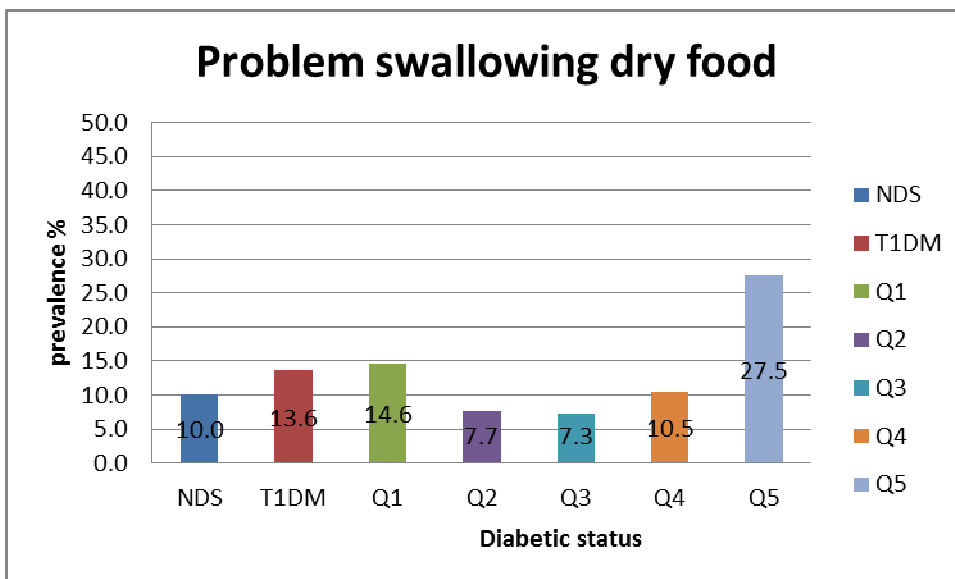


Figure 5-13 Bar chart showing the number of patients in the T1DM, Quartile 1-5 by HbA1c and NDS who reported that they felt that they problems swallowing dry food

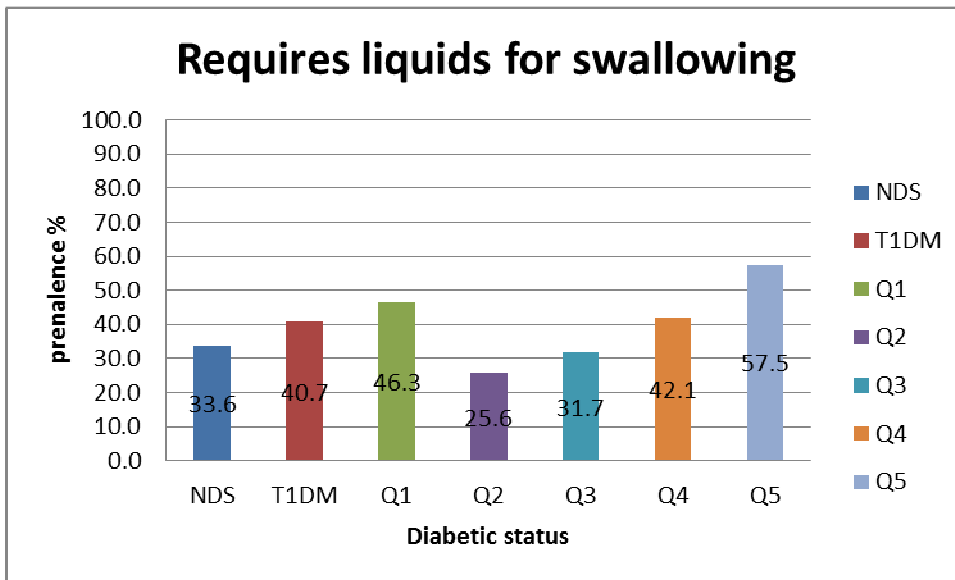


Figure 5-14 Bar chart showing the number of patients in the T1DM, Quintile 1-5 by HbA1c and NDS who reported that they required liquids for swallowing dry foods

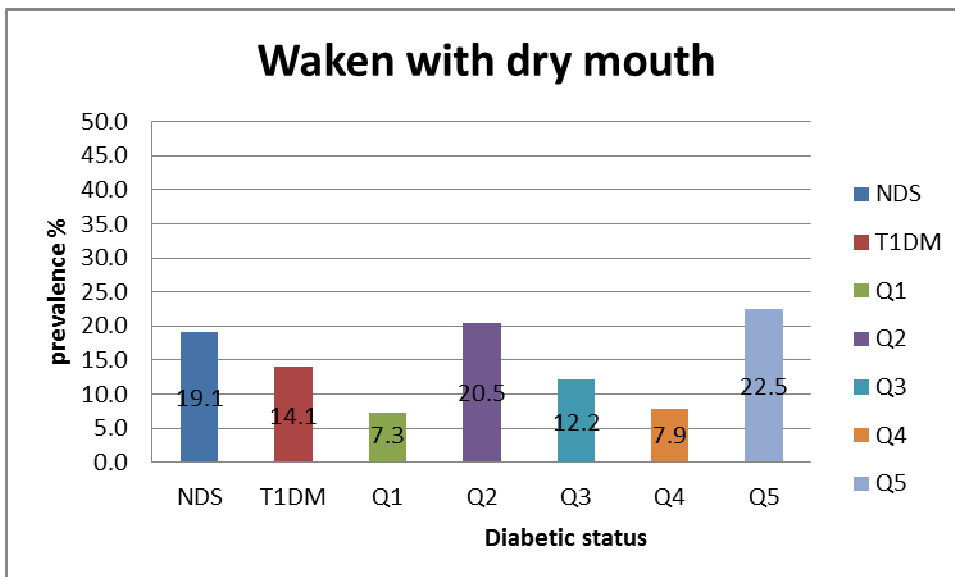


Figure 5-15 Bar chart showing the number of patients in the T1DM, Quintile 1-5 by HbA1c and NDS who reported that they felt that were woken during the night with a dry mouth

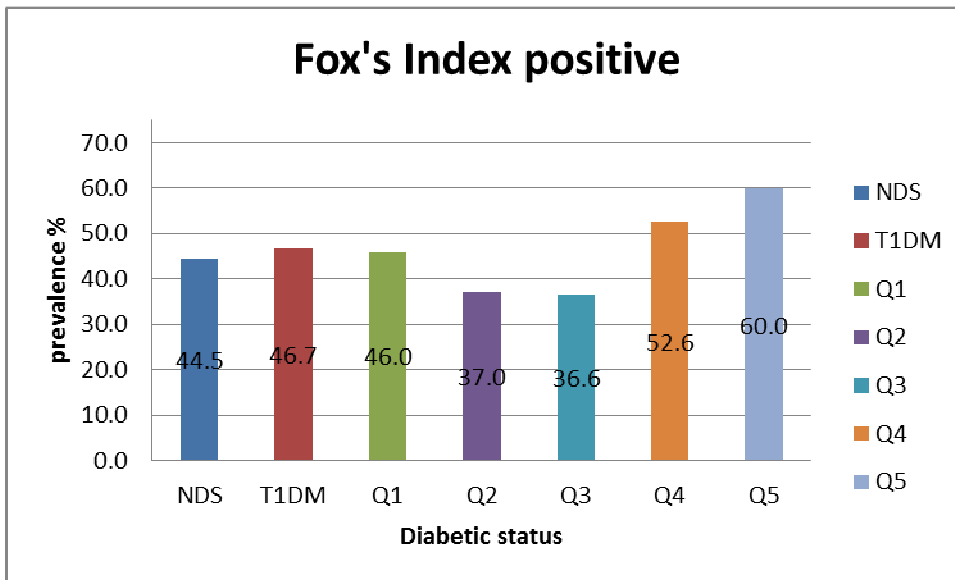


Figure 5-16 Bar chart showing the number of patients in the T1DM, Quintile 1-5 by HbA1c and NDS who answered positively to any one of the questions in the Fox's xerostomia index

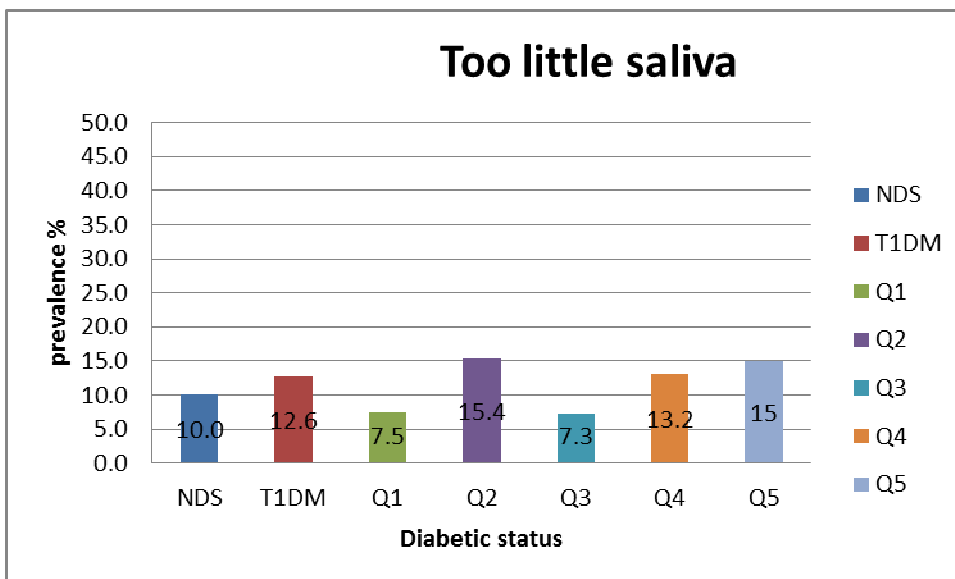


Figure 5-17 Bar chart showing the number of patients in the T1DM, Quintile 1-5 by HbA1c and NDS who reported that they felt that they had too little saliva

5.4 Discussion

5.4.1 Caries variables

5.4.1.1 Missing teeth

The median number of missing teeth in the present study was four in all groups. This is lower than the national average as shown in the ADH survey (Nunn et al, 2001). Surveys in other developed countries including Germany, Australia and the United states showed that the number of missing teeth was highest in the United Kingdom. In the adult dental health survey the mean number of missing teeth was 7.2 while comparable data from the above countries were much lower. German adults had only 2.5 missing teeth; Australians had 4.5 missing teeth and in the United States 5.3 in the 45-54 year old group, 2.4 in the 35-44 year old group and only 0.6 in the 25-34 year old group (Crocombe et al., 2009).

The lower number of missing teeth in the study reported here is a function of the inclusion criteria that stipulated that people should have a minimum number of 20 teeth and of course no edentulous patients were examined. This feature precludes further comparisons with other surveys but in no way limits the validity of comparisons between the groups as all participants were subject to the same inclusion criteria. In this study there were no differences in the number of teeth. This is in agreement with the studies in adolescents where there were no differences in the number of missing teeth presumably because the disease was minimal and they had not the condition long enough to experience tooth loss as a result (Do Amaral et al., 2006; Lalla et al., 2006a; Patino-marin et al., 2008; Tagelsir et al., 2011). Kaur and co-workers (2009) however reported that tooth loss was significantly associated with T1DM. Al Shammari (2006) reported that the number of missing teeth was significantly higher in diabetic patients of long duration compared with those who had had the disease for less than 5 years however this study did not contain a healthy control group for comparison. It is also possible that the group who had had diabetes longer were older. This may account for the relationship between tooth loss and duration. Moore and co-workers (1998) also found that T1DM patients did not have any more tooth loss than the healthy controls. The mean number of missing teeth in this study was only 2.5 teeth.

5.4.1.2 Decayed teeth

The number of decayed teeth in all three groups was high compared with the national average (Nunn et al., 2000). It is reasonable to compare with the ADH as we used the same level of caries as a cut off. While the use of the D3 level accepts that there is a

significant amount of decay that will go unreported this is not an issue. The absolute level of disease is less important than the fact that the same level was applied throughout the study across the groups. About three quarters of all patients had untreated decay (NDS 69%, WCD 71%, PCD 78%). There was a slight difference in the number of decayed surfaces between the two groups although this was not significant at the 1% level. Tagelsir et al. (2010) recently showed that there was no difference in the level of dental caries in type 1 diabetic children. Miko et al. (2010) reported that the DMFT in T1DM adolescents was higher but the decayed component was lower. Those subjects who had good oral hygiene and good glycaemic control were not at a higher risk of dental caries. This may reflect access to dental care which may be better for this group. In our study we showed that there was a tendency for the care index to be lower in T1DM rather than higher. A further study in T1DM adolescents showed that the level of untreated decay was higher in the T1DM group and that this could have been due to poorer dental attendance (Tagelsir et al., 2011). Saes-Busato and co-workers (2010) reported that the DMF in adolescents with T1DM was higher than NDS (1.5 NDS, T1DM 3.3 $p < 0.05$). Patino-Marin and co-workers (2008) concluded that there was no difference in the level of caries between T1DM and NDS.

A longitudinal study over a two year period (Siudikiene et al., 2008) reported that, in addition to the shared risk factor of poor oral hygiene, high levels of glucose in saliva in diabetic patients were associated with an increased caries increment. Do Amaral and co-workers (2006) reported that T1DM patients had less caries than the control group despite the fact that the diabetic subjects ate more frequently and had poorer oral hygiene. In another study (Miralles et al., 2006) in a group of Spanish T1DM adults aged 18-50 years old investigators reported that the diabetic group had more caries than the control group despite having similar levels of oral hygiene. Siudikiene and co-workers (2006) also reported that adolescents with T1DM had more caries than the control group. There was also a difference between WCD patients and the PCD subjects. Lalla et al. (2006a) also did not detect any differences in the level of decay in a group of American diabetic subjects.

The risk of caries was investigated by Twetman and co-workers (2005). They used the cariogram caries risk analysis tool and reported that those patients with a high caries risk were 7.3 times more likely to be poorly controlled compared with those who had a low risk of caries. Syrjala et al. (2004) showed that self efficacy was the best overall determinant of various health behaviour practices.

5.4.1.3 Implications of findings

This study has showed no significant increase in dental caries in the WCD or the PCD groups compared with NDS. The oral hygiene was poorer in both diabetic groups compared with the NDS and the PCD had the worst oral hygiene. These differences were only slight and any differences may have been reduced by the high level of disease in this population. The ubiquitous nature of dental caries in the adult population in the West of Scotland may have diluted the additive effect of diabetes mellitus. There was no difference in the number of meals and snacks per day between the three groups (mean number of meals NDS 2.92, WCD 2.92, PCD 3.0). Despite the fact that the PCD group had poorer oral hygiene, lower salivary flow and a lower level of education this did not appear to have a significant impact on caries experience. A low restorative index may be due to low dental attendance but also may be due to dental prescribing patterns. The carious lesions in question were clear unambiguous lesions that were normally cavitated. The frequency of dental attendance and the attitude towards health care may result in a reduction in the care index as there were a greater number of patients in the diabetic groups with a lower ratio of filled teeth to DMFT. This indicates that there are dental needs among diabetic subjects that are unmet at the present time. This is in contrast to at least one study that reported that the DMFT was the same between the T1DM group and the control group but that there were more filled teeth in the T1DM group and more decayed teeth in the control group. This may reflect the availability of services or the motivation of patients and carers. A recent paper demonstrated that 61.7% of all type 1 diabetic subjects had clinically significant apathy (Padala et al., 2008). Clinical expressions of apathy include the following three characteristics: reduced goal directed behaviour, reduced goal directed cognition and reduced emotional concomitants of goal directed behaviours. This apathy was associated with increased BMI, poor compliance with medical advice and poor compliance with an exercise regime. It is possible that this apathy could also lead to low dental aspiration, compliance with dental goal setting and concern (Knecht 1999a,b, 2000, 2001; Syrjala 1999, 2002). This could lead to apathetic diabetic subjects failing to comply with dental advice and failing to see the importance of dental treatment.

5.4.2 Soft tissue abnormalities

The examination of the oral mucosa of patients involved in this study revealed that there was a range of benign oral mucosal abnormalities noted in both groups.

5.4.2.1 Frictional keratosis

The most common of these was frictional keratosis, a normal response of the oral mucosa to mechanical irritation. This condition is often found in patients who suffer from bruxism

and cheek biting. It is an asymptomatic benign condition that confers no risk of malignant change. In combination with other signs of Temporomandibular Joint Dysfunction (TMD) it can be a sign of parafunction (Scully and Shotts, 2000). TMD can cause functional pain and this is often associated with psychosocial stress (Jones et al., 1997). There are no studies to demonstrate a link between TMD and diabetes mellitus. It could be hypothesised that the underlying stress of having a long term chronic, potentially debilitating condition could have contributed to an increase in TMD and frictional keratosis in the diabetic patients. However, there were no differences in the prevalence of frictional keratosis between the groups.

5.4.2.2 Abnormalities of the tongue

Abnormalities of the tongue were the second largest group of oral mucosal abnormalities. These included fissuring of the tongue, glossitis, depapillation, geographic and coated tongue and one case of median rhomboid glossitis. Fissuring, glossitis and depapillation are all associated with vitamin deficiencies including folate, vitamin B12 and iron (Field et al., 1995; Scully and Shotts, 2000; Thongprasom et al., 2001) although they can also be present in oral candidosis and xerostomia (Terai and Shimahara, 2005; Huguley, 1990). It is possible that candidal carriage and/or reduced salivary flow could have resulted in an increase in the prevalence of these conditions in T1DM. A large cross-sectional study on T1DM adults showed that median rhomboid glossitis and atrophy of the papillae of the tongue were more common in the T1DM group than the controls (NDS 0.4%, T1DM 7.2% NDS 2.2%, T1DM 8.9%) (Guggenheimer et al., 2000a, 2000b). There was no significant increase in these lesions in our study (NDS 1 lesion, T1DM 0% NDS 0%, T1DM 1%). The study by Guggenheimer had a larger sample size which would have increased the statistical power for detecting differences in these rare conditions. The prevalence of smoking was high in this study and this may have also affected the prevalence of oral mucosal abnormalities.

5.4.2.3 Oral mucosal lesions associated with Candidal infection

There was no difference in the prevalence of candida associated soft tissue lesions including denture stomatitis, angular cheilitis, median rhomboid glossitis or papillary hyperplasia. This was perhaps surprising given that there was a slightly higher prevalence of candidal carriage in the T1DM group. In addition, those diabetic patients that carried the yeast showed greater density of colonisation. The number of candidal lesions was very small in all groups. This finding is in agreement with Manfredi et al. (2002) who also reported that while candidal carriage was common (66%) the prevalence of candidal

lesions or symptoms was of the order of 3.6%. Willis and co-workers (2000) reported that candida species were recovered from the mouths of 77% of diabetic subjects. Soysa (2005) reported that in two comparable studies (Manfredi et al., 2002; Al-Karaawi et al., 2002) the prevalence of candidal carriage was higher in patients with diabetes mellitus.

5.4.2.4 Dental sepsis

There was also no difference between the groups in the number of patients with an acute dental abscess at examination. Historically, purulent oral infections were very common in diabetes and indeed can be the presenting feature of undiagnosed or poorly controlled diabetes. (Harrison et al., 1983; Ueta et al., 1993). T1DM patients who today would be regarded as being poorly controlled (HbA1c>7.5%) would be unlikely to have widespread suppuration. Diabetes continues however to be a risk factor for the development of spreading odontogenic infection of any cause a condition that can have fatal consequences (Huang et al., 2005). It has been shown that subjects who suffer from diabetes have a higher prevalence of periapical disease radiographically (Segura-Egea et al., 2005). Correspondingly there are reports of improved insulin sensitivity after endodontic treatment in patients with diabetes (Schulze et al., 2007)

5.4.2.5 Lichen planus

The prevalence of lichen planus /lichenoid reaction was slightly higher in T1DM patients. This difference was however not statistically significant which may have been due to the low numbers affected. The study was not powered to detect a difference in prevalence of conditions that are as rare as lichen planus. Lichen planus only affects (0.5-1.9%) of the population and it is more common in women than men (Brown et al., 1993; Jaques et al., 2003). Lichen planus is an autoimmune disorder and as such it has been suggested that there may be a common pathway linking lichen planus and diabetes. There are reports of higher levels of T1DM in patients with lichen planus (Nigam et al., 1987; Conte et al., 1990; Brown et al., 1993; Denli et al., 2004; Seyhan et al., 2007). There are also reports of higher levels of lichen planus in diabetes mellitus (Albrecht et al., 1992; Bagan-Sebastian, 1992; Borghelli, 1993). Quirino and co-workers (1995) and Guggenheimer and co-workers (2000b) did not however find any increase in the prevalence of oral lichen planus in patients with diabetes. Although in the current study there was a 3 times higher prevalence in the T1DM compared with the NDS group this difference may or may not have persisted in a larger sample with increased statistical power and further investigation would be required.

5.4.2.6 The impact of the absence of smoking on the oral mucosal findings

There was a variety of other oral mucosal lesions reported, but mostly in only one or two cases. The relative infrequency of oral mucosal lesions could be attributable to the fact that there were no smokers included in the study. Tobacco use has been associated with premalignant lesions in the mouth including leukoplakias and erythroplakias as well as other reactive lesions (Warnakulasuriya et al., 2010). A study conducted in India showed an increase in premalignant lesions in women with diabetes mellitus but this may have been due to the confounding effect of smoking (Dikshit et al., 2006). Similarly Ujpal and co-workers (2004) reported in a Hungarian study that the prevalence of oral premalignant lesions was higher than previous studies on non-diabetic subjects. Within the limits of this study it is concluded that healthy non-smoking T1DM patients do not have a higher prevalence of oral mucosal lesions than healthy controls. The autoimmune, inflammatory and infective nature of many oral mucosal conditions makes the likelihood of a connection possible however this has not been born out in this study. To detect minor differences in rare conditions a much greater sample size would have been required.

5.4.3 The oral microflora in Type 1 diabetes mellitus

5.4.3.1 Oral carriage of Yeast

Carriage of yeasts in the oral cavity was increased slightly in the T1DM and PCD groups compared with the NDS although the difference was not significant. The percentage of T1DM patients (59.5%) carrying *C albicans* was almost double the average described by Macfarlane et al. (1990) of 34%. Despite the high prevalence of candidal carriage in the T1DM group, the prevalence was 48.5% in the NDS group meaning that the difference in *C albicans* carriage between the groups was not significant. This is in agreement with a small number of studies that did not find an increase in candidal carriage in diabetes (Mehnert and Mehnert, 1958; Peters et al., 1966; Tapper-Jones et al., 1981; Phelan and Levin, 1986; Doroka-Bobkowska et al., 1996; Kadir et al., 2002; Manfredi et al., 2002). These studies are however in the minority. Many studies have shown that candidal carriage rate is increased in T1DM patients (Barlow and Chattawa, 1969; Bhatt et al, 1983; Bartholomew et al., 1987; Lamey et al., 1988; Fongsmut et al., 1998; Guggenheimer et al., 2000a). It is possible that previous studies did not adequately allow for the confounding effect of smoking. Guggenheimer (2000a) reported that *Candida* pseudohyphae were associated with cigarette smoking. Soysa et al., (2005) also reported in a review of the literature that smoking causes an increase in *Candida albicans* carriage. Manfredi et al. (2002), Budtz-Jorgensen (1990) and Guggenheimer et al. (2000a) all concluded that local

factors including denture wearing and tobacco smoking had a significant impact on carriage rates.

There was an increased density of *C. albicans* colonisation in T1DM and PCD, who carried the yeast, compared with NDS in the current study. This is in agreement with Odds et al., (1978) who also showed that candidal density was related to blood sugar levels and urinary glucose levels at the time of sampling. Tapper-Jones et al. (1981), Hill et al. (1989) and Aly and co-workers (1992) also reported that the density of candidal colonization was higher in diabetic subjects particularly in those who were poorly controlled.

A simplistic explanation for the increased carriage rates in diabetic patients is the increase in salivary glucose levels as a source of nutrients for the fungi. Yeast grow well in high nutrient environment and it is thought that since salivary levels of glucose reflect the systemic hyperglycaemia this will encourage fungal growth.

However, it is also possible that changes to the mucosal immune response affect the ability of candida to colonise the oral mucosa. Pathogen associated molecular pattern receptors (PAMPs) such as TLRs are fundamental to the innate immune systems ability to recognise bacterial, viral and fungal pathogens and initiate both the innate and adaptive host response. TLR 2 and 4 are responsible for initiating a response to yeasts through interaction with the fungal cell wall (Mogensen, 2009). There are a number of studies that have shown that polymorphisms in TLRs are present in patients with both type 1 and type 2 diabetes mellitus (Pirie et al., 2005; Devaraj et al., 2008). These defects could be responsible for an increased susceptibility to fungal infection although this has not been investigated.

The first stage of fungal colonisation is adhesion to epithelial cells and there is some evidence that this initial stage occurs with increased efficiency in patients with diabetes. It has been shown that buccal epithelial cells (BEC) from diabetic patients permit increased adhesion of *C. albicans* (Darwazeh, MacFarlane, and Lamey; Willis et al.). In one study the adhesion of *C. albicans* buccal epithelial cells of 50 diabetic patients was 55% higher than to the BEC of 50 age and sex matched non-diabetic subjects ($p < 0.0001$). This was confirmed by later workers. The mechanism of this binding is unknown but may be due to reduced salivary lysozyme (Pinducciu et al.), production of extracellular proteinases or upregulation of receptors to complement such as inactivated C3b (iC3b) in high glucose concentrations (Manfredi et al. 183-89). An alternative theory to explain the increased adhesion of *C. albicans* to epithelial cells when grown in high sugar media due to the development of a fibrofloccular layer on yeast cell surfaces when grown in high sugar media may also explain the increased adhesion of candida albicans to the oral mucosa.

Accumulation of glycosylation products in epithelial cells may increase numbers of receptors for *C. albicans* (Willis et al.). It is possible that accumulation of glycosylation products on buccal epithelial cells may increase the number of available receptors for *Candida* and thus increase initial adhesion.

It is however possible that the increased colonization is related to acute hyperglycaemia rather than chronically elevated blood glucose levels. In one study examining the oral flora of diabetic subjects admitted to hospital with acute diabetic ketoacidosis the levels of *candidal* carriage were highest in patients admitted for acute metabolic complications. This would tend to indicate that it is not directly related to long term structural changes such as the development of advanced glycation end products but is rather related to reversible changes in the oral mucosa or host response.

Hyphae formation is a critical virulence factor that is associated with candidal adhesion and colonisation. It has been shown in one study that hyphae formation occurred quicker in candidal species grown in sera from diabetic patients compared with controls although strangely a similar relationship was not seen in saliva (Kumar et al., 1980). Strains of *Candida albicans* isolated from patients with type 1 diabetes mellitus have been shown to be better at forming biofilms (Rajendran et al., 2010). This capability will greatly increase initial colonisation, protection from the host and subsequent resistance to treatment.

In addition, it has also been reported that high salivary and blood glucose concentrations may lead to increased resistance to intracellular killing by macrophages (Willis et al., 2000; Tsang et al., 2008). Defects in neutrophil maturation, chemotaxis, phagocytosis and cell killing have all been demonstrated in patients with type 1 diabetes (Manoucher-pour et al., 1981; Ueta et al., 1993). The inability of the innate and adaptive immune system to eliminate the fungal infections may lead to increased Candidal colonisation in T1DM.

Changes in salivary flow level have been shown to be associated with both diabetes and oral candidosis. Diabetic patients have been shown to suffer from hyposalivation and xerostomia more frequently than non-diabetic subjects. Saliva produces a mechanical flushing and lubricating effect on the oral mucosa limiting colonization. When this is reduced the prevalence of fungal infection increased. Ben-Aryeh et al. (1993) showed that salivary gland dysfunction was associated with oral candidosis in type 1 diabetic patients. Saliva also contains a myriad of antibacterial and antifungal molecules that will be reduced in patients with type 1 diabetes. Of particular relevance is secretory IgA which is important in preventing adhesion to the oral mucosa. It is likely that a decrease in salivary flow rate consequent to diabetes may enhance candidal colonization through a number of mechanisms.

The high prevalence of *C albicans* carriage in the NDS group could be due to the fact that they were generally a relatively deprived population and may not therefore be representative of the general population. It is also worth noting that the salivary flow rates in the NDS group were reduced compared with normal unstimulated salivary flow rates. The normal salivary flow rate is >0.2ml/minute however this level was only seen in 43% of all NDS patients. The median salivary flow rate for NDS was only 0.8ml/5 minute sample. The median salivary flow rate for the T1DM group was only 0.5ml/5 minute sample or 0.1ml/minute. This reduced salivary flow rate in both groups could account for the high level of candidal carriage in NDS and T1DM as salivary gland hypo-function for any reason reduces the cleansing effects of saliva. It is also possible that there are differences in the immune system that predispose T1DM patients to candidal colonisation (Wilson and Reeves, 1986). We have shown elsewhere that there were differences in the pathogenicity of *C albicans* isolates from T1DM in terms of their biofilm forming capacity and proteinase production (Rajendran et al., 2010). It is possible that the micro-environment in the mouths of T1DM patients leads to changes in the expression of virulence factors leading to improvements in biofilm formation, adhesion and subsequent colonisation (Tsang et al., 2009; Rajendran et al., 2010). The level of glucose in the saliva of T1DM is higher than NDS patients and this means that *C albicans* are constantly bathed in a nutrient rich broth which will increase growth (Odds et al., 1978; Darwazeh et al., 1991). It is also possible that there are more complex defects in the innate immune system including reduced antimicrobial peptide production or other differences in salivary composition that lead to inability of the T1DM patient to clear *C albicans* from the mouth. *C albicans* was the most common species recovered in accordance with all other studies however Willis et al. (2000) showed that there was a very high prevalence of *C. dubliniensis* in diabetic patients and HIV positive patients (58/318 of strains recovered (18%). We only reported *C dubliniensis* in 2% of all cases. This difference is difficult to explain but may represent geographic variation in candidal ecology. It is also possible that there were methodological differences in the way in which the samples were processed that increased the sensitivity of the culture method used.

5.4.3.2 Oral *staphylococcal* carriage

S. aureus carriage was high in both groups compared to studies from different centres. It has been suggested that *S. aureus* carriage is only a transient feature of the oral microflora and is not found in the same mouths on successive occasions (Smith et al., 2001). There have only been two studies that have reported *Staphylococcal* carriage in T1DM patients. Massler (1949) reported that diabetic patients had oral carriage of *S. aureus* and Sanchez-cordero (1979) reported that *S. aureus* was associated with periodontitis in T1DM.

Staphylococcus aureus can also be associated with a painful staphylococcal mucositis (Smith et al., 2001). Oral carriage of *S. aureus* could serve as a reservoir for nosocomial infection and T1DM patients have been shown to be at increased risk of staphylococcal skin infection (Graham et al., 2006). Jackson et al., (1999) reported that the prevalence of *S. aureus* carriage in a healthy population in Scotland was 24% and this increased with age and in medically compromised patients. The level of *S. aureus* carriage in T1DM was 33% which is slightly higher than that reported from the same lab in 1999. The healthy control group carried *S. aureus* in 28% of cases. This was again only slightly higher than the figures reported by Jackson et al., (1999). There are reports that increasing levels of MRSA are posing a problem in diabetic foot clinics however we only found one case of oral carriage of MRSA in all the patients. This would tend to indicate that the mouth is not a common source of nosocomial MRSA infection although it may be a source of methicillin sensitive *S. aureus* infection.

5.4.3.3 Oral carriage of coliforms

The carriage of coliforms was low in all three groups (NDS:3.6%, T1DM: 3.4%, PCD 3.5%). These gram negative bacteria are associated with many different serious diseases and although there are no conditions specifically associated with oral carriage it is possible that the oral cavity could act as a potential reservoir of infection. Carriage rates in the healthy population were recently reported as 1% to 4% from oral rinses and culture techniques and this figure is similar to the prevalence reported in both the T1DM and NDS groups (Sedgely et al., 2004). In T1DM patients, who are at an increased risk of infections due to being immuno-compromised, it is potentially worrying that they have oral carriage of such pathogens. There were no differences in the coliform carriage rates between NDS and T1DM.

Antimicrobial resistance was reported only infrequently and there was no difference between NDS and T1DM groups in their resistance profiles. Antifungal resistance was seen in only a small number of cases and the anti-fungal agent to which most species were resistant was itraconazole. There were no differences in the resistance profiles of species isolated from NDS compared with T1DM. This confirms that despite the fact that T1DM patients are more likely to be given antibiotics or antifungals to treat infection this has not had a detrimental effect on the resistance pattern of the microflora isolated from this group. Responsible prescribing of antibiotics and antifungal agents should however continue to be followed in T1DM patients as well as in the general population.

5.4.4 Salivary flow and xerostomia in T1DM

The unstimulated salivary flow rate was low in T1DM and particularly PCD patients. The numbers of patients with a severely reduced unstimulated salivary flow rate was highest in the PCD group. We also showed that the prevalence of very reduced unstimulated salivary flow (<0.1ml) was as high as 29% for the NDS and 41% in the PCD. This was significantly higher than that reported by Flink (2007) who measured salivary flow rates in adults between 20 and 70 years of age in a large study. The prevalence of very low unstimulated salivary flow rate ranged from 10.9-17.8% depending on age group. A number of papers have concluded that poorly controlled diabetes can lead to a reduction in salivary flow (Sreebny et al., 1992; Moore et al., 2001b; Siudikiene et al., 2006). This reduction in salivary flow can also be seen in children and adolescents although this finding is not universal (Siudikiene et al., 2006; Edblad et al., 2001). It has been hypothesised that the reduction in salivary flow rate may be due to dehydration as the body excretes excess glucose through the kidneys. Where patients are hyperglycaemic it is likely that patients may be slightly dehydrated as the body attempts to restore glucose homeostasis. It would have been interesting to see whether the salivary flow was related to the concurrent blood glucose level at the time of sampling. All patients had been instructed to have an early breakfast and had not had anything to eat for at least an hour prior to sampling so it is unlikely that they would have been hyperglycaemic when the saliva was collected. There is also a diurnal pattern to salivary flow however this is unlikely to have been an issue as all sampling was taken at around the same time during the morning (Ionescu et al., 1998; Navazesh and Kumar, 2008). Any variability in the time of sampling would be expected to be equally distributed across the groups. The number of patients who were aware of having too little saliva was relatively low and there was no difference between the groups. It has been shown that subjective assessment of reduced salivary flow is not an accurate measure of salivary gland hypo-function. Use of the Fox's index showed that there were very high numbers of subjects who answered positively to these questions. 47% of all T1DM and 45% of all NDS answered positively to at least one of the questions in the Fox's index. It is possible that the high percentage of patients reporting symptoms of xerostomia in the NDS group is masking the effect of diabetes on salivary function. When the responses to the Fox's questions were looked at according to the quintiles of HbA1c it was apparent that there were differences within the diabetic group. For every question, the most poorly controlled group had the most symptoms. However, quintile one which consisted of a group of very well controlled subjects were very aware of symptoms of a dry mouth despite having a median salivary flow rate that was higher than the other quintiles. An alternative explanation must be sought to explain

this unusual pattern. Salivary flow rate can be reduced by stress and anxiety and it is possible that the group of very well controlled diabetic patients may have awareness of a dry mouth because of this (Hugo et al., 2008). The data presented in this study cannot confirm this association and a future study with psychometric testing for personality traits, anxiety and depression may answer this question. It is also the case that the best controlled diabetic subjects were on average slightly older than the poorest controlled subjects. It is therefore possible that the long term effects of having diabetes may increase symptoms of a dry mouth as may the use of polypharmacy and xerostomic drugs although salivary flow rates were higher in WCD. In this analysis we have not accounted for the use of xerostomic medication as this would be on the causal pathway. Many patients were on medication that theoretically could result in reduced salivary flow mostly as a result of their diabetic condition. It has been reported that the hypo-salivation seen in type 2 diabetes mellitus is largely drug related (Meurman et al., 1998). This does not invalidate the finding that type 2 diabetic patients suffer from hyposalivation and are thus at increased risk of oral hard and soft tissue complications as a result. To correct for the possible causative agent would be to miss a real problem that exists regardless of the aetiology. The identification of the aetiology is secondary to the prevalence of the signs of hyposalivation. The high levels of xerostomia in the control group could possibly be related to the use of xerostomic medication although these were not commonly reported in the control group that was made up of healthy individuals. Salivary flow rates have also been shown to be reduced in patients who have an increased BMI (Flink, 2007). A significant proportion of both the T1DM and the NDS groups had elevated BMIs and this could be a possible explanation for the low salivary flow.

5.5 Conclusion

It appears that the caries risk of T1DM adults is not significantly increased compared with healthy controls. The number of restorations was also lower and the care index was correspondingly low in T1DM compared with NDS. Poorly controlled diabetic patients had the poorest oral hygiene, the largest number of decayed surfaces and the lowest number of filled teeth and the lowest care index. This may indicate that they have low dental interest and aspirations. The heterogeneity of results from studies across the world indicates that T1DM is not associated with caries in the absence of other risk factors. Local and systemic risk factors including the presence of dental plaque and the frequency of intake of processed sugars are more important than T1DM as an independent factor. Dietary advice to T1DM patients should be targeted to avoiding frequent processed sugars and maintaining a healthy diet. In addition advice on effective oral hygiene measures is

essential as this advice is clearly not being followed in either group. Consideration should be given to motivational methods encouraging T1DM patients to manage both their oral and systemic health (Rosenbek et al., 2011).

Within the limits of this study it is concluded that healthy non-smoking T1DM patients do not have a higher prevalence of oral mucosal lesions than healthy controls. The only lesion that was slightly increased in T1DM was the prevalence of lichen planus. To detect minor differences in a rare condition such as this would require a much greater sample size.

T1DM patients had only a slight increase in candidal carriage rates but a significant increase in the density of colonisation where it occurred. We have also shown that differences in the pathogenicity in a small number of strains isolated from T1DM may account in part for this increase (Rajendran et al., 2010). The literature to date has often mixed up T1DM and type 2 Diabetes and has also failed to account for smoking and denture wearing. In our non-smoking population, the majority of whom had 20 teeth, there was no difference in prevalence between the T1DM group and NDS despite poor glycaemic control. There were no differences in the carriage rates of *S. aureus* or coliforms. There was also no difference in the antibacterial or anti-fungal resistance pattern between NDS and T1DM.

The unstimulated salivary flow rate across the groups was low and the prevalence of self-reported symptoms of xerostomia were very high in all groups. T1DM and PCD subjects were shown to have reduced unstimulated salivary flow rates. This information on its own would lead us to conclude that these patients are at higher risk of dental caries, mucositis and candidal carriage. The mechanism by which the salivary flow rate is reduced is unknown. Further studies would be required to elucidate the connection between T1DM and reduced salivary flow. Possibilities include ultrastructural changes to the salivary glands themselves, the role of osmotic diuresis in causing dehydration and consequent reduced salivary flow or autonomic neuropathy. The impact of progressive neuropathy on the sympathetic and parasympathetic control of the salivary glands might also be another mechanism for hyposalivation in T1DM patients.

6 Oral and General Health Related Quality of life in Type 1 Diabetic patients

6.1 Introduction

T1DM has been shown in the preceding chapters to be associated with an increased prevalence of periodontal disease and xerostomia. The clinical data has shown that poorly controlled diabetic patients produce less saliva and have higher mean periodontal probing depths and clinical attachment level as well as an increased prevalence of moderate and severe periodontitis. There was also an increase in the total load of *C. albicans* with higher mean cfu/ml in those diabetic subjects that carried the fungus and a trend to an increase in dental caries. The impact that these clinical findings have on oral function and oral and general health related quality of life has not been examined in this group. It is not known whether the presence of diabetes alone is sufficient to significantly impact on the patient's perception of Oral Health Related Quality Of Life (OHRQOL). The aim of this chapter is to examine the relationship, if any, between type 1 diabetes and general and OHRQOL and to measure its impact.

The research questions are:

- Do patients with T1DM report a greater impact of oral diseases on OHRQOL?
- Does poor glycaemic control result in a greater impact of OHRQOL?
- Do measures of diabetes related quality of life correlate with OHRQOL measures?

6.2 Materials and Methods

Recruitment method and inclusion and exclusion criteria have been described in chapter 2. All patients were asked to complete two questionnaires during the visit for the clinical examination. The clinical research nurse was available to assist with completion of the questionnaires if required. A generic oral health questionnaire was also used to record demographic information as well as information about the subjects' medical and dental history.

6.2.1 OHIP-14 Questionnaire

The subjects were given all necessary information about the study and provided informed consent to participation. All subjects were asked by the clinical research nurse to complete the Oral Health Impact Profile (OHIP-14) questionnaire during the clinical examination visit. This instrument consists of 14 questions organized into 7 dimensions: functional limitation (2 questions), physical pain (2 questions), psychological discomfort (2

questions), physical disability (2 questions), psychological disability (2 questions), social disability (2 questions), and handicap (2 questions). Each question had 5 response categories from “never” to “very often.” The questions referred to the subject’s experience in the previous 12 months. The questions included in the OHIP-14 are listed in table 6-1 including their domains.

6.2.2 ADDQOL

At the same visit the T1DM subjects were asked to complete the Audit of Diabetes Dependent Quality of Life Questionnaire (ADDQOL). The ADDQOL questionnaire consists of two overview questions including, a global quality of life question, and a question about the impact of diabetes on their quality of life, followed by 18 questions about the impact of diabetes on specific aspects of their lives. Subjects were asked to complete the ADDQOL at the same visit. The ADDQOL questions are listed in table 6-2. Answers ranged from very much better/increased to very much worse/decreased. The maximum negative score was -3 and the maximum positive score was 3. For each separate question, subjects were asked to rate the importance of each domain to them. Importance was scored in the following way: very important (3), important (2), somewhat important (1), not at all important (0). The individual domain score was multiplied by the relative importance giving a maximum negative impact of -9 and a maximum positive impact of 9. Question 1 records the individual’s general quality of life. Question 2 records the patients’ perception of the impact that diabetes specifically has had on their quality of life. The average weighted impact (AWI) of the diabetes on the patient’s quality of life was calculated by adding up the scores for the individual domains and dividing by the number of valid domains. The maximum tolerable number of missing or non-applicable items was 6 maintaining an alpha >0.8 as calculated by Bradley et al. (1999). The ADDQOL was summarised using the two overview items and the AWI. Further exploratory analyses were also performed on the impact of the individual domains on the global, diabetes specific, and OHRQOL measures.

Table 6-1 OHIP-14 Questions and domains (Slade et al., 1997)

Functional Limitation	<p>Have you had trouble pronouncing any words because of problems with your teeth, mouth or dentures?</p> <p>Have you felt that your sense of taste has worsened because of problems with your teeth, mouth or dentures?</p>
Physical pain	<p>Have you had painful aching in your mouth?</p> <p>Have you found it uncomfortable to eat any foods because of problems with your teeth, mouth or dentures?</p>
Psychological discomfort	<p>Have you been self-conscious because of your teeth, mouth or dentures?</p> <p>Have you felt tense because of problems with your teeth, mouth or dentures?</p>
Physical disability	<p>Has your diet been unsatisfactory because of problems with your teeth, mouth or dentures?</p> <p>Have you had to interrupt meals because of problems with your teeth, mouth or dentures?</p>
Psychological disability	<p>Have you found it difficult to relax because of problems with your teeth, mouth or dentures?</p> <p>Have you been a bit embarrassed because of problems with your teeth, mouth or dentures?</p>
Social disability	<p>Have you been a bit irritable with other people because of problems with your teeth, mouth or dentures?</p> <p>Have you had difficulty doing your usual jobs because of problems with your teeth, mouth or dentures?</p>
Handicap	<p>Have you felt that life in general was less satisfying because of problems with your teeth, mouth or dentures?</p> <p>Have you been totally unable to function because of problems with your teeth, mouth or dentures?</p>

Table 6-2 ADDQOL questionnaire (Bradley, 1999)

ADDQOL

1. In general, my present quality of life is:
 2. If I did not have diabetes, my quality of life would be:
 3. If I did not have diabetes, my working life and work-related opportunities would be:
 4. If I did not have diabetes, my family life would be:
 5. If I did not have diabetes, my friendships and social life would be:
 6. If I did not have diabetes, my sex life would be:
 7. If I did not have diabetes, my physical appearance would be:
 8. If I did not have diabetes, the things I could do physically would be:
 9. If I did not have diabetes, my holidays or leisure activities would be:
 10. If I did not have diabetes, ease of travelling (local or long distance) would be:
 11. If I did not have diabetes, my finances would be:
 12. If I did not have diabetes, my confidence in my ability to do things would be:
 13. If I did not have diabetes, my motivation to achieve things would be:
 14. If I did not have diabetes, the way society at large reacts to me would be:
 15. If I did not have diabetes, my worries about the future would be:
 16. If I did not have diabetes, my need to depend on others for things I would like to do for myself would be:
 17. If I did not have diabetes, my living conditions would be:
 18. If I did not have diabetes, my freedom to eat as I wish would be:
 19. If I did not have diabetes, my enjoyment of food would be:
 20. If I did not have diabetes, my freedom to drink as I wish (e.g. sweetened hot and cold drinks, fruit juice, alcohol) would be:
-

6.2.3 Data management

The three questionnaires were filled in on paper and were later transcribed into a custom-made electronic form in Microsoft AccessTM. A unique identifier was used to link all questionnaire data to the clinical parameters. Full clinical dental examinations were carried out on all subjects as described in chapter 2 and stored electronically as described.

6.2.4 Statistical analysis

The OHIP-14 data was analysed using a number of different algorithms. The scores for the different domains (0-4) can simply be summed to give a score ranging from 0-56 (OHIP

Sum). In this method 0=Never, 1= Hardly ever, 2= occasionally, 3= Fairly often and 4=Very often. The simple count method counts the number of impacts above a certain threshold. In this study we used three levels; firstly, any impact in the last 12 months (OHIP count) secondly, any impact that occurred occasionally (OHIP count >1) - and the most frequent impacts of fairly often or very often (OHIP >2). The individual questions were also computed into a dichotomous record of an impact in each of the seven domains. The summaries of both OHIP-14 and ADDQOL were subsequently linked to the generic oral health questionnaire and the clinical data and loaded into PASW18 (SPSS inc.) for analysis.

OHIP sum and the three OHIP count scores and individual domains were calculated and tested for differences according to the demographic data and clinical disease. The demographic data included the following; age, gender, difficulty paying for dental treatment, dental anxiety, dental attendance, SIMD, alcohol consumption, frequency of tooth cleaning, access to own transport and educational status. The OHIP scores were also compared between patients in whom dental disease was reported including pain, dry mouth, altered taste, periodontal disease, plaque levels, bleeding on probing and dental caries. Where the disease was recorded as a dichotomous variable the Mann Whitney U test was used to calculate differences in the distributions between the OHIP scores for individuals with and without the disease or complaint. Where the dental disease was expressed as a continuous variable the Spearman rank correlation statistic was calculated. Spearman Rank correlations were calculated between OHIP scores and the severity of pain, percentage bleeding on probing, percentage of sites with plaque, mean clinical probing depths, mean clinical attachment loss, number of sites with CPD and CAL >3mm, 5mm and 7mm, DMFT, DMFS the number of missing teeth, number of filled surfaces and the number of decayed surfaces.

The distribution of OHIP-14 scores (sum, count and individual domains) were compared between NDS and T1DM, NDS and WCD and NDS and PCD. Because the data were not normally distributed the Mann Whitney U test was used to compare between two groups and the Kruskal-Wallis test was used to test for differences in the distributions between NDS:WCD:PCD.

The distribution of OHIP scores was also compared across the five quintiles of HbA1c using the Kruskal-Wallis test. OHIP scores were also tested for correlations with the average HbA1c using the Spearman Rank correlation

Data was extracted from the ADHS 1998 (Nuttal et al., 2001) and the number of impacts in this national dataset was compared with the data derived from the NDS and T1DM groups.

Differences in the frequency of oral health impacts between the three groups were calculated using the Chi squared test.

The ADDQOL scores were correlated with the demographic information in a similar way to the OHIP-14 scores using the Spearman Rank correlation. QOL, Diabetes related QOL and AWI were all correlated against the OHIP14 scores and the clinical variables. Further exploratory analyses were also performed on the impact of the individual domains on the global, diabetes specific and oral health related quality of life measures.

6.3 Results

The descriptive data for the three groups (NDS, WCD, PCD) were re-analysed based on those who had OHIP and ADDQOL data. OHIP scores were calculated for 110 NDS, 27 WCD and 157 PCD. There were no changes from the descriptive data reported in chapter 2 (Table 2-6).

In order to establish the construct validity of the OHIP-14 in this patient group the OHIP scores were examined for relationships between the summary measures and the demographic and clinical findings across all groups. The relationships between OHIP scores and these variables are shown in Table 6-3 and 6-4.

The median OHIP-14 scores were higher in individuals who were dentally anxious, brushed infrequently, who had difficulty paying for dental treatment and who did not have access to transport. Median OHIP-14 scores were also increased by the presence of certain oral diseases. Patients had higher OHIP-14 scores if they had any of the following; less than 18 sound teeth, more than 5 carious surfaces, symptoms of dry mouth, pain in the previous year and either metallic or unpleasant taste (Table 6-3). Strangely there was only a weak association with the presence of dental sepsis with an increase in the median number of frequent impacts in patients with clinically obvious sepsis ($p=0.039$). The severity of pain experienced, as measured on a scale of one to ten, was strongly correlated with OHIP sum, OHIP count, and OHIP count >2 (0.275-0.576, $p=0.000$).

When periodontal variables were examined there were only two statistically significant correlations. OHIP sum and OHIP count >1 were weakly correlated with bleeding on probing (Spearman $\rho=0.15$ $p=0.01$, 0.16 $p=0.006$) and plaque scores were correlated with OHIP sum and OHIP count >1 (Spearman $\rho=0.15$ $p=0.01$, 0.18 $p=0.002$). There were no other statistically significant correlations with the periodontal parameters although when the scores were compared between patients with and without severe periodontitis, as defined by the presence of CAL ≥ 5 mm, the median OHIP count was higher in those patients with severe periodontitis. This difference was not however statistically significant ($p=0.26$).

The caries related variables were positively correlated with the OHIP scores. OHIP sum, OHIP count, OHIP count >1 and OHIP count >2 were significantly correlated with DMFS (Spearman rho= 0.23 p<0.001, 0.222 p<0.001, 0.252 p<0.001, 0.216 p<0.001), DMFT (Spearman rho= 0.24 p<0.001, 0.231 p<0.001, 0.248 p<0.001, 0.223 p<0.001), the number of missing teeth (0.164 p=0.005, 0.152 p=0.009, 0.188 p<0.001, 0.141 p=0.016) and the number of filled surfaces (0.176 p=0.003, 0.169 p=0.004, 0.189 p=0.001, 0.224 p<0.001). OHIP-14 scores were significantly higher in patients who had answered positively to any of the xerostomia questions as shown in Table 6-3. In addition, there were differences in the distribution of OHIP sum, OHIP count, and OHIP count >2 in patients who reported having metallic or unpleasant taste.

OHIP-14 scores were not significantly affected by age, gender, SIMD, dental attendance, alcohol intake, education or saliva level.

Table 6-3 Relationship between OHIP outcomes and dental variables

	OHIP SUM	OHIP count	OHIP count>2	Direction
Infrequent tooth cleaning	0.018*	0.017*	0.007**	↑
Difficulty paying for dental treatment	0.000**	0.000**	0.000**	↑
Dental anxiety	0.000**	0.000**	0.069	↑
Access to own transport	0.002**	0.001**	0.36	↑
Pain in the last year	0.000**	0.000**	0.000**	↑
Dental sepsis	0.267	0.066	0.039	↑
<18 sound teeth	0.006**	0.005**	0.009**	↑
>5 decayed surfaces	0.022*	0.024*	0.87	↑
Dry mouth	0.029*	0.015*	0.007**	↑
Fox's positive	0.002**	0.002**	0.011*	↑
Problems swallowing	0.001**	0.001**	0.008**	↑
Needs liquids for swallowing	0.000**	0.000**	0.047*	↑
Woken with a dry mouth	0.002**	0.002**	0.009**	↑
Unpleasant taste	0.000**	0.000**	0.000**	↑
Metallic taste	0.000**	0.000**	0.000**	↑

- p<0.05, ** p<0.01 Mann Whitney U test. The OHIP scores were statistically significantly higher for those subjects in the above categories.

Table 6-4 Correlations between OHIP outcomes and dental variables

	Spearman's rho	OHIP SUM	OHIP count	OHIP count >1	OHIP count >2
DMFT	Corr Coeff	.240**	.231**	.248**	.223**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000
	N	290	290	290	290
DMFS	Corr Coeff	.234**	.222**	.252**	.216**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000
	N	290	290	290	290
Number of decayed teeth	Corr Coeff	0.105	0.109	0.096	-0.043
	Sig. (2-tailed)	0.073	0.063	0.101	0.464
	N	290	290	290	290
Number of missing teeth	Corr Coeff	.164**	.152**	.188**	.141*
	Sig. (2-tailed)	0.005	0.009	0.001	0.016
	N	290	290	290	290
Number of filled teeth	Corr Coeff	.151**	.143*	.165**	.220**
	Sig. (2-tailed)	0.01	0.015	0.005	0
	N	290	290	290	290
Number of decayed teeth	Corr Coeff	0.113	0.11	0.112	-0.022
	Sig. (2-tailed)	0.055	0.062	0.056	0.704
	N	290	290	290	290
Number of filled surfaces	Corr Coeff	.176**	.169**	.189**	.224**
	Sig. (2-tailed)	0.003	0.004	0.001	0.000
	N	290	290	290	290
Bleeding	Corr Coeff	.150*	.136*	.160**	0.034
	Sig. (2-tailed)	0.01	0.02	0.006	0.567
	N	294	294	294	292
Plaque	Corr Coeff	.151**	.136*	.180**	0.061
	Sig. (2-tailed)	0.01	0.02	0.002	0.299
	N	293	293	293	291
Severity of dental pain	Corr Coeff	.573**	.576**	.455**	.275**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000
	N	296	296	292	294

*p<0.05, **p<0.01 Spearman Rank Correlation.

There was no relationship between summary OHIP measures and diabetic status (Table 6-5). OHIP sum and OHIP count scores were in fact slightly lower in the well-controlled diabetic group than in the control group (Figures 6-1 - 6-4).

**Table 6-5 Median OHIP score by diabetic status. WCD
HbA1c \leq 7.5%, PCD HbA1c $>$ 7.5%**

Group (n)	NDS (108)	T1DM (184)	WCD (27)	PCD (157)	P value
OHIP Sum Median(IQR)	2 (1-7)	2 (0-7)	2 (0-7)	2 (0-7)	0.348
OHIP Count Median(IQR)	2 (1-4)	1.5 (0-4)	1 (0-4)	2 (0-4)	0.282
OHIP Count>1 Median(IQR)	1 (0-2)	0 (0-2)	0 (0-2)	0 (0-2)	0.693
OHIP Count>2 Median(IQR)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.111

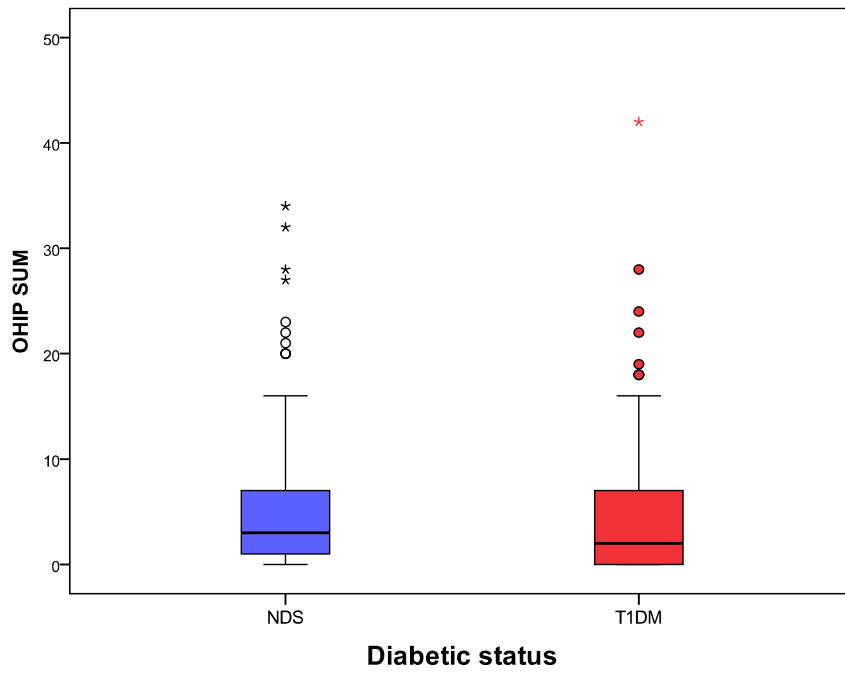


Figure 6-1 Box plot showing median and interquartile range of OHIP sum by diabetic status

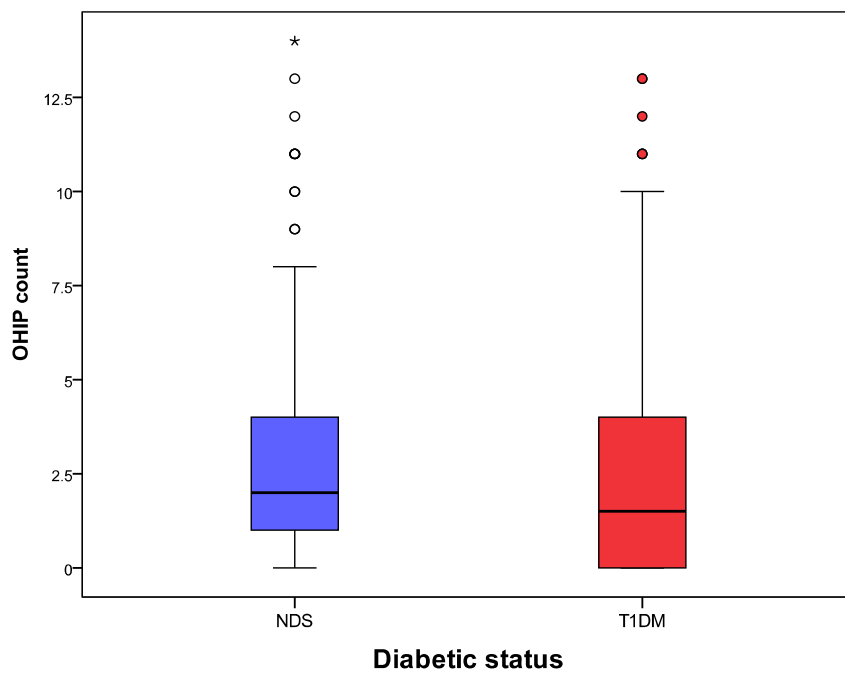


Figure 6-2 Box plot showing median and interquartile range of OHIP count by diabetic status

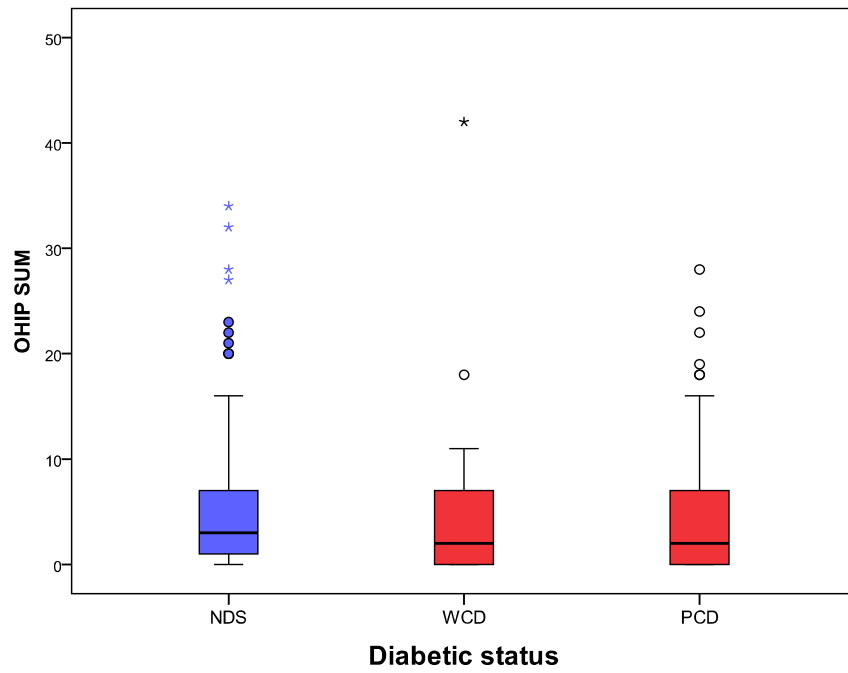


Figure 6-3 Box plot showing median and interquartile range of OHIP Sum by diabetic status (NDS, WCD HbA1c \leq 7.5%, PCD HbA1c $>$ 7.5%)

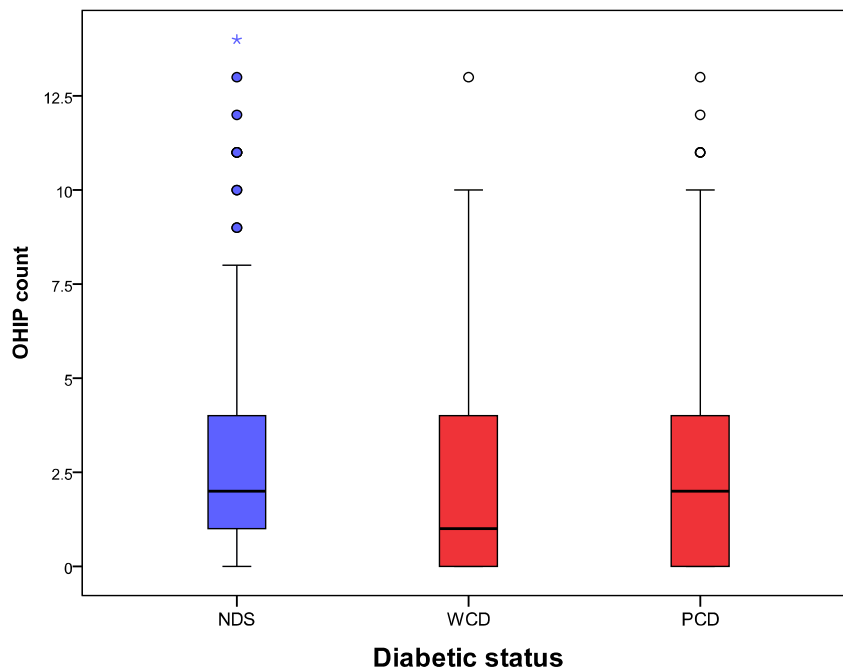


Figure 6-4 Box plot showing median and inter-quartile range of OHIP count by diabetic status (NDS, WCD HbA1c \leq 7.5%, PCD HbA1c $>$ 7.5%)

Table 6-6 Frequency of OHIP-14 scored cross-tabulated against and diabetic status

	Non Diabetic Subjects N=108	Diabetic Well controlled N=27	Poorly controlled diabetes N=157	P value
OHIP Sum (%)				
0	21.8	48.1	30.2	nsd
1	18.2	0	13.8	
2	8.2	7.4	10.1	
3	8.2	7.4	9.4	
4	2.7	7.4	3.8	
5	7.3	0	1.3	
>5	33.6	29.6	31.4	
OHIP count (%)				
0	21.8	48.1	30.2	nsd
1	23.6	3.7	18.9	
2	15.5	18.5	11.9	
3	2.7	0.0	8.8	
>3	36.4	29.6	30.2	
OHIP count >1 (%)				
0	48.2	51.9	55.3	nsd
1	20.0	11.1	14.5	
2	10.0	18.5	8.8	
3	8.2	0	5.7	
>3	13.6	18.5	15.7	
OHIP >2 (%)				
0	80.9	81.5	89.2	nsd
1	8.2	11.1	7.0	
2	6.4	3.7	0.6	
3	0.9	0	2.5	
>3	3.6	0.0	0.6	

Chi squared test - no significant differences

Because the OHIP data was skewed towards zero we also cross-tabulated OHIPSum scores for 0, 1, 2, 3, 4, 5 and >5. There were no differences in the proportion of NDS, WCD or PCD in any category (Table 6-6). The same analysis was performed for OHIP count, OHIP count >1 and OHIP count >2. This did not show any other differences between NDS, WCD or PCD in the proportion of subjects in any level of oral health impact (Table 6-6).

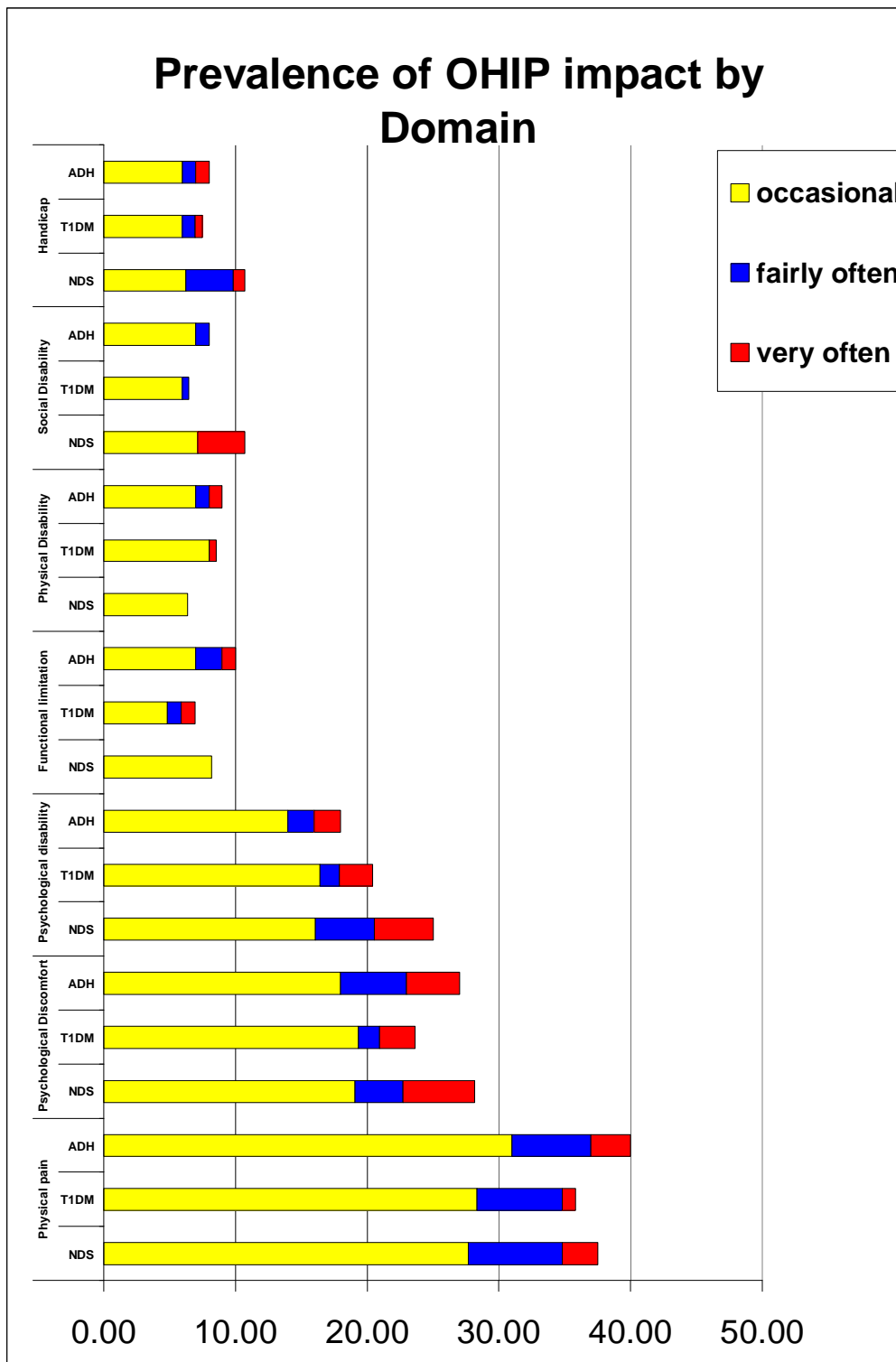


Figure 6-5 Bar chart showing the frequency of oral health impacts by domain comparing T1DM with NDS and with data extracted from the Adult Dental Health (ADH) survey representing the United Kingdom population (Nuttal et al., 2001)

Table 6-7 Frequency of oral health impacts by domain comparing T1DM with NDS and with data extracted from the Adult Dental Health survey representing the United Kingdom population (Nuttal et al., 2001)

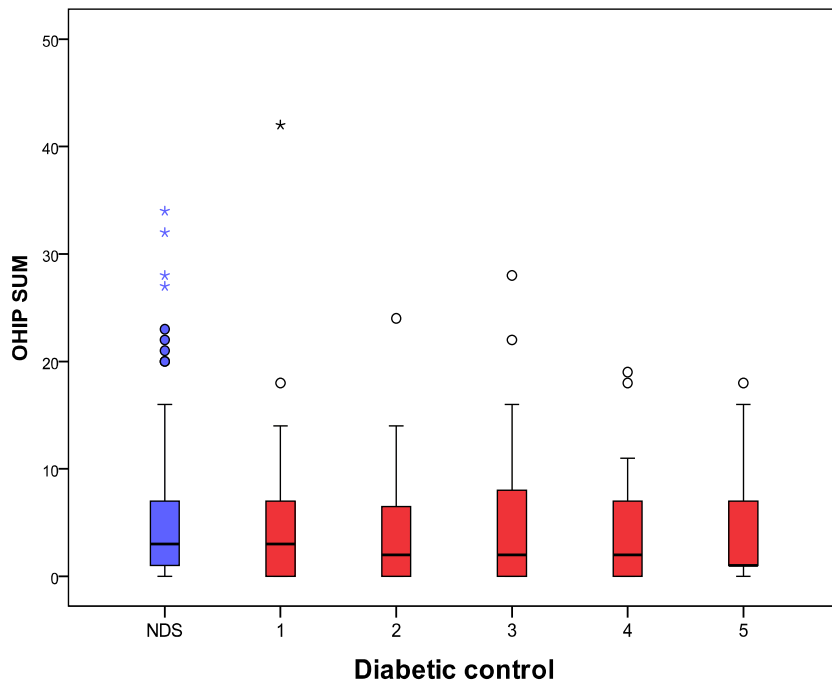
Proportion experiencing problem at threshold of occasional and above^a (%)	NDS (n=110)	T1DM (n=184)	ADH 1998 (n=6204)	P value NDS vs ADH	P value ADH vs T1DM
Physical pain (%)	37.5	35.8	40.0	0.485	0.01*
Psychological Discomfort (%)	28.2	23.7	27.0	0.829	0.356
Psychological disability (%)	25.0	20.4	18.0	0.063	0.403
Functional limitation (%)	8.2	6.9	10.0	0.631	0.213
Physical Disability (%)	6.3	8.6	9.0	0.402	1.0
Social Disability (%)	10.7	6.5	8.0	0.296	0.433
Handicap (%)	10.7	7.5	8.0	0.476	1.0

^aDomain proportion represents those who report an impact for at least one of the questions representing the domain

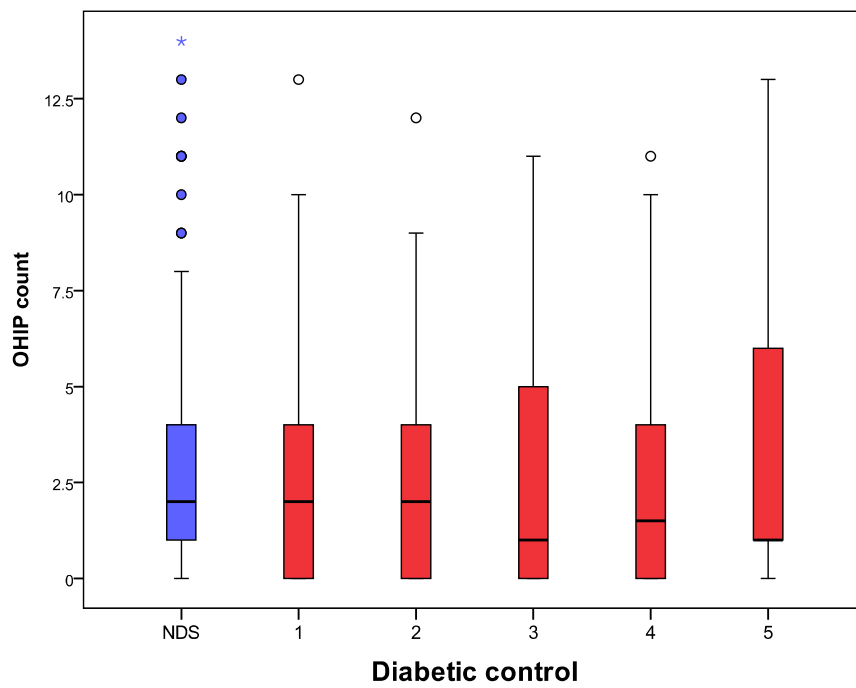
Both the NDS and the T1DM patients were also compared with the data extracted from the ADH survey (1998) (Nuttal et al., 2001). 37.5% of all NDS experienced dental pain at least occasionally in the previous year while the same frequency of discomfort affected 35.8% and 40% of T1DM and ADH respondents respectively NDS v ADH ($p = 0.485$; ADH v T1DM $p = 0.01$). ADH respondents therefore reported more frequent physical pain than T1DM subjects.

Subgroup analysis separating the diabetic subjects into five groups by average HbA1c did not reveal any significant trends relating either OHIP sum or count scores to the level of glycaemic control (Figure 6-6).

Figure 6-7 represents the OHIP domains by the three groups NDS, WCD and PCD. There was a lower prevalence of physical disability in the WCDs compared with the (NDS 19%, WCD 11%, PCD 22%) however this was not statistically significant. Similarly, there was a slightly lower prevalence of psychological discomfort among the WCDs compared with the other groups (NDS 43%, WCD 26%, PCD 35%) again this was not statistically significant. WCD had a slightly higher prevalence of handicap (NDS 16%, WCD 24%, PCD 18%). There were no significant differences in any of the OHIP domains.



a.



b.

Figure 6-6 Box plots comparing the distribution of (a) OHIP Sum and (b) OHIP count median and inter-quartile range between NDS and 5 quintiles of HbA1c

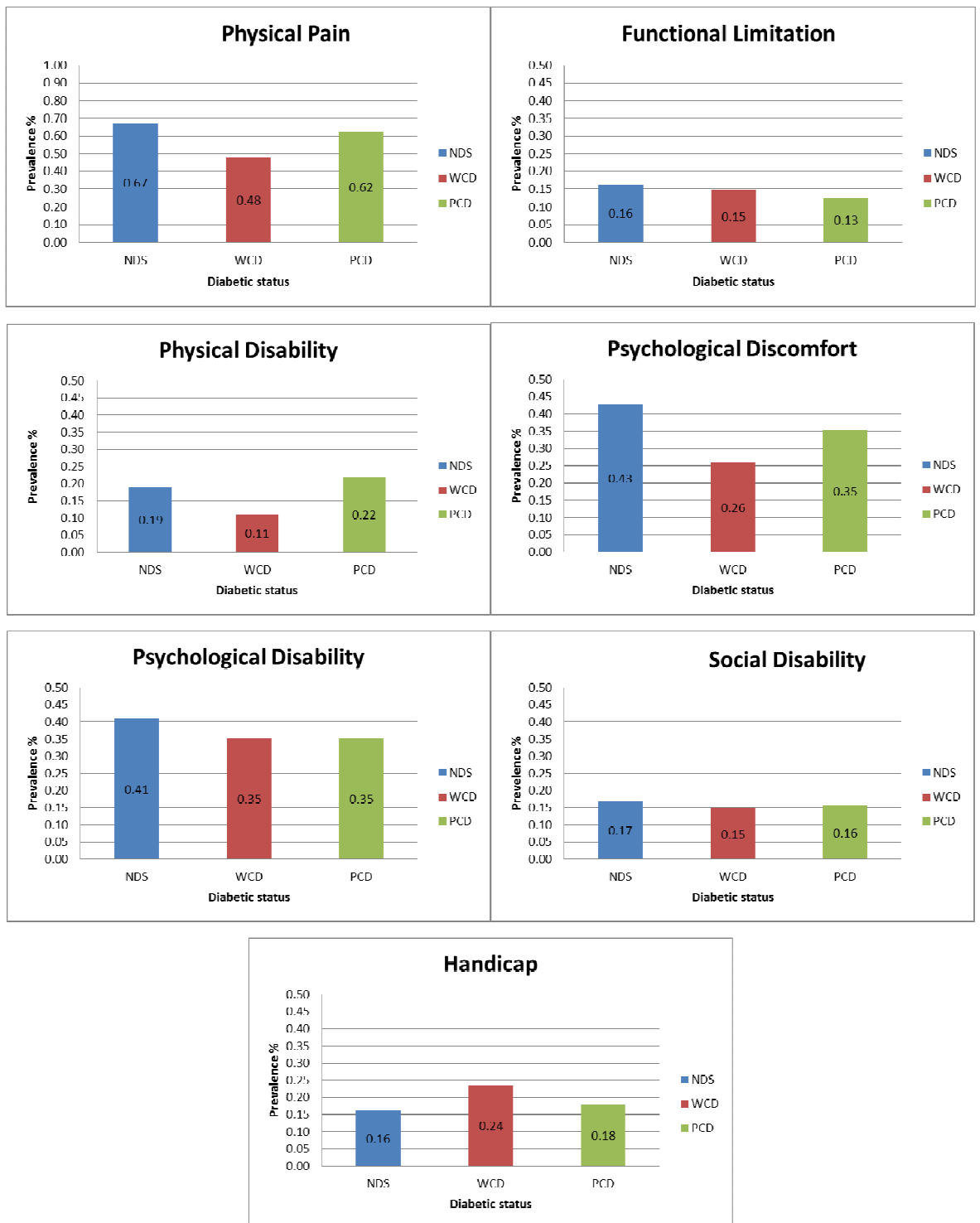


Figure 6-7 Bar charts comparing the proportion of NDS, WCD (HbA1c ≤ 7.5%) and PCD (HbA1c > 7.5) who experienced at least one impact in each of the seven domains. No significant differences in chi squared test

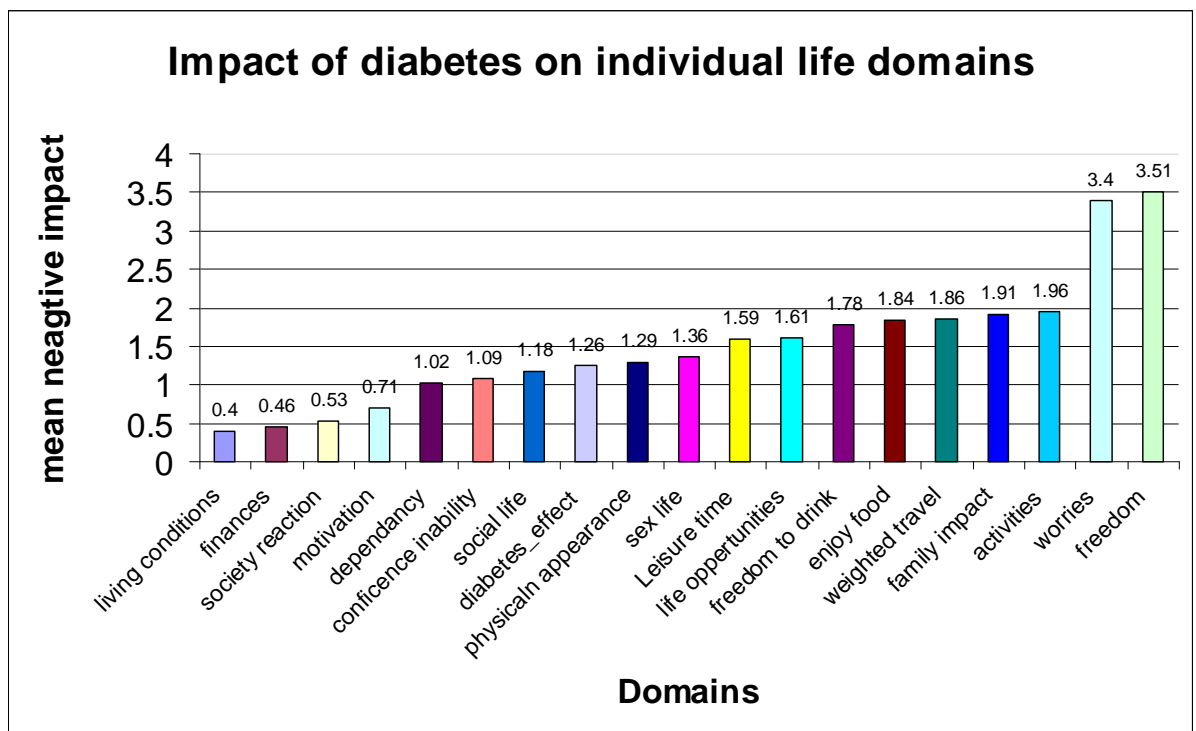


Figure 6-8 Bar chart showing the mean negative impact on different life domains represented in the ADDQOL

The ADDQOL items are arranged in order of increasing negative impact in figure 6-8. They range from the relatively rare items with minimal impact including living conditions and finances (-0.4/-9, -0.41/-9) through to those aspects of life where diabetes has a significant impact. The higher scoring life aspects were the impact on diabetes of their worries and their freedom (-3.4/-9, -3.51/-9).

The ADDQOL scores were correlated with the demographic data, the OHIP scores, and the clinical outcomes.

Table 6-8 Table of bivariate correlation between OHIP outcomes and summary measures of ADDQOL (Spearman correlation)

		OHIP SUM	OHIP count	OHIP count >1	OHIP count >2
QoL	Corr Coeff	-0.13	-0.139	-0.069	0.032
	Sig. (2-tailed)	0.089	0.07	0.37	0.674
	N	172	172	172	172
AWI	Corr Coeff	-.307**	-.298**	-.309**	-.179*
	Sig. (2-tailed)	0.000	0.000	0.000	0.024
	N	158	158	158	159
Diabetes effect	Corr Coeff	-.160*	-.164*	-0.138	-0.091
	Sig. (2-tailed)	0.035	0.031	0.071	0.233
	N	173	173	173	173

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

There were no correlations between QoL and any of the OHIP outcomes. There were highly significant correlations between the average weighted impact of diabetes and OHIP sum, OHIP count and OHIP count $t > 1$ (0.307 $p < 0.000$, 0.298 $p < 0.000$, 0.309 $p < 0.000$). OHIP sum and OHIP count were correlated with the negative impact of diabetes (-0.160 $p = 0.035$, -0.164 $p = 0.031$) however these did not reach significance at the 1% level (Table 6-8).

Table 6-9 shows the correlations between the ADDQOL summary items and the individual domains of the OHIP questionnaire. There were strong correlations between the AWI of diabetes and functional limitation (-0.219 $p = 0.006$), physical pain (-0.245 $p = 0.002$), psychological discomfort (-0.215 $p = 0.007$), psychological disability (-0.308 $p < 0.000$), physical disability (-0.218 $p = 0.006$) and handicap (-0.245 $p = 0.001$).

There were statistically significant correlations between OHIP sum and OHIP count and leisure (-0.26 $p = 0.001$, -0.276 $p < 0.000$), finances (-0.304 $p < 0.000$, -0.308 $p < 0.000$) and worries (0.29 $p < 0.000$, 0.28 $p < 0.000$). OHIP > 1 was also correlated with finances, enjoyment of food, freedom and dependency on others.

Table 6-9 Table showing bivariate correlation coefficients for OHIP domains and summary measures of ADDQOL (Spearman correlation)

		Functional limitation	Physical pain	Psych discomfort	Psych disability	Handicap	Social disability	Physical Disability
QoL	Corr Coeff	-0.059	-0.095	-0.076	-0.126	0.022	-0.07	-0.045
	Sig. (2-tailed)	0.439	0.217	0.324	0.09	0.769	0.352	0.555
	N	172	172	172	181	181	181	172
AWI	Corr Coeff	-0.219**	-0.245**	-0.215**	-0.308**	-0.245**	-0.144	-0.218**
	Sig. (2-tailed)	0.006	0.002	0.007	0.000	0.001	0.063	0.006
	N	158	158	158	167	167	167	158
Diabetes effect	Corr Coeff	-0.122	-0.151*	-0.087	-0.166*	-0.133	-0.047	-0.162*
	Sig. (2-tailed)	0.109	0.048	0.254	0.025	0.072	0.527	0.034
	N	173	173	173	182	182	182	173

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 6-10 shows the correlations between the individual items on the ADDQOL and the OHIP domains. There were statistically significant correlations between “Functional Limitation” and the impact of diabetes on family life (-0.209 $p=0.007$), physical appearance (-0.209 $p=0.006$), motivation (-0.248 $p=0.001$), enjoyment of food (-0.205 $p=0.009$) and finances (-0.205 $p=0.009$). The experience of dental pain was also highly significantly correlated with a negative impact on leisure (-0.278, $p < 0.001$), worries (-0.202 $p = 0.009$) and finance (-0.262 $p = 0.001$). Psychological discomfort was correlated with a negative impact on the social life (-0.215 $p < 0.001$) worries and a negative impact on finance (-0.249 $p < 0.001$). Psychological disability was correlated with a negative impact on leisure (-0.215 $p = 0.004$), worries (-0.312 $p < 0.001$) and finance (-0.328 $p < 0.001$). Physical disability correlated with worries (-0.283 $p < 0.001$). Oral handicap correlated with a negative impact on leisure (-0.218 $p = 0.003$), worries (-0.236 $p = 0.002$), and finance (-0.255 $p = 0.001$).

None of the ADDQOL summary scores correlated with age, gender, average HbA1c, duration, BMI, pack years, alcohol consumption or difficulty paying for dental treatment. SIMD was correlated with the average weighted impact (-0.183 $p=0.019$) and global quality of life (-0.186 $p=0.013$). Access to the patient’s own transport was also correlated with global quality of life (0.237 $p=0.001$) (Table 6-11)

There were also strong correlations between age and impact on sexual and family life (-0.29 $p<0.000$, -0.262 $p<0.000$). Gender was also correlated with impact on sex life (-0.219 $p=0.004$). Duration of diabetes was correlated with negative impacts on freedom to eat and drink (-0.218 $p=0.004$, -0.218 $p=0.004$). BMI was strongly correlated with negative impact of diabetes on physical appearance (-0.257 $p<0.000$). The number of pack years was correlated with the impact of diabetes on subjects motivation (-0.275 $p<0.000$). SIMD was correlated with negative impacts on freedom and a negative impact on the ability to be involved in different activities (-0.217 $p=0.005$, -0.203 $p=0.007$). There were no correlations between any other demographic variables and any other ADDQOL items (Table 6-11).

Table 6-10 Exploratory analysis of correlations between the OHIP domains and the individual ADDQOL items

ADDQOL Items	Functional limitation	Pain	Psychol. discomfort	Psychol. disability	Social disability	Physical disability	Handicap
life opportunities	-0.086	-0.114	-0.065	-0.135	0.115	-0.018	-0.069
Corr Coeff							
Sig. (2-tailed)	0.263	0.138	0.398	0.071	0.124	0.817	0.354
family impact	-0.209**	-0.124	-0.065	-0.127	0.002	-0.09	-0.092
Corr Coeff							
Sig. (2-tailed)	0.007	0.111	0.407	0.095	0.98	0.248	0.228
social life	-0.056	-0.062	0.001	-0.024	0.016	-0.003	0.025
Corr Coeff							
Sig. (2-tailed)	0.531	0.487	0.987	0.789	0.856	0.974	0.778
Sex	-0.121	-0.044	-0.098	-0.134	-0.046	-0.125	-0.122
Corr Coeff							
Sig. (2-tailed)	0.124	0.576	0.216	0.082	0.549	0.113	0.114
Physical	-0.209**	-0.151*	-0.129	-0.178*	-0.088	-0.131	-0.171*
Corr Coeff							
Sig. (2-tailed)	0.006	0.049	0.093	0.017	0.239	0.089	0.022
Activities	-0.143	-0.1	-0.117	-0.173*	-0.065	-0.157*	-0.142
Corr Coeff							
Sig. (2-tailed)	0.061	0.192	0.127	0.02	0.389	0.04	0.057
Leisure	-0.179*	-0.278**	-0.092	-0.215**	-0.071	-0.168*	-0.218**
Corr Coeff							
Sig. (2-tailed)	0.019	<0.001	0.231	0.004	0.344	0.028	0.003
Travel	-0.051	-0.131	-0.078	-0.169*	-0.05	-0.087	-0.126
Corr Coeff							
Sig. (2-tailed)	0.512	0.089	0.31	0.023	0.504	0.26	0.092
Ability	-0.052	-0.138	-0.109	-0.182*	-0.062	-0.094	-0.12
Corr Coeff							
Sig. (2-tailed)	0.502	0.074	0.159	0.014	0.412	0.225	0.109
Motivation	-0.248**	-0.127	0.023	-0.099	-0.044	-0.137	-0.14
Corr Coeff							
Sig. (2-tailed)	0.001	0.099	0.768	0.188	0.556	0.075	0.062
Social	-0.157*	-0.076	-0.215**	-0.185*	-0.004	-0.022	-0.156*
Corr Coeff							
Sig. (2-tailed)	0.046	0.332	0.006	0.015	0.961	0.782	0.042
Worries	-0.054	-0.202**	-0.331**	-0.312**	-0.187*	-0.283**	-0.236**
Corr Coeff							
Sig. (2-tailed)	0.483	0.009	<0.001	<0.001	0.013	<0.001	0.002
Dependence	-0.078	-0.07	-0.174*	-0.142	0.027	-0.131	-0.083
Corr Coeff							
Sig. (2-tailed)	0.328	0.384	0.029	0.068	0.724	0.101	0.284
living conditions	0.005	-0.105	-0.193*	-0.151*	-0.069	-0.196*	-0.184*
Corr Coeff							
Sig. (2-tailed)	0.947	0.186	0.014	0.049	0.372	0.013	0.017
Freedom	-0.160*	-0.172*	-0.113	-0.173*	-0.107	-0.144	-0.08
Corr Coeff							
Sig. (2-tailed)	0.042	0.028	0.151	0.024	0.162	0.068	0.299
enjoy food	-0.205**	-0.132	-0.073	-0.149	-0.193*	-0.151	-0.157*
Corr Coeff							
Sig. (2-tailed)	0.009	0.094	0.354	0.052	0.011	0.056	0.04
freedom drink	-0.106	-0.012	-0.114	-0.212**	-0.165*	-0.149	-0.165*
Corr Coeff							
Sig. (2-tailed)	0.18	0.884	0.149	0.005	0.031	0.059	0.032
Finance	-0.205**	-0.262**	-0.249**	-0.328**	-0.151*	-0.191*	-0.255**
Corr Coeff							
Sig. (2-tailed)	0.009	0.001	0.001	<0.001	0.049	0.015	0.001

** . Correlation is significant at the 0.01 level (2-tailed). * . Correlation is significant at the 0.05 level (2-tailed).

Table 6-11 Table of bivariate correlations between the ADDQOL summary measures and demographic data.

	<i>Spearman's rho</i>	<i>AWI</i>	<i>QoL</i>	<i>Diabetes effect</i>
Age	Correlation Coefficient	-0.111	0.056	-0.071
	Sig. (2-tailed)	0.15	0.447	0.338
	N	170	184	185
Gender	Correlation Coefficient	0.001	-0.008	0.09
	Sig. (2-tailed)	0.994	0.912	0.221
	N	170	184	185
Average HbA1c	Correlation Coefficient	-0.084	-0.138	0.023
	Sig. (2-tailed)	0.277	0.062	0.755
	N	170	184	185
Duration	Correlation Coefficient	0.083	0.088	0.033
	Sig. (2-tailed)	0.283	0.236	0.651
	N	170	184	185
BMI	Correlation Coefficient	-0.104	-0.091	-0.106
	Sig. (2-tailed)	0.175	0.217	0.152
	N	170	184	185
Pack Years	Correlation Coefficient	-0.112	-0.003	-0.103
	Sig. (2-tailed)	0.145	0.965	0.164
	N	170	184	185

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 6-11 cont.

	<i>Spearman's rho</i>	<i>AWI</i>	<i>QoL</i>	<i>Diabetes effect</i>
Alcohol Consumption	Correlation Coefficient	0.076	0.096	0.007
	Sig. (2-tailed)	0.326	0.195	0.93
	N	170	184	185
SIMD	Correlation Coefficient	-.183*	-.186*	-0.13
	Sig. (2-tailed)	0.019	0.013	0.083
	N	164	178	179
Difficulty paying for treatment	Correlation Coefficient	-0.149	-0.131	-0.015
	Sig. (2-tailed)	0.054	0.078	0.844
	N	168	182	183
Educational status	Correlation Coefficient	.175*	0.117	0.103
	Sig. (2-tailed)	0.023	0.116	0.165
	N	168	182	183
Availability of transport.	Correlation Coefficient	0.076	.237**	0.006
	Sig. (2-tailed)	0.326	0.001	0.931
	N	168	182	183

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 6-12 Table of summary ADDQOL measures and oral disease

	QoL			AWI			Diabetes effect		
	Corr Coeff	Sig. (2-tailed)	N	Corr Coeff	Sig. (2-tailed)	N	Corr Coeff	Sig. (2-tailed)	N
Number missing teeth	-0.012	0.868	184	-0.118	0.127	170	-0.095	0.196	185
Number of decayed teeth	0.106	0.155	182	-0.056	0.468	168	-0.031	0.676	183
Number of decayed surfaces	-0.118	0.114	182	0.044	0.575	168	0.082	0.269	183
Decay>1	-0.091	0.222	182	0.085	0.275	168	0.039	0.6	183
Decay>3	-0.126	0.089	182	0.015	0.847	168	0.05	0.506	183
Decay>5	-0.127	0.087	182	0.011	0.885	168	0.055	0.462	183
Bleeding	-.177*	0.017	181	-0.046	0.558	167	0.062	0.403	182
Attach >3mm	0.03	0.685	181	-0.078	0.314	167	0.128	0.084	182
Attach >5mm	-0.123	0.098	181	-0.017	0.829	167	0.068	0.36	182
Sepsis	-0.017	0.819	183	-0.069	0.375	169	0.049	0.513	184
Pain year	-0.09	0.225	182	-.307**	0.000	168	.196**	0.008	183
Pain scale	-0.07	0.351	182	-.270**	0.000	168	-.179*	0.015	183
Altered metallic	-0.087	0.253	173	-.188*	0.018	159	0.071	0.351	174
Altered taste	-0.137	0.071	174	-.221**	0.005	160	0.147	0.052	175
FOX'S yes/no	-.157*	0.034	182	-.195*	0.011	168	-0.14	0.058	183

** Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

The ADDQOL summary items were correlated with signs and symptoms of dental disease. The results of these correlations are summarised in Tables 6-12. The average weighted impact was highly significantly correlated with pain in the last year, severity of pain and altered taste (-0.307 $p < 0.000$, -0.270 $p < 0.000$, -0.221 $p = 0.005$). The effect of diabetes on quality of life was weakly correlated with pain experience (-0.196 $P = 0.08$).

The number of missing teeth was weakly correlated with the impact of diabetes on motivation (-0.192 $p = 0.009$). There were no other statistically significant correlations between ADDQOL items and dental caries. Bleeding on probing was correlated with the impact of diabetes on finances (-0.233 $p = 0.002$) and the number of sites with attachment loss of ≥ 3 mm was also correlated with those patients who felt that diabetes had a negative impact on their family lives (-0.251 $p = 0.001$). Three of the individual items (finance -0.274 $p < 0.000$, freedom -0.256 $p = 0.001$, and leisure -0.292 $p < 0.000$) were significantly correlated with pain in the last year. There were also significant correlations between pain scale and finance (-0.251 $p = 0.001$), leisure (-0.312 $p < 0.000$), worries (-0.204 $p = 0.006$),

ability (-0.192 $p=0.01$) and motivation (-0.215 $p=0.004$). Altered taste was correlated with impact on family life (-0.261 $p=0.001$) and finance (-0.218 $p=0.005$) as well as dependence (-0.211 $p=0.008$), physical appearance (-0.272 $p<0.000$) and motivation (-0.267 $p<0.000$). The Fox's xerostomia index was also correlated with impact on family (-0.195 $p=0.01$) and leisure (-0.241 $p=0.001$).

The Global quality of life measure was positively correlated at the 5% level with SIMD ($p=0.013$) as well as bleeding on probing ($p=0.017$), the Fox's index ($p=0.034$) and frequency of tooth cleaning ($p = 0.028$). The score for the impact of diabetes on QOL correlated with mean clinical attachment level ($p=0.012$), furcation involvement ($p=0.028$) and mobility ($p = 0.036$). The average weighted impact was correlated with SIMD ($p=0.019$), education ($p = 0.023$), the percentage plaque score ($p=0.01$), and the Fox's index ($p=0.011$).

6.4 Discussion

The OHIP scores were not increased in the T1DM groups despite the fact that they had generally higher levels of periodontal disease, reduced salivary flow rate, increased density of candidal colonisation and slightly higher levels of caries. The lack of difference in OHIP scores could be accounted for in a number of possible ways. One possibility is that OHRQOL is worse in T1DM but that the NDS were not representative of the general population with regard to OHRQOL. Secondly, it could be that OHIP is not a sensitive enough instrument to detect differences in OHRQOL caused by periodontal disease, caries and xerostomia. Thirdly, it is possible that T1DM patients experience no difference in impact on quality of life as a result of higher levels of oral disease.

6.4.1 Poor control selection

With regard to the first possibility, the control group for some reason could have had particularly poor oral health or disproportionately poorer OHRQOL. In order to investigate this possibility, the results of the ADH survey OHIP data were compared with both the control group and the diabetic group. This showed that the pattern of impacts was similar and the overall differences in scores were not statistically significant. Therefore poor control selection cannot be the explanation for the lack of differences.

6.4.2 Poor tool

The second hypothesis is a feasible explanation. In the preliminary analysis, it was shown that OHIP summary measures correlated significantly with oral symptoms of disease, pain, dry mouth, some but not all measures of caries. However, there was little relationship between OHIP and periodontal parameters. Many of the studies validating the OHIP and

OHIP-14 have used correlations between oral disease and OHIP summary measures. These correlations can be highly statistically significant despite only being <0.1 if there is enough data. Although the p values were extremely low, the correlations were quite weak. Aslund reported that there was a strong correlation between B.P.E. and OHRQOL however in fact the correlation was only 0.295. This means that there is a significant part of this apparent relationship that cannot be explained by the B.P.E. score. In most studies it is acknowledged that the correlations are low to moderate (Robinson et al., 2003). Even the correlation with dental pain was only moderate at 0.43-0.49 in this study. It is therefore to be expected that less obvious dental disease would have less of an impact and therefore the correlations would be correspondingly poorer.

It is possible that the OHIP-14 is not a sensitive enough measure of the signs of oral disease such as mild to moderate periodontitis and dental caries detected during a detailed clinical examination. In the study reported by Drummond-Santana and co-workers (2007) they conclude that OHIP scores are increased in patients with periodontitis. 21.4% of these patients had advanced periodontal disease which was likely to be impacting on their oral health. Cunha-Cruz showed a similar increase in oral health impact due to periodontal disease however again this study was extremely large and the levels of periodontal disease were severe (Cunha-Cruz et al., 2007). Nine teeth with CPD > 5 mm or three teeth with CPD ≥ 8 mm were statistically significantly associated with the oral health impact. Busato and co-workers (2009) showed that OHRQOL was associated with xerostomia and hyposalivation. This association was also confirmed in this study but did not lead to higher overall OHIP scores for patients with T1DM.

6.4.2.1 Other explanatory variables

It may also be that there are other factors that determine an individual's response to the OHIP questions for example social, or psychological factors as well as dental factors that may have an overriding impact over the presence of moderate asymptomatic periodontal disease. It has been shown in this population that there is a statistically significant difference in OHIP outcomes with dental disease although this was not as great as might have been expected. All tests were in the expected direction but the differences were minimal and not statistically significant. This is perhaps due to the fact that the OHIP is designed to measure the impact of disease on an individual's OHRQOL, therefore diseases that do not cause significant problems will have less of an impact. While correlations with pain, missing teeth or caries confirm the validity of the tool as these change in the presence of oral disease, this does not mean that this is all that is being measured. Caries will only impact on an individual's quality of life if it gives them halitosis, pulpitis or a periapical abscess. Similarly, periodontal disease will only impact on an individual's OHRQOL if or

when they develop obvious bleeding, halitosis, mobility, pain or when it leads to tooth loss. Both pain and missing teeth, the ultimate outcome of caries and periodontal disease, were strongly correlated with OHIP summary measures. It is worth noting that bleeding on probing was more closely correlated to OHIP outcomes than the presence of severe periodontitis as defined by 1 site with ≥ 6 mm CAL. It is possible that patients would be more aware of multiple bleeding sites during brushing than the asymptomatic presence of one site that has ≥ 6 mm CAL. The presence of the signs of the disease may not be noted by the patient, but rather it is the symptoms appearing later that ultimately impact on OHRQOL.

A longitudinal study of T1DM patients with a cohort of control subjects whose OHIP-14 score was measured over a period of time would be required to demonstrate whether the OHIP score would change as the sequelae of caries and periodontal disease manifest themselves as the disease progresses with early tooth loss and subsequent loss of function. Studies that have shown a relationship between OHRQOL and periodontal disease have been in patients who have severe periodontitis and have been seen in a specialist centre for their periodontal treatment. It is possible that there is a difference between such patients and those who were recruited into the current study. Patients who had more severe disease including mobility and tooth loss would be more likely to report an impact on their OHRQOL while those with less severe disease would be less likely to do so. In addition, the impact of diagnosed disease, the implications and long term sequelae of which have been explained to the patient, would likely be different from those with undiagnosed disease. If an individual is aware of bleeding on brushing, but does not know that they may lose their teeth as a result of this, then the impact of this symptom on the individual will be different from on a recently well informed patient. The patients in the study by Needleman had been referred by their general dental practitioners to a specialist in periodontology with a view to receiving periodontal care. The private referral process is necessarily biased toward the recruitment of subjects who are more affluent and this will, in turn, affect dental awareness and motivation. The patient's knowledge of the disease process, the severity of disease and the socioeconomic status of these patients mean that the subjects in these studies may not be comparable with the general population (Needleman et al., 2004).

6.4.3 Altered perception of oral health impacts

The third explanation is that patients with diabetes genuinely do not suffer from additional impacts on their OHRQOL for other reasons. It may be that diabetic patients do not perceive that their oral health is worse, even though it can be shown to be so. One possibility is that T1DM patients are less concerned about their oral health due to the fact

that they have a long term chronic condition that threatens their eyesight, their kidneys and their heart and it may be that this affects their outlook on oral health. It is interesting that this study is the second to show that the OHIP is not increased in patients with diabetes. The study in Ireland was on only a small number of T1DM patients and there were no detailed chartings carried out, although they were obviously a dentally neglected group. 34% of the patients had not seen a dentist in 5 years and just over 40% of them had seen a dentist in the previous years. It is probably safe to assume that they would have had some dental disease and yet their OHIP-20 scores were lower than those reported by a population attending the local dental school. It could however, also be argued that patients attending a dental school may not be dentally fit either and so we cannot draw any firm conclusions from this study based on the information available (Allen et al., 2008).

When the diabetic patients were asked about their general QOL only 4 out of the whole diabetic group (182) stated that their quality of life was bad or very bad. It seems that despite having a significant chronic disease the large majority of diabetic patients do not perceive this as having a negative impact on their quality of life. It is one of the aims of diabetes management that patients are not made to feel that they are limited in their ambitions and lifestyle by their diabetic condition. It is possible that in the same group poorer oral health may have a similarly insignificant effect.

The ADDQOL showed that diabetes impacts on an individual's quality of life in different ways for different life domains. The areas of life that were least affected by diabetes were living conditions, finances and society reaction. Patients did not feel that diabetes had a significant impact on their finances or their living condition, which indicates that patients do not feel materially disadvantaged by their condition. The fact that T1DM patients do not feel stigmatised by their condition is a good thing for them and for society. The areas of life where diabetes has the greatest negative impact are in "worries about life" and "freedom to eat as they pleased." These data are only exploratory and no firm conclusions can be drawn from this study due to the number of comparisons and the relatively low sample size which lacked statistical power. The individual domains and the AWI are all negative indicating that diabetes has a significant negative impact on the life of most T1DM.

The question was whether it could be shown that there was any relationship between the summary measures for the ADDQOL and OHIP scores and dental disease. The aim was to identify whether poor OHRQOL and poor oral health could impact on diabetes specific or general quality of life measures. The average weighted impact of diabetes on an individual's quality of life was correlated with the OHIP sum and count measures. This was highly significant ($p < 0.001$) although the correlation coefficient was only 0.307 and

0.298. This could indicate that patients who have worse overall perception of their OHRQOL also have a lower perception of their diabetes specific quality of life. In addition the AWI was also significantly correlated with functional limitation, physical pain, psychological discomfort, psychological disability, handicap and physical disability. These correlations were only 0.218-0.308. In order to assess whether this relationship was caused by dental disease, bivariate correlations were calculated between the AWI and dental signs and symptoms. There was no strong relationship between caries or periodontal disease although there was a correlation between pain and altered taste and AWI. It is therefore possible that OHIP and AWI scores are not related directly through the presence of dental disease but may be influenced by the underlying psychological characteristics of the patients. There are no other studies to date that have investigated the possibility of a relationship between global, diabetes specific and oral health related quality of life measures.

6.5 Conclusions

The oral health related quality of life of patients with T1DM does not seem to be worse than NDS. This could be seen as a positive finding on its own however the lack of correlation between either oral disease or diabetes and OHIP-14 scores may give some cause for concern. The mechanism by which T1DM patients who have slightly more dental disease have lower OHIP scores is unknown. Further studies using qualitative methods for example focussed groups or structured interviews could be helpful in exploring this relationship. OHIP measures were higher with increased ADDQOL AWI. This is unlikely to reflect a causal relationship but may represent a shared psychological characteristic. This study has generated a lot of data surrounding the OHRQOL of T1DM as well as their Diabetes specific QOL. The relationships that have been discovered in this exploratory analysis could form the basis of a further study to confirm these initial findings.

7 General conclusions

7.1 Conclusions

This was the first study of its kind to examine the oral and dental health of non-smoking T1DM adults. The conclusions from the clinical data support the view that patients with T1DM should be targeted with preventative oral and dental health advice. Patients with T1DM had a greater extent and severity of periodontitis. There was also evidence that the prevalence was increased as well although due to limitations in sample size this effect was not statistically significant. There was little difference in the dental hard tissues of patients with T1DM and NDS. Candidal density, reduced salivary flow rates and symptoms of xerostomia were higher in T1DM. T1DM subjects did not have any greater frequency or severity of oral health impacts on their quality of life as measured using the OHIP-14. The study examining the bone biochemistry in periodontitis and T1DM showed that the ratio of RANKL:OPG was reduced. This would tend to suggest that excessive bone loss is less of an issue in diabetes. Reduced osteocalcin in T1DM suggests that T1DM patients may have a reduced capacity for bone healing and regeneration and this uncoupling may lead to net bone loss in response to bacterial stimulation.

Encouragingly, the prevalence of periodontitis was lower in well controlled diabetic subjects suggesting that the effect of T1DM on the oral cavity can be ameliorated by good glycaemic control even though logistic regression analysis did not show a linear relationship. It is important that health professionals work together in order to prevent and manage the oral complications of T1DM in the same way that there are preventive and screening programmes for other diabetic complications. These findings highlight the need for incorporation of oral health education into T1DM care pathways, particularly since the majority of T1DM patients both in this study and in the United Kingdom generally have poor glycaemic control and knowledge of the link between diabetes and oral diseases is low. Early screening and intervention for periodontal disease at diagnosis of diabetes, is of particular importance. In addition, dentists and dental care professionals should be informed that patients with poor control of their diabetes have worse periodontitis. The importance of contacting medical colleagues when caring for T1DM patients should be stressed because of the bi-directional relationship between general and oral health in T1DM.

7.2 Further work

Future studies are required to confirm the effect of periodontal disease including gingivitis and periodontitis on glycaemic control in patients suffering from T1DM. The bi-directional relationship between T1DM and oral health should be investigated to understand whether the oral cavity is a possible source of systemic inflammatory burden leading to poor glycaemic control. Studies are also required to understand the mechanism of the increased risk of oral complications in diabetic patients.

7.2.1 Pathogenesis and treatment of diabetes associated periodontitis

The impact of T1DM on poor wound and bone healing could also be an avenue of interesting research in the light of the findings presented in chapter 4. Other areas which require further work include the hyper-inflammatory theory; the possibility exists that T1DM patients are more prone to inflammation and develop an inappropriate immunoinflammatory response to dental plaque (Salvi et al., 2010). The mechanisms behind the altered innate and adaptive immune response including toll-like receptor activation and host defence peptides as well as defects in the adaptive immune response are all possible contributing factors to T1DM susceptibility. The role of TLRs in diabetes and periodontal disease could be further investigated with a view to exploring whether this is one of the underlying explanatory mechanisms.

7.2.2 Pathogenesis of diabetes associated oral candidosis

In addition micro-biological investigation is warranted into the virulence factors possessed by species of *C albicans* found in T1DM and the mechanism by which their increased density of colonisation is achieved. We have shown in a small study that *C. albicans* species taken from the T1DM population have different virulence factors. Biofilm formation was significantly increased in the PCD group compared to NDS and BCD (better controlled diabetic) groups ($P < 0.05$). Both haemolysin and proteinase levels were significantly greater in the diabetes groups than in the healthy control group ($P < 0.05$) (Rajendran et al., 2010). Further investigation of these virulence factors in a larger study could confirm or refute these findings.

7.2.3 Oral health education and improvement

Further research to investigate the most appropriate delivery of oral health messages and to audit and evaluate the introduction of services should be carried out to ensure that this is done with maximum efficiency. Education and training of individuals involved in

providing care for patients with T1DM should be instigated. The implication of these public health and targeting measures should be monitored through prospective studies to demonstrate the health benefits achieved in providing this preventive and therapeutic approach to the oral complications of T1DM.

7.2.4 Measuring oral health impact

The lack of impact of the oral complications of T1DM on oral health related quality of life measures is interesting. It is increasingly important for researchers, clinicians and public policy makers to be able to measure quality of life and patient centred outcomes as valid forms of outcome. What matters to the patient must feed into the way in which they are treated and the shared goals of treatment. This study is the second in diabetic subjects to show that the OHIP questionnaire outcomes are not higher in diabetic subjects despite the evidence that they have more oral disease. Further investigation of this phenomenon could include qualitative research methods including focus groups and structured interviews. Differences in psychosocial behaviour and perception of disease due to prolonged exposure to a serious chronic condition may account for the differences or targeting questionnaires in a qualitative fashion may be able to explain the reasons why T1DM patients do not suffer poorer levels of oral health related quality of life as a result of their increased prevalence of disease.

One possible explanation for the fact that individuals suffering from periodontal disease and T1DM did not report poorer oral health related quality of life maybe due to the fact that current indexes do not record the impact of periodontal disease particularly well. The development of measures specifically designed to record the impact of periodontal disease on oral health related quality of life may be required. Future work should involve focus groups, input from professionals and from subjects suffering from periodontitis in order to develop a valid tool that reliably measures differences in OHRQOL between groups and changes with treatment.

There is a need for on-going research into the oral complications of T1DM. As the number of people suffering from this disease increases in the coming years the oral health impact both economically and on individuals will continue to increase. Methods to ameliorate this effect and research to inform our understanding of the pathogenesis are required in order to understand, plan for, prevent and treat the oral complications of T1DM in the future.

Appendices

Appendix 1 letter confirming ethical approval for study

North Glasgow University Hospitals
Division

Glasgow Royal Infirmary LREC (2)
4th floor, Walton Building
Glasgow Royal Infirmary
84 Castle Street
GLASGOW
G4 0SF



Telephone: 0141 211 4020
Facsimile: 0141 232 0752

22 September 2005

Dr Penny Hodge
Clinical Lecturer/ Hon. Staff Grade
Glasgow Dental Hospital and School
378 Sauchiehall Street
Glasgow
G2 3JZ

Dear Dr Hodge

Full title of study: An investigation of the prevalence of chronic periodontitis and other oral complications in non smoking patients with type 1 diabetes mellitus
REC reference number: 05/S0705/70

Thank you for your letter of 09 September 2005, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Application	1	05 August 2005
Investigator CV		20 June 2005
Protocol	3	16 June 2005
Covering Letter		04 August 2005
Summary/Synopsis Sheet 1 Diabetic Patients	5	09 September



Appendix 2 List of amendments and approved documentation

05/S0705/70

Page 2

Summary/Synopsis Sheet 2 Non-Diabetic Patients	5	2005 09 September 2005
GP/Consultant Information Sheets	2 letters - Version 1	16 June 2005
Participant Information Sheet	5	09 September 2005
Participant Consent Form	4	09 September 2005
Response to Request for Further Information		09 September 2005
Letter from CSO - referee reports		18 October 2004
Summary Sheet 1 - Diabetic Patients	4	30 June 2005
Summary Sheet 2 - Non-Diabetic Patients	4	30 June 2005
Medical History Questionnaire	1	10 May 2005
ADDQoI		
Oral Health Questionnaire	2	26 May 2005

Research governance approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final research governance approval from the R&D Department for the relevant NHS care organisation.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

05/S0705/70	Please quote this number on all correspondence
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With the Committee's best wishes for the success of this project

Yours sincerely

R Gallacher

Dr Malcolm Booth
Chair

Email: rose.gallacher@northglasgow.scot.nhs.uk

Enclosures:

Standard approval conditions
Site approval form

Copy to: Dr Caroline Watson
*North Glasgow University Hospitals Division
Research & Development Manager
4th Floor Walton Building,
Glasgow Royal Infirmary
84 Castle Street
Glasgow G4 0SF

SF1 list of approved sites

Appendix 3 Confirmation of approval for recruitment from Glasgow Royal Infirmary

Glasgow Royal Infirmary LREC (2)				
LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION				
For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.				
REC reference number:	Issue number:	Date of issue:		
C5/S0705/70	1	22 September 2005		
Chief Investigator:	Dr Penny Hodge			
Full title of study:	An investigation of the prevalence of chronic periodontitis and other oral complications in non smoking patients with type 1 diabetes mellitus			
This study was given a favourable ethical opinion by Glasgow Royal Infirmary LREC (2) on 15 September 2005. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.				
Principal Investigator	Post	Research site	Site assessor	Date of favourable opinion for this site
Dr Kenneth Paterson	Consultant Physician and Hon Clin Senior Lecturer	Glasgow Royal Infirmary	Glasgow Royal Infirmary LREC (2)	22/09/2005
Approved by the Chair on behalf of the REC: <i>R Gellacher</i> (Signature of Chair/Administrator) (delete as applicable) <i>ROSE GALLACHER</i> (Name)				

(1) The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.

Appendix 4 Oral Health Questionnaire

1. Are you registered with a general dental practitioner? Yes No

2. If the answer to question 1 is "Yes" how often do you attend your general dental practitioner?

At least once a year Occasionally Only when having trouble with your teeth

3. What do you do if you have a dental emergency?

—

4. Are you anxious about visiting the dentist? Yes No

5. Would you have difficulty paying for dental services? Yes No

6. What is the highest level of education you have received?

No formal education Primary school Secondary school University or college

7. Many people own cars nowadays. Is there a car or van normally available for use by you or your family?

Yes No

8. How often do you clean your teeth?

Never Less than once/day Once a day Twice a day More than twice a day

9. If you do clean your teeth, do you use a toothpaste containing fluoride?

Yes No

10. Nowadays, there are more things available in chemist shops to help with dental hygiene. Do you use anything other than an ordinary toothbrush and toothpaste for dental hygiene purposes?

Yes No

11. If the answer to question 10 is "Yes" do you use?

Electric toothbrush
Other

Floss

Interdental brushes

Toothpicks/sticks

12. Do you use a mouthwash? Yes No

If the answer is "Yes" which one?

13. In the last 12 months have you had any painful aching or discomfort in your mouth?

Never

Hardly ever

Occasionally

Fairly often

Very often

If you have experienced any pain or discomfort in the last 12 months please answer questions 14 to 21. Otherwise please move to question 22.

14. What type of pain or discomfort do/did you have?

Sharp
sensation

Throbbing

Dull ache

Burning hot or scalded

Tingling

15. Whereabouts in the mouth do/did you feel the pain or discomfort?

16. How long has the pain or discomfort been present for (days)?

17. Is it present all the time? Yes No

18. If the answer to question 17 is "No" describe the pattern of pain or discomfort?

19. Does anything relieve the pain, if so what?

20. Does anything make the pain worse, if so what?

21. How severe is the pain on a scale of zero to 10? (please circle)

no pain

intolerable pain

1	2	3	4	5	6	7	8
---	---	---	---	---	---	---	---

22. Does your mouth feel dry when eating a meal? Yes No

23. Do you find it difficult to swallow dry foods? Yes No

24. Do you sip liquids to help swallow dry foods? Yes No

25. Do you wake during the night because your mouth is dry? Yes No

26. Do you feel as if you have too little or too much saliva or, you are not aware of it?

Too little

Too much

Unaware of saliva

27. How many units of alcohol do you consume per week?

28. What type of alcohol do you drink (i.e. beer, wine, spirits and mixers)?

29. In the last 12 months have you felt that your sense of taste has altered because of problems with your teeth, mouth or dentures?

Never

Hardly ever

Occasionally

Fairly often

Very often

If you have noticed an alteration in your sense of taste:

30. Did/do you have an unpleasant taste? Yes No

31. Did/do you have a metallic taste? Yes No

32. In the last 12 months have you found it difficult to talk because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

33. In the last 12 months have you found it uncomfortable to eat any foods because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

34. In the last 12 months have you felt self-conscious because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

35. In the last 12 months have you felt tense because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

36. In the last 12 months has your diet been unsatisfactory because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

37. In the last 12 months have you had to interrupt meals because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

38. How many meals do you eat per day?

39. How many between meal snacks do you eat per day?

40. In the last 12 months have you found it difficult to relax because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

41. In the last 12 months have you been a bit embarrassed because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

42. In the last 12 months have you been a bit irritable with other people because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

43. In the last 12 months have you had difficulty doing your usual jobs because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

44. In the last 12 months have you felt that life in general was less satisfying because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

45. In the last 12 months have you been totally unable to function because of problems with your teeth mouth or dentures?

Never Hardly ever Occasionally Fairly often Very often

Thank you for taking the time to complete this questionnaire.



University
of Glasgow



Appendix 5 ADDQOL© questionnaire (copyright professor Claire Bradley) do not use without permission

ADDQoL

This questionnaire asks about your quality of life and the effects of your diabetes on your quality of life. Your quality of life is how good or bad you feel your life to be.

Please shade the circle which best indicates your response on each scale.

There are no right or wrong answers; we just want to know how you feel about your life now.

I) In general, my present quality of life is:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
excellent	very good	good	neither good nor bad	bad	very bad	extremely bad

For the next statement please consider the effects of your diabetes, its management and any complications you may have.

II) If I did not have diabetes, my quality of life would be:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
very much better	much better	a little better	the same	a little worse	much worse	very much worse

Please respond to the 18 more specific statements on the pages that follow.

For each statement, please consider the effects of your diabetes, its management and any complications you may have on the aspect of life described by the statement.

<p>In each of the following boxes:</p> <p>a) shade a circle to show how diabetes affects this aspect of your life;</p> <p>b) shade a circle to show how important this aspect of your life is to your quality of life.</p> <p>Some statements have a "not applicable" option. Please shade this "not applicable" circle if that aspect of life does not apply to you.</p>

1a) If I did not have diabetes, my working life and work-related opportunities would be:							<input type="radio"/> not applicable
<input type="radio"/> very much better	<input type="radio"/> much better	<input type="radio"/> a little better	<input type="radio"/> the same	<input type="radio"/> a little worse	<input type="radio"/> much worse	<input type="radio"/> very much worse	
1b) This aspect of my life is:							
<input type="radio"/> very important		<input type="radio"/> important	<input type="radio"/> somewhat important		<input type="radio"/> not at all important		

2a) If I did not have diabetes, my family life would be:							<input type="radio"/> not applicable
<input type="radio"/> very much better	<input type="radio"/> much better	<input type="radio"/> a little better	<input type="radio"/> the same	<input type="radio"/> a little worse	<input type="radio"/> much worse	<input type="radio"/> very much worse	
2b) This aspect of my life is:							
<input type="radio"/> very important		<input type="radio"/> important	<input type="radio"/> somewhat important		<input type="radio"/> not at all important		

3a) If I did not have diabetes, my friendships and social life would be:						
<input type="radio"/> very much better	<input type="radio"/> much better	<input type="radio"/> a little better	<input type="radio"/> the same	<input type="radio"/> a little worse	<input type="radio"/> much worse	<input type="radio"/> very much worse
3b) This aspect of my life is:						
<input type="radio"/> very important		<input type="radio"/> important	<input type="radio"/> somewhat important		<input type="radio"/> not at all important	

4a) If I did not have diabetes, my sex life would be:							<input type="radio"/> not applicable
<input type="radio"/> very much better	<input type="radio"/> much better	<input type="radio"/> a little better	<input type="radio"/> the same	<input type="radio"/> a little worse	<input type="radio"/> much worse	<input type="radio"/> very much worse	
4b) This aspect of my life is:							
<input type="radio"/> very important		<input type="radio"/> important	<input type="radio"/> somewhat important		<input type="radio"/> not at all important		

5a)	If I did not have diabetes, my physical appearance would be:						
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	very much better	much better	a little better	the same	a little worse	much worse	very much worse
5b)	This aspect of my life is:						
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	very important	important	somewhat important	not at all important			

6a)	If I did not have diabetes, the things I could do physically would be:						
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	very much increased	much increased	a little increased	the same	a little decreased	much decreased	very much decreased
6b)	This aspect of my life is:						
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	very important	important	somewhat important	not at all important			

7a)	If I did not have diabetes, my holidays or leisure activities would be:						
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	very much better	much better	a little better	the same	a little worse	much worse	very much worse
7b)	This aspect of my life is:						
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	very important	important	somewhat important	not at all important			

8a)	If I did not have diabetes, ease of travelling (local or long distance) would be:						
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	very much better	much better	a little better	the same	a little worse	much worse	very much worse
8b)	This aspect of my life is:						
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	very important	important	somewhat important	not at all important			

9a) If I did not have diabetes, my confidence in my ability to do things would be:

very much increased much increased a little increased the same a little decreased much decreased very much decreased

9b) This aspect of my life is:

very important important somewhat important not at all important

10a) If I did not have diabetes, my motivation to achieve things would be:

very much increased much increased a little increased the same a little decreased much decreased very much decreased

10b) This aspect of my life is:

very important important somewhat important not at all important

11a) If I did not have diabetes, the way society at large reacts to me would be:

very much better much better a little better the same a little worse much worse very much worse

11b) This aspect of my life is:

very important important somewhat important not at all important

12a) If I did not have diabetes, my worries about the future would be:

very much decreased much decreased a little decreased the same a little increased much increased very much increased

12b) This aspect of my life is:

very important important somewhat important not at all important

13a) If I did not have diabetes, my finances would be:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
very much better	much better	a little better	the same	a little worse	much worse	very much worse
13b) This aspect of my life is:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>			
very important	important	somewhat important	not at all important			

14a) If I did not have diabetes, my need to depend on others for things I would like to do for myself would be:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
very much decreased	much decreased	a little decreased	the same	a little increased	much increased	very much increased
14b) This aspect of my life is:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>			
very important	important	somewhat important	not at all important			

15a) If I did not have diabetes, my living conditions would be:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
very much better	much better	a little better	the same	a little worse	much worse	very much worse
15b) This aspect of my life is:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>			
very important	important	somewhat important	not at all important			

16a) If I did not have diabetes, my freedom to eat as I wish would be:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
very much increased	much increased	a little increased	the same	a little decreased	much decreased	very much decreased
16b) This aspect of my life is:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>			
very important	important	somewhat important	not at all important			

17a) If I did not have diabetes, my enjoyment of food would be:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
very much increased	much increased	a little increased	the same	a little decreased	much decreased	very much decreased
17b) This aspect of my life is:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
very important	important	somewhat important	somewhat important	not at all important	not at all important	not at all important

18a) If I did not have diabetes, my freedom to drink as I wish (e.g. sweetened hot and cold drinks, fruit juice, alcohol) would be:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
very much increased	much increased	a little increased	the same	a little decreased	much decreased	very much decreased
18b) This aspect of my life is:						
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
very important	important	somewhat important	somewhat important	not at all important	not at all important	not at all important

If there are any other ways in which diabetes, its management and any complications affect your quality of life, please say what they are below:

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Rajendran R, Robertson D, Hodge PJ, Lappin DF, Ramage G. Hydrolytic enzyme production is associated with *Candida albicans* biofilm formation from type 1 diabetes patients. *Mycopathologia*. 2010;

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Published abstracts

M. Murad, G. Ramage, D. Lappin, , D. Robertson, P. Hodge, CXCL5/ENA-78/IL-6 are increased in T1 DM Patients with& without Periodontitis, IADR 2011

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