PROGNOSTIC FACTORS IN ORAL CANCER

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Thesis

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TO MY

PARENTS, BROTHERS AND SISTERS

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DECLARATION

This thesis is the original work of the author

Sabah M H Saka

PREFACE

The work reported in this thesis was undertaken while the author was completing postgraduate studies in the University of Glasgow, supported by a grant from the **Mosul University - Ministry of Higher Education and Scientific Research of the Republic of Iraq.**

The research was undertaken in the Department of Oral Medicine and Pathology, Glasgow Dental Hospital and School, from August, 1987 to July, 1990. The clinical and follow-up data used for the study were obtained from patients with primary squamous cell carcinoma of the oral cavity who were admitted and treated surgically in the Plastic Surgery Unit of Canniesburn Hospital, Glasgow, and supplemented with postoperative radiotherapy in the Radiotherapy Unit of the Western Infirmary, Glasgow, between January, 1982 and December, 1987. The histological material used in the study was obtained from initial biopsy, local resection and neck dissection specimens of the patients involved in the study.

Some of the techniques used in this thesis are modifications of previously published work and some are techniques developed in the Oral Pathology Unit of Glasgow Dental Hospital and School. The applications of the techniques described in the present study were undertaken by the author personally. The preparation of histological sections was carried out partly by the technical staff under direct supervision of the author and his supervisor, and partly by the author himself.

Material based on the review of the literature discussed in Chapter 1 has been presented in The Royal Society of Medicine Symposium, November, 1988, London, and will appear as "Chapter 8, Structural Indicators of the High Risk Lesion" in a book titled Oral Cancer: Detection of Patients and Lesions at Risk, Volume 2. edited by N W Johnson, Cambridge University Press (in press).

Part of the data reported in Chapter 3 of this thesis has been presented at the annual meeting of the British Society for Oral Pathology, September, 1989, Newcastle Upon Tyne, and at the Scientific Forum in Glasgow Dental Hospital and School, June, 1989.

SUMMARY

This study was undertaken to assess several prognostic factors in oral squamous cell carcinoma. Structural histological features encountered in the initial biopsy and local resection of these carcinomas were evaluated in relation to nodal metastasis and clinical outcome of the patients. Only patients with carcinomas arising in one of three intra-oral anatomical sites namely, floor of the mouth, tongue and tongue plus floor of mouth were used for the evaluation of the parameters in question.

On the histological sections obtained from the initial biopsy of primary tumours, the parameters mitotic phase distribution and the silver stained nucleolar organizer regions (AgNORs) count were evaluated. The histological sections obtained from the local resection specimens of the primary tumours were used for the evaluation of tumour thickness, clearance of surgical margins and the type of tissue associated with positive margins and also for the assessment of malignancy grading using the system which was recently recommended by Anneroth et al. (1987). In patients who underwent neck surgery, the neck dissection specimens were used for the histological examination of the lymph nodes and whether or not these were involved with metastasis.

The work presented in this thesis is in two parts. In the first part attention is directed toward assessment of the structural histological features in relation to nodal metastasis of intra-oral carcinoma. The results presented in Chapter 3 describe the value of the parameters in predicting nodal metastasis. The mitotic phase

distribution parameters, namely the four apparently normal mitotic phases, abnormal mitoses, apoptotic mitoses and apoptotic cells were found not to be helpful in the prediction of nodal metastasis of carcinomas arising from floor of mouth, tongue or tongue plus floor of mouth.

The AgNORs count was found to be an important predictive factor of nodal metastasis in carcinomas arising from floor of mouth and an 85 per cent accurate assessment was possible. This same parameter however, was found not to be of value in predicting nodal metastasis in carcinoma of tongue and carcinomas involving tongue plus floor of mouth.

The malignancy grading parameters were assessed comparing two groups with and without nodal metastasis in all the three sites. The total malignancy grade was found not to differ significantly in each site separately or in combinations. The pattern of invasion and tumour-host interaction, by contrast, were found to differ significantly between the group with positive nodes and the group with negative nodes in floor of mouth carcinomas but not in carcinomas of tongue or carcinomas involving tongue plus floor of mouth.

Tumour thickness was found to be of predictive value for nodal metastasis in carcinoma of floor of mouth. In carcinomas of tongue and carcinomas involving tongue plus floor of mouth, tumour thickness did not show the same predictive accuracy. However, when comparison was made between the groups with positive nodes and the groups with negative nodes in all the three sites combined, tumour thickness values differed significantly.

The second part of the work presented in this thesis was directed towards evaluation of the structural histological features in relation to the clinical outcome of the patients following treatment of their intra-oral squamous carcinomas. Two groups of patients were created in each of the three anatomical sites. One group comprised patients who developed tumour recurrence locally, in the neck or at both sites in a follow-up period of not less than four months and within 24 months. The second group included patients who survived, disease-free a minimum of 24 months following treatment.

In Chapters 4, 5 and 6 the three anatomical sites are examined separately. In patients with floor of mouth carcinoma and patients with tongue carcinoma, the AgNORs count did not differ significantly between the groups. This parameter was not compared between the two groups of patients with carcinomas involving tongue plus floor of mouth due to the small number of cases which were suitable for assessment of AgNORs count.

Tumour thickness values were significantly greater in patients with carcinoma of floor of mouth or the tongue who developed recurrence. This difference was not apparent in tumours involving tongue plus floor of mouth. Positive surgical margins were more frequent in patients who developed recurrence and this was statistically significant in patients with floor of mouth carcinoma.

Total malignancy grade showed no association with the likelihood of recurrence at any of the three sites. Similarly the tumour factor and the three histological features comprising the tumour factor were found not to differ significantly between the groups with and without

recurrence. However the stage of invasion, lymphoplasmacytic infiltration and tumour-host interaction scored higher in floor of mouth cases with recurrence. In patients with carcinoma of the tongue only the feature, tumour-host interaction, was found to differ significantly between the groups. In patients with carcinomas involving tongue plus floor of mouth none of the malignancy grade parameters was significantly related to recurrence.

In Chapter 7 the data are related to the clinical outcome in all the three sites combined. Positive surgical margins were more common in patients who developed recurrence. Tumour thickness values demonstrated a highly significant difference between the group of patients who developed recurrence and those who survived, disease-free a minimum of 24 months. The pattern of invasion, stage of invasion and tumour-host interaction also showed significant differences between the groups. Involvement of lymph nodes with metastatic tumour was significantly more frequent in patients who developed tumour recurrence. Extracapsular spread of metastatic tumour was also more common in patients who developed recurrence than in patients without recurrence, but the difference in frequency was not significant.

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 INTRODUCTION

Oral cancer is a disease of elderly people, having a peak incidence in the 60-70 year age group. Oral cancer in developed countries is uncommon when compared with cancer at other sites. In the United Kingdom and the United States of America, oral cancer accounts for 2-3 per cent of all malignancies. However, regional variations exist. In the Indian subcontinent the figure reaches 40 per cent of all malignancies (Langdon, 1985).

Squamous cell carcinomas are the most common malignant tumours of the oral cavity, representing approximately 90 per cent of all oral malignancies.

The cause of oral carcinoma remains unclear, although there are some known direct relationships such as with tobacco chewing and the use of betel. The risk of mouth cancer in heavy smokers and heavy alcohol drinkers may be as much as 15 times greater than in those who neither smoke nor drink. Batsakis (1979) stated that more than 70 per cent of oral cancers occur in the dependent parts most exposed to food and saliva. The reason might be due to longer duration of exposure of these parts to a potential carcinogen which might be produced as a results of interaction of the many ingredients within the oral environment.

It has been suggested that one third of those who develop an intra-oral cancer will die within a five year period after the

diagnosis. Although the mouth is readily accessible for inspection, biopsy and radiotherapy, the outstanding feature of these neoplasms is the poor prognosis if the tumours are not diagnosed and treated early.

Batsakis (1979) stated that approximately 60 per cent of oral carcinomas are well advanced at the time of diagnosis. Several factors might contribute to this delay in detecting the carcinomas. In developed countries the majority of the patients are over the age of 60 years and almost all are edentulous. If such patients wear satisfactorily functioning dentures or if they do not have dentures, they assume that no examination of the mouth is necessary (Storer, 1972). A second factor might be that general dental and medical practitioners are unaware of the features of early oral lesions. Another factor is the overall lack of screening techniques that could help in early diagnosis. In this context it will be worthwhile to mention some details on what are known as precancerous lesions, since these lesions show a greater liability for cancer development than apparently normal mucosa.

The decision to treat an oral precancerous lesion depends upon the outcome of histological examination, the aetiology and the clinical appearance of the lesion. Subsequent attention should therefore be directed towards treatment of the higher risk lesions in order to limit the operative extent which would be required if established carcinomas are to be treated. This would definitely also allow a better prognosis and outcome.

1.2 PRECANCEROUS LESIONS

An oral precancerous lesion is a "morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart" (Axell et al., 1984). The high risk lesion is the one with the greatest likelihood of progressing to squamous cell carcinoma. There are two precancerous lesions, namely erythroplakia and leukoplakia. It is universally accepted that squamous cell carcinoma can develop from leukoplakia and erythroplakia, but there is a wide divergence of views on the frequency with which individual tumours are either preceded by or co-exist with precancerous lesions. There is probably a considerable geographical variation in this. Tumours in high risk countries such as India, for example, are said to always be associated with precancerous lesions, but in countries with low oral cancer prevalence, the association with precancerous lesions is less frequent. Bouquot et al. (1988) reported from the United States that 62 per cent of lip cancers were associated with leukoplakia, but only 36 per cent of intraoral cancers showed such association. In a more recent study Hogewind et al. (1989) reported from the Netherland that in a series of 212 patients with intra-oral carcinoma, 48 per cent were associated with white lesions and this co-existence was significantly more often in women than men.

Malignant transformation of oral precancerous lesions has been reported in various studies. Figures between 0.13 and 17.5 per cent have been documented (Silverman et al., 1984). The incidence of malignant transformation of precancerous lesions has been found to be influenced by the lenghth of follow-up period. Einhorn and Wersall

(1967) studied 782 patients with leukoplakia in Sweden over a prolonged period and noted that oral carcinomas developed in 2.4 per cent after 10 years and in 4 per cent within 20 years of follow-up.

A high proportion of oral cancers in the UK and USA appear to arise in mucosa which, clinically, does not show a precancerous lesion. Interestingly, Bouquot et al. (1988) in their extensive studies were able to indicate differences in the prognosis of cancer in which there was a related precancerous lesion. The tumours preceded by, or co-existing with, leukoplakia had a better prognosis in that they were less aggressive. However, Shibuya et al. (1986) reported that in tongue cases there was a greater likelihood of multiple tumours developing in patients with leukoplakia.

1.2.1 Clinical Indicators of High Risk Lesions

The highest risk amongst the apparent clinical precancers is the case in which a carcinoma is already present in a lesion which seems clinically to be no more than precancerous. Pain associated with a leukoplakic lesion, absence of tobacco habits, long duration and clinically non-homogeneous leukoplakia are all features associated with high risk lesions (Silverman et al., 1984). Location of a precancerous lesion in the floor of the mouth or on the ventral aspect of the tongue should also be considered high risk (Kramer et al., 1978).

An erythroplakic lesion appears to be the earliest visible sign of asymptomatic oral squamous cell carcinoma, either invasive or in situ. The presence of a white component does not appear to be significant unless it is accompanied by erythroplakia (Mashberg et

al., 1973).

In a study of clinical criteria for identifying early oral carcinoma in populations of tobacco and alcohol users, Mashberg and Feldman (1988) reported that the traditional clinical characteristics of ulceration, induration, elevation, bleeding and associated cervical lymphadenopathy were not usually present in these early lesions. These authors found that the presence of leukoplakia was a variable characteristic whereas the presence of erythroplakia was almost constant. Granularity of erythroplakic lesions was also found to be more common in early invasive carcinoma.

1.2.2 Histological Indicators of High Risk Lesions

Although the clinical appearance and site of presentation of precancerous lesions can be predictors of prognosis in terms of malignant transformation, the structural indicators which are evident microscopically can more accurately predict lesions in which there is a greater risk of malignant transformation.

There is a generally held view that assessment of dysplasia in premalignant lesions is important because dysplastic lesions are more likely to undergo malignant change and because it is felt that the chances of malignant transformation increase with increasing severity of dysplasia. However, the grade of dysplasia alone is not a reliable predictor of prognosis since carcinoma can actually develop from epithelium showing only mild or no dysplasia (Lind, 1987).

It is well recognised that there is great interobserver variation in the assessment of epithelial dysplasia. This is because

diagnostic pathologists assess dysplasia subjectively. This was strikingly illustrated in the report of Pindborg et al. (1985) who concluded that their study illustrated a need for uniform criteria for the diagnosis of dysplasia.

The main requirement for improved diagnosis and assessment of dysplasia is to improve objectivity. Smith and Pindborg (1969) attempted this with their publication of photographic standards against which the individual new case is evaluated. The use of such a system is valuable for standardisation (Katz et al., 1985), but is too time consuming for everyday use (Pindborg et al., 1985).

Few studies have addressed the question of identifying which features of dysplasia are the best predictors of high risk. Banoczy and Csiba (1976) recorded the frequency of occurrence of individual dysplastic feature in 120 cases of dysplasia, and their results showed that disturbed maturation of the epithelium was the most frequent feature, but was not a predictor for the high risk lesion. Wright and Shear (1985) adopted a different approach and studied the features seen in dysplasia immediately adjacent to established carcinomas. Their results showed that basal cell hyperplasia, disturbed epithelial maturation, nuclear hyperchromatism and loss of cellular cohesion were the features associated with more ominous prognosis. Kramer et al. (1970) used discriminant analysis to try and determine which histological features best separated leukoplakias which underwent malignant transformation from those which did not. Their results showed that nuclear hyperchromatism, loss of polarity of basal cells, increased number of mitoses, abnormal mitoses, intraepithelial

keratinization, cellular pleomorphism and enlarged nucleoli were the strongest indicators for ominous prognosis.

However, it is also important to stress that not all dysplastic lesions inevitably progress to carcinoma. In a follow-up study of 61 dysplastic lesions, Pindborg et al. (1977) showed that in a 7 year period only 6.6 per cent underwent malignant transformation, while 14.8 per cent showed spontaneous regression evident clinically. In some of the patients regression was also shown on repeated biopsy.

1.3 PATIENT FACTORS AND PROGNOSIS OF ORAL CANCER

A large number of factors can influence prognosis in oral cancer. Among these are the age and sex of the patient.

1.3.1 Age of Patients

Squamous cell carcinoma of the oral cavity is seen most frequently in patients in the fifth, sixth and seventh decades of life. However, McGuirt (1986) drew attention to an expected change in this concept in future due to the fact that more people, especially women under 20 years of age, are now tobacco smokers and/or alcohol drinkers. It is known that there are strong relationships between tobacco and alcohol and carcinoma of the upper aerodigestive tract. Consequently carcinomas may increase in younger age groups. In the future therefore, cancer will have to be considered more seriously in the differential diagnosis of head and neck lesions especially in young women.

In their study of prognostic factors in oral cancer, Easson and Palmer (1976) noticed that their results showed quite clearly that

the older patients had a higher risk of presenting with advanced cancer of the mouth. These authors stated that it was difficult to explain this finding as it was not known whether older patients simply delayed longer in seeking advice or the tumours grew more rapidly in older patients. In contrast, malignancies are said to behave more aggressively in younger patients than in older ones (Slotman et al., 1983).

Byers (1975) reported an increased proportion of histologically aggressive tumours in his series of tongue carcinoma patients who were younger than 30 years of age. This report also showed a poorer survival rate in patients with poorly differentiated lesions. On the other hand, McGregor et al. (1983) reported an improved survival rate in younger patients with oral tongue carcinomas, but 80 per cent of the lesions in their series were less than 4cm in size and only 14 per cent had palpable nodes at presentation. However, McGregor and Rennie (1987) concluded from their study of intra-oral carcinomas in patients under 40 years of age, that young patients did not generally behave differently from similarly stage matched lesions in older patients. These authors reported that local recurrence was the major problem as nine out of 13 patients in their series developed local recurrence shortly after initial treatment and died within two years of first presentation. In a recent study of carcinoma of the oral cavity and oropharynx in young adults, Cusumano and Persky (1988) stated that these oral carcinomas lacked the usual aetiological factors and seemed to represent a unique disease entity.

During analysis of the prognostic parameters in a large series of patients with oral carcinoma, Platz et al. (1983) found that there was a relationship between the age of patients and five year survival. Statistically significant differences were noted between three age groups. Prognosis worsened as the patient's age increased from less than 50 years, 60-70 and more than 70 years respectively. Cachin (1975) reported that survival was significantly better in patients less than 45 years of age. A slightly different age limit was suggested by Lash and Erich (1961) in relating patient age in carcinomas of the tongue to duration of survival. These authors found that 51 per cent of 37 patient less than 50 years of age were alive after five years as compared to only 38 per cent out of 151 patients 50 years of age or more. Langdon (1985) also found that patients with squamous cell carcinoma who presented before 60 years of age had a 59.5 per cent five year survival rate, in contrast to those over 60 who showed only a 22 per cent survival rate.

1.3.2 Sex of Patients

It is widely reported that females appear to have a better prognosis and longer survival than males in many malignant diseases. This seems to be accurate in tumours of the aerodigestive tract, bone (especially of the skull and face), nervous system and endocrine organs (Langdon and Rapidis, 1979).

In cancer of the oral cavity this view has been confirmed by many authors (Easson and Russell 1968; Smith 1973; Easson and Palmer 1976; and Langdon et al. 1977). However, Binnie et al. (1972) doubted that there was any real difference in survival of the two sexes in the

various sites which they studied. In contrast, Farr and Arthur (1972) actually found a slightly better survival for males.

An explanation for a better prognosis in females was suggested by Smith (1973) who claimed it was because females with tumours presented for treatment at an earlier stage. However, Easson and Palmer (1976) demonstrated that for each stage at presentation, females had better survival figures. In an attempt to answer the question why females have a better prognosis, Langdon and Rapidis (1979) concluded from their study that the better survival was not due to their presenting younger, with well differentiated, smaller tumours that arose at more favourable sites but to the possible differences of host response of females to intra-oral cancer.

In carcinoma of the tongue, Lash and Erich (1961) stated that differences in five year survival were noticed in analysing their patients for sex and survival. Out of 142 male patients, 37 per cent were alive for five years, whereas 52 per cent of 46 females with infiltrating disease were alive for five years or more. In contrast when analysing factors affecting treatment failure at the primary site and neck in patients with carcinoma of the oral cavity Shah et al. (1976) found that female patients who had advanced disease did poorly compared to male patients in terms of local and regional control of the disease.

In summary, a better survival for female patients has been observed in many studies, but so far no definite explanation has been proved. It has been suggested that the difference in alcohol or tobacco consumption might affect the prognosis. Other suggestions of

hormonal differences, genetic factors and differences in the immunological mechanisms between males and females might contribute to the explanation of the better survival in females (Langdon and Rapidis, 1979).

1.4 CLINICAL PARAMETERS AND PROGNOSIS

For clinical research and prognostic determination, acceptable classification systems for carcinomas of the oral cavity are required. The aim of such classifications is to obtain homogeneous, statistically equivalent groups of patients for the purpose of assessing, evaluating and comparing various therapeutic approaches. Another equally important factor is the determination of the efficacy of different methods of treatment in patients attending various hospitals, through exchange of information about the responses of patients to various methods of treatment.

At present, there exist two main systems for the classification of oral carcinoma. These are the TNM classification of the International Union Against Cancer (UICC) (1978) and the American Joint Committee (AJC) (1978). The parameters involved in these classifications are the size of the tumour (T), the clinical nodal status (N) and metastasis beyond the clavicle (M). The two systems are essentially very similar, differing only in the classification of lymph node status. In the AJC classification importance is attached to the number, size and localization of palpable nodes in the neck.

The four size (T) categories are the same in both classifications. T1 tumours are less than 2cm in greatest diameter; T2 tumours more than 2cm, but less than 4cm. Tumours greater than 4cm

are categorised T3. Tumours extending directly to muscle, bone, skin or antrum are considered T4 irrespective of size.

The clinical nodal status (N) categories show slight differences. N0 refers to the absence of palpable nodes. This is similar in both classifications. The N1 category in the UICC classification refers to homolateral, mobile nodes. N2 refers to contralateral or bilateral mobile nodes, whereas N3 refers to fixed nodes. The Nx category indicates regional nodes where the minimum requirement for their evaluation is not met. In the AJC classification, the size and the number of palpable nodes are considered. The N1 category indicates one or more palpable, equal to or less than 3cm single homolateral node. The N2 category is further subclassified to N2a which refers to a homolateral single palpable node greater than 3cm but less than 6cm and N2b indicates multiple homolateral nodes less than or equal to 6cm. The N3 category is also subcategorised to N3a for palpable nodes greater than 6cm, homolateral single or multiple. N3b refers to palpable bilateral nodes and N3c to indicate contralateral, single or multiple palpable nodes.

The (M) parameter is categorised to M0 when there are no distant metastases clinically at the presentation, and M1 when metastases are detected either clinically or radiographically.

Individual patient's neoplasms can be assigned to clinical stages dependent upon the TNM findings of the UICC (1978) or the AJC (1978) classifications. Four stages are recognised which are thought to represent different prognostic groups of patients. The clinical stages are similar in both TNM classification. The use of a, b and c

cervical node classification of the AJC (1978) is not required, but just recommended. Thus the stage grouping is done exactly the same way as in UICC (1978) classification. Stage I comprises T1 N0 M0 while stage II indicates T2 N0 M0. In the case of stage III it is either T3 N0 M0 or any of T1, T2, T3 combined with N1 M0. Stage IV indicates either T4 N0 or N1 M0 or any T with N2 or N3 M0; or any T and N with M1.

Platz et al. (1982) in their analysis of 802 oral cancer patients according to the most frequently used clinical TNM classifications, found that none of the classifications was able to create prognostically homogeneous groups of patients. In order to achieve a more exact assessment of the prognosis of patients with oral carcinomas, these authors suggested that multidimensional analysis of prognostically relevant factors, with a prognosis index as a goal, were necessary.

On the grounds of clinical experience Rapidis et al. (1977) suggested that in order to make any reasonable assessment of a tumour in terms of its treatment and prognosis it is necessary to take into account the site and the histology of the tumour in addition to the more conventional TNM criteria. Hence, these authors proposed a new system namely STNMP for the clinicopathological classification and differentiation of intra-oral carcinomas. In addition to the conventional TNM the S and P represent the anatomic site and histological differentiation of the tumour respectively. This system was applied to a group of patients and was compared to the conventional TNM system. The results showed that the STNMP system was

appreciably better at predicting those patients whose prognosis was good (Rapidis et al., 1977). However, in a study of 118 oral cancer patients who were staged according to both TNM and STNMP, Rich and Radden (1984) found that although both staging systems separated patients into those with a good prognosis and those with a poorer prognosis, the more sophisticated system (STNMP) did not provide any additional information over the conventional TNM.

1.4.1 Site of the Primary Tumour

Considerable prognostic significance has been attributed to the sites of primary tumours involving the oral cavity. However, conflicting views can be found in reviewing the literature. The tumour site is an important initial prognostic factor, but further variables related to both tumour extent and nodal involvement complicate interpretation. Bilateral nodal involvement is more common in cancers located near the midline and this feature worsens the prognosis (Feind and Cole, 1969).

In the reports of Fries et al. (1979) the oral cavity was divided into anatomical sites by levels and areas. Two vertical levels were defined. Level I was located below the occlusal plane and level II above this plane. Three areas were defined; namely the precanine, postcanine and retromolar areas. These authors attempted to study the prognostic significance of the primary tumour site by levels and areas in patients with oral carcinoma. Their conclusion was that given an identical extension and analogous metastasising rate, no computationally demonstrable difference in prognosis was found. The only difference found was between precanine and retromolar sites in

cases with T1 tumours in which the minimum requirement to assess the regional lymph nodes was not met (T1Nx).

The difference in prognosis of the same types of carcinomas in different sites often is explained by a difference in blood and lymph supply. However, Broders (1941) stated that this explanation was inadequate. The highly malignant nature of squamous cell carcinoma of the tonsil, posterior half of the tongue and oesophagus as compared to the lesser aggressiveness of the same type of carcinoma of the anterior half of the tongue, buccal surface of the cheek and lips, most certainly cannot be explained on the basis of a difference in blood and lymph supply. Also this does not explain the aggressive nature of melanocytic neoplasms of the skin by contrast with basal cell carcinomas of the same tissue. Broders (1941) stated that it was the differentiation of the carcinoma and not its location that influenced the prognosis. In a study of patients with carcinoma of the tongue however, Flamant et al. (1964) reported that the position of the primary tumours along the anteroposterior axis was of principle importance in determining the course of the disease.

On the other hand the anatomical site of any particular malignant tumour at presentation is said to be closely related to its prognosis, thus affecting the survival of the patients. Rapidis et al. (1977) realized the importance of site in relation to prognosis in patients with carcinoma of the oral cavity. These authors stated that a carcinoma arising in the posterior third of the tongue had a graver prognosis than a carcinoma of the same dimension arising on the lower lip. Similarly, antral lesions with clinically palpable nodes, although relatively small in dimension, had a hopeless prognosis.

In carcinoma of the mouth and pharynx, Arthur and Farr (1972) found that the overall cure rates in patients with tumours arising anterior to the faucial arch were markedly better than those located posteriorly. These authors also found that the anteriorly located lesions had a tendency for better histological differentiation compared to posteriorly located lesions.

1.4.2 Size of the Primary Tumour

The size of a neoplasm has been widely used as a major indicator of tumour aggressiveness; the larger the tumour the greater the threat to life. In patients with carcinoma of the floor of the mouth, Feind and Cole (1968) found a very striking correlation between size and the incidence of lymph node metastasis. Lesions smaller than 2cm had an incidence of 38 per cent of cervical node involvement whereas lesions more than 2cm had an incidence of cervical node metastasis of 62 per cent. Consequently it is reasonable to claim that the size of a lesion influences the survival rate as it is accepted that lymph node involvement worsens the prognosis.

Spiro and Strong (1973) and Spiro and Frazell (1968) found that the size of the primary tumour in patients with carcinoma of the oral cavity and oropharynx influenced survival. Spiro and Frazell (1968) also stated that when the lesions were 3cm or less, 26 per cent of patients survived whereas when the lesions were 6cm or more fewer than five per cent survived for five years after aggressive treatment.

It is well known that fixation of the cervical nodes is a sign of a sinister prognosis in patients with carcinoma of the head

and neck. Stell et al. (1984) found that fixed nodes became more common with increasing T stage of the lesion, from 4 per cent for T1 to 18 per cent for T4.

In an elegant study of evaluating the size of primary tumours correlated with the prognosis of patients with oral carcinoma, Moore et al. (1986a) concluded that very small surface size, less than 2cm correlated well with good survival whereas when the size was greater than 2cm it did not correlate well with survival or metastasis. These authors also suggested that tumour thickness might be a more reliable factor than size for predicting the outcome and survival of patients with oral carcinoma. Tumour thickness is discussed in more details in Section 1.7.

In a study assessing the efficacy of TN categories as clinically available data in relation to prognosis of patients with oral carcinoma, Fries et al. (1976) found that the exclusive consideration of the extent of the primary tumour in the TNM classification only allows for differentiation between slightly extensive T1 and incurable T4. On the other hand Hibbert et al. (1983) found that the five year survival figures were not significantly related to tumour size and they stated a possible reason for worse prognosis in stage IV disease was not the size of the tumour itself, but that the larger tumours were more likely to give rise to cervical lymph node deposits and it was this which reduced the five year survival.

Langdon et al. (1977) found that the dimensions of tumours at presentation were inversely related to the five year survival in

patients with oral carcinoma. Early lesions and those less than 4cm, had a 39 per cent five year survival, whereas late lesions, 4cm or more had a five year survival of 21 per cent.

Dividing oral cancer patients into three different groups according to the size of their primary lesion, namely those with less than 2cm, 2-4cm and those with more than 4cm, and correlating this with prognosis, Platz et al. (1983) stated that the analysis showed that the prognosis was significantly worse when the size was more than 4cm compared to less than 2cm lesions. In squamous cell carcinoma of the hard palate, Ratzer et al. (1970) demonstrated a significant relation between the size of the tumour and the cure rate of patients. They reported a 54 per cent five year cure rate with lesions less than 3cm diameter, as compared to 16 per cent for those greater than 3cm.

1.4.3 Clinical Nodal Status and Prognosis

Oral carcinomas are not localized diseases. Depending on the biological behaviour of a particular tumour, they invade the adjacent tissues and metastasise mainly through lymphatic vessels that drain to the regional lymph nodes. The involved regional lymph nodes are subjected to certain changes that can be identified clinically as enlarged size, palpability, location and fixation to the adjacent soft tissues. These features seem to be of prognostic importance in relation to the cure rate of the patients. The presence or absence of lymph node metastasis plays a predominent role in determining the prognosis of patients with squamous cell carcinoma of the mouth. However, controversies exist about the prognostic significance of various clinical parameters of cervical node metastasis.

The correlation between palpability of regional lymph nodes and five year survival of patients with oral cancer has been studied by many investigators, who noted a significant decrease in survival in those patients with palpable nodes. Noone et al. (1974); Hibbert et al. (1983) and Grandi et al. (1985) reported that a range of 65-70 per cent of oral cancer patients with clinically negative nodes enjoyed a five year survival, whereas in patients with palpable nodes a range of 43-46 per cent remained alive for five years. In their series of oral cancer patients, Spiro et al. (1974) and Cachin et al. (1979) reported that about 50 per cent of patients without palpable nodes remained alive after five years compared to those patients with palpable nodes of whom only 24-26 per cent survived five years. In the series of Hibbert et al. (1983) only 20 per cent of oral cancer patients with palpable nodes survived five years. The reason for such variable results in these studies might relate to the site and the stage of tumours at first presentation. For example in the report by Noone et al. (1974) carcinomas of the lip were included. Grandi et al. (1985) included many head and neck carcinomas but nasopharyngeal and tonsillar carcinomas which are known to behave badly, were excluded. It is important also to mention that in the study by Grandi et al. (1985) more than one third of their patients were N0 and have had elective neck dissection. This might contribute partly to the high survival of their series since Spiro and Strong (1973) reported less recurrences and higher five years survival in oral carcinoma patients who underwent elective neck dissection compared to those in whom the neck dissection was carried out therapeutically. The lower survival rates in the series reported by Spiro et al. (1974) and by Cachin et

al. (1979) might be due to the facts that in Spiro et al's study all the T1 and majority of T2 tumours were excluded and about 75 per cent of the patients had advanced tumours and had en bloc resection. In the study of Cachin et al. (1979) only patients with oropharynx carcinomas, the majority of whom, had tumours located in the tonsillar regions, were evaluated. These differences in tumour sites and clinical stages can most likely explain why various survival rates were obtained. However, it is not clear why in Hibbert et al's (1983) report the survival rate was so low in cases with palpable nodes as a whole. Although varying figures have been reported, palpable nodes are undoubtedly of prognostic significance.

Not only palpability of nodes has been found to have an influence on the cure rate of patients with oral carcinoma, but features, like the number, site and anatomical level in the neck at which the palpable node has been detected, all have been found to significantly affect the prognosis. Hibbert et al. (1983) emphasised the number of palpable nodes related to prognosis in oral cancer patients. They reported a dramatic decrease in cure rate of patients with multiple palpable nodes. They recorded five year survival in 65 per cent of N0 patients with oral cancer, 52 per cent in N1 single node patients but only 17 per cent in N1 patients with multiple enlarged nodes. In N2 and N3 patients no instances of five year survival were found. On the other hand Spiro et al. (1974) reported slightly different figures. Only 51 per cent of N0 patients survived five years after treatment, as opposed to 37 per cent of patients thought to have a solitary enlarged ipsilateral node. When multiple ipsilateral nodes became enlarged only 28 per cent of patients were

alive after five years. In patients with bilateral nodal involvement only 8 per cent remained alive five years after treatment. In contrast, Snow et al. (1982) reported that among the clinical parameters of cervical node metastasis, only nodal fixation was found to be an important prognostic parameter. None of the other clinical parameters of the neck nodes such as nodal size, number of clinically involved nodes and presence of homolateral versus bilateral or contralateral involvement showed a statistically significant correlation with prognosis.

1.4.4 Distant Metastases

The advanced stages of carcinoma of the head and neck pose major problems to even the most experienced oncologists. These problems become more complex when cancer is controlled after intensive treatment with irradiation. Although these carcinomas are slow in spreading outside the confines of the head and neck, some of them do metastasise to distant organs. Jesse and Sugarbaker (1976) stated that distant metastasis as the only manifestation of treatment failure becomes an increasing problem as more patients are successfully treated for the cancer above the clavicle. These authors suggested an additional treatment modality other than surgery and radiation therapy to kill the subclinical deposits of cancer that must be present at distant sites at the time of treatment of the primary tumour.

It is not common for patients with head and neck cancer to die from distant metastatic disease alone (O'Brien, 1986). The incidence is usually said to be less than 10 per cent, although a rate of 20 per cent or more has been reported among groups of surgically

treated patients with advanced head and neck cancer by Trez et al. (1981); Vikram et al. (1984a) and Byers (1985).

Death from distant metastasis alone is generally more common among patients treated with combined surgery and radiotherapy; the mortality rate ranging from 10 to 27 per cent (Schuller et al. 1979; Vikram et al. 1984a and Byers, 1985). There are also other reports by Trez et al. (1981) and DeSanto et al. (1982) in which the incidence of distant metastasis was similar for patients treated with surgery alone and those treated with combined surgery and radiotherapy.

With advances in treatment modalities for patients with head and neck cancer, control of lesions at the primary sites and regional nodes has become more successful. However, Jesse and Lindberg (1975) stated that a greater numbers of patients survive only to become victims of distant metastasis.

In a study of the prognostic relevance of various factors at the time of the first admission of patients with carcinoma of the oral cavity Platz et al. (1983) concluded from their study that the influence of evidence of distant metastases on the prognosis should be considered one of the strongest indicators of poor prognosis of all the factors that had been analysed.

Shah et al. (1976) studied factors affecting treatment failure in a large series of patients with oral carcinoma and found that among 54 patients in the series who developed distant metastases 52 (96 per cent) died of disease before five years and 49 of these 54 patients with distant metastases also had recurrent disease at the

primary site, the neck or both sites.

Oral cancer is usually regarded as a disease which kills by uncontrollable local tumour, recurrence and metastatic disease above the clavicles. However, following analysis of the causes of death on autopsies of patients with oral carcinoma, Thoma (1970) found that 61 per cent of those whose deaths were attributable to their first primary malignant lesions died with metastatic disease beyond the neck.

1.5 HISTOLOGICAL FEATURES RELATING TO PROGNOSIS

1.5.1 Summary of the Relative Merits of Features

Following establishing of the final diagnosis that the tumour being squamous cell carcinoma type, the details of the histological features which have impaction on the prognosis and outcome of the patient should be fully studied. There is relationship to some extent between tumour grade and the site of the primary lesion, the stage of disease, lymph node involvement and prognosis (Arthur and Farr, 1972). Anaplastic tumours usually contain an aneuploid chromosome population and metastasise earlier than those neoplasms that are more differentiated.

The mode of invasion in which malignant tumour invades the stroma of the host has prognostic significance (Yamamoto et al., 1984). Tumours composed of large cohesive masses of cells with pushing borders are less likely to metastasise. In contrast, neoplasms that invade diffusely in single and small aggregates of cells have a greater incidence of regional lymph nodes and distant

metastases.

It is apparent that some patients with squamous cell carcinoma of the head and neck can evoke an immunological response to their tumour and that the greater the inflammatory reaction the better is the prognosis. The vast majority of inflammatory cells are T-lymphocytes subpopulations (Guo et al., 1987). There is also preliminary evidence indicating that patients with squamous cell carcinoma that are associated with marked tissue eosinophilia may have an improved survival (Goldsmith et al., 1987).

Perineural invasion by squamous cell carcinoma is more common than previously thought and is an ominous sign that correlate with an increased incidence of local recurrence, regional lymph node metastasis and decreased survival (Soo et al., 1986). Perineural invasion is a histological sign of biological aggressiveness and for mucosal squamous cell carcinoma appears to be independent of tumour size. Once perineural invasion is identified, postoperative irradiation to the site of primary tumour and neck, even if it is clinically negative, appears warranted (Soo et al., 1986).

Vascular invasion denotes both blood vessels and lymphatic invasion. Access of tumour cells to blood vessels and the subsequent development of metastasis is dose dependent and involves a clonal selection of cells that are able to overcome mechanical and immunological barriers. The merely presence of tumour emboli within blood vessels, although worrisome, cannot always be equated with assumed dissemination. Poleksic and Kalwaic (1978) however, have observed a statistical correlation between blood vessel invasion and

lymph node metastasis in patients with squamous cell carcinoma of the oral cavity and pharynx.

Tumour invasion of bone, presence of tumour at surgical margins, deeply invasive tumours through submucosal and muscular layers are all structural features that indicate poor prognosis. The details of most of the features will be dealt with in the following sections.

1.5.2 Degree of Differentiation of the Primary Tumour

Histological factors relating to prognosis of patients with carcinomas have been studied by many investigators. Broders (1920, 1925) first used a monofactorial grading system based upon the relative number of undifferentiated neoplasic cells in carcinomas of the head and neck region. The number of mitotic figures and the number of cells with single large deeply staining nucleoli play an important part in the grading. On the basis of percentage of differentiated neoplastic cells, 4 grades were defined. Grade 1 describes tumours showing differentiation in more than 75 per cent of neoplastic cells. When 50-75 per cent of neoplastic cells show differentiation, it is graded 2. Grade 3 indicates tumours with 25-50 per cent of neoplastic cells exhibiting differentiation. Tumours with less than 25 per cent differentiated neoplastic cells are graded 4. Using his system of differentiation, Broders (1941) was able to show in cases of lip cancer that grades 3 and 4 were higher risk lesions associated with poorer postoperative results. This system has rarely been used in diagnostic pathology services in its complete form. Usually, three poorly defined categories are used. These are well

differentiated, moderately well differentiated and poorly differentiated carcinomas. Interpretation is very subjective which makes this system an unreliable predictor of prognosis in individual cases although there is undoubtedly evidence to support the contention that poorly differentiated tumours are higher risk lesions.

The relationship between tumour differentiation and regional lymph node metastases has been studied by many investigators. McGavran et al. (1961); Feind and Cole (1968) and Ali et al. (1985) reported that patients with poorly differentiated lesions had a significantly greater incidence of metastasis than did those with better differentiated lesions. McGavran et al. (1961) found that 49 per cent with poorly differentiated carcinomas of the larynx developed nodal metastases, in contrast with metastases in only 22 per cent and 11 per cent of patients with moderately differentiated tumours and well differentiated tumours respectively.

Five year survival figures have also been reported as proportional to the degree of differentiation. Langdon et al. (1977) recorded figures of 40 per cent for well differentiated tumours, 26 per cent for moderately differentiated and only 8 per cent for those with poorly differentiated tumours. Rapidis et al. (1977) also found that the patients with poorly differentiated squamous cell carcinoma of the oral cavity had a shorter survival than those with well differentiated lesions, and that the degree of differentiation was directly proportional to the survival rate of the patients.

1.5.3 Histological Malignancy Grading and Concepts of Scores

In Broder's grading scheme, as discussed in Section 1.5.2, a number of histological features are assessed subjectively in arriving at a decision on the grade of an individual tumour. Arthur and Fenner (1966) have introduced the concept of scoring for the histological grading of squamous cell carcinoma of the oral cavity. The concept actually is a modification of scoring concept developed by Bloom and Richardson (1957) for the histological grading of carcinoma of the breast. Arthur and Fenner (1966) studied three criteria in an individual tumour. These are, first, the degree of keratinization which represents tumour differentiation. The second parameter was assessing number of mitosis or hyperchromatic nuclei. This factor was thought to describe the rate of tumour growth. The last criterion was to study the irregularity of the cells and nuclear size. The system of scoring based on awarding 1, 2 or 3 marks for each of the criteria. A tumour showing well marked keratin pearl formation scored 1. Tumours with keratin formation but without pearls scored 2. Score 3 was given for tumours with no keratin formation or at most occasional cells showing individual keratinization. For the mitotic and hyperchromatic nuclei criterion, the score of 1 was given to tumours with 0-1 mitosis or hyperchromatic nuclei per high power field of the light microscope. Tumours with 2-3 of either, score 2, and score 3 was given for tumours with more than 3 such nuclei in a high power field. The scores given for cells and nuclei irregularity in individual tumour were as follow. Tumours with no significant irregularity in cells or nuclear size scored 1. Scores of 2 and 3 were given to tumours exhibiting moderate and marked cellular and

nuclear irregularity respectively. Addition of the scores for the three criteria gave the final grade of individual tumour. Score of 3-4 were graded I, 5-6 graded II and 7-9 graded III. Arthur and Fenner (1966) had applied this system of grading on large series of 299 patients with squamous cell carcinoma of the tongue and found that there was a good correlation between the tumour grades and the survival of the patients.

Jacobsson et al. (1973) described a more elaborate multifactorial grading system for squamous cell carcinomas which incorporated multiple observations describing histological parameters of both tumour cell populations and the tumour-host interface. The parameters describing tumour cell populations include structure and growth of neoplasm, degree of keratinization, nuclear polymorphism and frequency of mitoses. The histological parameters used to describe the tumour-host interface include mode of invasion, stage of invasion, identification of vascular invasion and evaluation of lymphoplasmacytic cellular response.

The initial approach for the developing of a quantitative score for determination of tumour grade was mentioned in Jacobsson et al's (1973) original report. For each of the 8 parameters a numerical scores of 1 to 4 was given and the sum of the scores of these parameters represents the total malignancy grade for an individual lesion. This system was applied first to the material of glottic carcinoma. Jacobsson (1973) found an evident correlation between the sum of the scores of the 8 parameters and the prognosis. Lund et al. (1975a) modified the grading system developed by Jacobsson et al.

(1973) by presenting a more exact definition of each parameter and grade and by introducing a histological score, defined as the total sum of points divided by the number of parameters evaluated. Using this modified system, Lund et al. (1975a) found a statistically significant correlation between the histological score and the death rate as well as the frequency of local recurrences and regional node metastases in a series of 438 patients with squamous cell carcinoma of the lip.

Total malignancy scores as originally proposed by Jacobsson et al. (1973) however, were evaluated by Crissman et al. (1984) on patients with carcinoma of the oropharynx. Two features namely structure and growth of tumour, and vascular invasion were excluded. These authors had separated the patients into two groups according to the total point scores of the six histological parameters. Group I represents patients with total scores of 12 or less, whereas group II involves patients with total score of 13 to 24. Comparison of the survival of patients between the two groups indicated no significant differences. Many investigators applied this malignancy grading system in both its original and modified version on cases with oral squamous cell carcinomas. Conflict results however, had been obtained. Thereafter, many malignancy grading systems involving various histological parameters had been elaborated.

More recently, Anneroth et al. (1987) have comprehensively reviewed malignancy grading systems and recommended a new system. The main conclusions of their review focused on the lack of uniformity and the exact definitions of the criteria for evaluating the histological parameters. Additionally, most of the investigators had evaluated

malignancy grading systems on heterogeneous group of oral cancer patients. Therefore, these authors stated that for histological malignancy grading systems to be able to predict with accurracy the prognosis in patients with oral carcinoma should fulfill a number of clinical and histological requirement, hence Anneroth et al. (1987) recommended a new system for malignacy grading. Six histological features are involved in the scheme. Three tumour features are degree of keratinization, nuclear polymorphism and number of mitoses above the basal layer. The three tumour-host relationship features are pattern of invasion which describes the nature of growing front, the stage of invasion is a reflection of the depth of penetration within underlying tissues, and lymphoplasmacytic infiltration reflects the immunological responses against the tumour. The criteria described for evaluation the histological features in Anneroth et al. (1987) system are well defined but assessment of some of them is very subjective and evaluation of others is very dependent on sampling of the resection specimems. Furthermore, application of the system is time consuming and may not be appropriate for routine diagnostic use.

1.5.4 Pattern of Invasion

This histological feature describes the nature of the growing fronts of the tumour. This has been included in the majority of grading systems. In these systems the pattern of invasion is graded in four categories. Grade 1 is given for carcinoma with pushing margins and well defined borderlines. When the borderline is less marked and with solid cords this is categorised as grade 2. In cases with no distinct borderline and with groups of invading cells

and those with diffuse growth the categories of grade 3 and 4 respectively are attached.

The mode of invasion across the tumour-host boundary described by Jacobsson et al. (1973) was applied on squamous cell carcinoma of the tongue by Yamamoto et al. (1983). Following investigation of the clinical course of diffuse invasive growths (grade 4) and comparing it with the other grade groups (grades 1-3), these authors found that metastasis to regional lymph nodes and local recurrence after surgery were extremely common in grade 4. Primary and secondary metastases were present in 8 out of 12 (67 per cent) cases with grade 4 group, but only 3 out of 24 (13 per cent) in the other grade groups. Furthermore, the local recurrences in grade 4 group led to the death of 5 out of 7 patients. On the basis of malignant nature, these authors also further subclassified grade 4 into a cord-like type (grade 4C) and a diffuse type (grade 4D) because the later was found to be more malignant than the former.

When investigating the prognostic value of histopathological parameters in squamous cell carcinoma of the oropharynx, Crissman et al. (1984) stated that following an analysis of various tumour parameters, the results demonstrated that pattern of invasion was the only histological factor which predicted five year survival. Patients with grade 1 and 2 had a five year survival of 60 per cent in contrast with 37.5 per cent for patients with grade 3 and 4 pattern of invasion.

Anneroth et al. (1987) reported a comprehensive review of histological malignancy grading systems and recommended a modified

system that could predict the prognosis. Pattern of invasion; to express the infiltrative characteristic of a particular tumour was included in the scheme and was precisely defined. Grade 1 corresponds to neoplasms with pushing and well-delineated borders. Grade 2 refers to neoplasm with infiltrating solid cords, bands and strands. Neoplasms with small groups of cells, not less than 15, or thin infiltrating cords are categorised as grade 3. Grade 4 refers to neoplasms with a marked diffuse, widespread cellular invasion in single neoplastic cells or in small groups of cells not more than 15 in number.

Pattern of invasion in relation to cervical node metastases has been recently studied by Shingaki et al. (1988) on 57 patients with oral carcinoma. These authors divided pattern of invasion into three grades. Grade 1 is for lesions with pushing and well delineated borders. Tumours with less distinct borderline with small nest or infiltrating cords are grade 2 and those tumours with diffuse growth and small cords or aggregates are graded 3. It was found that 71.4 per cent of grade 3 group had nodal metastases whereas grade 2 and grade 1 groups have 44.4 and 5.2 per cent nodal metastases respectively.

1.5.5 Stage of Invasion

Stage of invasion, which represents the level of tumour spread into the underlying tissues has been correlated with tumour prognosis. Investigators working on skin tumours, particularly cutaneous melanomas were first to define stage of invasion of a tumour and its correlation with prognosis in terms of metastasis, recurrence

and survival. Breslow (1970) studied the parameters, stage of invasion and thickness of the tumour, on patients with cutaneous melanoma and found that stage of invasion was of value in assessing prognosis. When this was combined with the thickness of the tumour it was possible to identify a high risk group of patients who were liable to develop recurrence or lymph nodes metastasis.

In Jacobsson et al's (1973) multifactorial system for malignancy grading of squamous cell carcinomas of the head and neck region, stage of invasion was also considered. Four grades were described. In cases where the presence of invasion was in doubt grade 1 is used. Grade 2 describes microinvasion. Grade 3 is for carcinomas showing invasion into submucosa, whereas cases showing massive invasion are graded 4. Jacobsson (1973) applied the system on glottic carcinomas and the validity of the system was clearly demonstrated. Statistically significant correlation was found between the total malignancy grading of 8 parameters and recurrence as well as survival.

The histological parameters described by Jacobsson et al. (1973) were evaluated on squamous cell carcinomas of floor of the mouth by Crissman et al. (1980). Among those parameters only stage of invasion was found to be of value in predicting lymph node metastasis. These authors stated that T1 and T2 neoplasms with only superficial or microinvasion of submucosa had little or no propensity for lymph node metastasis, whereas in T2 carcinomas with nodular or vertical invasion deep into and through the submucosa, 44 per cent developed lymph node metastasis.

In a study by Thompson (1986) for assessment of the depth of invasion of tongue carcinomas and carcinomas affecting other oral sites that demonstrated confirmed histological cervical node metastases, the conclusion was reached that tongue carcinomas had a propensity to infiltrate more than carcinomas of other oral sites. Thompson (1986) found in tongue carcinoma there was no significant difference in the prognosis whether the tumour invasion was confined to deep lamina propria or into underlying skeletal muscle. However, this study was undertaken on only 10 cases of tongue carcinoma. For such studies larger numbers of cases should assessed in order the statistical conclusions can be meaningful and more reliable.

1.5.6 Lymphoplasmacytic Infiltration

Oral carcinomas frequently evoke an inflammatory response. The intensity of the infiltrate varies widely from one case to another. Some deeply infiltrative tumours show almost no inflammatory response, whereas at the other extreme, verrucous carcinomas, which do not infiltrate, characteristically have a dense subepithelial accumulation of lymphocytes, plasma cells and macrophages. Lesions between these two extremes show considerable variation in the composition and density of the inflammatory response and in its proximity to the tumour cells (Johnson, 1977).

Histological host response in patients with squamous cell carcinoma of the larynx and hypopharynx was studied by Bennett et al. (1971) who concluded that lymphocytic infiltration at the tumour margin was of prognostic significance only in those patients with positive regional lymph nodes. Such patients with moderate to marked

lymphoid infiltrate at the tumour margin had a five year survival of 38 per cent as compared to a five year survival of 15 per cent for those with minimal infiltration. In patients with negative regional nodes however, Bennett et al. (1971) found no relation between the amount of inflammatory infiltrate and survival.

The intensity of lymphocytic infiltrate has been found to reflect improved survival in lip cancer. Jones and Coyle (1969) found that most tumours which metastasised had little surrounding lymphocytic infiltrate. In contrast, no metastases were noted in patients whose tumour showed considerable density of inflammatory reaction. Lund et al. (1975a) also found almost the same relation between inflammatory infiltrate and prognosis of patients with carcinoma of the lower lip.

Inflammatory infiltrate evoked by oral carcinoma has been evaluated in three grades defined as minimal, moderate and marked. Noone et al. (1974) found a distinct correlation of the intensity of inflammatory infiltrate with prognosis. Patients with minimal lymphoplasmacytic infiltration showed 41 per cent five year survival. When the infiltrate was thought to be moderate, 61 per cent survived five years. On the other hand all patients with marked inflammatory infiltrate were five year survivors, and none had nodal metastases.

In a recent study of the prognostic significance of histological parameters evaluated on squamous carcinoma of the oesophagus, Edwards et al. (1989) found that tumours with moderate or severe inflammatory infiltrate were significantly associated with improved survival time compared with tumour which had elicited no or

little inflammatory response.

Crissman (1986) has comprehensively reviewed the tumour-host interaction as a prognostic factor in the histological assessment of different carcinomas in various anatomical sites and he stated that not only was the intensity of inflammatory infiltrate important, but the specific types of cells involved in the infiltrate surrounding the tumour and the venules could reveal better prognostic information. In this review, the author has cited the results of the relation between lymphocytic infiltrate and five year survival in patients with squamous cell carcinoma of the uterine cervix. In a series of 125 patients an 80 per cent of five year survival was reported in patients with moderate to marked lymphocytic infiltration in contrast to only 39 per cent of five year survival in patients lacking lymphocytic infiltrates. Similar results were demonstrated in a comprehensive study of 718 patients and five year survival of 55.6 per cent was reported in patients with no inflammatory infiltrates compared to 87.8 per cent of five year survival in patients with heavy inflammatory infiltrates.

Immunohistochemical studies of infiltrate in cancer tissue from 30 patients with squamous cell carcinoma of the oral cavity were carried out by Hiratsuka et al. (1984). Subpopulations of the infiltrating lymphocytes in cancer tissues were identified on paraffin-embedded sections by a modified indirect immunoperoxidase technique. These authors found that the grade of T-cell infiltration correlated well with the size of the tumour and was significantly more marked in patients without cervical lymph node metastases than in those with lymph node metastases. Hiratsuka et al. (1984) also

suggested that T-cells might inhibit the development and spreading of the cancer cells, and that the T-cell infiltrate correlated with the clinical course and prognosis of oral cancer patients.

In the new system for histological malignancy grading proposed by Anneroth et al. (1987) lymphoplasmacytic infiltration as a prognostic parameter was included and was considered to reflect the immunological reaction to the neoplasm. These authors stated that the occurrence of an infiltration of plasma cells and lymphocytes in close relation to invasive neoplastic cells should be considered marked in grade 1, moderate in grade 2, and slight in grade 3. Grade 4 represents cases where no inflammatory infiltrate is found.

1.5.7 Prognostic Values of Combined Clinical and Pathological Parameters

For many years variations of TNM staging systems have been used on squamous cell carcinomas of the oral cavity to clinically predict the likely response to therapy and survival. The T designation has been found to be an especially reliable prognostic factor. In early cases of oral squamous cell carcinomas, however, there are many patients who die despite the fact that their neoplasms were considered clinically to be stage I or II and were treated accordingly. In such patients a combined assessment of clinical staging and histopathological features of the neoplasm might serve as a more precise measure for predicting the outcome and for determining treatment.

There are a number of studies of squamous cell carcinomas correlating histological malignancy grading with different clinical parameters such as clinical staging, recurrence and prognosis. Rapidis et al. (1977) were the first who combined with the conventional TNM classification, parameters like site (S) and pathology (P). Thus a system STNMP was developed. This system is said to be better than TNM alone in predicting prognosis in oral cancer patients. Crissman et al. (1984) evaluated clinical and histological parameters to determine which factors might predict prognosis and survival in patients with carcinoma of the oropharynx. It was found that in T2 and T3 neoplasms, both frequency of mitoses and pattern of invasion were statistically significant in predicting which patients in these groups would die of the disease. Crissman et al. (1984) found also that the clinical parameters that determine tumour stage were the most important factors in predicting patient outcome, and the assessment of pattern of invasion was an additional independent variable. In evaluating T3 and T4 carcinomas the histological parameters frequency of mitoses and pattern of invasion were highly statistically significant in predicting survival.

Lund et al. (1975b) applied the malignancy grading system to patients with carcinoma of the tongue and their results suggested that the clinical TNM classification supplemented by microscopical grading of the primary tumour can facilitate the separation of special risk groups in which regional lymph node metastasis may be expected. This is a feature that consequently leads to a poorer prognosis.

1.6 MICROSCOPIC MARGINS OF RESECTED PRIMARY TUMOURS AND PROGNOSIS

Carcinomas of the head and neck are often treated by surgery, radiation or combinations of both modalities sometimes also supplemented by chemotherapy. Most of the operable carcinomas however, are best treated by resection of tumour. The probability of obtaining a cure is believed to be much higher if the tumour is excised completely. Jesse and Sugarbaker (1976) stated that failure to eradicate the cancer at the primary site remains the largest reason for patient's demise. There is no way of being certain at a gross level that malignant cells do not remain at the resection margins following surgery. Therefore, the adequacy of resection margins must be determined microscopically.

Many investigators have attempted to study surgical margins and the correlation of these with recurrences and cure rates. In patients with squamous cell carcinoma of the skin, mainly the face, Glass and Perez-Mesa (1974) found that in 15 carcinomas termed inadequately excised by the pathologist, a recurrence of about 50 per cent was observed with subsequent mortality of about 25 per cent from uncontrolled carcinoma. These authors also reported that re-excision following pathological examination could significantly reduce the recurrence rate as well as the ultimate mortality rate.

In two groups of patients with squamous cell carcinoma of the upper aerodigestive tract with same staging, same site and distribution, and the same mode of initial treatment who had inadequate surgical margins, Lee (1974) found that the two year

survival rate was 90 per cent for those who were immediately subjected to re-excision compared to 48 per cent for similar cases not immediately treated. However, Bauer et al. (1975) in their study of the significance of positive surgical margins in patients with laryngeal carcinoma, felt that only conservative management and adequate follow-up for such patients was necessary. These authors also stated that immediate treatment was not required as it did not influence the outcome of these patients in terms of survival. This is not unexpected due to the possibility that small numbers of cancer cells left behind at the local site were dealt with quite adequately by the host's defense mechanisms. Other possibility which is equally important is that even with gross tumour at the margins in some cases, the last cancer cell might have removed and that the only cancer around is that seen in the microscopic sections. Furthermore, on the basis of specimen examination it is not possible to predict which patients with positive margins will develop recurrence since 82 per cent of patients with positive margins in Bauer et al's (1975) series did not developed recurrences.

Following analysis of factors that were thought to have an influence on treatment failure at the primary site and the neck in patients with oral carcinoma, Shah et al. (1976) stated that a majority of patients with pathology reports showing tumour at the margins of resection developed recurrent tumour locally and/or at the neck lymph nodes. Out of 54 patients with histologically positive margins, 42 recurred locally and/or at the regional lymph nodes.

The significance of positive histological margins in surgically resected squamous cell carcinoma of the oral cavity was studied by Looser et al. (1978). These authors reported that there was a significant correlation between the surgical margins and recurrence of the lesion. Patients with positive margins had a higher rate of local recurrence than those with negative margins (71 versus 32 per cent) which reflected an increased possibility of patient mortality. However, Looser et al. (1978) also stated that the absence of microscopically positive margins did not guarantee local control of tumour. Clearly this is in part a function of how the pathologist samples the margins. Also the presence of negative margins did not in any way predict the biological behaviour of the tumours.

Scholl et al. (1986) studied frozen sections of surgical margins in patients with carcinoma of the tongue and correlated these with local recurrence and survival rates. They found that those patients with initially positive margins who were rendered negative at the completion of the procedure and treated with surgery only, had a significantly increased local recurrence and reduced survival rate compared with patients similarly treated who had initially negative margins.

As a matter of controversy however, Slaughter et al. (1953) proposed a theory of field cancerization which holds that a majority of oral squamous carcinomas have much greater linear extent than depth. These authors also postulated that such tumours had microscopically multicentric origins which grew independently. Additionally they proposed that unknown carcinogens can alter an area of epithelium giving it the potential to form multiple cancers. If

this is the case, the significance of negative margins would become less important in terms of recurrence, simply because either what look adequate resection margins may be inadequate due to microscopic foci of carcinoma being left distant to the site of surgical resection, or the recurrence might develop at the same field since it would be preconditioned epithelium.

1.7 THICKNESS OF THE TUMOUR AND PROGNOSIS

Most human carcinomas run an unpredictable course in terms of prognosis and survival of the patients. The marked variations in prognosis are probably a function of many variables, one of which is the size of the tumour. Although there is a roughly inverse relationship between the diameter of the lesion and survival, very small lesions may recur and metastasise. In cutaneous melanoma Breslow (1970) stated that one possible reason for lack of reliability of tumour size in estimating prognosis was probably that conventional consideration of size is in only two dimensions and neglects tumour volume. Considering the thickness of the tumour in 98 cutaneous melanomas Breslow (1970) found that tumour thickness was of value in assessing prognosis as it was possible to identify a good prognosis of 45 patients with tumours at depth of invasion in stages II or III and a thickness of 0.76mm or less, only one of whom developed recurrence or metastasis.

The prognostic and therapeutic use of microstaging of cutaneous squamous cell carcinoma of the trunk and extremities was studied by Friedman et al. (1985). These authors found that the thickness and level of invasion of these carcinomas appeared to

represent important prognostic factors and may be relevant indicators for wide resections and for elective node dissections.

In studying prognostic factors in squamous cell carcinoma of lower lip, Frierson and Cooper (1986) carried out a comparison of histologic parameters between two groups of patients, a group with negative cervical nodes and another with nodal metastases. These authors found a statistically significant difference between the mean thickness of primary tumours in the two groups. The mean thickness of the primary tumours in the group with negative nodes was 2.5mm whereas for those with node metastasis it was 7.5mm.

Thickness of the tumour as a prognostic parameter has been studied recently in patients with squamous cell carcinoma of the oral cavity. Spiro et al. (1986) studied the predictive value of tumour thickness in tumours confined to tongue and floor of the mouth in 105 patients with negative nodes. According to the thickness of the tumours, three groups of patients were considered. Those with less than 3mm, 3-8mm and more than 8mm respectively. Their results indicated that thickness had the greatest impact on survival, although the difference between the patients with primary tumours 3-8mm in thickness and those with primary tumours that exceeded 8mm in thickness was not significant. These authors also stated that, the results had strongly suggested that treatment failure and survival may depend more on tumour thickness than on clinical stage. The prognosis was excellent in patients with thin oral tumours, 2mm or less, even when they exceed 2cm in greatest surface diameter (T2 and T3).

Moore et al. (1986b) studied tumour thickness in squamous cell carcinoma of the oral cavity, pharynx and larynx. Their results indicated that thickness and depth of invasion of the tumour seemed to represent its aggressiveness both because of good correlation with survival and with regional node metastasis, and also from the fact that two-thirds of failures in their series were associated with local recurrence. Corresponding to the four clinical stages of tumour size (T), these authors suggest a substitution by thickness of tumour. Tumours with 1-6mm to correspond T1, 7-12mm as T2, 12-18mm and more than 18mm are designated T3 and T4 respectively.

Moore et al. (1986b) also stated that the sites most suitable for thickness measurement are those with some soft tissue beneath the mucosa, such as floor of the mouth, tongue, buccal mucosa and soft palate. Anatomical sites like hard palate, gingiva and retromolar trigone seem less adaptable to thickness measurement. This is probably because these sites lack soft tissue beneath the mucosa.

Tumour thickness has been evaluated as a prognostic parameter in more homogeneous groups of patients with squamous cell carcinomas arising from one anatomical site of oral mucosa. Mohit-Tabatabia et al. (1986) studied a series of 84 patients with stage I and II tumours arising from the mucosa of the floor of mouth. These authors concluded that the thickness of the tumour showed a strong correlation with the appearance of later metastasis in the neck. Less than 2 per cent of patients with their tumour less than 1.5mm in thickness developed nodal metastasis during the mean follow-up period of 69 months. On the other hand, this incidence increased to 33 per cent

when the tumour was 1.6 to 3.5mm in thickness and to 60 per cent for those tumours thicker than 3.6mm. Moreover this was true regardless of the tumour size.

Urist et al. (1987) analysed prognostic factors in 89 patients with squamous cell carcinoma arising from the buccal mucosa. Tumour thickness was one of the factors that was evaluated. These authors concluded that analysis of survival by tumour thickness showed a significantly worse prognosis for tumours with a thickness of 6mm or greater regardless of tumour stage. Urist et al. (1987) also added that measurement of tumour thickness should be included in estimating prognosis, planning therapy and comparing results in patients with squamous cell carcinoma of the buccal mucosa.

Shingaki et al. (1988) were able to correlate tumour thickness with the cervical node metastases in a group of 57 patients with squamous cell carcinoma arose at various sites of the mouth and oropharynx. Their results showed that tumours with deeper stromal invasion had a higher tendency towards lymph node metastases. In tumours less than 4mm thick only 8.3 per cent had positive nodes. In contrast, with tumours 4-8mm thick and more than 8mm thick, lymph node metastasis was sharply increased and amounted to 35.3 and 83.3 per cent respectively.

In a more recent and the only prospective study at Tata Memorial Hospital in India, Fakih et al. (1989) found that in tongue, tumour thickness of 4mm was a dividing line for separation of patients with and without nodal metastasis and good and poor survival as well. Only 24 per cent of patients with tumour thickness less than 4mm

developed nodal metastasis in contrast to 78 per cent of patients with tumour thickness greater than 4mm. When the survival (median 30 months follow-up) was compared with respect to a tumour depth of 4mm, it was found that 81 per cent of those with tumour thickness less than 4mm compared to only 43 per cent for those with tumour thickness greater than 4mm were alive.

1.8 HISTOLOGICAL STATUS OF REGIONAL LYMPH NODES AND PROGNOSIS

1.8.1 Histologically Confirmed Metastasis in Regional Lymph Nodes

The prognostic importance of clinical nodal status in patients with squamous cell carcinoma of the oral cavity has been discussed earlier in section 1.4.3. However, clinical evaluation of lymph nodes is not absolutely reliable since many factors can affect the criteria that have been put forward by clinicians for the evaluation of regional lymph node status. The clinical features of lymph nodes do not always reflect the histological features. Lymph nodes of small size may contain metastatic carcinoma while large nodes may not.

Many investigators have recognised the fact of false negative (clinically negative but histologically positive) , and false positive (clinically positive but histologically negative) nodes. Sako et al. (1964); Spiro et al. (1974); Noone et al. (1974); Martis et al. (1979) and Ali et al. (1985) recorded figures varying between 10-53 per cent false positive and 10.8-38.1 per cent false negative clinical evaluation. Because of this magnitude of false interpretation, attempts to correlate the histology of regional lymph nodes and prognosis in patients with oral cancer have been undertaken

(Noone et al., 1974).

It has been universally accepted that histologically confirmed lymph node involvement by carcinoma is an important prognostic factor. The number of histologically positive nodes and their location in the neck also correlate with prognosis in patients with oral cancer. Kalnins et al. (1977) noticed a sharp decrease in five year survival as the number of positive nodes increased and they recorded a five year survival of 39 per cent in patients with a single positive node and 25 per cent in patients with two positive nodes. In 108 patients with three or more positive nodes only 11 per cent were alive five years after treatment. Schuller et al. (1980) stated that the single most important feature which affects the prognosis of oral cancer patients seems to be documentation of the presence of metastatic nodal disease rather than particular features, such as number or size, of the involved nodes.

The prognosis of patients with oral cancer has been evaluated on the basis of anatomical location of histologically involved nodes. The lower the level of location in the neck, the worse the prognosis. Spiro et al. (1974); Kalnins et al. (1977) and Schuller et al. (1980) stated that the prognosis was directly influenced by the level of location of the involved nodes. When such nodes were located at the lowest anatomical level in the neck the prognosis was terminal. Other histological features of the involved regional nodes were studied and a significant correlation was observed between some of these features and the outcome of patients in terms of survival. Tumour involvement of the capsule of a node can lead to

extracapsular invasion of carcinoma and open new routes for spread through the adjacent structures such as loose connective tissue and blood vessels. Invasion of veins leads to distant metastasis which dramatically influences the prognosis of oral cancer patients. Zoller et al. (1978) and Cachin et al. (1979) stated that extracapsular spread of carcinoma indicated a poorer prognosis. Johnson et al. (1981) commented that a significant difference existed when comparing the numbers of patients whose lesion showed no extracapsular spread with those showing extracapsular spread. Snow et al. (1982) stated that, among the histological parameters of involved nodes that influenced the prognosis of patients with oral cancer, extracapsular spread was the single most important factor.

As carcinoma of the oral cavity has a tendency to breach the nodal capsule and spread through the adjacent soft tissues, this feature has also been studied and correlated with the likelihood of tumour recurrence. Shah et al. (1976) commented that when extracapsular or soft tissue involvement was demonstrated histologically, a significantly higher number of patients developed recurrent tumour in the neck . This finding was confirmed by Carter et al. (1985) who stated from their study on stage III & IV oral cancer patients, that the recurrence rate on the ipsilateral side within 12 months after radical neck dissection was related to the degree of capsular involvement. These authors recorded that 44 per cent of patients with macroscopic extracapsular spread had recurrence, by contrast with those patients with microscopic extracapsular spread in whom only 25 per cent developed recurrence.

1.8.2 Reactivity of Regional Lymph Nodes and Prognosis

Patients treated for oral carcinoma often follow an unpredictable course in terms of survival. This variable response may be due to an interaction between the patient and his tumour possibly mediated through immunologic host defence mechanisms.

Morphological changes in lymph nodes draining tumours have been documented in many anatomical sites involved by carcinoma, particularly breast cancer, (Tsakraklides et al., 1974), colorectal carcinoma, (Tsakraklides et al., 1975) and head and neck cancer, (Berlinger et al., 1976 and Tanner et al., 1980). These authors concluded that patients whose lymph nodes demonstrated active immunological responses in the form of expanded inner cortex or increased number of germinal centers had five year survival rates significantly greater than those patients whose regional lymph nodes showed an unstimulated pattern or showed lymphocyte depletion.

Sinus histiocytosis is said to be an immune defence mechanism of lymph nodes subjected to certain antigens. This feature has been studied and correlated with the rate of survival in patients with carcinoma. Black & Speer (1958) stated that sinus histiocytosis in regional lymph nodes draining carcinoma of breast was a favourable prognostic sign and axillary metastases were less common in patients with sinus histiocytosis.

Friedell et al. (1974) also concluded from their study on regional lymph nodes of 86 Japanese cases of breast cancer and 86 British cases of breast cancer that sinus histiocytosis was present in

more than half of the Japanese cases and was rare in British cases. These findings were correlated with the fact that breast cancer runs a less aggressively malignant course in Japan.

The morphological features of reactive regional lymph nodes as prognostic indicators in patients with squamous cell carcinoma of the larynx and hypopharynx were studied by Bennett et al. (1971). These authors found that only extensive germinal centre hyperplasia was a favourable prognostic sign. This correlation was found only in those patients with positive nodes. None of the other morphological features related to lymph node reactivity showed significant correlation with prognosis.

Regional lymph node responses and the correlation of this with prognosis in carcinoma of the head and neck has recently been studied by Ortega et al. (1987). These authors reported that the proportion of patients with nodal metastases increased progressively as the response in regional lymph nodes was shown to be lymphocyte predominance, germinal centre hyperplasia and non-stimulated nodes respectively. This was also true for patient's survival. The number of patients surviving with lymphocyte predominance nodes was greater than that of patients who showed germinal centre hyperplasia or unstimulated lymph nodes.

From an outstanding study based on a very homogeneous group of 43 patients with squamous cell carcinoma arising from the floor of the mouth, Ring et al. (1985) demonstrated a statistically significant prognostic value of the regional lymph node patterns of immune response. These authors found that patients who showed lymphocyte

predominance or sinus histiocytosis patterns had a better prognosis than those with either germinal centre predominance or an unstimulated pattern. Previously, Noone et al. (1974) had demonstrated that sinus histiocytosis and follicular hyperplasia in lymph nodes draining oral carcinoma suggested a more favourable prognosis in those patients with positive lymph nodes during the first years of follow-up, but had no demonstrable effect on overall five year survival.

1.9 TREATMENT MODALITIES AND PROGNOSIS

Until the middle of this century, irradiation or surgery were considered to be the only choices for the oncologist in the management of head and neck cancers. Squamous cell carcinomas of the oral cavity were treated either by irradiation or by surgical excision. Neck disease was treated by radical neck dissection unless the nodes were unresectable. During the last two decades, a flexible interplay of irradiation and surgery has evolved. There are lesions best treated by irradiation or by surgery alone. On the other hand there are other lesions best treated by combining the two modalities.

Little argument exists about the treatment policy for stage I and II carcinomas of the head and neck, as either surgery or radiotherapy can give almost the same results in terms of survival. However, for stage III and IV carcinomas, many controversies exist about the appropriate treatment modalities and considerably different results have been obtained by different investigators.

Different policies of treatment have been adopted in different centres for patients with stage III and stage IV carcinomas. Consequently, detailed comparison between the results obtained would be

meaningless. It is possible, however, to review some results which have been obtained by different workers.

In a series of patients with tumours arising from various sites in the head and neck region, the superiority of combined therapy to either surgery or radiotherapy alone was noted by Barkely et al. (1972) Krause et al. (1973) and Fletcher and Jessee (1977). Furthermore, Krause et al. (1973) reported that except for carcinoma of the buccal mucosa, combined therapy was superior. The five year survival was 76 per cent with combined therapy, 45 per cent with surgery alone and 37 per cent with radiation therapy.

Langdon et al. (1977) stated that better results in terms of survival were obtained when preoperative radiation was adopted. Patients who received preoperative radiation showed 32.5 per cent five year survival, in contrast, those having postoperative radiotherapy had only 25 per cent showing five year survival. The advanced stage of tumours in the majority of patients involved in this study might explain the low percentages of five year survival.

On the other hand Vandenbrouck et al. (1977); Vikram et al. (1980); Robertson et al. (1985) and Kramer et al. (1987) noticed better results in terms of survival in patients with carcinoma of the head and neck region when they were treated by radical surgery followed by radical radiotherapy. Robertson et al. (1985) stated that when radical surgery followed by radical radiotherapy was adopted in patients with advanced squamous cell carcinoma of the tongue and those with carcinoma of the floor of the mouth, five year survival was 44 per cent for tongue cases and three year survival was 41 per cent in

cases with floor of mouth carcinoma. These authors found also, when treating similar lesions by minimal surgery plus radical radiotherapy, only five per cent of tongue cases survived five years, and 10 per cent of floor of mouth cases survived three years.

Different views have been documented in other series concerning treatment policy for patients with carcinoma of the head and neck region. Trez et al. (1981) and O'Brien et al. (1986) reported that there was no difference in five year survival between a group of patients who had been treated by surgery alone, and those treated by combined therapy. However, a group of patients who received preoperative irradiation was shown to have a lower incidence of local recurrences.

In contrast, Schuller et al. (1979) reported an increased survival in patients with carcinoma of the head and neck region when only radical surgery was adopted compared to those who received combined therapy involving preoperative radiotherapy.

Attention have been given recently to combining chemotherapy with other treatment modalities in patients with inoperable squamous cell carcinoma of the head and neck. Al-Sarraf et al. (1987) stated that concurrent use of radiotherapy and chemotherapy with cisplatinum seemed to be effective and safe. Clavel et al. (1987) tried combination chemotherapy in a total of 185 patients with inoperable, advanced squamous cell carcinoma of the head and neck region. A combination of methotraxate, bleomycine and vincristine with cisplatinum was tried on a group of patients, whereas in another group, combinations lacking cisplatinum were tried. Although no

impact on ultimate survival was demonstrated the results underlined the potential role of cisplatinum in advanced head and neck carcinoma and also supported the superiority of combined chemotherapy over single agent treatment for such cases.

From the Memorial Sloan-Ketering Cancer Center Vikram et al. (1984a) reported that failure at distant sites become a problem in patients with advanced but operable carcinomas of the head and neck regions who were treated by multimodalities. These authors have studied retrospectively 114 patients with clinical stages III and IV lesions. Their results showed that 20 per cent of the patients developed distant metastasis as a first site of failure within 2 years after the surgery. It was found that the most sensitive prognostic indicator for distant metastasis is the pathologic extent of nodal metastasis at neck dissection. Carr and Langdon (1989) stated that it seems the successful locoregional control in the treatment of oral cancer has been achieved at the expense of an increase in the occurrence of distant metastasis and the emergence of second primary tumours. These authors reported a 14.5 per cent of 105 patients developed multiple primaries within an average period of 25.6 months after the diagnosis of the first primary. It seems likely that even if a cure were discovered for oral cancer, the actual gain in survival would be modest due to the emergence of second primary tumours in organs that carry a poor prognosis particularly lung and oesophageal cancer (Vikram, 1984).

1.10 AIMS OF PRESENT STUDY

As noted in the preceding review two of the major problems in patients with oral cancer are lymph node metastasis and recurrence of tumour following treatment. If it were possible to predict the likelihood of these problems in individual cases, this might help by allowing treatment to be modified.

The first part of the study, reported in Chapter 3 was concerned with the prediction of the presence of lymph node metastasis from examination of the biopsy and excision specimens of primary tumours.

The second part of the study, reported in Chapters 4 to 7, was concerned with the follow-up of patients after treatment and examined whether features evident at the time of treatment were predictors of later outcome. General discussion and conclusions are presented in Chapter 8.

CHAPTER 2

GENERAL MATERIAL AND METHODS

2.1 INTRODUCTION

An essential goal of clinical cancer research is the creation of guidelines for giving a pre-therapeutic prediction of prognosis which is as accurate as possible. The achievement of this goal has being attempted worldwide with the help of the TNM classification systems.

During practical use of the TNM system in many retrospective studies of carcinomas of the oral cavity, the classifications were found to be inadequate for accurate prediction of the prognosis in terms of survival, cure rate and recurrences.

Many patients with oral squamous cell carcinomas die in spite of receiving treatment at an early stage of the disease. In such patients a combined assessment of the clinical staging and of cytomorphology of the neoplasms might serve as a more precise measure for predicting the outcome of the neoplasms and for determining their treatment. Therefore, the present study was designed to assess the prognostic potential of histological factors and some clinical factors in oral squamous cell carcinomas. The results of these analyses should finally lead to the definition of prognostic indicators which might be useable in clinical practice. For such studies, which require analysis of large numbers of factors, it was felt that it would be most convenient to store the data on computer.

A regular follow-up of patients is necessary for monitoring of the outcome of tumours in regard to lymph node metastasis, recurrences, the development of second primary tumours, cure rate and survival. The questions asked for this study were, firstly, whether it would be possible following the assessment of certain histological features on primary tumours to accurately predict cervical lymph node metastasis which is a feature of great importance concerning cure rate and survival in patients with oral carcinomas. The second question asked was how accurately cure rate and survival could be predicted following assessment of clinical and histological features of primary tumours.

2.2 GENERAL MATERIALS AND METHODS

2.2.1 Patient Selection for Main Study

The Oral Pathology Unit in Glasgow Dental Hospital and School, maintains a disease index of reported cases. From this it was possible to retrieve the pathology numbers of cases diagnosed as oral squamous cell carcinomas that were received and reported between 1st January 1982 and 31st December 1987. From the case numbers retrieved, it was possible to obtain the pathology report form of each case and this provided the name of the patient and the hospital record numbers. Having this information it was possible to look at the patient name index, as for each patient a separate card with all the related pathology report numbers is maintained. Thus it was possible to access all the pathology reports related to individual patients.

Depending on a particular case and the stage at which it was referred, the report numbers in the card system might include reports of biopsy, resection, neck dissection and recurrence or only some of these.

2.2.2 Coding the Cases According to Site of Origin

It was planned to study different clinical and histological factors in relation to prognosis and outcome of the patients. It was felt that assessing these parameters on a reasonable numbers of cases was required to give meaningful statistical results.

Initially one large database file including records of all the patients with oral squamous cell carcinoma was created. A separate record was created for each patient. The basic patient clinical details were obtained from the pathology report. These involved details like the sex, date of birth, dates of surgery, the Consultant in charge and the hospital record numbers. These were recorded and stored in the computer using a Dbase III plus programme. A file of 402 individual patients was created.

For such studies it was felt that it would be reasonable to group together cases that had tumours arising from similar well-defined intra-oral anatomical sites. Therefore, the oral cavity was divided into ten anatomical sites for recording the locations of the tumours. These were tongue, floor of mouth, combined tongue plus floor of mouth, buccal mucosa, lower alveolar mucosa, lower lip, upper alveolar mucosa, retromolar area, palate and the complex site where the tumour was so extensive to involve all together the area of posterior part of tongue, tonsillar mucosa, faucial pillars, pharynx

and soft palate. Code numbers were given for each individual well defined anatomical site. For example cases with carcinoma of the tongue were coded 1, those with floor of mouth tumour were coded 2, code 3 was given to cases which had tumour involving tongue plus floor of mouth and so on for each of the ten anatomical intra-oral sites. Following the consultation of the original pathology reports of the 402 cases, a hundred and four cases were found not suitable to be coded for the anatomical sites, hence they were excluded. For various reasons these cases were excluded. Firstly, some of the cases had carcinomas arising outwith the oral cavity. Secondly, some cases had carcinoma, but not of squamous cell type. The third reason was that some cases had records only for the metastatic tumour and no data available for the primary tumour. In other instances, the records available were only for second primary or recurrent tumours, but not for first primary tumour. Finally in some cases it was found that they were actually receiving treatment before 1st January 1982. According to the code given for the remaining 298 cases it was possible to group together cases which had tumours arising from well-defined sites. Hence, it became possible to extract sorted files from the initial large file according to the codes given for each anatomical site. Thus the individual sorted files should include only records of cases of squamous cell carcinomas that had tumours arising from similar anatomical intra-oral sites. Table 2.1 summarises the number of cases for each of the individual anatomical intra-oral sites. The first three sites; that is the tongue, floor of mouth and the combined tongue plus floor of mouth were found to have the largest numbers of cases. Therefore, it was decided to restrict the study to

the cases of these three sites.

By the means of creating additional files according to the site of the primary tumours, the process of adding more data related to each case during the course of the study and the follow-up became easier and more convenient.

2.2.3 Clinical Data Obtained from Pathology Forms

It was possible to obtain basic data related to each case from the pathology request forms. This included the sex, date of birth, and hospital record numbers. The study was restricted to cases that were admitted and treated in Canniesburn Hospital, Glasgow, because it was felt that the cases treated in this hospital had been subjected to the same treatment regimens. Depending on how extensive the tumour was, treatment was most frequently by surgery supplemented by radical radiotherapy within 6 weeks postoperatively. Cases which had been treated elsewhere apart from Canniesburn Hospital, were not included in the detailed study.

The maximum extension (T) of the primary tumours and the clinical status of the cervical nodes of the individual case were also obtained from the pathology request forms. In cases where not all of these data were clear on the pathology form, reference was made to patient's original hospital record. These records were retrieved from the record office of Canniesburn Hospital, Glasgow.

2.2.4 Histological Data Obtained from Pathology Reports

The pathology reports of the cases selected for the study were reviewed for the histological data on the primary tumour and on

the neck dissection if it had been performed. Concerning the primary tumours, the initial biopsies were reviewed for the types of squamous cell carcinomas. If the report showed that the tumour was a recurrence or it was of a verrucous carcinoma type, then the case was excluded from the study. This is because if the tumour was a recurrence that would mean it had been previously subjected to treatment. Therefore such a case would not meet the criterion of selecting only cases with primary tumours. In cases of verrucous carcinomas of which there were only a few cases, on the other hand, the biological difference with a good prognosis in contrast to the more aggressive nature of squamous cell carcinomas, led such cases to be excluded.

In cases which had undergone neck dissection, the histological status of cervical lymph nodes was reviewed. The features of the nodes included the number of the nodes that had been sampled from the neck dissection specimen. These were recorded according to the anatomical location within the neck. The anatomical locations of the neck were submental, submandibular, jugulo-digastric, upper deep cervical, lower deep cervical and the posterior cervical triangle.

Generally the features of the lymph nodes reviewed were the histological status of the lymph nodes for metastases.

When the node was positive for metastases, it was noted whether the metastasis was extracapsular or was contained only within the node. The numbers and the anatomical locations of the nodes that contained secondary tumours were

particularly studied and the data recorded.

Occasionally, the lymph node features were not clearly indicated in the pathology reports. This was mainly due to a group of nodes in a particular anatomical area in the neck being clinically and histologically matted together because of their extensive involvement with secondary tumour. In such cases therefore, there was a problem in assessing the nodal features, like number and the extension of metastasis. Re-examination of the histological sections of the neck dissection specimens for such cases was mandatory to re-assess the lymph node features of the number and the extension of metastasis.

2.3 MATERIAL SELECTED FOR EVALUATING MITOTIC PHASE DISTRIBUTION

2.3.1 General Considerations

Aspects of mitosis form some of the criteria for the diagnosis of epithelial dysplasia and malignancy in various epithelial linings of the body. There is difficulty in evaluating mitosis, because dividing cells can be found in normal, benign and malignant lesions of oral epithelium. There are no standardized mitotic parameters that distinguish one lesion from another.

Mitotic figures in histopathology sections are interpreted as cells undergoing division, and thus indicative of cellular proliferation. It is generally accepted that in malignant neoplastic lesions the number of cells entering mitosis is more than in the cells of the corresponding normal or benign neoplastic tissues. Mitotic figures seen in histology sections can be categorised in one of four mitotic phases. These are prometaphase, where the nuclei are

stained darker than normal and in which there is loss at least part of the nuclear membrane allowing the recognition of the knobbly outline of one or more individual chromosomes. The next mitotic phase is metaphase in which the chromosomes are aligned into the elongated metaphase plate. Anaphase which is the third mitotic phase is characterized by chromatid separation and migration away from the metaphase plate. The last phase of the mitotic process is telophase in which the two daughter nuclei form and the cells separate. The question asked in this study was whether the mitotic phase distribution, that is the proportions of mitoses in the four phases, evaluated on the original biopsy of primary oral squamous cell carcinomas could contribute to the prediction of outcome of patients in terms of nodal metastasis, recurrences, cure rate and survival.

2.3.2 Criteria for Material Selection

Part of the data obtained from the cases selected for the main study as discussed in the earlier sections of this Chapter, was biopsy status of particular cases. Cases that had primary carcinomas which arose from mucosa of the tongue, floor of the mouth or from a combination of these two sites; tongue plus floor of mouth, were reviewed to see whether they had biopsy specimens diagnosed in the Oral Pathology Unit.

Selection was restricted to those cases in which an initial biopsy had been submitted for histological interpretation. This was done to exclude recurrent cases that had been treated once by any means in order to avoid what effects the treatment might have had on the specimens. The criteria for selection of the cases were that the

case should have been treated in Canniesburn Hospital, Glasgow, and have resection specimen of the primary tumour preferably supplemented with neck dissection. Cases which had neck dissections were particularly selected because part of the study was planned to correlate the mitotic phase distribution in the original biopsy, with the histological status of cervical nodes. The histology slides of the biopsy should show an unequivocal invasion of squamous cell carcinoma within the connective tissue underneath the surface mucosa. Another criterion was that there should be enough tissue material remaining in the paraffin blocks of the cases, for further sections to be prepared. Cases which fulfilled these criteria formed the material on which the study was carried out.

2.3.3 Preparation of Histological Sections

The histological sections of the biopsy specimens of the cases selected for the study were retrieved from the files of the department. These sections were re-examined to confirm the suitability of the tissue sample for the study. The original paraffin blocks of the selected cases were retrieved from the files for recutting.

The requirements of the histological techniques to make mitoses more easily recognisable were described in the studies of mitotic phase distribution in oral mucosal hyperplasia and neoplasia by Ojo (1985). This entailed the use of different haematoxylin dyes to bring out mitotic figures clearly in the sections. The use of different section thicknesses to display the best morphological features of mitosis was also examined. Ojo (1985) found that a

section thickness of 5μm and staining with Harris's haematoxylin and one per cent eosin gave the optimal preparation for identification of mitotic figures.

It was planned in this study to count 100 apparently normal looking mitotic figures in each case. This number was selected in order to have the exact percentage of the frequecy of each mitotic phase. It was felt that a single section might not be sufficient for counting 100 mitotic figures in some cases, and that extra sections should be prepared.

Occasionally in histological sections, only part of the mitotic figure is revealed and in such instances it can be difficult to recognise the mitotic phase. To overcome such problems a short ribbon of three serial sections can be used. The main analysis can be undertaken on the middle section and in cases of doubt it is then possible to examine the adjacent serial sections in order to clarify the mitotic phase.

The principle of mitotic counting in this study requires that the individual mitotic figure is only counted once. Therefore, it was thought that for preparing extra sections, some sections had to be discarded in between the sections to be mounted on the slides. Thus, eight sections were discarded in between the three short ribbon of serial sections prepared.

2.3.4 Delineation of Phases of Normal Mitosis

The process of cell division is probably a continuous process which has been artificially divided into the four recognisable phases

of mitosis. Ojo and MacDonald (1988) discussed the problem of defining the transition points of one phase to the next. They utilised photographic standards against which the mitosis to be categorised could be compared. The same principles were used in the present study as shown in Fig. 2.1. The textbook description of mitosis starts with prophase, metaphase, anaphase and telophase. The prophase actually is not identifiable on conventional histological sections. Therefore, the prometaphase which is identifiable on histological sections was considered the first phase of cell division.

2.3.5 Delineation of Abnormal Mitoses

On simple haematoxylin and eosin stained histological sections, the abnormal mitoses are most easily recognised when the chromosomes appear in a variety of configurations that do not coform to any of the four normal mitotic phases. Abnormal mitoses can take the form of a clumped mitotic figure with extensive chromatin resembling multinucleated cell, but the "hairy" processes of chromosomes allow it to be identified. Other forms which abnormal mitosis can take are, for example, tripolar mitoses, where the chromosomes are arranged in such a manner as to point to three directions from a common central point Fig. 2.2.

2.3.6 Delineation of Apoptotic Mitoses and Apoptotic cells

Apoptosis which is a physiologic or pathologic process of cell loss can be identified in histological sections stained by haematoxylin and eosin. Initial identification of apoptotic cells is by the characteristic eosinophilic cytoplasm due perhaps to swollen mitochondria that takes the acidic stain. Apoptosis in tumours,

sometimes appeared to be cells in mitosis. In this study two categories of apoptosis therefore, were delineated. These were the apoptotic mitoses and other apoptotic cells.

The apoptotic mitoses were recognised in this study as cells with characteristic apoptotic cytoplasm but with the nucleus appearing to consist of chromosomes suggestive of mitosis (Fig. 2.3). The apoptotic cells which represent the process of necrosis, on the other hand, have been recognised to consist of nuclear fragments scattered in eosinophilic cytoplasm. Not uncommonly, the apoptotic cells were recognised as consisting of a condensed, shrunken nucleus surrounded by a clear halo in an eosinophilic cytoplasm Fig. 2.4.

2.3.7 Examination of Sections and Mitosis Counting Techniques

The histological sections were examined using a conventional light microscope. The x12.5 eyepieces were used and in one eyepiece a square graticule was fitted in order to restrict the part of the field within which the mitoses were counted. A high power objective of x40 was used giving a total magnification of x500. This magnification was found to reveal the features of mitotic figures clearly.

The middle section of each ribbon of three was selected and was scanned systematically. The technique of counting was carried out by starting from one side of the section and moving in only one direction at a time. All mitotic figures falling within the rectangle of the graticule were recorded. Care was taken not to allow the fields demarcated by the rectangular line to overlap areas already counted. This was necessary so that no mitotic figure was counted

twice. With back and forth movement of the sections, counts were made for histologically normal mitotic figures encountered in the invasive neoplastic keratinocytes until the required 100 mitoses was achieved.

In cases where fewer than 100 mitoses were seen in the sections from the first level of each block, sections of the further levels were examined until the total of 100 mitoses was assessed. In cases where there was difficulty in interpretation of an individual mitotic figure this was resolved by examination of the immediately adjacent serial sections.

In the areas where the 100 histologically normal mitotic figures were counted, the categories of abnormal mitoses, and apoptotic neoplastic cells and mitoses were also counted. The count for each of these categories was recorded separately for each case involved in the study.

2.4 MATERIAL AND METHODS FOR ASSESSMENT OF SILVER STAINED NUCLEOLAR ORGANIZER REGIONS (AgNORs)

2.4.1 AgNORs - What are they?

Nucleolar organizer regions (NORs) are loops of DNA which occur in the nucleoli of cells that possess ribosomal RNA genes. These genes are transcribed by RNA polymerase I for the ultimate synthesis of proteins. In human cells these are located on each of the short arms of chromosomes 13, 14, 15, 21 and 22 and therefore 20 may be seen in a normal diploid intact cell.

The exact biochemical nature of the NORs proteins is still controversial. A number of suggestions of the possible nature of

these proteins have been made. Williams et al. (1982) stated that the proteins may consist of large subunits of the RNA polymerase I. In subsequent studies Ochs and Busch (1984) stated that the proteins associated with NORs are C23 proteins. These are a nucleolar phosphoprotein which appears as band C23 in gel studies. Whatever the nature of NORs proteins is, Buys and Osinga (1980) reported that these proteins probably have a regulatory role in ribosomal DNA transcription and keep them in the extended form. Thus Morton et al. (1983) suggested that the NORs proteins could serve as preferential cytochemical markers of both the ribosomal DNA and its actual or potential transcription.

Howell and Black (1980) were able to develop a one-step simple silver staining method for the detection of the NORs at the ultrastructural level by using a protective colloidal developer in combination with especially prepared aqueous silver nitrate solution. Hence, Howell (1982) stated that silver staining is useful for the study of nucleolus and of the nucleolar activity. Further modifications and improvement in the staining and visualization of NORs proteins at the light microscope level were obtained by Ploton et al. (1986). These authors were able to apply the technique on thin paraffin embedded tissue sections of human prostatic cancer and to visualize the AgNORs as black dots and clusters. Further, they suggested that the number of AgNORs in the nuclei may reflect their state of activation and be related to malignancy.

2.4.2 Practical Applications of AgNORs in Histopathology

AgNORs have been studied for some years by Cytogeneticists who have made use of their demonstration in the analysis of various genetic defects. Consequently, in recent years a one-step silver staining method for demonstrating NORs on tissue sections has found widespread applications in the field of tumour pathology.

On the basis of AgNORs counts on formalin-fixed, paraffin embedded histological sections, Crocker and Nar (1987) were able to separate low grade from high grade non-Hodgkin's lymphomas. Separation between some cutaneous melanotic lesions was also possible (Crocker and Skilbeck, 1987). In assessing the prognostic relevance of AgNORs, however, Howat et al. (1988) found that AgNORs counts were of no prognostic value in skin malignant melanomas. Similarly, Griffiths et al. (1989) reported the absence of prognostic relevance of AgNORs counts in adenocarcinoma of the rectum. Nonetheless, it has been generally observed that the number of AgNORs in malignant cells is significantly greater than in their normal or benign counterparts. The purpose of the present study was to investigate, on biopsy material of primary squamous cell carcinoma of the oral cavity, the AgNORs count, and to find out whether the mean number of AgNORs in a particular case could help in the prediction of prognosis in terms of lymph node metastasis, cure rate and survival.

2.4.3 Selection of Material

The material on which the mitotic phase distribution studies was carried out, as discussed in section 2.3.2 was essentially the same as was selected for the assessment of AgNORs. Only cases with

carcinoma of the tongue, floor of the mouth or tongue plus floor of mouth which had biopsy, resection and preferably neck dissection specimens were involved in the study. As it was planned to correlate the AgNORs counts with the survival of individual patients, cases that had initial biopsy and resection but no neck dissection specimens were also involved in the study.

Because histological sections from multiple levels were prepared for the evaluation of mitotic phase distribution, in some cases there was not enough tissue remained in the paraffin blocks of the initial biopsy material. Such cases were not included in this study.

Occasionally, it seemed that biopsy material of some cases selected for this study were postfixed with fixative containing mercuric chloride. This chemical, if present in biopsy material, reacts with silver ions within the staining solution resulting in a nonspecific background reaction. This influences the resolution of AgNORs and makes interpretation of silver grains unreliable. Therefore such cases were also discarded from the study in order to get more consistent and reproducible results.

2.4.4 Preparation of Working Solutions

The techniques for staining AgNORs were essentially those described by Crocker and Nar (1987). The working solution was a combination of two basic solutions A and B. Solution A was prepared by dissolving powdered gelatin at a concentration of 2 per cent w/v in deionized water over a water bath at 65 -70°C. Pure formic acid was

then added to a final concentration of 1 per cent. Solution B was simply a preparation of 50 per cent aqueous silver nitrate in deionized water.

Deionized water was used for the preparation of the solutions to avoid non-specific precipitation that might result from silver ions reacting with different ions in tap or distilled water. The solutions were then stored in separate containers and kept in the fridge to be ready for use when needed. Solution B was stored in a dark coloured glass container in order to be protected from a direct light which might reduce the silver ions within the solution.

2.4.5 Section Preparation and Staining Techniques

Formalin-fixed, paraffin embedded blocks of the biopsies of selected cases were obtained from the storage files. One section of 3μm thickness was cut from each block. These sections were mounted on glass slides, dewaxed in xylene, hydrated through decreasing concentrations of ethanol and washed thoroughly in deionized water for 10 minutes. In the meantime the required amount of solutions A and B for a single run was filtered through 0.22μm millipore filter in a separate measuring cylinder.

Sections were then placed in a Coplin jar and one volume of solution A and 2 volumes of solution B were poured simultaneously over the sections to give a working solution. Sections were immediately incubated in the dark for 30-35 minutes at room temprature. This was found to give optimal staining after attempts at 20, 25, 30, 35, 40, 45 minutes. Sections were then washed thoroughly in 2 changes of deionized water for 10-15 minutes, dehydrated in ascending ethanol

* In each microscopic field the AgNORs in all cells at the growing front were counted. A range of 0 - 58 AgNOR dots per nucleus was found. Where no AgNORs were found in a nuclear profile in the section, it was thought that this could either be because no AgNORs were present in that nucleus as a whole or in the segment viewed.

concentrations, cleared in xylene and resin mounted in Harelco Synthetic Resin (HSR).

Lymphocytic infiltration within the sections proved to be an effective internal control as the majority of these infiltrative lymphocytes were found to contain 1-2 silver grains. Initially, a number of counterstains were assessed but none of these assisted in interpretation. Sections with no counterstain gave the best results.

2.4.6 Examination and Counting Procedure

Sections were examined by using the light microscope with a x100 oil immersion objective and x12.5 eyepiece, giving a total magnification of x1250. Careful focussing allowed the AgNORs to be visualized as black dots arranged in clusters, clumps or as individual satellites within the nucleus. Fig. 2.5 illustrates the photomicrographic appearance of silver stained nucleolar organizer region proteins. To avoid double counting of a particular nucleus, an eyepiece with graticule was used in order to restrict the field on which the count would be undertaken.

Attempts were made to decide the number of the neoplastic cells on which the count of AgNORs should be made in order to be considered as representative for a particular case. The count of AgNORs in 200 neoplastic cells per section in 5 cases was undertaken. Only those cells in the growing front of the invading part of the tumour were counted.* In cases where tumour cells formed keratin pearls in this front, counting was restricted to the basal layer of cells of the pearl. Applying the cumulative means test, it was found

that a minimum of 60 neoplastic cells could be considered representative for each case. Therefore to be on the safe side AgNORs in 80 neoplastic cells from each section were involved in the counting.

The counting of the 56 cases involved in the study was then undertaken. There were 42 cases with biopsy, resection and neck dissection specimens. The remaining 14 cases had only biopsy and local resection but no neck dissection specimens. In each section the mean number of discernible AgNOR dots was calculated and the data were stored on the computer with the records of each case.

2.5 MATERIAL SELECTED FOR EVALUATING HISTOLOGICAL PARAMETERS FOR MALIGNANCY GRADING

Following the analysis of the general material selected for the main study, it was possible to separate all the cases in which tumours arose from mucosa of the tongue, floor of the mouth and tongue plus floor of mouth. The cases selected for the evaluation of the histological features were those in which a primary squamous cell carcinoma had been resected and representative histological sections related to the resection specimens were available in the files of the Oral Pathology Unit.

2.5.1 Histological Parameters Evaluated for Malignancy Grading

The histological features evaluated in the study were the same as those recommended by Anneroth et al. (1987) in their malignancy grading system. Six histological features were assessed. Three of the features relating to tumour factor were degree of

keratinization, nuclear polymorphism and number of mitoses. The other three features relating to tumour-host interaction were, pattern of invasion, stage of invasion and lymphoplasmacytic infiltration.

Histological sections related to the resection specimens of the selected cases were retrieved from the files. The histological features were evaluated on the representative sections using an ordinary light microscope.

2.5.2 Grading and Scores for Histological Features

For each histological feature studied, using the Anneroth et al's system a numerical score of one to four was given. A score of one is said to indicate a good sign of prognosis. Gradually toward the worse prognosis were scores 2, 3, and 4 respectively. In each section for a particular case, screening all over the section was carried out and for each histological feature the apparently worst looking area in the section that gave the highest score for each parameter was assessed. The criteria followed for evaluating each grade within a particular histological feature were essentially those of Anneroth et al. (1987). The only modification considered, was concerning the assessment of the number of mitoses. In the Anneroth et al. (1987) malignancy grading system, the number of mitoses was assessed by counting all mitoses above the basal layer in as many as possible high power fields of x400. In this study it was found occasionally that squamous cell carcinoma invaded through the connective tissue in a thin cords or small groups of cells or as single cells. Thus no supra-basal cell layer was evident in such cases. Therefore, it seemed reasonable to assess the feature of mitoses by counting all the mitoses within randomely selected 10 high

power fields, at a magnification of x500, from the invading neoplastic cells. The mean number of mitoses per high power field was then calculated and the score for each case was given accordingly. It is worthy to mention that a decision was made to count mitoses in 10 high power microscopic field after an attempt of counting the mitoses all over the histological sections in 5 cases. It was found that although there was a slight difference in the mean number of mitoses comparing the two methods of counting, this seemed not to influence the overall grade of a particular case on the basis of the mitosis score.

The high risk indicators related to tumour factor features were thought to be no keratinization within or around tumour cells, marked nuclear polymorphism and frequent mitoses (Fig. 2.6). Whereas the good prognostic indicators related to tumour factor were thought to be abundent keratinization within or around the neoplastic cells and keratin pearl formation, no or very little variation in the nuclear sizes and no or scant mitoses (Fig. 2.7). The high risk indicators for tumour-host interaction were thought to be diffuse and deep invasion of neoplastic cells which excited little or no lymphocytic or plasma cell infiltrate (Fig. 2.8). The good prognostic indicators related to tumour-host interaction were thought to be pushing and well defined growing margins, superficial invasion and the presence of marked lymphoplasmacytic infiltration (Fig. 2.9).

Initially the scores related to the features considered in the study were recorded on a specially designed form. The sum of the scores of the six features in individual case represented the total malignancy grade. The sum of the scores of the first three features and the sum of the second three features represented respectively the

tumour factor and the tumour-host interaction. The mean malignancy grade was calculated by dividing the total malignancy grade by the number of features studied. These were also recorded in the forms. Then all the data achieved for an individual case was transferred to the computer.

2.6 MATERIAL SELECTED FOR THE ASSESSMENT OF THICKNESS AND MICROSCOPIC SURGICAL MARGINS OF THE TUMOURS

The cases used for the assessment of histological parameters for total malignancy grading discussed in Section 2.5 were the same cases on which the thickness of the tumours and microscopic surgical margins evaluated.

The cases selected were those which had their primary tumours resected. From the resection specimens however, many histological sections were often prepared in order to evaluate the tumour thoroughly and enable the diagnostic pathologist to issue a comprehensive pathology report.

It was felt that assessing tumour thickness on resection specimens would be more accurate than on the biopsy specimens, because the biopsy specimens were unlikely to contain the thickest part of the tumour. Therefore, all the histological sections prepared from surgical specimens of a particular case were examined and the section that showed the greater tumour thickness was selected for the study. Sections selected for the evaluation of microscopic surgical margins were those indicated on the drawing or photographs of surgical specimens made during the initial sampling and cut up procedures.

This was also double checked by re-examining the particular sections under the light microscope.

2.6.1 Techniques for Evaluating Thickness of Tumours

The one histological section which had been obtained from the resection specimen for measurement of thickness was assessed. The thickness of the tumour was considered to represent the distance between the surface epithelium of the mucosa from which the invasion of neoplastic cells started and the deepest growing part of the tumour. The measurement was performed by using a micro-occulometer device. The sections were placed on an illuminated viewer and were examined with the micro-occulometer. The thickness was considered to the nearest millimeter and this was recorded.

In cases where the mucosa was intact and the growing edge was within the level of normal adjacent mucosa, the thickness was considered from surface of the epithelium including the keratin layer, when it was present, to the deepest growing part of the tumour. When the tumour showed ulceration the thickness was regarded from the base of the ulcer including the necrotic surface to the deepest invading part of the tumour.

Tumours occasionally showed exophytic growth. In such cases, the thickness was assessed as the mean of two measurements. The first was taken between the epithelium surface and the deepest invading point of the tumour. The second measurement was taken between an arbitrary line where the original surface would have been, and the deepest invading front of the tumour. The data obtained were recorded on special forms and then transferred into the computer.

2.6.2 Techniques for Evaluating Microscopic Surgical Margins

Resection specimens when delivered for histopathological study should always be oriented for histological evaluation of the surgical margins. Certain anatomical landmarks involved in the specimen are quiet helpful for the accurate orientation. Occasionally however, it is quiet difficult to orientate the specimen, particularly in cases where no anatomical landmarks are evident. In such cases the surgeons mostly indicate the site of specimen and mark it in a way that makes the orientation apparent.

Before sampling the resection specimen, a drawing and or a photograph of the specimen had usually been prepared. The tissue blocks taken from the specimen were always labelled to correspond to the similar labels marked on the drawing and the photograph. These tissue blocks often involved the surgical margins of the specimens.

The histological sections related to the surgical margins of a particular case were examined microscopically for their clearance of tumour cells. The closest margins were then examined using a micro-occulometer and illuminated viewer. The mucosal and the deep margins were evaluated and the distance between the particular surgical margin and the neoplastic growth at the same side was measured. The deep surgical margins and type of tissue into which the tumour was invaded were also noted. The measurement were recorded to the nearest millimeter. Codes were given for the type of tissues forming the surgical margins of the resection. Data were recorded on specially designed forms and then were stored in the computer.

Tumour Site	Number of Cases
Tongue	62
Floor of Mouth	68
Tongue Plus Floor of Mouth	33
Buccal Mucosa	15
Lower Alveolar Mucosa	27
Lower Lip	13
Retromolar Area	23
Upper Alveolar Mucosa	11
Palatal Mucosa	11
Complex Site (Tumour involved posterior tongue, tonsil, faucial arch, soft palate, oropharynx)	35
Total	298

Table 2.1. Number of the cases according to the intra-oral sites on which the study was planned initially.

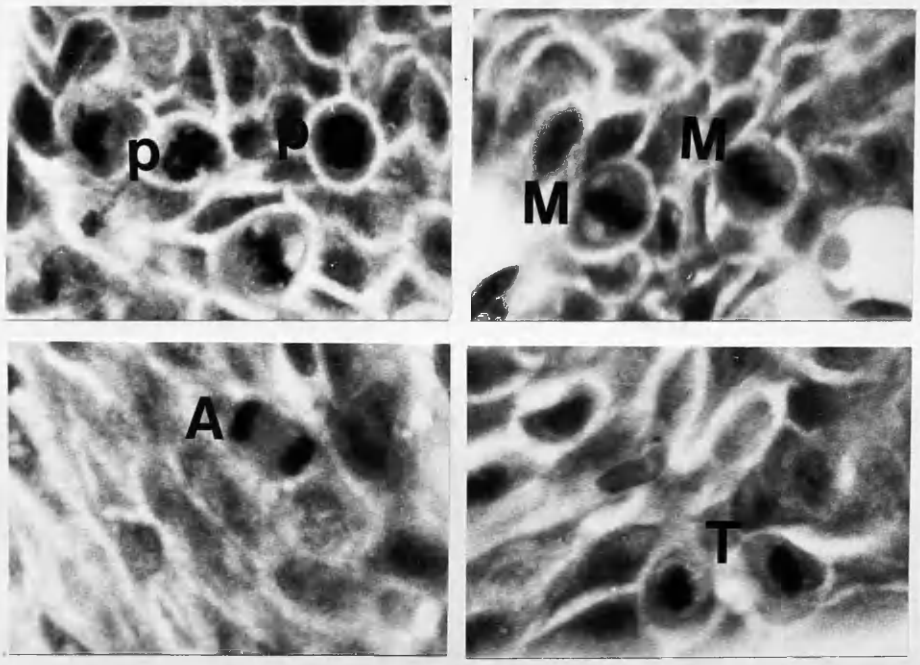


Fig. 2.1. Haematoxylin and eosin stained sections illustrating the four apparently normal phases of mitosis, prometaphase (P) x970, metaphase (M) x1000, anaphase (A) x960 and telophase (T) x800.

Fig. 2.2. Illustration of a tripolar form of abnormal mitosis H & E x840

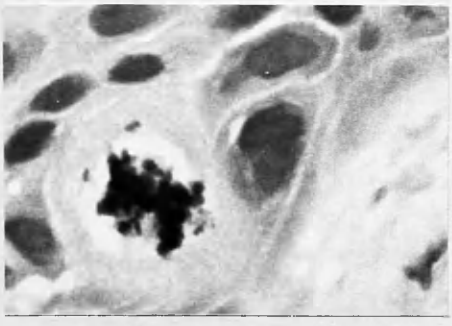


Fig. 2.3. Apoptotic mitosis showing masses of chromatin scattered in the cytoplasm. H & E x875.

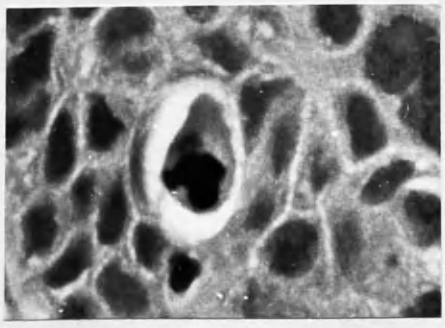


Fig. 2.4. Apoptotic cell evident with shrunken nucleus surrounded by a clear halo. H & E x930.

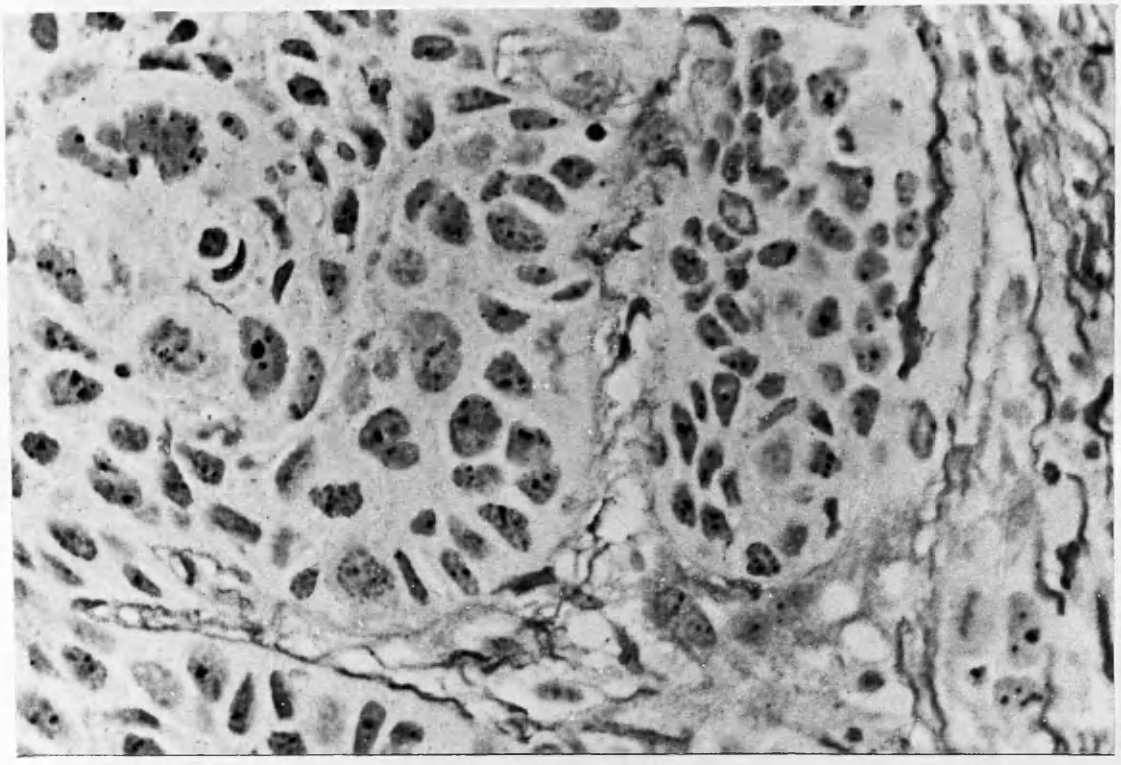


Fig. 2.5. Silver stained nucleolar organizer regions (AgNORs) appearing as black dots within nuclei. Silver-stained section, x590.

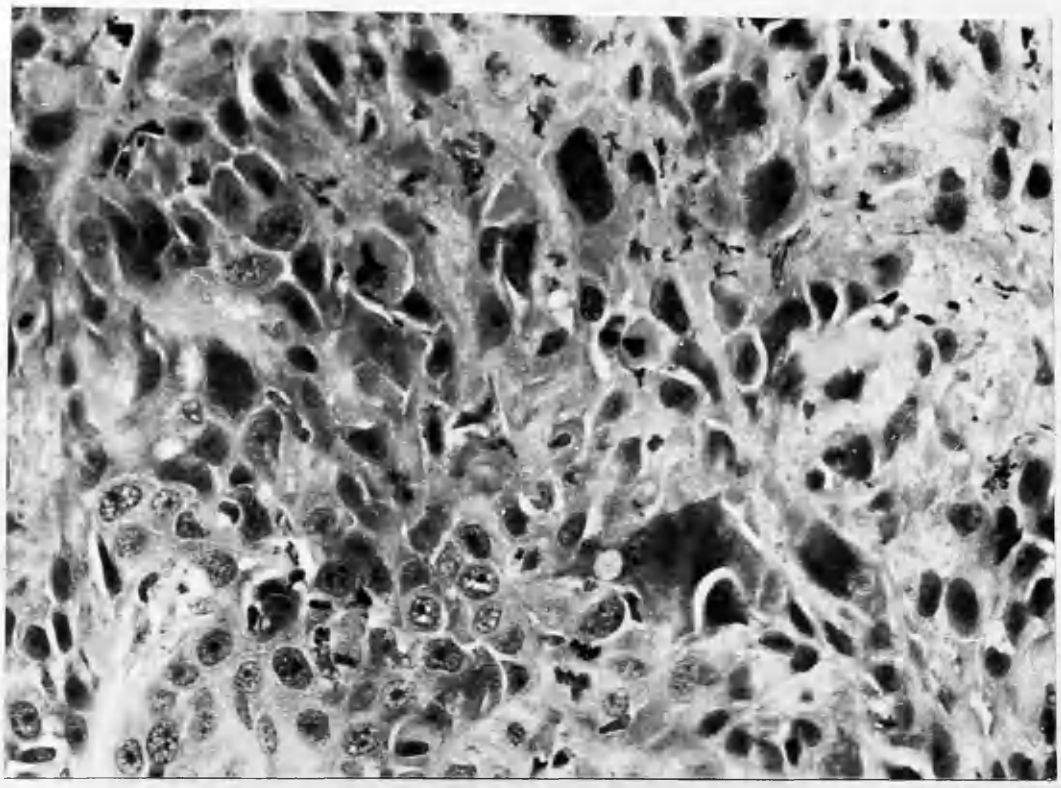


Fig. 2.6. Squamous cell carcinoma demonstrating the high risk indicators related to tumour factor (no keratinization, marked nuclear polymorphism and frequent mitoses). H & E x365.

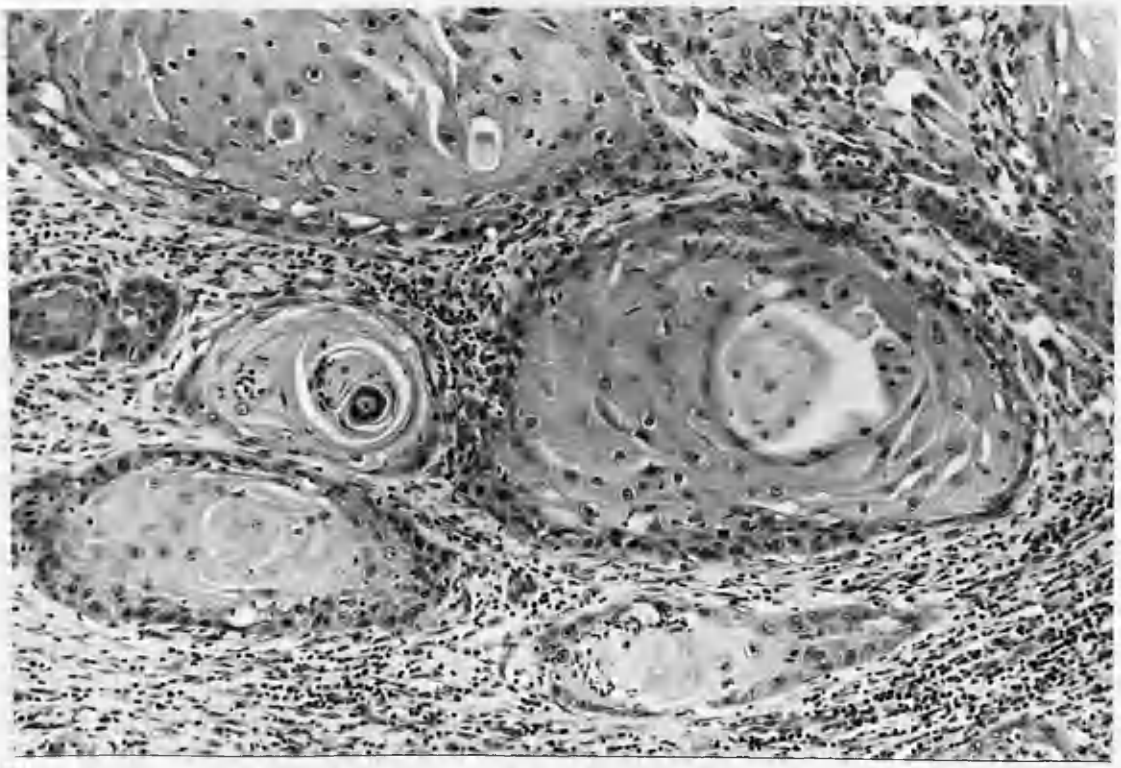


Fig. 2.7. Illustration of good prognostic indicators related to tumour factor in squamous cell carcinoma (abundant keratinization, very little variation in nuclear sizes and no mitosis). H & E x140.

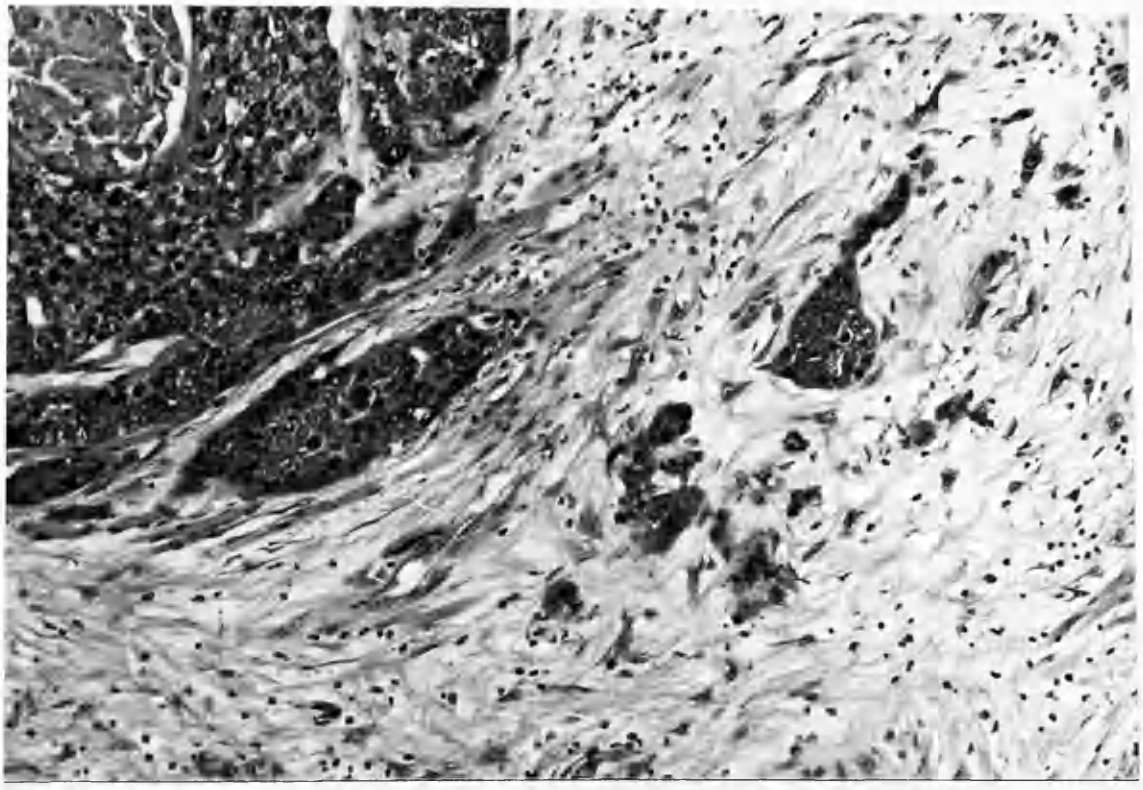


Fig. 2.8. Squamous cell carcinoma demonstrating the high risk indicators of tumour-host interaction (diffuse and deep invasion and excited little lymphoplasmacytic infiltration). H & E x140.

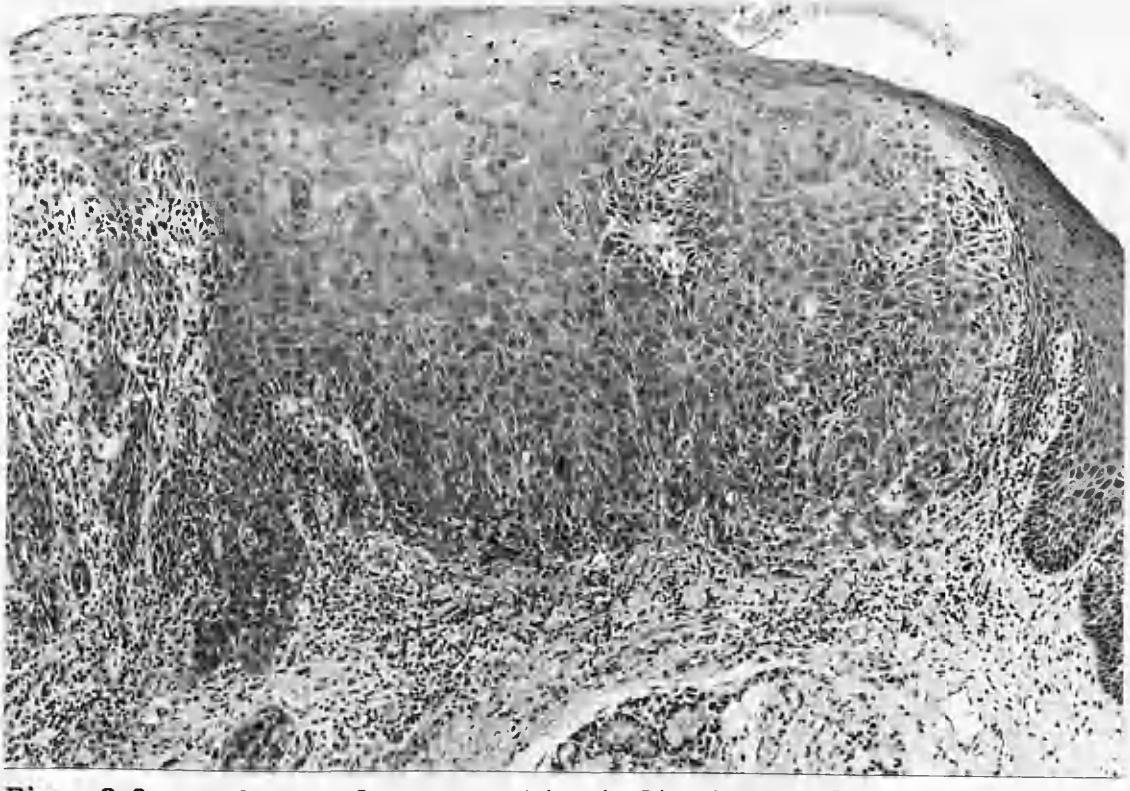


Fig. 2.9. The good prognostic indicators of squamous cell carcinoma related to tumour-host interaction (well defined invading margins, superficial invasion and marked lymphoplasmacytic infiltration). H & E x140.

CHAPTER 3

PREDICTION OF METASTASES IN ORAL CARCINOMAS

3.1 INTRODUCTION

Involvement of lymph nodes with secondary tumour is undoubtedly a bad prognostic sign adversely affecting cure rate and survival of patients. Five-year survival has been reported in up to 70 per cent of patients when the neck was negative, whereas in positive necks 5-year survival was down to 21 per cent (Noone et al. 1974; Grandi et al. 1985 and Cachin et al. 1979).

Clinical evaluation of cervical lymph node involvement is unreliable. Clinically lymph nodes are considered positive when the nodes are enlarged, palpable or fixed to adjacent tissues.

Many workers have recognised the fact of false positive and false negative clinical assessment of cervical lymph nodes and figures up to 53 per cent false positive and 38 per cent false negative have been documented (Sako et al. 1964; Spiro et al. 1974; Noone et al. 1974 and Ali et al. 1985). Thus patients with clinically, apparently positive necks may have been treated erroneously in up to 53 per cent of cases when in fact these operations were not required. On the other hand, if clinically negative nodes are left without treatment until tumour presents clinically there is a poor prognosis. In a group of 32 patients with oral cancer who were left under observation until palpable nodes appeared, a possibility of a 60 per cent 5-year survival was reduced to 30 per cent because of the delay in treatment (Mendelson et al., 1976). Therefore, it was felt that there is a need

for predictors of lymph node metastases which are more accurate than simple clinical assessment.

With the development of computerised tomographic (CT) scanning, there has been considerable enthusiasm that this might provide an effective non-invasive method of assessing nodal metastases. However, conflicting results have been documented in different reports. Friedman et al. (1984) and Stevens et al. (1985) reported that CT scanning of the neck was more accurate than clinical examination in predicting metastasis in cervical lymph nodes. On the other hand, Feinmesser et al. (1987) have questioned the sensitivity of CT scanning in the prediction of nodal metastasis and, in fact, they suggested that it offered no advantage over the physical examination of the neck for the presence of nodal metastasis.

Recently, a CT scan evaluation of regional lymph node involvement in cancer of the oral cavity and oropharynx was carried out by Close et al. (1989) as a prospective study on 61 previously untreated patients with T2 or larger squamous cell carcinomas. These patients underwent neck dissection within one month following the computed tomography. The authors found that there was a significant relation between the pathological status of lymph nodes and the CT categorisation of nodes according to shape, size, appearance and multiplicity. Although this technique was found to be superior to physical examination in predicting nodal metastasis, its reliability is still under question. The overall efficacy for computed tomography in evaluating necks for regional node metastasis was 80.7 versus 68.7 per cent for physical examination (Close et al., 1989). This is because lymph nodes can be confused with, or obscured by, the multiple

anatomical structures in the neck region, making interpretation of the CT scan difficult and thus not totally reliable. Another problem is that not all nodes involved by tumour are obviously enlarged.

Excisional biopsy of clinically suspicious lymph nodes for histological assessment of possible metastasis is undoubtedly more accurate than simple clinical assessment. However, such an approach carries the risk of postsurgical complications. McGuirt and McCabe (1978) noted a significant increase in wound necrosis, regional recurrences and distant metastases in those patients who had excisional node biopsy before the definitive treatment, in contrast with those patients who had no biopsy or had biopsy at the time of definitive treatment. Therefore excision biopsy for assessment of nodal involvement is contra-indicated.

Fine needle biopsy may provide an alternative method for predicting metastasis in the cervical lymph nodes in patients with oral carcinoma. This technique in the hands of experienced surgeons can provide a meaningful sampling without interfering with the ultimate treatment and disturbing tissues in the manner of an excisional biopsy (Frable and Frable, 1979). The technique is of particular value in debilitated patients or those with serious cardiorespiratory diseases who can be poor surgical risks. The technique can be useful also in diagnosis of a possible recurrence of previously treated tumours.

The aspirates of clinically suspected lymph nodes fall into three general diagnostic categories: metastatic carcinoma, benign lymphadenopathy and malignant lymphoma. Perhaps the most frequent

application of fine needle biopsy is the aspiration of lymph nodes for diagnosis of metastatic carcinoma. Within the head and neck region, squamous cell carcinoma represents the most commonly diagnosed tumour. Metastatic squamous cell carcinoma is not usually difficult to diagnose. Most aspirates are said to contain abundant squamous cells demonstrating cytological criteria of malignancy and evidence of keratinization on Papanicolaou stain. However, necrosis and cystic degeneration are common elements in metastatic squamous carcinoma. In such instances aspiration from the centre of a necrotic tumour can yield mostly debris with only a few squamous cells exhibiting karyolysis or pyknosis. If these findings are present on initial aspirates during rapid assessment, re-aspiration of the edge of the mass has been recommended (Bedrossian et al., 1988).

Clinically enlarged lymph nodes occasionally contain only a small focus of metastatic tumour. Thus there is a chance of a blind needle aspiration biopsy missing the target lesional tissue, and giving a false negative result. It is crucial therefore, that the needle must make contact with the target lesion. Such a limitation, and the fact that in oral cancer patients, cervical lymph nodes which are neither enlarged nor palpable may contain metastasis, have mitigated against the widespread use of aspiration biopsy.

In this study it was planned to tackle the problem of predicting nodal metastasis in patients with intra-oral carcinoma by assessing certain histological features on biopsy and on local excision of the primary tumour. The attempt was then made to correlate these features with the histological status of the lymph

nodes in neck dissection specimens.

3.2 MATERIAL AND METHODS

3.2.1 Details of Case Selection

The bulk of the material used in this study was extracted from the general material used for the main study and the general criteria for case selection are discussed thoroughly in Chapter 2. The study was restricted to cases of squamous cell carcinoma that arose from mucosa of the floor of mouth, the tongue or a combination of both sites.

The purpose of this retrospective study was to evaluate in oral squamous cell carcinoma certain histological parameters on original biopsy and on resection specimens and to correlate the results to the histological status of the lymph nodes in individual cases. Therefore, to evaluate the histology of lymph nodes for metastasis, availability of neck dissection specimens was a crucial criterion for the case to be selected. The other two criteria were that a preliminary biopsy of the tumour was available and that there was a resection specimen of the primary tumour.

3.2.2 The Parameters Studied and the Techniques of Assessment

The parameters evaluated on the biopsy material were the mitotic phase distribution and the nucleolar organizer region (AgNORs) counts. On the resection specimens of the primary tumour, the histological features for assessing malignancy grading as recommended by Anneroth et al. (1987) were studied. The three features related to tumour factor were, degree of keratinization, nuclear polymorphism and

number of mitoses. The other three features related to tumour-host interaction were pattern of invasion, stage of invasion and lymphoplasmacytic infiltration. Tumour thickness for individual cases was also assessed on the histology sections that were obtained from the resection specimens.

The techniques involved in the evaluation of mitotic phase distribution are thoroughly discussed in Section 2.3 and the related subsections. Assessment of histological parameters for malignancy grading and the related techniques are discussed in Section 2.4 and the related subsections. The techniques for evaluating tumour thickness are discussed in subsection 2.5.1. All the techniques involved in assessing the nucleolar organizer regions are discussed in Section 2.6 and the related subsections.

3.3 RESULTS

The results will be presented in the following subsections in a way that each predictor parameter is dealt with separately. The numbers of cases on which each parameter in question was studied varied. This was because the mitotic phase distribution and AgNORs were assessed on biopsy material whereas malignancy grading and tumour thickness were assessed on the resection specimens. There were more cases with resection specimens than cases with preliminary biopsies suitable for analysis.

The cases on which the mitotic phase distribution was carried out were numbered in each site. The same case numbers are used in the presentation of data on other parameters like AgNORs, malignancy

grading and tumour thickness.

3.3.1 Mitotic Phase Counts and Prediction of Nodal Metastasis for Floor of Mouth Carcinoma

Twenty-three cases of floor of mouth carcinoma were studied. Of these 11 had metastatic tumour in nodes and 12 were node negative. The normal mitotic figures evaluated in the biopsies were categorised into phases of cell division using the criteria described by Ojo (1985) and Ojo and MacDonald (1988).

The percentages of the four normal mitotic phases namely prometaphase (P), metaphase (M), anaphase (A) and telophase (T) are shown in Table 3.1. This table also shows the numbers of abnormal mitoses (Abm), apoptotic mitoses (Apm) and apoptotic cells (Apc) counted in the same fields as the 100 normal mitoses were assessed. The ratios P:M and P+M:A+T in individual case are also shown. The means and the standard deviations (SD) for each mitotic parameter in the group with negative and the group with positive nodes are also shown in Table 3.1.

It was found that in the majority of cases the percentage of prometaphases was higher than the rest of the mitotic phases. The next most frequent phase was metaphase. Mann-Whitney U-tests were carried out for all the statistical analyses. The percentages of each of the phases were compared between the group with negative nodes and the group with positive nodes. Although there were slight differences in the means in the mitotic phases between the groups, no statistically significant differences were noted.

The P:M ratio in the node positive cases was slightly lower than in the node negative cases, but the difference was not statistically significant. Similarly the ratio of early to late phases of mitoses (P+M:A+T) did not differ significantly between groups.

The abnormal mitoses and apoptotic mitoses and cells were then compared and no differences in incidence between the groups was evident. The values for abnormal mitoses plus apoptotic mitoses were combined to give an indication of aberrant mitotic activity, but again no difference was evident between groups. Similarly apoptotic mitoses and apoptotic cells were combined to give a total indication of this form of cell loss, but comparison showed no difference between groups.

3.3.2 Mitotic Phase Counts and Prediction of Nodal Metastasis for Tongue Carcinoma

Eighteen cases of tongue carcinoma were assessed for mitotic phase distribution. Of these 13 showed metastatic tumour and five were node negative.

As in cases of floor of mouth carcinoma, the percentages of the four normal mitotic phases, abnormal mitoses, apoptotic mitoses and apoptotic cells were studied. The results are shown in Table 3.2, categorised into two groups, with either negative or with positive nodes. The ratios of P:M and P+M:A+T in individual case are also shown in Table 3.2. Similar trends to those described in the floor of mouth carcinoma were noticed. The percentages of prometaphases were higher than the rest of the mitotic phases.

Statistical analyses were carried out in the same manner as in cases of floor of mouth carcinoma. Similar parameters were compared between the group with negative and the group with positive nodes. There were slight differences in the means but no statistically significant differences between groups were demonstrated.

3.3.3 Mitotic Phase Counts and Prediction of Nodal Metastasis for Carcinomas Involving Tongue Plus Floor of Mouth

Ten cases of carcinoma involving tongue plus floor of mouth were studied. Six were node positive and four were node negative.

The same mitotic parameters and the ratios that were assessed in cases of floor of mouth carcinoma and cases of tongue carcinoma, were also studied on the cases at this anatomical site. The results of these mitotic parameters and the ratios are shown in Table 3.3.

Similar statistical analyses to those described in Section 3.3.1 were carried out to compare the mitotic parameters between node negative and node positive groups at this site. Slight differences in the means between the groups were noted, but the differences were not statistically significant.

3.3.4 Combination of Cases at Different Sites

It was felt that the numbers of cases at each site were relatively small and that this might have influenced the results. Accordingly further comparisons were undertaken contrasting the data on all node negative cases with that from node positive cases. Table

3.4 summarises the data showing the means and standard deviations of the combined groups. Comparisons were made using Mann-Whitney U-tests and these showed no significant differences in any of the parameters between the node positive and node negative cases.

3.3.5 Statistical Comparison of Mitotic Phase Counts Between the Sites

An attempt was made also to compare the mitotic parameters between the sites to determine whether there might be differences in the mitotic phase distribution of carcinomas in these three anatomical sites. Table 3.5 summarises the data on normal mitoses for the three sites studied. Mann-Whitney U-tests gave apparently significant differences between floor of mouth and tongue cases in the percentage of prometaphases and the percentage of telophases (P= 0.046 and P= 0.005, respectively). Because 69 separate Mann-Whitney U-tests had been undertaken, there was a risk that these were not real differences. When a Bonferroni adjusted probability was derived neither difference appeared significant. No significant differences were found in the data from floor of mouth compared to cases involving tongue plus floor of mouth. When the tongue alone was contrasted with cases involving tongue plus floor of mouth the percentage of telophases initially appeared significantly different but was found not to be so following Bonferroni adjustment.

3.3.6 Comparisons of Abnormal Mitoses and Apoptotic Cells and Mitoses

The data on abnormal mitoses and apoptosis are summarised in Table 3.6. Abnormal mitoses were combined with apoptotic mitoses to give a total value for aberrant mitoses. Apoptotic mitoses were combined with apoptotic cells to give a total count for apoptosis. The only significant differences apparent following Bonferroni adjustment were between the floor of mouth tumours and the tongue tumours ($P = 0.028$). Abnormal mitoses and apoptotic mitoses were more frequent in tongue cases. The combination of apoptotic mitoses and apoptotic cells was also more frequent in tongue cases. The value for apoptotic cells was not significantly different but the value of the combined data on apoptotic cells and mitoses showed a significantly higher value in tongue tumours ($P = 0.014$, Bonferroni adjusted).

In all comparisons the data for tumours of tongue plus floor of mouth were intermediate between floor of mouth alone and tongue alone and did not differ significantly from these other groups.

3.4 AgNORs AND PREDICTION OF NODAL METASTASIS

The data for the three sites studied are shown in Table 3.7. The case numbers are the same as those used in the corresponding analyses of mitotic phase data shown in Tables 3.1 to 3.3. The tumour size (T) and the clinical nodal status (N) for the cases in the three sites are also shown in Table 3.7.

3.4.1 Nodal Status

The groupings in Table 3.7 indicates the cases with histologically proven node metastases. Comparison with the clinical node evaluation showed only one instance in the 15 node negative cases where the neck was thought to be positive clinically. This is a 6.7 per cent false positive assessment. By contrast more cases assessed as N0 clinically, were found to have nodal metastases. Seven of the 27 node positive cases were in this category, representing a 26 per cent false negative assessment. All but two of the cases in this category involved the tongue tumours.

3.4.2 Prediction of Nodal Metastasis in Floor of Mouth Carcinoma

Twenty cases of floor of mouth carcinoma were studied. Of these, 11 had metastatic tumour in the nodes and nine were node negative.

The mean numbers of AgNORs per nucleus in each individual case is evident in Table 3.7. These are categorised according to whether the individual case had metastatic tumour in the nodes or not. Three cases in the group with negative nodes are marked with an asterisk. This is to indicate that cases with the same case number shown in Table 3.1 where the data of mitotic phase distribution are presented, were not suitable for assessment of AgNORs. The reason was due either to the biopsy material being completely used following the preparation of histological sections through multiple levels for assessment of mitotic phase distribution or the biopsy specimens had been postfixed with mercuric chloride which was found to interfere

with the silver staining for AgNORs.

It was found that the mean AgNORs counts were generally lower in the cases with negative nodes than in cases with positive nodes. The mean numbers of AgNORs were compared between the two groups using the Mann-Whitney U-test. The differences were significant (P = 0.019).

Following the histological study of the lymph nodes it was found that the clinical assessment of the nodes was not accurate in only two cases. One case in the node negative group and one case in the node positive group. Case number 2 in the group with negative nodes, clinically was T4N1, but scored a low mean AgNORs count (9.96). Case number 11 in the group with positive nodes, clinically was T2N0, but scored a high mean AgNORs count (20.43). However, there were two cases, number 5 and number 8 in the group with negative nodes, which gave high AgNORs counts but were correctly assessed as N0 clinically.

Fig. 3.1 demonstrates the dot plot of the two groups to show the distribution of the mean numbers of AgNORs. Contrasting of the dot plots reveals that although there is some overlapping in the values, the differences are quite obvious. Out of nine cases with negative nodes only two cases showed mean numbers of AgNORs greater than 12.6. In contrast, all the 11 cases with positive nodes showed mean numbers greater than 12.6.

Linear discriminant analysis was then undertaken and it was found that analysis of the AgNORs count correctly classified 17 of the 20 cases (85%). The misclassified cases were 5 and 8 in the node negative group and case 3 in the node positive group. Because there

was no other group of floor of mouth cases upon which to test the discriminant function, cross validation was undertaken by omitting each individual case in turn, recalculating the classification on the remaining cases and then applying this to the omitted case. This showed no loss of discrimination giving strong support to the validity of the discriminant ability of the AgNORs count in predicting metastasis.

3.4.3 Prediction of Nodal Metastasis in Tongue Carcinoma

Fourteen cases of tongue carcinoma were studied for AgNORs assessment. Of these 11 cases had metastatic tumour in the nodes and three cases were node negative.

The mean numbers of AgNORs are shown in Table 3.7. These are grouped according to the histological nodal status, whether or not the case had metastatic tumour.

The number of cases in the group with negative nodes was small. The statistical analyses described in Section 3.4.1 were carried out. Although slight differences were noted in the means between the two groups, no statistically significant differences were demonstrated.

3.4.4 Prediction of Nodal Metastasis in Carcinomas Involving Tongue Plus Floor of Mouth

Only eight cases of carcinoma involving tongue plus floor of mouth were used for the assessment of AgNORs. Of these, five had metastatic tumour in the nodes and three were with tumour-free nodes.

Table 3.7 shows the mean numbers of AgNORs for the group with negative and the group with positive nodes. Slight differences were noted in the means of the numbers of AgNORs between the two groups. As in the cases of tongue carcinoma, statistical analyses were carried out to compare the mean numbers of AgNORs. The results did not differ significantly between the groups.

3.4.5 Comparison of Cases in All Sites Combined

The numbers of cases in the tongue and tongue plus floor of mouth groups were small, particularly of node negative cases. All node negative cases, from the three sites were combined and contrasted with the data from all node positive cases. The mean AgNORs count for node negative cases was 11.49 and for node positive cases was 16.32. The cases were compared using the Mann-Whitney U-test and the difference was significant (P= 0.029).

Further, discriminant analysis was undertaken using the AgNORs count in all the 42 cases. This resulted in assigning only 27 cases correctly to either the negative or positive group. This is a 64.3 per cent accurate assessment which indicated that AgNORs count was not a particularly good discriminator for the cases as a whole.

3.4.6 Statistical Comparison of AgNORs Counts in Combined Floor of Mouth and the Tongue cases

Cases of floor of mouth carcinoma in the group with negative nodes were combined with cases of tongue carcinoma in the group with negative nodes. The groups with positive nodes in these two sites were also combined. The mean numbers of AgNORs in the node negative

group was 11.66 and for the group with positive nodes was 16.5. It was found that the difference was significant (P= 0.0052, Mann-Whitney U-test).

3.4.7 Statistical Comparison of AgNORs Counts in Combined Floor of Mouth and Tongue Plus Floor of Mouth Cases

Cases of floor of mouth carcinoma in the group with negative nodes were combined with cases of tongue plus floor of mouth carcinoma in the group with negative nodes. Similarly the groups with positive nodes were combined. The mean numbers of AgNORs in the node negative group was 14.78 and for the node positive group was 16.26. There was a slight difference between the mean values, but this was not significant statistically.

3.4.8 Statistical Comparison of AgNORs Counts in Combined Tongue and Tongue Plus Floor of Mouth

Cases of tongue carcinoma in the group with negative nodes were combined with cases of tongue plus floor of mouth carcinoma in the group with negative nodes. Similarly the groups with positive nodes were combined. The mean numbers of AgNORs in the node negative group was 14.41 and for the node positive group was 15.74. There was a slight difference in the means, but overall the difference was not statistically significant.

3.5 MALIGNANCY GRADING AND PREDICTION OF NODAL METASTASIS

The number of cases involved in the assessment of malignancy grading in each anatomical site included the same cases on which the

mitoses and AgNORs were assessed, plus also cases that had local resection and neck dissection specimens, but no preliminary biopsy specimens. The individual case numbers are the same as used for the mitotic phase distribution and AgNORs assessments.

3.5.1 Prediction of Nodal Metastasis in Floor of Mouth Carcinoma

Thirty-five cases of carcinoma of floor of mouth were studied for the assessment of malignancy grading using the system described by Anneroth et al. (1987). Of these 20 cases had negative nodes and the other 15 cases had metastatic tumour in the nodes.

Table 3.8 summarises the scores of the six individual histological features involved in the system. The scores of the tumour factor which is a summation of the three cellular features, and the scores of the tumour-host interaction which is a summation of three tumour-host interaction features are also shown. The total malignancy grades for individual cases are the sum of all six features. These are presented according to the histological nodal status.

Statistical analyses using Mann-Whitney U-tests were carried out to compare the individual parameters between the group with negative and group with positive nodes. There were slight differences in the scores of total malignancy grades, but statistical analyses showed that the differences between the groups were not significant. Similarly, the total scores of tumour factor were compared and again no significant differences were demonstrated between the groups. However, when the total scores of the tumour-host interaction were compared, statistical analysis showed that the difference was significant ($P= 0.024$).

An attempt was made also to compare each individual feature between the groups. Statistical analyses showed that the pattern of invasion differed significantly between the groups (P= 0.014). None of the other individual features demonstrated significant differences between the groups.

3.5.2 Prediction of Nodal Metastasis in Carcinoma of Tongue

Forty-one cases of tongue carcinoma were studied for the assessment of malignancy grades. Of these, 27 cases had positive nodes and 14 had negative nodes.

Total malignancy grades, total tumour factor and total tumour-host interaction are shown in Table 3.9. The scores of the individual features as well as the means and standard deviations for each parameter are also shown in Table 3.9. These are presented according to whether or not the individual cases had metastatic tumour in the nodes.

As in the cases of floor of mouth carcinoma, similar statistical analyses were carried out to compare the parameters between the groups. Slight differences were noted, but in no instance were significant differences demonstrated.

3.5.3 Prediction of Nodal Metastasis in Carcinomas Involving Tongue Plus Floor of Mouth

Twenty-seven cases of carcinoma involving both tongue plus floor of the mouth were studied for the assessment by the malignancy grading system. Out of these, 15 cases had metastatic tumour in the

nodes whereas the other 12 cases were node negative.

Table 3.10 summarises the total malignancy grades, total tumour factor and total tumour-host interaction. The scores of the six individual features as well as the means and standard deviation for each parameter are also shown in Table 3.10. These are presented in two groups according to histological nodal status.

As in the cases of the other two anatomical sites, similar statistical analyses were carried out. No significant differences were demonstrated between the groups.

3.5.4 Comparison of Cases in All Sites Combined

In order to increase the size of the material studied, it was felt that it would be sensible to compare the cases in node negative groups combining all the three sites together and contrasted with the corresponding data of node positive groups in all sites combined. The mean scores of the total malignancy grade in node negative cases in all the three sites was 16.93 and for the node positive group was 17.23. Only slight differences were noted and these were not statistically significant. The data of the mean tumour factor were also contrasted between the node negative group (mean = 7.21) and the node positive group (mean = 7.09). It was found that the mean score of the tumour factor in the node positive group was slightly less than in the group with negative nodes. No significant differences were demonstrated. The mean score of tumour-host interaction in the node negative group was 9.72 and for the group with positive nodes was 10.16. Again no statistically significant differences were

demonstrated. No instance of statistically significant differences were demonstrated when comparison between the groups was undertaken for each of the six individual features.

3.5.5 Statistical Comparison of Malignancy Grades in Combined Floor of Mouth and the Tongue Cases

An attempt was made to compare the malignancy grade parameters between the node negative groups and the node positive groups in combined cases of floor of mouth and cases of the tongue. The mean of the total malignancy grade in the group with negative nodes was 16.95 and for the node positive group was 17.15. The Mann-Whitney U-test was used and no statistically significant differences were demonstrated. The mean tumour factor scores in the group with negative nodes was 7.23 and in the group with positive nodes was 6.91. This difference was not statistically significant. Similarly the mean tumour-host interaction in the group with negative and the group with positive nodes, 9.72 and 10.23 respectively, were compared and no statistically significnat difference was demonstrated. The same trend also was noticed when the mean scores of each of the individual features were compared between the groups.

3.5.6 Statistical Comparison of Malignancy Grades in Combined Floor of Mouth and Tongue Plus Floor of Mouth Cases

As in the previous Section 3.5.5, it was attempted here to combine node negative group cases in floor of mouth with node negative group cases in tongue plus floor of mouth and to compare the malignancy grades data of these cases with the data in corresponding combinations of node positive groups. The mean of the total

malignancy grade of the node negative group was 16.75 and for the node positive group was 17.7. Although there were slight differences, no statistically significant differences were evident. Similarly the mean scores of tumour factor in the group with negative nodes (7.14) and the group with positive nodes (7.38) were compared and again no statistically significant differences were demonstrated. The mean scores of tumour-host interaction of the group with negative nodes and the group with positive nodes, 9.62 and 10.3 respectively, were compared and a statistically significant difference was demonstrated (P= 0.034 Mann-Whitney U-test). When the mean scores of each individual feature were compared, only the pattern of invasion (3.19 node negative group vs 3.63 node positive group) was found to be statistically significant (P= 0.027 Mann-Whitney U-test). In no other instance, were statistically significant differences demonstrated.

3.5.7 Statistical Comparison of Malignancy Grades in Combined Tongue and Tongue Plus Floor of Mouth Cases

An attempt was made to compare the mean scores of the malignancy grades of the node negative groups in combined cases of tongue and tongue plus floor of mouth with the data of node positive groups in the correspoding combination. Interestingly the mean scores of the total malignancy grades in the group with negative nodes (17.1) was slightly greater than in the node positive group (16.85) but the difference was not statistically significant. The mean scores of tumour factor of the node negative group and the group with positive nodes, 7.27 and 6.99 respectively, were compared and again no statistically significant difference was evident. Similarly the mean

scores of tumour-host interaction in the two groups (9.83 node negative vs 9.86 node positive) did not differ significantly. The same trends were noticed between the groups when comparisons were restricted to the mean scores of each feature individually. No instance demonstrated statistically significant differences.

3.6 TUMOUR THICKNESS AND PREDICTION OF NODAL METASTASIS

Exactly the same cases as for assessment of malignancy grades, were involved for the assessment of tumour thickness. The same case numbers as described for the malignancy grade data presented in Tables 3.8, 3.9 and 3.10 in each anatomical site were also used for presenting tumour thickness values in an individual case. Data of the tumour size (T) and the clinical nodal status (N) as well as tumour thickness values in an individual cases are shown in Table 3.11.

Overall there were 46 cases with negative lymph nodes of which 14 had been thought clinically to show metastatic tumour. This represents a 30 per cent false positive clinical assessment. In the 57 cases with histologically proven nodal metastases 15 were felt clinically to be N0. This is a 26 per cent false negative clinical assessment.

3.6.1 Prediction of Nodal Metastasis in Floor of Mouth Carcinoma

Thirty-five cases of floor of mouth carcinoma were studied for the evaluation of tumour thickness of the primary carcinoma. Of these, 20 cases had negative nodes whereas the other 15 cases had metastatic tumour in the nodes. Tumour thickness values of these cases are shown in Table 3.11. The values of tumour thickness are

presented in two groups according to the histological nodal status for metastasis

Statistical analyses using Mann-Whitney U-tests were carried out. There was a difference in the values between the group with negative and the group with positive nodes. Tumours in the group with positive nodes generally had greater thickness than in the group with negative nodes. This difference was significant (P= 0.0046).

3.6.2 Prediction of Nodal Metastasis in Tongue Carcinoma

Forty-one cases of tongue carcinoma were studied for the assessment of tumour thickness. Of these 27 cases had metastatic tumour in the nodes and 14 had negative nodes.

Table 3.11 summarises the tumour thickness values as well as the means and standard deviations. There were very slight differences in the means of the two groups and statistical analyses indicated that the differences were not significant.

3.6.3 Prediction of Nodal Metastasis in Carcinomas Involving Tongue Plus Floor of Mouth

Twenty-seven cases of carcinomas involving tongue and floor of mouth were studied for the assessment of tumour thickness of the primary carcinoma. Out of these, 15 had metastatic tumour in the nodes and 12 cases were node-negative.

Tumour thickness values of these cases are shown in Table 3.11. Similar statistical analyses to those described in Section 3.6.1 were carried out. There was a slight difference in the means of

tumour thickness values between the groups, but the difference did not reach statistical significance.

3.6.4 Comparison of Cases in All Sites Combined

All node negative cases, from the three sites were combined and contrasted with the data from all node positive cases. The mean tumour thickness value for node-negative cases was 8.65 and for node-positive cases was 12.17. The cases were compared using the Mann-Whitney U-test and the difference was significant (P= 0.0044).

3.6.5 Statistical Comparison of Tumour Thickness Values in Combined Floor of Mouth and the Tongue cases

The node-negative cases of floor of mouth and the node-negative tongue cases were combined into one group. In a second group, node-positive cases of the same sites were also combined. The mean tumour thickness in the node-negative group was 7.84 and for the node-positive group was 10.96. These data were compared statistically and were found to differ significantly (P= 0.027 Mann-Whitney U-test).

3.6.6 Statistical Comparison of Tumour Thickness Values in Combined Floor of Mouth and Tongue Plus Floor of Mouth Cases

The node-negative cases of floor of mouth carcinoma were combined with the node-negative cases of carcinoma involving tongue plus floor of mouth. The node-positive cases in these two sites were also grouped together. The mean tumour thickness value in the group with negative nodes was 7.94 and for node-positive cases was 12.7. The data of the mean tumour thickness values in these two groups were

contrasted. Statistical analysis showed the difference was highly significant (P= 0.0003 Mann-Whitney U-test).

3.6.7 Statistical Comparison of Tumour Thickness Values in Combined Tongue and Tongue Plus Floor of Mouth Cases

The node-negative cases of tongue and node-negative cases of tongue plus floor of mouth were combined. Node-positive cases in these two sites were also grouped together. The mean value of tumour thickness in the node-negative group was 10.17 and for node-positive group was 12.86. Analysis showed that this difference did not reach statistical significance.

3.6.8 Discriminant Analyses of Tumour Thickness

Linear discriminant analysis was undertaken contrasting the tumour thickness values of node negative and node positive cases at each of the three sites studied.

In the floor of the mouth 74 per cent of cases were correctly assigned to node negative or node positive groups using a thickness value of 8mm as the dividing line between the groups. There were seven misclassified observations. The node negative cases 2, 10, 14 and 16 were wrongly assigned to the node positive group. The node positive cases 2, 5 and 7 were wrongly assigned to the node negative group. If a thickness value of 5mm were chosen as the dividing line for allocation to groups, none of the node positive cases would have been wrongly assigned. However, 11 of the 20 node negative cases would have been wrongly allocated to the node positive group.

In the tongue cases, linear discriminant analysis gave poor results in assigning cases to the correct groups. Only 49 per cent of the 41 cases were correctly assigned. The dividing line for thickness between groups was 8mm. Fourteen node-positive cases were wrongly assigned to the node negative group and seven node negative cases were wrongly assigned to the node positive group. If a thickness value of 5mm had been chosen as the dividing line between groups, four of the node positive cases, 9, 16, 17, 25 would have been wrongly assigned. Of these, the thinnest case showed a thickness value of only 1mm. Using 5mm as the dividing value, 12 of the 14 node negative cases would have been wrongly assigned to the node positive group.

In the tongue plus floor of mouth cases a 74 per cent correct allocation to groups was obtained by linear discriminant analysis. The dividing line between groups was thicker; 10mm. Four node negative cases were wrongly assigned to the node positive group and three node positive cases were wrongly assigned. If the division between groups was assessed at 5mm, no false negative assessments would have been made but 10 node negative cases would have been wrongly assigned to the node positive group.

3.7 STATISTICAL COMPARISON OF TUMOUR THICKNESS RESTRICTED TO FLOOR OF MOUTH CASES ON WHICH AgNORs WAS ASSESSED

An attempt was made to examine the tumour thickness values on the same 20 cases on which the AgNORs count was assessed. That is the nine cases 1 to 5, 8, and 10 to 12 in the group with negative nodes and the first 11 cases in the group with positive nodes presented in Table 3.11. As discussed in Section 3.4.2, there was a statistically

significant difference in the AgNORs count between these two groups (P = 0.019). The statistical comparison of tumour thickness also indicated a significant difference (P= 0.033 Mann-Whitney U-test).

The dot plot of tumour thickness values in two groups of the 20 cases is shown in Fig. 3.2. There is some overlap in the distribution of the values in the groups. However, the differences are obvious, as only one case out of nine in the negative node group showed a tumour thickness greater than 6mm in contrast with nine out of 11 cases in the group with positive nodes.

3.8 LINEAR DISCRIMINANT ANALYSES ON FLOOR OF MOUTH CASES

The predictive value of AgNORs and tumour thickness seemed best on floor of mouth cases. A more detailed analysis was therefore undertaken on these floor of mouth cases for which data on the three parameters AgNORs, thickness and malignancy grading were available. There were 20 such cases and the data concerning the three parameters are shown in Table 3.12.

As a first stage the data were assessed to see if there was a correlation between the three parameters. Spearman rank correlation co-efficients were calculated for AgNORs and tumour thickness, AgNORS and malignancy grading score, and for tumour thickness and malignancy grading score. No significant correlations were apparent. It was concluded that these three parameters could be considered as independent variables.

Dot plots for the three parameters were prepared separately for node positive and node negative cases. The distribution of the

thickness data was skewed and a square root transformation was used to normalise the data (Fig. 3.3).

The linear discriminant analysis of the AgNORs data correctly assigned 17 of the 20 cases. This is an 85 per cent correct assessment. There were three instances of misclassified observations. The node negative cases 5 and 8 were wrongly assigned to the node positive group. The node positive case 3 was wrongly assigned to the node negative group.

Linear discriminant analysis was then undertaken using tumour thickness alone. It was found that this gave poorer prediction than AgNORs and only 15 cases were correctly allocated to the node negative and node positive groups. This is a 75 per cent correct prediction. There were five misclassified observation. Three node positive cases 2, 5 and 7 were wrongly assigned to the node negative group. Cases 2 and 10 in the node negative group were wrongly assigned to the node positive group.

Similarly total malignancy grade was used alone for dicriminant analysis. The result showed that only 10 cases were correctly allocated to the node negative or node positive groups, giving only 50 per cent correct prediction. Cases 2, 4, 6, 10 and 11 in the node positive group were misclassified to the node negative group. Also the node negative cases 1, 3, 8, 10 and 11 were wrongly allocated to the node positive group.

An attempt was made to undertake linear discriminant analysis on various possible combinations of the three parameters. It was concluded that in no instances of any possible combination did the

linear discriminant analyses show prediction of nodal metastasis to be better than the AgNORs alone. Therefore, AgNORs was considered as the best predictor for the nodal metastasis.

3.9 LINEAR DISCRIMINANT ANALYSES INCLUDING OTHER SITES

Because the number of cases available for assessment of the possible predictors of nodal metastasis for the carcinomas of the other sites was small, no discriminant analyses was attempted. However, further discriminant analysis was carried out on all the 42 cases on which the AgNORs count was assessed. The variables tumour thickness, tumour factor, tumour-host interaction and total malignancy grades were also used for discriminant analysis. Using each variable separately in the analysis, it was found that none of them, individually gave a good discrimination. Although tumour thickness as well as tumour factor could individually correctly assign 30/42 (71.4%) cases to either node negative or node positive group, this was not accurate enough for a good prediction of nodal metastasis. When the remaining parameters were used separately, discriminant analysis gave poorer separation than tumour thickness or tumour factor. Discriminant analysis on various combinations of variables were then attempted. Analysis showed that using a combination of AgNORs, tumour thickness and tumour factor gave the best discrimination. This combination correctly assigned 32/42 (76.2%) cases to node negative or node positive groups. These results however, indicated that these variables as assessed in the present study are unlikely to predict nodal metastasis with a sufficiently high degree of accuracy at sites other than the floor of mouth, to be clinically useful.

3.10 DISCUSSION

It was felt that it would be more convenient to discuss each of the parameters studied in this Chapter separately for a better understanding. Therefore, the following subsections will deal with the discussion of individual parameters.

3.10.1 Mitotic Phase Distributions

It is not known whether any work on mitotic phase distribution in relation to metastases in carcinomas of oral cavity has been previously undertaken. No published data have been observed. Ojo and MacDonald (1988) assessed mitotic phase distribution in hyperplastic and neoplastic oral epithelia and their results indicated that squamous cell carcinomas showed a significantly different mitotic phase distribution from non-neoplastic lesions, with an increased proportion of metaphases. The idea was further extended in this study to assess the mitotic phase distribution in oral carcinomas to find whether the percentage of individual mitotic phases could predict nodal metastases in a particular case. Analyses of the results in the three anatomical sites studied revealed that mitotic phase distribution could not help in the prediction of metastases. The attempt was made to find out if there were any differences in the mitotic phase distribution in the tumours of the three sites studied, which might indicate differences in the proliferative parameters in the tumours at these sites. Interpretation of this is somewhat tentative because the data are expressed as proportions of mitotic phases and ratios of other factors such as apoptotic cells to mitoses.

The data may not relate to absolute mitotic activity at the different sites. Initially it appeared that there might be differences between floor of mouth and tongue cases in the percentages of metaphases and telophases. These phases were more frequent in tumours of the tongue than in tumours of floor of mouth. However, when Bonferroni adjusted probability was derived neither difference remained significant. Whether this observation would have indicated that tongue tumours are more actively proliferative than floor of mouth tumours is not certain. It could be argued that the apparent duration of these mitotic phases might seem longer in tongue tumours, possibly due to arrest of mitosis more frequently at these phases in tongue tumours. Interestingly also there were significant differences in the ratios of aberrant cells (abnormal mitoses plus apoptotic mitoses) between floor of mouth cases and tongue cases. These were more frequent in tongue cases. Similarly the total cell loss (apoptotic mitoses plus apoptotic cells), appeared greater in tongue cases than in floor of mouth cases. This might indicate that tumours of the tongue were possibly less differentiated and, as well, more proliferative than tumours of floor of the mouth. The intermediate values of these features in the tumours of tongue plus floor of mouth might also support the above possibility. However, none of these features seemed to help in the prediction of nodal metastasis.

3.10.2 AgNORs

Concerning the AgNORs, it was found that the mean numbers for a particular case, proved to be a strong predictive factor for nodal metastases in floor of mouth carcinoma. However, it could be argued that the clinical assessment of the cervical lymph nodes in the 20

cases of floor of mouth carcinoma studied was as accurate as the prediction of metastasis on the basis of AgNORs count. In this respect it would be reasonable to emphasise the value of AgNORs in picking up accurately the two cases that were misinterpreted clinically. Case number 2 in the group with negative nodes, clinically was T4N1 and the AgNORs score was low (9.96). Case number 11 in the group with positive nodes, clinically was T2N0 and the AgNORs count was high (20.43). However, on the basis of AgNORs, cases number 5 and 8 in the group with negative nodes were predicted as being node positive. Both cases scored a high mean AgNORs count but were clinically accurately predicted. It is possible of course that cases scoring high AgNORs values could still be considered high risk for nodal metastasis. The nodes might actually have had small foci of metastatic tumour which were not disclosed histologically due to the limitation of conventional methods of lymph node sampling. Some authors have suggested that there is a probability of 30 per cent for missing such small foci of metastatic tumour in nodes (Wilkinson and Hause, 1974). The practicalities of this have been considered further in relation to oral cancer by Saka and MacDonald (1989) who recommended that consideration should be given to a more extensive sampling technique for enlarged lymph nodes.

The AgNORs counts did not differ significantly between node negative and node positive cases of tongue and cases of tongue plus floor of mouth. It was felt that this was probably influenced by the small numbers especially in the node-negative groups. The AgNORs count was found to differ significantly when combinations of cases at different sites were studied. It is noteworthy that significant

differences were demonstrated when cases of floor of mouth were combined with cases of tongue, and also when the cases in all sites were combined. Whether these results in the combined sites were due to the impact of significant differences demonstrated in cases of floor of mouth or the AgNORs were really an indication of nodal metastasis for all the sites, will require to be further researched by including greater numbers of cases for each individual site.

The counting of AgNORs can pose difficulties. The AgNORs dots are not generally identical in size. Some of the dots might be aggregated to each other within the nucleus forming a cluster. Accurate counting of the dots within the cluster might be very difficult without careful focussing. It was thought that considering such clusters as a single dot in cases where no small discernible dots within the cluster were identified was reasonable. The problems in the counting of AgNORs are fully discussed by Crocker et al. (1989) and a suggestion has been made for a standard approach to the counting. This includes two approaches. Firstly, all silver-stained structures could be counted, but when lying in groups each cluster is treated as one structure. Secondly, where AgNORs can be seen separately within a nucleolus, each AgNOR could be counted as a unit, together with the smaller AgNORs seen outside the nucleolus. Both of these techniques have a rational basis and are by no means mutually exclusive. Indeed, if the AgNORs count represents nucleolar disaggregation, which in turn reflects cellular activity, it is important therefore, to assess whether AgNORs can be resolved within nucleoli. It should be remembered that in some resting cells it will not be possible to resolve separate AgNORs within the nucleoli which

are wholly aggregated (Boldy et al., 1989). Overall, Crocker et al. (1989) suggest that total AgNOR dots both intra- and extra-nucleolar, be enumerated. In the present study, this approach was followed, and was found not to be an impossible exercise. Attention should also be directed to a meticulous technique of staining and reaction timing.

In this study it was found that the number of AgNORs reflected the biological behaviour of oral cancer. This is in agreement with the results obtained by Crocker and Nar (1987) in non-Hodgkin's lymphomas. These authors were able, on the basis of AgNORs counts, to separate the high grade from the low grade tumours. High grade lymphomas showed increased numbers of AgNORs.

Studies performed on biopsy material are of advantage in the sense that surgeons could be informed about, for example, the AgNORs count and its impact in relation on nodal metastases before initiation of any more extensive surgery, especially in the cases where nodal involvement clinically was in doubt.

3.10.3 Malignancy Gradings

The six histological features assessed in the study of malignancy grading were recommended by Anneroth et al. (1987) and the same criteria were followed, with the exception of a minor modification in assessing the numbers of mitoses. The sum of the scores for these features was suggested as representing the malignancy grading for each case. The sum of the first three features was considered as the tumour factor or tumour differentiation. The sum of the other three features represents tumour-host interaction.

These were not calculated separately in the report by Anneroth et al. (1987). In the present study, it was found that the pattern of invasion was significantly different between the cases with positive nodes and those with negative nodes in floor of mouth carcinomas. The same held true when the total scores of the tumour-host interaction feature were compared. However, these features singly or in combination did not differ significantly in carcinoma of tongue and carcinoma involving tongue plus floor of mouth.

Interestingly, the tumour-host interaction features as a total, had not been statistically correlated in previous studies. The pattern of invasion had been found to be an important prognostic parameter in carcinomas of the tongue (Yamamoto et al., 1983) and in carcinomas of the oropharynx (Crissman et al., 1984).

In the present study the differences in the total tumour-host interaction and pattern of invasion were shown to be significant when they were compared between combined groups of floor of mouth and the groups involving tongue plus floor of mouth. It might be that the significant differences between the groups with negative and positive nodes of floor of mouth influenced the statistical results when combined with cases involving tongue plus floor of mouth.

Stage of invasion, one of the tumour-host interaction features, which in some studies had been found to be of prognostic importance, in this study showed no relationship to nodal metastases. Lymphoplasmacytic infiltration has previously been found to be associated with nodal metastases in carcinoma of the lip. Jones and Coyle (1969) noted that most tumours with little lymphoplasmacytic

infiltration metastasised to cervical nodes. In contrast, no nodal metastases were noted in patients whose tumours evoked marked infiltrates. The intensity of infiltrate in the primary tumours involved in the present study, however, did not show any relation to nodal metastases.

Total malignancy grading which represents the sum of the scores of all the six features failed to predict nodal metastases. It is likely that evaluation of the histological features following the Anneroth et al. (1987) scheme is very much dependent on the sampling of the specimen. Furthermore, interpretation and evaluation of some of the features involved in the system is very subjective. It is also very time-consuming and unlikely to be acceptable in clinical practice.

3.10.4 Tumour Thickness

In the present study tumour thickness as a single independent factor, proved to be of predictive value for nodal metastases in floor of mouth carcinomas. The thickness was also of predictive value when cases of floor of mouth were combined with cases of tongue or with cases involving tongue plus floor of mouth. It was of predictive value again when cases of all sites were combined together. These findings are in agreement with several workers who have evaluated thickness of tumours in different anatomical sites and found that a thickness greater than 6mm was a predictor of high risk for nodal metastases (Frierson and Cooper, 1986; Moore et al. 1986b and Urist et al. 1987). However, Mohit-Tabatabai et al. (1986) evaluated thickness of locally excised early carcinomas of floor of mouth. The cervical

lymph nodes were clinically free from metastases in their series. The thickness of the tumours was correlated to subsequent development of nodal metastases. The conclusion was made that tumours greater than 1.5mm should be considered high risk and elective neck surgery should be undertaken. In the present study it was not possible to perform such analyses because the patients had already undergone neck dissection. Tumour thickness, however, failed to predict nodal metastasis accurately in cases of tongue and cases involving tongue plus floor of mouth either separately or in combination with each other.

Although tumour thickness, pattern of invasion and total score of tumour-host interaction have been shown to be possible predictors of metastases in floor of mouth carcinomas, their usefulness might be limited because the measurements require resection of the tumours. For those patients clinically assessed as N0 and undergoing resection alone, thickness could be used as a method of deciding on elective regional node dissection as a second procedure in the course of treatment.

In this respect, the AgNORs count should be considered superior to other predictive factors for two reasons. Firstly, the AgNORs count proved to more accurately predict nodal metastases. Secondly the assessment can be carried out on biopsy material. This would allow the required surgery to be undertaken as a single procedure.

Failure to confirm the discriminant potential of AgNORs count and tumour thickness in predicting metastases in the studies of

carcinoma of tongue and carcinomas involving tongue plus floor of mouth was thought to be most likely related to the different anatomical site. It is well known that biological behaviour of carcinomas varies in relation to the anatomical sites. Also the small numbers of cases studied in these two sites, especially in the node negative groups, might partly explain the failure of AgNORs and tumour thickness to help in prediction of nodal metastasis.

The findings from this study, although preliminary in nature, give some encouragement that particularly in floor of the mouth tumours, improved prediction of nodal metastasis may be possible.

	Case Number	Prometa-phase	Metaphase	Anaphase	Telophase	Abnormal Mitoses	Apoptotic Mitoses	Apoptotic Cells	P:M	P+M:A+T
	1	64	29	4	3	3	22	22	2.21	13.29
	2	39	45	7	9	4	15	20	0.87	5.25
	3	46	40	7	7	9	37	7	1.15	6.14
	4	64	24	5	7	3	32	11	2.67	7.33
NODE	5	57	35	3	5	14	19	20	1.63	11.50
NEGA-	6	70	21	3	6	3	34	15	3.33	10.11
TIVE	7	59	32	6	3	9	20	20	1.84	10.11
	8	62	25	7	6	14	46	21	2.48	6.69
	9	60	17	9	14	2	19	8	3.53	3.35
	10	50	43	4	3	1	5	6	1.16	13.28
	11	49	34	15	2	4	36	18	1.44	4.88
	12	61	30	7	2	3	27	12	2.03	10.11
	Mean	**56.75**	**31.25**	**6.42**	**5.58**	**5.75**	**26**	**15**	**2.03**	**8.50**
	SD	**8.94**	**8.70**	**3.29**	**3.48**	**4.58**	**11.42**	**5.94**	**0.85**	**3.34**
	1	69	19	6	6	7	21	55	3.63	7.33
	2	60	23	7	10	10	25	25	2.61	4.88
	3	57	35	5	3	1	17	16	1.63	11.50
	4	46	33	8	13	5	17	14	1.39	3.76
NODE	5	64	23	6	7	11	14	8	2.78	6.69
POSI-	6	50	36	4	10	4	34	29	1.39	6.14
TIVE	7	31	57	7	5	5	10	3	0.54	7.33
	8	43	49	3	5	8	31	3	0.88	11.50
	9	35	46	7	12	5	16	13	0.76	4.26
	10	68	25	6	1	6	20	30	2.72	13.29
	11	50	41	4	5	2	32	19	1.22	10.11
	Mean	**52.09**	**35.18**	**5.73**	**7**	**5.82**	**21.55**	**19.55**	**1.78**	**7.89**
	SD	**12.78**	**12.19**	**1.56**	**3.79**	**3.06**	**7.94**	**15.01**	**1.00**	**3.23**

Table 3.1 Percentage distribution of the phases of mitosis and numbers of abnormal mitoses and apoptotic cells in individual cases of floor of the mouth carcinoma studied, tabulated according to nodal status.

	Case Number	Prometa-phase	Metaphase	Anaphase	Telophase	Abnormal Mitoses	Apoptotic Mitoses	Apoptotic Cells	P:M	P+M:A+T
	1	43	44	4	9	9	83	22	0.98	6.69
NODE	2	60	30	3	7	6	39	13	2.00	9.00
NEGA-	3	45	37	9	9	5	42	67	1.22	4.56
TIVE	4	46	32	6	16	8	31	22	1.44	3.54
	5	47	27	13	13	11	34	66	1.74	2.85
	Mean	**48.20**	**34.00**	**7.00**	**10.80**	**7.80**	**45.80**	**38.00**	**1.47**	**5.33**
	SD	**6.76**	**6.67**	**4.06**	**3.63**	**2.39**	**21.23**	**26.30**	**0.41**	**2.51**
	1	48	31	6	15	9	40	82	1.55	3.76
	2	38	40	6	16	11	45	27	0.95	3.55
	3	62	27	3	8	8	47	23	2.30	8.09
	4	40	38	5	17	17	73	56	1.05	3.54
NODE	5	54	36	3	7	4	38	40	1.50	9.00
POSI-	6	43	43	3	11	2	30	35	1.00	6.14
TIVE	7	45	46	5	4	20	69	26	0.98	10.11
	8	66	30	2	2	8	13	1	2.20	24.00
	9	45	45	3	7	7	80	56	1.00	9.00
	10	47	22	10	21	9	39	12	2.14	2.23
	11	56	30	4	10	3	19	16	1.87	6.14
	12	41	48	3	8	2	24	11	0.85	8.09
	13	52	42	0	6	9	98	24	1.24	15.67
	Mean	**49.00**	**36.77**	**4.07**	**10.15**	**8.38**	**47.31**	**31.46**	**1.43**	**8.41**
	SD	**8.56**	**8.15**	**2.43**	**5.58**	**5.39**	**25.50**	**22.33**	**0.53**	**5.89**

Table 3.2 Percentage distribution of the phases of mitosis and numbers of abnormal mitoses and apoptotic cells in individual cases of tongue carcinoma studied, tabulated according to nodal status.

	Case Number	Prometa-phase	Metaphase	Anaphase	Telophase	Abnormal Mitoses	Apoptotic Mitoses	Apoptotic Cells	P:M	P+M:A+T
NODE NEGA-TIVE	1	52	34	10	4	20	37	30	1.53	6.14
	2	53	38	6	3	3	14	22	1.39	10.11
	3	53	43	1	3	3	16	15	1.23	24.00
	4	51	32	7	10	10	24	19	1.59	4.88
	Mean	**52.25**	**36.75**	**6.00**	**5.00**	**9.00**	**22.75**	**21.50**	**1.44**	**11.28**
	SD	**0.98**	**4.86**	**3.74**	**3.37**	**8.04**	**10.44**	**6.35**	**0.16**	**8.77**
NODE POSI-TIVE	1	50	31	10	9	6	48	26	1.61	4.26
	2	63	30	3	4	3	15	13	2.10	13.29
	3	66	31	1	2	0	18	16	2.13	32.33
	4	42	47	5	6	8	53	24	0.89	8.09
	5	66	20	8	6	2	8	45	3.30	6.14
	6	52	39	6	3	8	19	27	1.33	10.11
	Mean	**56.5**	**33.00**	**5.00**	**5**	**4.5**	**26.83**	**25.17**	**1.89**	**12.37**
	SD	**9.95**	**9.14**	**3.27**	**2.53**	**3.33**	**18.80**	**11.23**	**0.83**	**10.27**

Table 3.3 Percentage distribution of phases of mitosis and numbers of abnormal mitoses and apoptotic cells in individual cases of carcinoma involving tongue plus floor of mouth, tabulated according to nodal status.

		P	M	A	T	P:M	P+M:A+T	Abm	Apm	Apc
NODE NEGATIVE	Mean	53.86	32.95	6.48	6.71	1.78	8.28	6.86	30.1	21.7
	SD	10.57	7.68	3.39	4.06	0.72	4.79	4.92	16.15	16.03
NODE POSITIVE	Mean	51.63	35.43	4.97	7.97	1.65	9.01	6.67	33.77	25.83
	SD	10.57	9.75	2.4	4.82	0.79	6.26	4.44	22.37	18.31

Table 3.4. The means and standard deviations (SD) of all mitotic parameters in combined sites tabulated according to histological nodal status.

Site		P	M	A	T	P:M	P+M:A+T
Floor of Mouth (FOM)	Mean	54.52	33.31	6.09	6.26	1.91	8.21
	SD	10.95	10.46	2.58	3.62	0.91	3.23
Tongue	Mean	48.78	36.0	4.89	10.33	1.44	7.55
	SD	7.92	7.68	3.14	5.02	0.49	5.29
Tongue plus FOM	Mean	54.8	34.5	5.7	5.0	1.71	11.94
	SD	7.76	7.62	3.27	2.71	0.67	9.91

Table 3.5. The means and standard deviations (SD) of normal mitoses parameters according to the site of the tumours.

Site		Abm	Apm	Abm+Apm	Apc	Apm+Apc
FOM	Mean	5.78	23.87	29.65	17.17	41.04
	SD	3.84	9.95	11.45	11.2	15.88
Tongue	Mean	8.22	46.89	55.11	33.28	80.17
	SD	4.68	23.78	26.42	22.88	36.23
Tongue plus FOM	Mean	6.3	25.2	31.5	23.7	48.9
	SD	5.76	15.4	18.99	9.33	18.11

Significant differences following Bonferroni adjustment

FOM vs Tongue	Abnormal mitoses	$P < 0.001$
	Apoptotic mitoses	$P = 0.028$
	Abm + Apm	$P = 0.028$
	Apm + Apc	$P = 0.014$

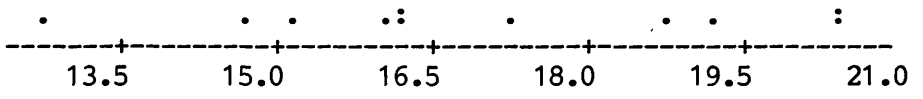
Table 3.6. The means and (SD) of abnormal mitoses, apoptotic mitoses and cells according to the sites of tumours.

	Case Number	Floor of Mouth		Tongue		Tongue plus Floor of Mouth	
		AgNORs	TN	AgNORs	TN	AgNORs	TN
	1	12.61	T1N0	13.35	T2N0	17.95	T2N0
	2	9.96	T4N1	*		*	
	3	11.00	T1N0	*		16.66	T1N0
NODE	4	8.83	T2N0	9.93	T2N0	17.96	T3N0
NEGA-	5	17.23	T2N0	11.18	T1N0		
TIVE	6	*					
	7	*					
	8	21.55	T1N0				
	9	*					
	10	7.20	T3N0				
	11	11.41	T4N0				
	12	8.45	T3N0				
	Mean	**12.03**		**11.29**		**17.52**	
	SD	**4.61**		**2.00**		**0.75**	
	1	14.75	T4N1	9.85	T2N1	21.78	T4N2
	2	16.24	T2N1	23.33	T2N1	9.86	T3N1
	3	12.74	T4N1	*		21.35	T3N0
	4	15.98	T2N3	24.94	T2N1	12.75	T2N1
NODE	5	17.18	T1N1	12.49	T1N2	11.75	T4N1
POSI-	6	19.23	T2N1	11.50	T1N0	*	
TIVE	7	15.30	T2N1	19.33	T4N0		
	8	18.76	T2N1	*			
	9	16.15	T4N3	13.03	T1N0		
	10	20.46	T2N1	7.69	T4N0		
	11	20.43	T2N0	22.60	T3N1		
	12			14.03	T2N1		
	13			17.04	T2N0		
	Mean	**17.02**		**15.98**		**15.50**	
	SD	**2.45**		**5.84**		**5.64**	

* Cases were not included for assessment of AgNORs but were included for mitotic phase count.

Table 3.7 The mean numbers of AgNORs per nucleus assessed on biopsy material of squamous cell carcinoma, tumour size (T) and clinical nodal status (N).

AgNORs count in node positive cases



AgNORs count in node negative cases

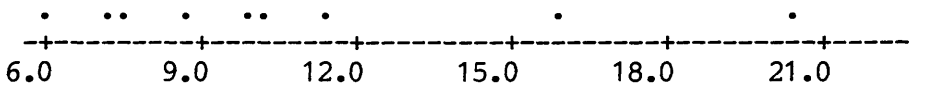
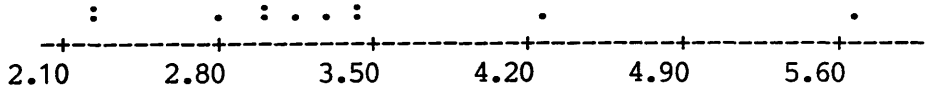


Fig. 3.1. Dot plot of the AgNORs count in cases of floor of mouth carcinoma with and without nodal metastasis.

Square root of tumour thickness values in node positive cases



Square root of tumour thickness values in node negative cases

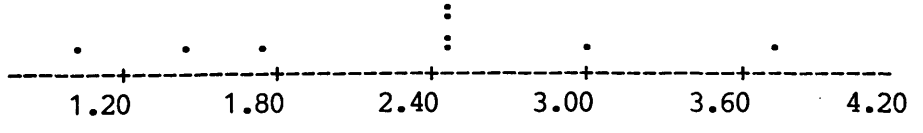


Fig. 3.2. Dot plots following square root transformation of tumour thickness values in cases with squamous cell carcinoma of the floor of the mouth on which the AgNORs count was evaluated.

	Case Number	Degree of Keratinization	Nuclear Polymorphism	Number of Mitosis	Tumour Factor	Pattern of Invasion	Stage of Invasion	Lymphoplasmacytic Infiltration	Tumour-Host Interaction	Total Malignancy Grade
	1	3	4	3	10	4	3	2	9	19
	2	2	2	2	6	4	4	3	11	17
	3	2	3	4	9	4	4	3	11	20
	4	2	2	1	5	3	3	2	8	13
	5	3	2	3	8	3	3	3	9	17
	6	3	2	2	7	3	4	3	10	17
	7	3	2	2	7	2	3	1	6	13
	8	3	4	3	10	3	3	2	8	18
NODE	9	1	2	1	4	2	3	3	8	12
NEGA-	10	3	3	4	10	3	4	3	10	20
TIVE	11	3	3	2	8	4	3	3	10	18
	12	2	3	1	6	3	4	3	10	16
	13	2	2	1	5	4	4	3	11	16
	14	2	2	3	7	4	4	4	12	19
	15	3	3	2	8	3	3	3	9	17
	16	1	2	3	6	2	4	2	8	14
	17	3	3	2	8	4	4	4	12	20
	18	2	2	3	7	4	4	3	11	18
	19	1	1	2	4	2	4	3	9	13
	20	2	2	2	6	3	3	2	8	14
	Mean	2.3	2.5	2.3	7.1	3.2	3.6	2.8	9.5	16.6
	SD	0.7	0.76	0.9	1.85	0.77	0.5	0.72	1.57	2.58
	1	3	3	4	10	4	4	3	11	21
	2	2	3	2	7	4	4	2	10	18
	3	3	3	1	7	4	4	3	11	18
	4	1	2	3	6	3	4	2	9	15
	5	3	3	2	8	4	4	2	10	18
NODE	6	2	2	1	5	4	4	4	12	17
POSI-	7	3	4	4	11	3	3	4	10	21
TIVE	8	3	3	3	9	4	4	3	11	20
	9	4	4	3	11	4	4	3	11	22
	10	2	2	3	7	4	4	2	10	18
	11	1	2	2	5	4	4	2	10	15
	12	3	3	2	8	4	4	3	11	19
	13	2	3	2	7	4	4	3	11	18
	14	1	1	2	4	4	4	4	12	16
	15	1	2	2	5	4	4	3	11	16
	Mean	2.27	2.67	2.4	7.3	3.86	3.9	2.87	10.67	18.0
	SD	0.96	0.82	0.9	2.2	0.35	0.26	0.74	0.82	2.2

Table 3.8 Scores of six histological features, tumour factor, tumour-host interaction and total malignancy grades in floor of mouth carcinomas categorised according to nodal status.

	Case Number	Degree of Keratinization	Nuclear Polymorphism	Number of Mitosis	Tumour Factor	Pattern of Invasion	Stage of Invasion	Lympho-plasmacytic Infiltration	Tumour-Host Interaction	Total Malignancy Grade
	1	3	3	4	10	4	4	4	12	22
	2	4	4	2	10	4	4	3	11	21
	3	2	2	2	6	4	4	2	10	16
	4	3	4	2	9	3	3	2	8	17
	5	1	2	2	5	3	4	3	10	15
NODE	6	3	3	1	7	3	4	2	9	16
NEGA-	7	1	1	1	3	3	4	1	8	11
TIVE	8	1	2	1	4	4	4	3	11	15
	9	3	3	4	10	4	4	3	11	21
	10	4	2	1	7	4	4	4	12	19
	11	1	1	2	4	3	4	2	9	13
	12	4	4	4	12	3	4	3	10	22
	13	2	2	2	6	2	4	2	8	14
	14	3	3	4	10	2	4	4	10	20
	Mean	**2.5**	**2.57**	**2.3**	**7.36**	**3.3**	**3.93**	**2.7**	**9.93**	**17.3**
	SD	**1.16**	**1.02**	**1.2**	**2.82**	**0.73**	**0.27**	**0.9**	**1.38**	**3.56**
	1	2	3	2	7	4	4	2	10	17
	2	1	1	1	3	4	4	2	10	13
	3	2	2	1	5	4	4	4	12	17
	4	2	2	2	6	3	4	3	10	16
	5	2	2	2	6	3	3	3	9	15
	6	2	2	2	6	3	4	2	9	15
	7	1	2	2	5	4	4	3	11	16
	8	4	4	3	11	3	4	3	10	21
	9	1	2	1	4	3	4	2	9	13
	10	2	2	2	6	4	4	2	10	16
	11	2	2	3	7	4	4	2	10	17
	12	1	1	2	4	2	4	1	7	11
NODE	13	4	4	2	10	4	4	3	11	21
POSI-	14	2	3	2	7	4	4	3	11	18
TIVE	15	1	1	1	3	3	4	2	9	12
	16	2	2	2	6	4	4	2	10	16
	17	1	1	1	3	1	1	1	3	6
	18	3	3	4	10	4	4	3	11	21
	19	2	3	3	8	4	4	3	11	19
	20	2	2	4	8	2	4	3	9	17
	21	2	2	3	7	3	4	3	10	17
	22	2	2	2	6	4	4	3	11	17
	23	3	3	1	7	3	4	3	10	17
	24	2	2	1	5	3	4	3	10	15
	25	4	3	2	9	4	4	3	11	20
	26	3	2	1	6	4	4	3	11	17
	27	3	4	4	11	2	4	3	9	20
	Mean	**2.15**	**2.3**	**2.07**	**6.52**	**3.3**	**3.85**	**2.59**	**9.78**	**16.29**
	SD	**0.9**	**0.87**	**0.96**	**2.28**	**0.8**	**0.6**	**0.69**	**1.69**	**3.3**

Table 3.9 Scores of six histological features, tumour factor, tumour-host interaction and total malignancy grades in carcinomas of the tongue categorised according to nodal status.

	Case Number	Degree of Keratinization	Nuclear Polymorphism	Number of Mitosis	Tumour Factor	Pattern of Invasion	Stage of Invasion	Lymphoplasmacytic Infiltration	Tumour-Host Interaction	Total Malignancy Grade
	1	2	4	2	8	4	4	2	10	18
	2	2	2	2	6	4	4	2	10	16
	3	2	3	4	9	3	4	3	10	19
	4	3	4	4	11	3	4	3	10	21
NODE	5	2	2	2	6	3	4	3	10	16
NEGA-	6	2	3	1	6	3	4	3	10	16
TIVE	7	1	1	1	3	2	4	2	8	11
	8	4	4	3	11	2	4	3	9	20
	9	1	1	2	4	3	4	2	9	13
	10	2	2	2	6	4	4	3	11	17
	11	3	3	3	9	4	4	2	10	19
	12	2	2	2	6	4	4	3	11	17
	Mean	**2.18**	**2.64**	**2.36**	**7.18**	**3.18**	**4.00**	**2.54**	**9.73**	**16.9**
	SD	**0.87**	**1.12**	**1.02**	**2.64**	**0.75**	**0.00**	**0.52**	**0.79**	**2.98**
	1	3	2	1	6	3	4	3	10	16
	2	3	3	2	8	4	4	4	12	20
	3	3	3	3	9	2	4	3	9	18
	4	2	2	3	7	3	4	3	10	17
	5	2	2	2	6	4	4	2	10	16
	6	3	3	3	9	4	4	3	11	20
NODE	7	3	3	4	10	3	4	3	10	20
POSI-	8	4	2	3	9	2	4	3	9	18
TIVE	9	2	2	3	7	4	4	3	11	18
	10	3	2	2	7	4	4	2	10	17
	11	3	4	3	10	4	4	3	11	21
	12	1	2	2	5	4	4	1	9	14
	13	2	3	3	8	3	3	2	8	16
	14	2	2	1	5	4	4	2	10	15
	15	2	2	2	6	3	4	2	9	15
	Mean	**2.53**	**2.46**	**2.46**	**7.46**	**3.4**	**3.93**	**2.6**	**9.93**	**17.4**
	SD	**0.74**	**0.64**	**0.83**	**1.68**	**0.74**	**0.26**	**0.74**	**1.03**	**2.13**

Table 3.10 Scores of six histological features, tumour factor, tumour-host interaction and total malignancy grades in carcinomas involving tongue plus floor of mouth, categorised according to histological nodal status.

	Case Number	Floor of Mouth		Tongue		Tongue plus Floor of Mouth	
		Thickness	TN	Thickness	TN	Thickness	TN
	1	2	T1N0	5	T2N0	15	T2N0
	2	14	T4N1	9	T1N0	12	T1N0
	3	6	T1N0	9	T2N0	5	T1N0
NODE	4	1	T2N0	2	T2N0	8	T3N0
NEGA-	5	6	T2N0	5	T1N0	14	T1N1
TIVE	6	4	T1N1	12	T1N0	6	T1N0
	7	4	T2N0	14	T2N1	9	T3N1
	8	6	T1N0	18	T4N0	15	T4N1
	9	3	T1N0	4	T2N1	9	T2N1
	10	9	T3N0	12	T4N0	4	T4N1
	11	3	T4N0	10	T1N0	16	T4N0
	12	6	T3N0	11	T2N1	3	T2N1
	13	7	T2N0	14	T2N0		
	14	12	T4N0	16	T2N2		
	15	2	T1N0				
	16	11	T1N1				
	17	5	T1N0				
	18	3	T1N0				
	19	6	T2N2				
	20	2	T2N0				
	Mean	**5.6**		**10.07**		**10.27**	
	SD	**3.56**		**4.75**		**4.34**	
	1	9	T4N1	6	T2N1	15	T4N2
	2	5	T2N1	14	T2N1	17	T3N1
	3	12	T4N1	5	T1N1	15	T3N0
	4	10	T2N3	8	T2N1	15	T2N1
	5	5	T1N1	5	T1N2	19	T4N1
	6	11	T2N1	5	T1N0	22	T2N1
	7	8	T2N1	11	T4N0	8	T4N1
	8	9	T2N1	22	T2N1	8	T3N1
	9	32	T4N3	4	T1N0	13	T2N0
	10	12	T2N1	13	T4N0	24	T4N1
NODE	11	18	T2N0	9	T3N1	14	T4N1
POSI-	12	5	T1N1	13	T2N1	7	T4N1
TIVE	13	6	T1N0	6	T2N0	14	T2N0
	14	10	T3N1	9	T2N0	15	T3N2
	15	10	T2N1	26	T4N3	13	T3N2
	16			3	T1N1		
	17			1	T1N0		
	18			5	T2N1		
	19			27	T4N1		
	20			12	T1N1		
	21			10	T4N0		
	22			13	T2N1		
	23			28	T2N0		
	24			15	T4N0		
	25			4	T2N1		
	26			14	T2N2		
	27			12	T2N1		
	Mean	**10.8**		**11.11**		**14.6**	
	SD	**6.78**		**7.37**		**4.78**	

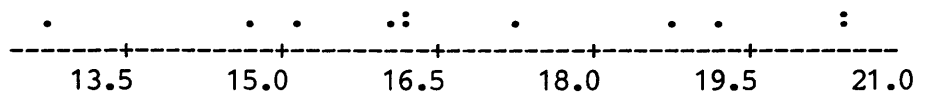
Table 3.11 Tumour thickness (mm) in cases of squamous cell carcinoma assessed on resection specimens, tumour size (T) and clinical nodal status (N).

	Case Number	AgNORs	Tumour Thickness	Total Malignancy Grade
	1	12.61	2	19
	2	9.96	14	17
	3	11.00	6	20
	4	8.83	1	13
	5	17.23	6	17
NODE	6	*	*	*
NEGA-	7	*	*	*
TIVE	8	21.55	6	18
	9	*	*	*
	10	7.20	9	16
	11	11.41	3	19
	12	8.45	6	17
	Mean	**12.03**	**5.89**	**17.56**
	SD	**4.61**	**3.92**	**2.18**
	1	14.75	9	21
	2	16.24	5	18
	3	12.74	12	18
	4	15.98	10	15
NODE	5	17.18	5	18
POSI-	6	19.23	11	17
TIVE	7	15.30	8	21
	8	18.76	9	20
	9	16.15	32	22
	10	20.46	12	18
	11	20.43	18	15
	Mean	**17.02**	**11.91**	**18.27**
	SD	**2.45**	**7.57**	**2.41**

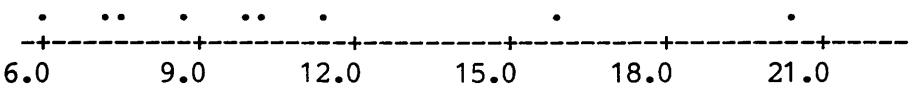
* AgNORs count was not evaluated

Table 3.12. Twenty cases of floor of mouth carcinoma in which the scores of the AgNORs count, tumour thickness and total malignancy grades were evaluated.

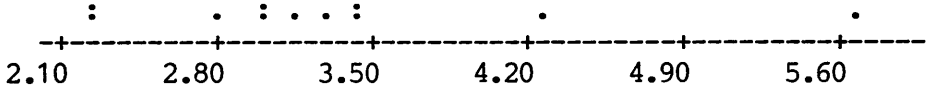
AgNORs count in node positive cases



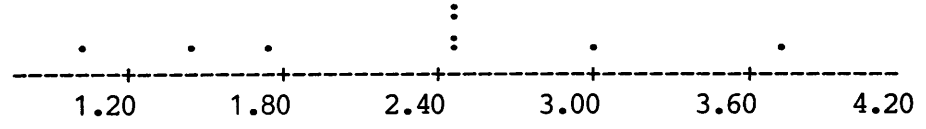
AgNORs count in node negative cases



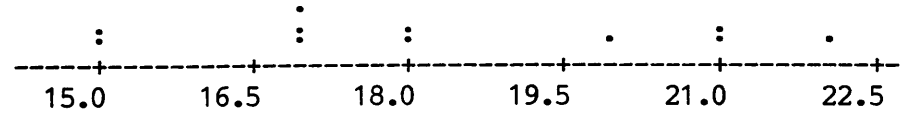
Square root of tumour thickness values in node positive cases



Square root of tumour thickness values in node negative cases



Total malignancy grades of tumours in node positive cases



Total malignancy grades of tumours in node negative cases

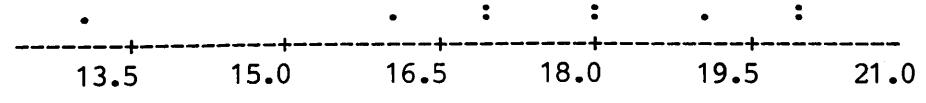


Fig. 3.3 Illustrations of the dot plots of AgNORs count, square root of tumour thickness values and total malignancy grades in the twenty cases of floor of mouth carcinoma where these parameters evaluated.

CHAPTER 4

SQUAMOUS CELL CARCINOMA OF THE FLOOR OF THE MOUTH - FOLLOW-UP STUDIES

4.1 INTRODUCTION

Of the group of 402 patients with oral squamous cell carcinoma that were initially recorded for the main study, 68 had their tumour arising from the mucosa of the floor of the mouth. Cases that did not meet the criteria discussed in Chapter 2, Sections 2.2.1 and 2.2.2 for case selection were excluded from the study and these were 14 cases. There were another five cases who were actually included mainly for the studies of AgNORs counts in order to increase the size of the material for evaluating the prediction of nodal metastasis which were discussed thoroughly in Chapter 3. The follow-up period for these five cases was less than 24 months. Hence, these were also excluded. Therefore, there were 49 cases of the floor of the mouth on which follow-up studies could be examined.

In the studies described in this Chapter it was planned to give an account related to clinical follow-up of the patients after treatment. The recurrences and survival were the factors related to which certain clinical features at initial presentation of individual patients as well as certain structural histological features were examined.

In order to provide clearly defined groups upon which comparison could be made, the patients were classed as surviving without recurrence at 24 months or as having recurrence prior to 24

months.

The group with recurrences comprised patients who developed recurrence at the primary site, the neck or both sites in not less than four months and within 24 months of the follow-up period. There were 12 such patients. Of these, nine died within 24 months, whereas the remaining three survived more than 24 months. In the cases of a further six patients death occurred before 24 months and the patients were either known to be disease free or the situation was not known with certainty. These six patients were not included in the study. The second group included patients who survived and were disease-free for a minimum of 24 months following treatment of the primary and the neck where it was involved. These patients who were 31 in number would form another group in this study. Therefore, of the original 49 cases 34 patients survived for over 24 months following treatment of their squamous cell carcinoma of the floor of mouth.

Data concerning radiotherapy was available for the majority of patients forming the two defined groups. All but one of the 12 patients in the group with recurrence had received radiotherapy postoperatively. Out of 31 patients in the second group, 15 had received radiotherapy postoperatively and six were not subjected to radiation therapy. No data concerning radiation therapy was available in the other 10 patients of this group.

4.2 GENERAL OVERVIEW

4.2.1 Age and Sex

Forty-three patients were eligible for studies reported in this Chapter. These were analysed in relation to age and sex. The results showed that only five were female, in contrast with the males that formed the majority of the cases. That is a female to male ratio equal to 1:7.6. The age range of the female group varied between 48 and 71 years. The mean age was 62 and the median 65.5. The age range of the male group on the other hand, varied between 35 and 75 years. The mean age was 59.9 and the median age was 60.5. Figure 4.1 shows the histogram of the male group distribution according to age. It was found that the majority of patients were in the fifties and sixties age groups. The number of the female group was too small to be usefully presented as a histogram. When the sex of patients was related to recurrence of the tumour, it was found that one out of five females, aged 67, developed recurrence. This is a 20 per cent incidence of recurrence. By contrast 11 out of 38 male patients developed recurrences. This is a 28.9 per cent incidence of recurrence.

4.2.2 Clinical TN in Patients with Recurrence

The tumour size (T) and the clinical nodal status (N) as well as the site of recurrence are shown in Table 4.1. The recurrence in the majority of the patients was locally. There were only three patients who developed recurrence in the neck alone. However, there were two patients who developed recurrence involving both the primary

site and the neck. Interestingly, both of these patients were of tumour size T4 category and one of them had clinically palpable nodes. This was confirmed histologically and two nodes were found positive for metastasis.

4.2.3 Type of Neck Surgery in Patients with Recurrence

All but two of 12 patients who developed recurrent tumours had some sort of neck surgery as part of the treatment management. The type of neck surgery however, varied among these patients. This was presumably due to the variations in the extent of the disease at first presentation of the patients. The extent of the neck surgery in this group of patients ranged from simple unilateral submandibular dissection to more extensive surgery which involved neck dissection on both sides of the neck. The nature of the neck surgery in individual patients is shown in Table 4.2.

4.2.4 Histological Nodal Status in Patients with Recurrence

Although the types of neck surgery in 10 patients who developed recurrences were different, it was presumed that this had involved the removal of the groups of the nodes which were suspected clinically as having metastasis. The number of the nodes cleared from the neck dissection specimens of individual patients varied. It would be meaningless therefore, to present the numbers of the nodes cleared from individual patients. However, it was felt that it would be reasonable to present data on the histological status of cleared nodes irrespective of the numbers. Data on whether or not the nodes were positive for metastasis and involvement of the nodal capsule are shown in Table 4.2. Combining the data in Tables 4.1 and 4.2 for evaluation

of false clinical assessment, it was found that there was no instance of false negative clinical assessment. However, no neck surgery was performed in cases number 7 and 11 who were clinically N0. By contrast 2 out of 8 cases (case 6 and 9) were thought clinically to have nodal metastasis, but histological examination revealed that the nodes were negative for metastsis. This is a 25 per cent incidence of false positive clinical assessment.

4.2.5 Clinical TN in Patients Without Recurrence

There were 31 patients in this group. These patients had survived for a minimum of 24 months without developing recurrence. Data of the TN at first presentation related to these patients are shown in Table 4.3. It was found that all but one patient presented with an early tumour of either T1 or T2 category. There was no patient in this group with T4 tumour. Only five patients in this group had palpable nodes of clinical N1 category.

4.2.6 Type of Neck Surgery in Patients Without Recurrence

Neck surgery was performed in 17 of the 31 cases. As discussed in Section 4.2.3 the pattern of neck surgery in this group was also varied. This ranged between a very conservative unilateral submandibular dissection and a much more extensive radical neck dissection plus submandibular dissection of the opposite side of the neck. The types of neck surgery that were performed on this group of patients are shown in Table 4.3.

4.2.7 Histological Nodal Status in Patients Without Recurrence

The histological status of the nodes in the 17 patients who did not develop recurrence but in whom the neck was treated surgically are shown in Table 4.3. Comparing the clinical assessment with the actual histological status of the nodes, it was found that one out of five instances where the neck was thought to have nodal metastasis turned out histologically to be tumour-free. This is a 20 per cent false positive clinical assessment. By contrast there were less incidences of false negative clinical assessment, as only one out of the remaining 12 cases who had neck surgery and was N0 clinically turned out histologically to have metastasis in the nodes. However, this case was noted to have extracapsular spread.

4.3 PREDICTION OF TUMOUR RECURRENCES

In the following subsections the data concerning the AgNORs count, tumour thickness, microscopic surgical margins and the scores of the malignancy gradings will be compared between the two groups of patients, those who developed recurrence and those who did not.

4.3.1 AgNORs Count and Prediction of Recurrence

There were 21 patients in whom the AgNORs count was assessed on the initial biopsy. Six cases were in the group who developed recurrence and 15 were in the group without recurrence.

Data of the AgNORs count as well as the means and standard deviations in the two groups of patients are summarised in Table 4.4. Cases which are marked with an asterisk are those in which the AgNORs

count was not evaluated. An attempt was made to compare the data of AgNORs count between the two groups. There was a slight difference in the mean of the AgNORs count between the two groups, the mean being greater in the group without recurrence. Statistical analysis using the Mann-Whitney U-test showed that the difference was not significant.

4.3.2 Tumour Thickness and Prediction of Recurrence

All the 43 cases involved in this Chapter were used for the evaluation of tumour thickness. There were 12 cases in the group who developed recurrence and 31 cases in the group without recurrence.

Data on the tumour thickness as well as the means and standard deviations are evident in Table 4.4. There was an obvious difference in the means between the groups. Overall, tumours in the group of patients who developed recurrence were thicker than tumours in the group without recurrence. The Mann-Whitney U-test was carried out and statistical analysis showed that the difference was highly significant ($P = 0.001$).

Linear discriminant analysis of thickness between the groups with and without recurrence gave a 79 per cent correct allocation of cases to the two groups. The dividing line for thickness between the groups was between 7mm and 8mm. If a thickness of 5mm had been used, one of the cases with recurrence (case 9) would have been wrongly categorised and 12 of the cases without recurrence would have been wrongly predicted as having recurrence.

An alternative way of looking at thickness and recurrence would be to determine the proportions of tumours of differing thickness values which recurred. Table 4.5 shows the number of tumours in thickness categories with the percentage recurring. No tumours of 3mm or less in thickness recurred, but a progressive increase in the proportion of recurrences was seen up to the 13-15mm group. The two thicker cases which did not recurr (cases 6 and 14) were re-examined. On the basis of tumour thickness, malignancy gradings and surgical margins, a poor prognosis would be expected in these two cases. The tumour thickness and total malignancy grade in case number 6 were 23mm and 23 respectively. The tumour in case number 14 was 16mm thick with a total malignancy grade 22. The growth pattern of the tumour in both cases was endophytic with very similar histological features manifesting infiltrative neoplastic epithelial islands which showed central necrosis (Fig. 4.2). Perineural invasion, a feature which is known to indicate aggressiveness of tumour was evident in case number 14 (Fig. 4.3). The surgical margins of the resections were positive and no neck surgery was performed in both cases. The two cases however, had received radiotherapy postoperatively. It seemed that the tumour in the two cases had most of the features which showed frequent association with a poor cure rate and yet, the locoregional control of the tumours was achieved for a minimum of 24 months following treatment. Later follow-up data beyond 24 months from treatment, was available and the two patients died with distant metastases in the lung. These distant metastases were discovered at the time of the death. Case number 6 died 26 months after treatment and case number 14 died 40 months after treatment. It is not known for certainty why these two patients

showed such a different behaviour pattern. However, it might be that tumours with such a characteristic histology are very sensitive to radiotherapy and that all the locoregional area related to tumour was effectively treated.

4.3.3 Microscopic Surgical Margins and Prediction of Recurrence

For all the 43 cases the surgical margins were studied for tumour clearance. The mucosal and the deep margins were evaluated. These surgical margins were summarised in two categories. The first was termed positive (+ve) and this was in cases where tumour was found actually at a margin. The second was termed negative (-ve) and this was where the margins were tumour free. Originally it had been intended to examine the marginal clearance in cases whose tumour approached close to margins (Section 2.6.2) but it was felt that the numbers in individual categories would be too small for meaningful analysis.

Table 4.4 shows the condition of the surgical margins as well as the tissue type at the closest margin to the tumour. Of the 43 patients in both groups, there were nine in whom the surgical margins were positive. Five of these developed recurrence and the other four were in the group without recurrence. When the surgical margins were analysed in each group separately, it was shown that five out of 12 (41.7%) patients who developed recurrence, had positive margins. By contrast only four out of 31 (12.9%) of the patients without recurrence had positive margins. Statistical comparison using the Fisher exact probability test shows this is significant (P = 0.044).

The tissue type at the positive margins was also evaluated. It was found that positive margins were located in connective tissue in six cases. Three of these showed recurrence, in two instances in the neck and the other one at the local site. The tissue type of the positive margins in the remaining three cases was muscle in two instances and salivary tissue in the other. The cases where the positive margin was located in muscle, both developed recurrence locally at the primary site.

4.3.4 Malignancy Grades and Prediction of Recurrence

All 43 cases were used for the assessment of malignancy gradings. Twelve cases were in the group with recurrence and 31 in the group without recurrence. The scores of the six histological features, tumour factor, tumour-host interaction and total malignancy grades as well as the means and the standard deviations for each parameter are summarised in Table 4.6.

An attempt was made to compare the parameters between the two groups. The Mann-Whitney U-test was used in all the comparisons. Although there were slight differences in the means of degree of keratinization, nuclear polymorphism, number of mitoses and tumour factor between the two groups, in no instance were these differences shown to be statistically significant. Similarly, pattern of invasion was compared between the two groups and was shown not to differ significantly. However, when the stage of invasion was compared between the two groups, a statistically significant difference was evident ($P = 0.039$). All but one of the lesions which recurred were graded 4 whereas only 16 of the 31 cases without recurrence were

* However, the most recent incidence of floor of mouth carcinoma in Scotland shows the male to female ratio as 2.9:1 (data from Cancer Registry Branch, S.H.H.D, Edinburgh, 1982-1986).

graded 4. Similarly lymphoplasmacytic infiltration was compared between the groups and again a statistically significant difference was noted (P = 0.032). The difference was mainly due to the numbers of cases graded 1 or 2 in the group without recurrence. An attempt was made also to compare the tumour-host interaction between the groups and this showed also a statistically significant difference (P = 0.019). The scores of the total malignancy grade were compared between the groups. Although there was a slight difference in the means, statistical analysis showed that the difference was not significant.

4.4 Discussion

Only a brief discussion is presented in relation to patients follow-up for individual intra-oral anatomical sites included in the studies. A comment on certain points related to tumours of floor of mouth were presented in this Chapter. More detailed discussion will follow in Chapter 8 where each of the parameters studied will be discussed separately. Previous studies have documented the overwhelming proportion of male patients with floor of mouth carcinoma by contrast to female patients. Flynn et al. (1973) reported, on a series from Kentucky, USA, a ratio of male to female patients with floor of mouth carcinoma to be 6.7:1 with an average age of 58 years. However, Shaha et al. (1984) reporting on 320 patients from New York, with floor of mouth carcinoma noted a male to female ratio of 2.2:1. In the present study, the results were in agreement with the Flynn et al. (1973) report and a ratio of 7.6:1 male to female patients was observed with a median age of 65.5 years in the female group and 60.5 years in the male group.*

In the present study the overall incidence of treatment failure was 12/41 (29.3%) within a follow-up period of 24 months. These results were comparable with the Shaha et al. (1984) study which reported an incidence of 33 per cent treatment failure locally or in the neck or both sites in a follow-up period of five years. The great majority of treatment failure in carcinomas of floor of mouth occurs within the first two years following treatment (Nason et al., 1989). There was a striking increase in the incidence of recurrences in relation to tumour size (T). From T1 through T4, 10.5 per cent, 27.8 per cent, 50 per cent and 100 per cent incidences of treatment failure respectively were encountered in the present study. Failure in the neck relating to clinical nodal (N) status showed an increasing incidence with increasing (N) category. Only 2 of 30 (6.7%) cases with N0 category showed neck recurrence by contrast with 3 of 12 (25%) of cases with N1 category who developed recurrence in the neck. There was no patient in the material studied with N2 category. The only patient categorised as N3 developed recurrence locally at the primary site, but not in the neck. These results were in agreement with the Shaha et al. (1984) report which noted a direct relatioship of tumour stage with locoregional failure following treatment.

From the previous sections related to prediction of recurrences, it would be possible to select patients with floor of mouth carcinoma who are at a higher risk. A comparative statistical analysis of the factors studied revealed that, those patients with higher tumour thickness values and those who had positive margins after surgical resection of the primary site had a significantly

higher incidence of local, and, or, regional failure. Patients whose tumour exhibited deep invasion and excited little or no lymphoplasmacytic infiltration with a higher total tumour-host interaction score also had a significantly higher incidence of tumour recurrence.

The AgNORs count was found not to differ significantly between the group of patients who developed recurrence and the group without recurrence. In the results presented in Chapter 3 related to prediction of nodal metastasis however, this parameter, the AgNORs count, was a good predictor with 85 per cent accuracy.

The parameters tumour thickness and total tumour-host interaction values were also helpful in prediction of nodal metastasis in patients with carcinoma of floor of mouth and were found also to be potential predictors for tumour recurrence. Therefore, in diagnostic pathology practice it would be recommended to evaluate these two factors on surgical specimens of local resections since these two factors showed predictive value for both nodal metastasis and tumour recurrence. If the detailed information concerning these two factors was to be provided to the clinician, a modification of the subsequent plan of treatment might be considered.

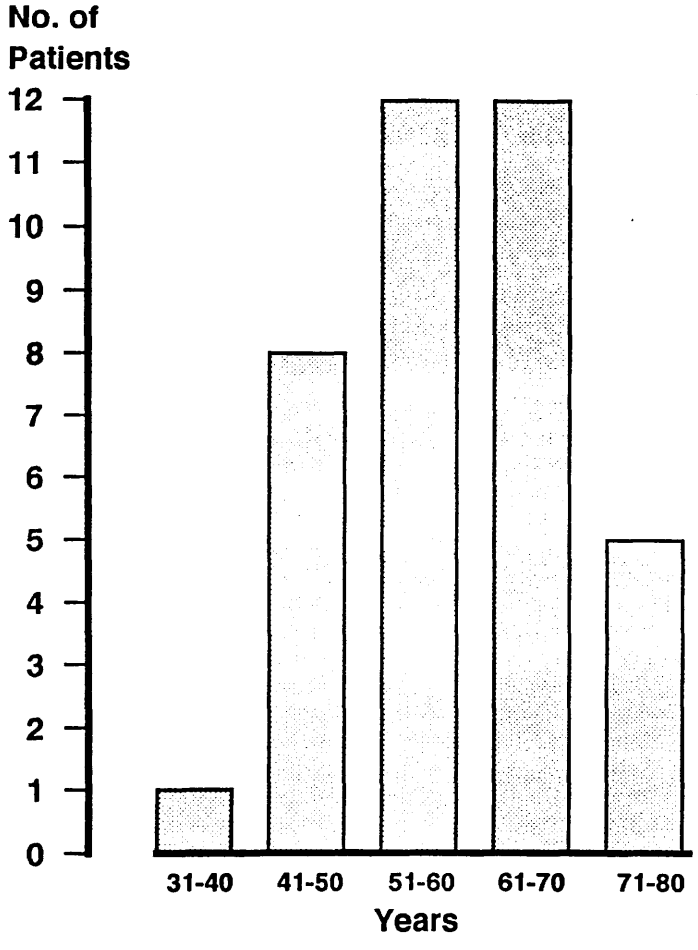


Fig. 4.1 **Age distribution (years) in male patients with carcinoma of floor of mouth.**

Case Number	Recurrence Site	Clinical TN
1	L	T2N0
2	L	T4N1
3	L+N	T4N1
4	L	T2N3
5	L+N	T4N0
6	L	T4N1
7	L	T2N0
8	N	T3N1
9	L	T1N1
10	N	T2N1
11	N	T1N0
12	L	T2N1

L = Local
N = Neck
L + N = Local and Neck

Table 4.1. Site of tumour recurrence and the clinical TN of patients who developed recurrence.

Case Number	Type of Neck Surgery	Number of Positive Nodes	Extracapsular Involvement
1	FND	0	0
2	FND + SHO	1	1
3	USMD	2	0
4	RND	1	0
5	USMD	0	0
6	RND	0	0
7	*	*	*
8	FND	2	0
9	BSMD	0	0
10	FND	1	0
11	*	*	*
12	RND + SMDO	3	3

FND = Functional neck dissection
SHO = Suprahyoid opposite side
USMD = Unilateral submandibular dissection
RND = Radical neck dissection
BSMD = Bilateral submandibular dissection
SMDO = Submandibular dissection opposite side

* Neck surgery was not performed

Table 4.2 Type of neck surgery and the histological nodal status in patients who developed recurrence.

Case Number	Clinical TN	Type of Neck Surgery	Number of Positive Nodes	Extracapsular Involvement
1	T1N1	RND	1	0
2	T2N1	BFND	2	0
3	T1N0	RND	0	0
4	T1N0	FND	0	0
5	T2N0	*	*	*
6	T2N0	*	*	*
7	T1N0	RND + SMDO	1	1
8	T2N0	*	*	*
9	T1N0	FND + SMDO	0	0
10	T1N1	FND	1	0
11	T2N0	BSMD	0	0
12	T1N0	*	*	*
13	T2N0	*	*	*
14	T2N0	BSMD	0	0
15	T2N1	RND	3	1
16	T2N0	*	*	*
17	T1N0	*	*	*
18	T2N0	*	*	*
19	T1N0	FND + SMDO	0	0
20	T1N1	RND + SMDO	0	0
21	T1N0	BSMD	0	0
22	T1N0	*	*	*
23	T1N0	FND + SMDO	0	0
24	T1N0	*	*	*
25	T2N0	*	*	*
26	T1N0	*	*	*
27	T1N0	FND + SMDO	0	0
28	T3N0	FND	0	0
29	T1N0	*	*	*
30	T2N0	*	*	*
31	T2N0	BSMD	0	0

RND = Radical neck dissection
BFND = Bilateral functional neck dissection
FND = Functional neck dissection
SMDO = Submandibular dissection opposite side
BSMD = Bilateral submandibular dissection

* Neck surgery was not performed

Table 4.3. Clinical TN, type of neck surgery and histological nodal status in patients who did not develop recurrence.

	Case Number	AgNORs	Tumour Thickness	Surgical Margins	Tissue Type	Recurrence Site
	1	*	7	+ve	muscle	L
	2	14.75	9	+ve	muscle	L
	3	12.74	12	-ve		
	4	15.98	10	-ve		
	5	*	12	-ve		
	6	9.96	14	-ve		
PATIENTS	7	*	13	+ve	connective	L
WITH	8	*	10	+ve	connective	N
RECURRENCE	9	*	4	-ve		
	10	15.30	8	-ve		
	11	19.84	5	+ve	connective	N
	12	*	10	-ve		
	Mean	**14.76**	**9.5**			
	SD	**3.31**	**3.09**			
	1	*	5	-ve		
	2	16.24	5	-ve		
	3	12.61	2	-ve		
	4	*	2	-ve		
	5	14.91	2	-ve		
	6	*	23	+ve	connective	
	7	*	6	+ve	connective	
	8	*	3	-ve		
	9	11.00	6	-ve		
	10	17.18	5	-ve		
	11	17.23	6	-ve		
	12	9.31	3	-ve		
	13	9.65	16	+ve	connective	
PATIENTS	14	*	4	-ve		
WITHOUT	15	18.76	9	-ve		
RECURRENCE	16	18.24	2	-ve		
	17	*	2	-ve		
	18	19.96	7	-ve		
	19	21.55	6	-ve		
	20	*	11	-ve		
	21	*	5	-ve		
	22	*	1	-ve		
	23	*	3	-ve		
	24	21.21	4	-ve		
	25	*	8	-ve		
	26	*	7	+ve	salivary	
	27	*	3	-ve		
	28	7.20	9	-ve		
	29	*	2	-ve		
	30	17.31	4	-ve		
	31	*	2	-ve		
	Mean	**15.49**	**5.58**			
	SD	**4.53**	**4.55**			

* AgNORs count was not evaluated

Recurrence site L = local, N = neck

Table 4.4. AgNORs, tumour thickness and clearance of surgical margins with tissue types at positive margins in patients with and without recurrence.

Thickness (mm)	Total Number of Cases	No Recurrence	Recurrence	% Recurrence
1 - 3	12	12	0	0
4 - 6	13	11	2	15
7 - 9	8	5	3	38
10 - 12	6	1	5	83
13 - 15	2	0	2	100
> 15	2	2	0	0
	43	**31**	**12**	

Table 4.5. Number of cases according to tumour thickness grouping showing the percentage of recurrences in each group.

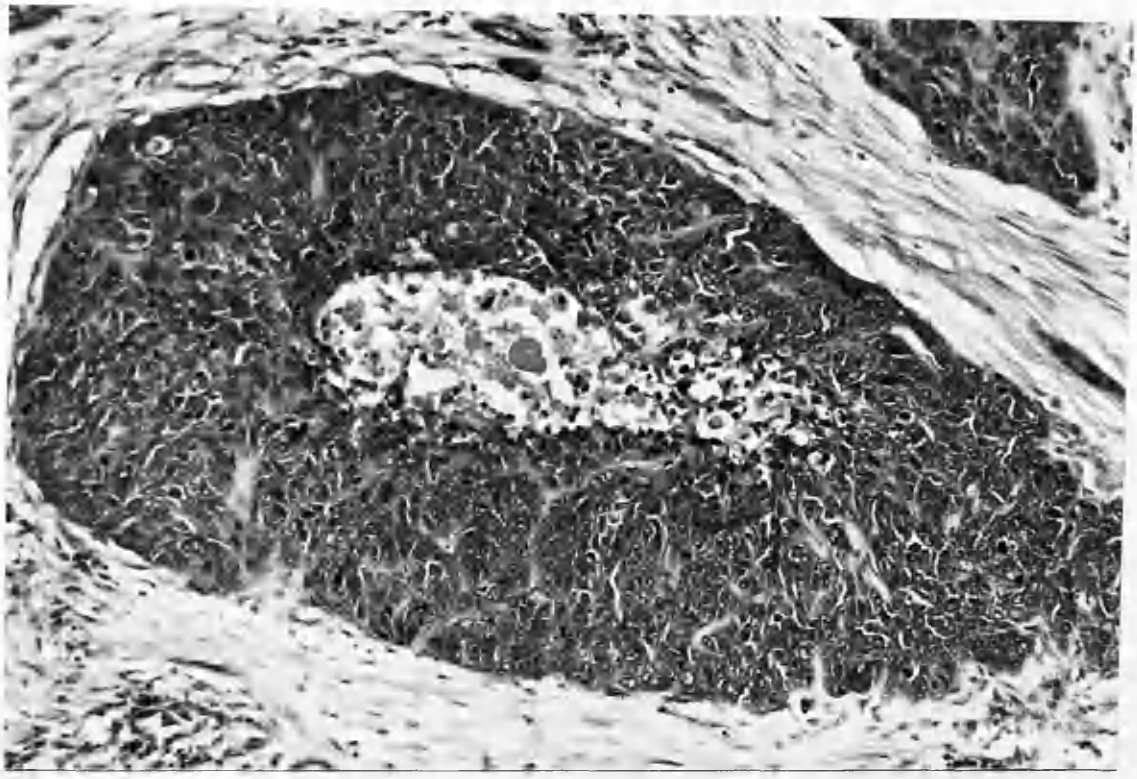


Fig. 4.2. Histological features of infiltrating neoplastic epithelial island manifesting central necrosis, seen in cases 6 and 14 in the group without recurrence but which later showed haematogenous spread. H & E x150.

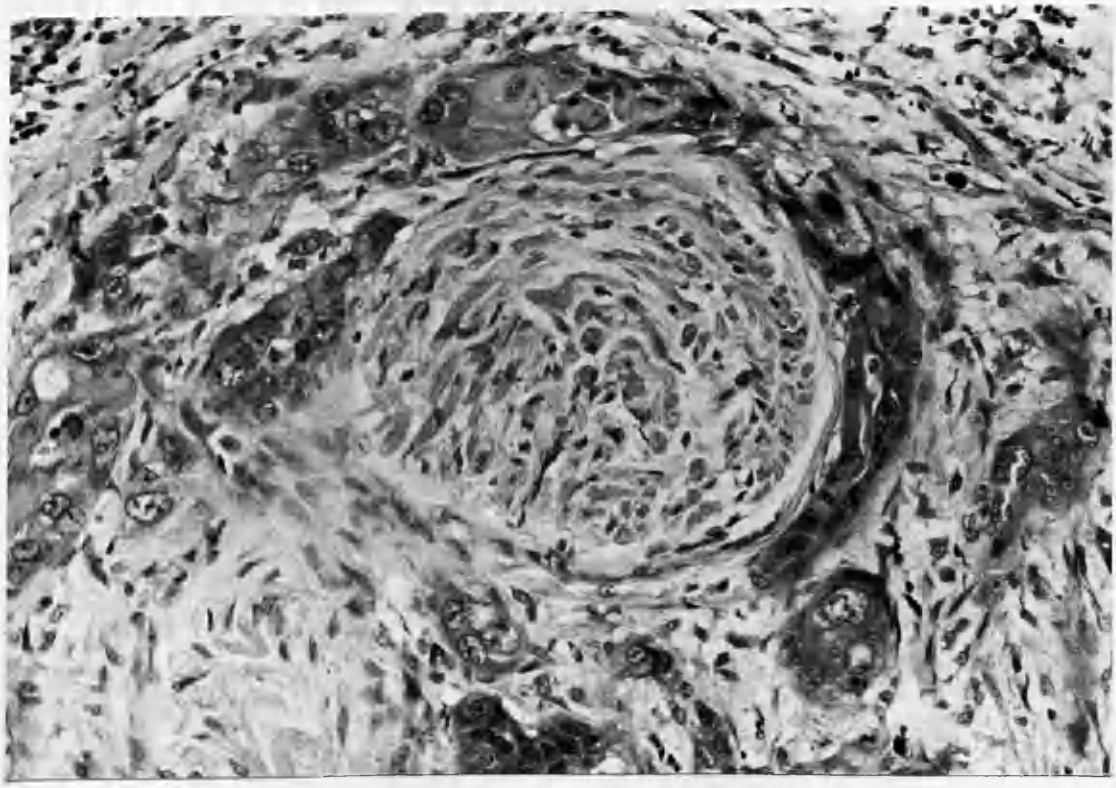


Fig. 4.3. Perineural invasion of squamous carcinoma in case 14 in the group without recurrence. H & E x270.

	Case Number	Degree of Keratinization	Nuclear Polymorphism	Number of Mitoses	Tumour Factor	Pattern of Invasion	Stage of Invasion	Lymphoplasmacytic Infiltration	Tumour-host Interaction	Total Malignancy Grade
PATIENTS WITH RECURRENCE	1	2	2	1	5	4	4	3	11	16
	2	3	3	4	10	4	4	3	11	21
	3	3	3	1	7	4	4	3	11	18
	4	1	2	3	6	3	4	2	9	15
	5	2	2	3	7	4	4	4	12	19
	6	2	2	2	6	4	4	3	11	17
	7	2	3	2	7	4	4	3	11	18
	8	1	1	2	4	4	4	4	12	16
	9	3	2	2	7	3	4	3	10	17
	10	3	4	4	11	3	3	4	10	21
	11	3	4	4	11	2	4	3	9	20
	12	1	2	2	5	4	4	3	11	16
	Mean	2.17	2.5	2.5	7.17	3.58	3.92	3.12	10.67	17.83
	SD	0.84	0.91	1.09	2.33	0.67	0.29	0.58	0.98	2.04
PATIENTS WITHOUT RECURRENCE	1	3	3	2	8	4	4	3	11	19
	2	2	3	2	7	4	4	2	10	17
	3	3	4	3	10	4	3	2	9	19
	4	3	3	2	8	3	3	3	9	17
	5	1	2	1	4	3	3	2	8	12
	6	4	3	4	11	4	4	4	12	23
	7	2	3	2	7	4	4	3	11	18
	8	3	3	2	8	3	3	3	9	17
	9	2	3	4	9	4	4	3	11	20
	10	3	3	2	8	4	4	2	10	18
	11	3	2	3	8	3	3	3	9	17
	12	3	4	2	9	3	4	3	10	19
	13	4	4	3	11	4	4	3	11	22
	14	3	2	2	7	2	3	1	6	13
	15	3	3	3	9	4	4	3	11	20
	16	3	3	2	8	3	4	2	9	17
	17	1	1	1	3	2	2	1	5	8
	18	2	3	3	8	4	3	2	9	17
	19	3	4	3	10	3	3	2	8	18
	20	1	2	3	6	2	4	2	8	14
	21	3	3	2	8	4	4	4	12	20
	22	2	1	1	4	1	1	2	4	8
	23	1	2	1	4	2	3	3	8	12
	24	2	2	2	6	2	2	3	7	13
	25	4	4	4	12	4	4	3	11	23
	26	4	4	4	12	4	3	3	10	22
	27	2	2	3	7	4	4	3	11	18
	28	3	3	4	10	3	4	3	10	20
	29	1	1	1	3	1	1	2	4	7
	30	2	3	2	7	4	4	3	11	18
	31	2	2	2	6	3	3	2	8	14
	Mean	2.52	2.74	2.42	7.68	3.19	3.32	2.58	9.97	16.77
	SD	0.93	0.89	0.96	2.43	0.95	0.87	0.72	2.15	4.23

Table 4.6. Scores of the malignancy grading features, tumour factor, tumour-host interaction and total malignancy grades in patients with and without recurrence.

CHAPTER 5

SQUAMOUS CELL CARCINOMA OF THE TONGUE - FOLLOW-UP STUDIES

5.1 INTRODUCTION

The criteria for inclusion of cases in the analysis of the results in this Chapter were the same as those described in Chapter 4. These were the criteria required for the general case selection as discussed in Chapter 2, Sections 2.2.1 and 2.2.2 plus the additional two criteria that were discussed in Chapter 4, Section 4.1. Four cases were excluded because they did not fulfil the criteria described in Sections 2.2.1 and 2.2.2. A further nine cases were excluded because they did not fulfil the criteria described in Section 4.1. Four of these patients survived without disease but the follow-up period was less than 24 months and the other five died within less than 24 months of follow-up without recurrence or the situation was not known with certainty. Therefore, from the original 62 cases with tongue carcinoma, 49 were eligible for the studies presented in this Chapter.

As in the previous Chapter, it was planned here to give an account related to clinical follow-up and outcome of patients after treatment. The recurrence and the survival were the end points for discussing and relating certain clinical and structural histological features obtained from the primary tumours of these patients. It was crucial for such studies to create clearly defined groups of patients upon which comparison could be made. The patients were grouped as surviving without recurrence at 24 months or as having recurrence prior to 24 months.

There were 25 patients who developed recurrence. Of these, 21 developed recurrence within a follow-up period of not less than four months, but within 24 months. These patients formed the first group in the following analyses. The remaining four patients developed recurrence at a later period of follow-up and this was more than 24 months. They formed a subgroup in the analyses and were termed a late recurrence subgroup where it was appropriate. The other group in the analyses comprised patients who did not develop recurrence during the whole period of follow-up and there were 24 such patients.

Out of all 25 patients who developed early or late recurrence, 21 had received radiotherapy as part of the treatment management and in three patients no radiotherapy was attempted. In another one patient, data concerning radiotherapy was not available. On the other hand in the 24 patients who did not develop recurrence, 10 had received radiotherapy and in another six patients no radiotherapy was attempted. There were eight patients in this group for whom no data concerning radiotherapy was available.

Seventeen patients in the group with recurrence died within less than 24 months of the follow-up period. Therefore, of the original 49 patients who were followed-up for the studies presented in this Chapter, 32 survived for over 24 months following treatment of their squamous cell carcinoma.

5.2 GENERAL OVERVIEW

5.2.1 Age and Sex

Analysis in relation to age and sex was carried out for all the 49 patients grouped together. There were 22 female patients and 27 male patients. This is a female to male ratio of 1:1.2. The age range in the female group was between 41 and 88. The mean was 66.7 and the median was 66. The age range in the male group on the other hand, varied between 28 and 83. The mean age was 59.4 and the median was 59. Figure 5.1 shows the histogram of the female and male ditribution according to age. It was evident that the peak was in the fifties and sixties age group in males, whereas in females the peak was in the seventies age group. Fourteen out of 22 (63.6%) of the female patients developed recurrence. By contrast 11 out of 27 (40.7%) of the male patients developed recurrence. The mean age of 25 patients who developed recurrence was 65 whereas in those who did not develop recurrence it was 60.

5.2.2 Clinical TN in Patients with Recurrence

The data will be presented in relation to whether or not the patients developed recurrence. Patients who developed late recurrence were regarded as a subgroup within the group of patients who developed recurrence. The same case number in each group is given for presenting data throughout this Chapter.

The tumour size (T) and clinical nodal status as well as the site of recurrence are shown in Table 5.1. The last four cases (22, 23, 24 and 25) in Table 5.1 formed the subgroup of patients with late

recurrence. The recurrence was found locally in nine patients and in the neck in another 11 patients. In four patients recurrences were found both in local and neck sites. The last patient, case 14, in whom bilateral neck dissections were performed at the planned initial treatment, developed recurrence in the lung 23 months after the treatment. Interestingly all the four patients who developed late recurrence were in T1N0 category and three of them had radical neck dissection as part of the treatment.

5.2.3 Type of Neck Surgery in Patients with Recurrence

Out of the 25 patients who developed recurrence whether early or late, 19 had neck surgery as part of the treatment management. The type of neck surgery in this group of patients was either radical or functional neck dissection. Occasionally this was associated with submandibular dissection of the opposite side. Table 5.2 summarises the pattern of neck surgery in this group of patients. The neck was not treated in one patient of the late recurrence subgroup.

5.2.4 Histological Nodal Status in Patients with Recurrence

The histological status of the nodes in patients who had neck surgery is evident in Table 5.2. This indicates the number of positive nodes as well as data on whether or not extranodal spread of the tumour was noted in individual cases. Two-thirds of the cases with histologically proven positive nodes had extracapsular spread either in single or multiple nodes. In the subgroup of late recurrences, one of the cases had 3 positive nodes all of which showed extracapsular involvement.

It was possible to evaluate the false clinical assessment in the 19 cases who had neck surgery through contrasting the data on clinical nodal status described in Table 5.1 with the data on histological nodal status presented in Table 5.2. Interestingly in the 10 cases who had neck surgery and who were assessed clinically as N0, seven actually showed nodal metastases on histological examination. This is a 70 per cent false negative clinical assessment. By contrast, only one out of nine cases thought clinically to have metastasis, was found to have negative nodes histologically. This is only an 11.1 per cent false positive clinical assessment.

5.2.5 Clinical TN in Patients without Recurrence

The patients included in this group were those who did not develop recurrence throughout the whole period of follow-up and survived disease free for a minimum of 24 months. Twenty four patients comprised this group. Data of the tumour size (T) and clinical nodal status (N) at initial presentation are shown in Table 5.3. It was found that all the patients, but one presented with early tumour size of either T1 or T2 category. More than half, had nodes assessed clinically as N0.

5.2.6 Type of Neck Surgery in Patients without Recurrence

Neck surgery was not performed in seven patients of this group. These were T1N0, with only one of T2N0 category. The type of neck surgery performed in all but one, was radical neck dissection. In more than half, this was associated with submandibular dissection

of the other side of the neck. Neck surgery types in individual patients in this group are shown in Table 5.3.

5.2.7 Histological Nodal Status in Patients without Recurrence

The histological status of the nodes in patients who had neck surgery is evident in Table 5.3. This indicates the number of positive nodes in individual patients. Data on whether or not these positive nodes showed extracapsular involvement are also indicated. Interestingly, eight of the patients showed histologically positive nodes and in seven of these the metastases were in more than one node. Only two cases with multiple positive nodes showed tumour spread beyond the capsule. The data on the clinical and histological assessments of the nodes were contrasted. It was found that out of eight cases assessed clinically as N0, two had histologically proven nodal metastases and one showed extracapsular spread in two nodes. This is a 25 per cent false negative clinical assessment. On the other hand three out of nine cases thought clinically to be positive for metastasis were found to be node negative histologically. This is a 33.3 per cent false positive clinical assessment.

5.3 PREDICTION OF TUMOUR RECURRENCE

It was realized that it could be appropriate to combine the four cases which formed the late recurrence subgroup with either the group of patients who developed recurrences or with the group of patients who survived a minimum of 24 months without recurrence. Therefore in the following analyses particularly in the comparison of structural histological features between the groups, the data in the late recurrence subgroup will be dealt with once as within the group

with recurrences and once as within the group without recurrence.

5.3.1 AgNORs Count and Prediction of Recurrence

There were 16 cases in which the AgNORs count was assessed on the original biopsy of the primary tumour. Of these eight cases were in the group of patients who developed recurrence and the other eight cases were in the group without recurrence.

Table 5.4 shows the data concerning the AgNORs. The means and standard deviations are shown as well. Cases which are marked with an asterisk are those in which the AgNORs count was not evaluated. None of the four cases forming the late recurrence subgroup was used for the evaluation of the AgNORs count. Therefore, they were not included in the statistical comparison.

Comparison of the data on the AgNORs between the two groups, showed a difference between the means in the two groups, this being greater in the group with recurrence. Statistical analysis using the Mann-Whitney U-test, showed that the difference was not significant.

5.3.2 Tumour Thickness and Prediction of Recurrence

All the 49 cases forming the two groups as well as the subgroup of late recurrence were used for the assessment of tumour thickness. There were 21 cases in the group with recurrence, four cases in the subgroup of late recurrence and 24 cases in the group without recurrence. The tumour thickness values as well as the means and the standard deviations in the groups are shown in Table 5.4. There was an obvious difference in the means between the groups.

Tumour thickness values were generally greater in patients who developed recurrence within 24 months. Interestingly the mean of the thickness values in the subgroup with late recurrence was less than the mean thickness values in the group without recurrence. The Mann-Whitney U-test was used for the comparison of the data between the groups. Statistical analysis shows that the difference in the means between the 21 cases with recurrence and the 24 cases without recurrence was significant (P = 0.024). However, when the tumour thickness values in all the patients who developed recurrence whether early or late, were compared with the thickness values in the group without recurrence, no statistically significant difference was demonstrated. Alternatively, the four cases in the subgroup of late recurrence were combined with the group without recurrence. Comparison of the thickness values of the resulting group with the values in the group with early recurrence was then undertaken. Analysis showed that the mean thickness values were different and this difference was statistically significant (P = 0.012).

Linear discriminant analysis was then undertaken. The results showed that tumour thickness alone was not a good discriminator. Only a 62 per cent correct prediction was obtained if late recurrences were ignored and 65 per cent correct prediction if the late recurrences were included with the group without recurrence.

Alternatively, it was felt that it would be reasonable to look at tumour thickness and recurrence to determine the proportions of tumours of differing thickness values which recurred. Table 5.5 shows the number of tumours in thickness categories with the percentages recurring. The four cases with late recurrences were

excluded. Although tumours of less than 6mm showed a lower incidences of recurrence than tumours of greater than 6mm thickness, again no clear dividing line between the tumour thickness categories and recurrence was evident.

5.3.3 Microscopic Surgical Margins and Prediction of Recurrence

The surgical margins were studied in all the 49 cases. The mucosal and the deep margins were examined for the tumour clearance. Cases which showed tumour actually at a margin, were termed positive (+ve) and those with tumour-free margins were termed negative (-ve). The condition of the microscopic surgical margins as well as the tissue type in cases with positive margins are shown in Table 5.4. Only four out of all the 49 cases showed positive margins. Three of these had developed recurrence and were actually in the group with early recurrence. Another one case with a positive margin was in the group without recurrence. When the surgical margins were considered in each group separately, it was evident that 3 out of 21 (14.29%) patients with early recurrence had positive margins. By contrast a positive margin was seen in only one out of the 24 (4.17%) patients without recurrence. The incidence of positive surgical margins of course would be changed if the cases in the late recurrence subgroup were combined to either of the other groups. This would show a 3 out of 25 (12%) incidence if they were combined with the group with recurrence and one out of 28 (3.57%) if they were combined with the group without recurrence.

The tissue type at the positive margins was also evaluated. It was found that positive margins were located in muscle in three

cases. Two of these developed recurrence, one in the local site and the other in the neck. The tissue type in the other case with positive margins was connective tissue in which the regrowth was established at the local site.

5.3.4 Malignancy Grades and Prediction of Recurrence

As for the tumour thickness and the microscopic surgical margins, all the 49 cases were used for the assessment of malignancy grades. The scores of the six histological features, tumour factor, tumour-host interaction and total malignancy grades as well as the means and the standard deviations for each parameter are summarised in Table 5.6.

The Mann-Whitney U-test was used in the statistical comparisons of the scores between groups. Firstly comparisons of the parameters between the group with early recurrence and the group without recurrence were undertaken. There were slight differences in the means of degree of keratinization, nuclear polymorphism, number of mitoses and tumour factor between the two groups. In no instances, however, were these differences shown to be statistically significant. Similarly pattern of invasion, stage of invasion and lymphoplasmacytic infiltrations, although showing slight differences in the means between the two groups, were not found to statistically significant. However, when the tumour-host interaction was compared between the two groups a statistically significant difference was evident ($P = 0.033$). Total malignancy grade was then compared between the groups. There was a slight difference in the means, but statistically this was not significant.

* The recent incidence data on tongue carcinoma in Scotland shows the male to female ratio as 1.6:1 (data from Cancer Registry Branch, S.H.H.D, Edinburgh, 1982-1986).

An attempt was then made to compare the malignancy grade parameters between the group with recurrence plus the subgroup with late recurrence against the group without recurrence. It was found that in no instances of statistical comparison were significant differences demonstrated. However, when malignancy grade parameters were compared between the group with recurrence versus the combined group without recurrence plus the subgroup with late recurrence, the tumour-host interaction parameter was found to differ significantly between the two groups (0.028). In no other instances of malignancy grade parameters was the difference shown to be statistically significant.

5.4 DISCUSSION

Certain points will be briefly discussed in this Chapter giving a general idea of the follow-up studies in patients with carcinoma of the tongue. More detailed discussion will be presented in Chapter 8 where all the predictor parameters are discussed in relation to tumour recurrence in carcinomas of individual anatomical site separately and in combination of all the cases as well.

Callery et al. (1984) in their series of 412 patients with squamous cell carcinoma of the tongue had reported a male to female ratio of 2.2:1 with a median age of 61 years. Although the number of the male patients was more than the number of the female patients in the present study, the ratio actually was less than that cited by Callery et al. (1984) and this was 1.2:1. The median age was 59 years in the male patients and 66 years in the female patients. The

overall 5-year cure rate for patients treated for carcinoma of the tongue reported by Callery et al. (1984) was 44 per cent. Due to the limited follow-up period in the present study, a 2-year cure rate of patients was calculated, and this was 28/49 (57%). The majority of treatment failures are said to occur within the first two years after treatment (Nason et al., 1989). Hence it would be not unreasonable to say that the cure rate obtained in the Glasgow cases was comparable to the results drawn from Callery et al's (1984) report.

Local recurrence of the tumours of the tongue increases in relation to tumour size (Spiro and Strong, 1971). This seemed to be true in the present study as well. Of 41 patients with tumour size T1 or T2, seven (17%) developed local recurrence by contrast to eight patients with tumour size T3 or T4 of whom six (75%) developed recurrence. The clinical nodal status was found not to influence the incidence of recurrences in the neck. Nine of 31 (29%) patients with N0 developed recurrence in the neck and five of 18 (27%) patients with palpable nodes developed recurrence in the neck. These findings might be comparable to the results reported by Vikram et al. (1984c) who stated that there was no significant difference in the frequency of tumour recurrence in the neck in patients with N0 or N1 compared to those with N2 or N3. However, Vikram et al. (1984c) had combined N0 and N1 categories together as a group. This might have influenced the overall data on incidence of tumour recurrence in the neck.

Analysis of the factors studied in relation to prediction of tumour recurrence revealed that tumour thickness and tumour-host interaction were the only parameters which showed significant differences between the group with recurrence and the group without

recurrence. However, when discriminant analysis was carried out using tumour thickness alone, only 62 per cent of the cases were correctly allocated to the groups with and without recurrence. In patients with carcinoma of the tongue, factors related to prediction of nodal metastasis were analysed earlier in Chapter 3, and the results revealed that neither tumour thickness nor tumour-host interaction were found helpful in predicting nodal metastasis.

Although these parameters were of no help in prediction of nodal metastasis and they were relatively helpful in predicting tumour recurrence. This suggests that consideration might be given to further treatment modalities or modifying initial treatment plans at least in patients with thick tumours that show diffuse and deep invasion without exciting lymphoplasmacytic infiltration.

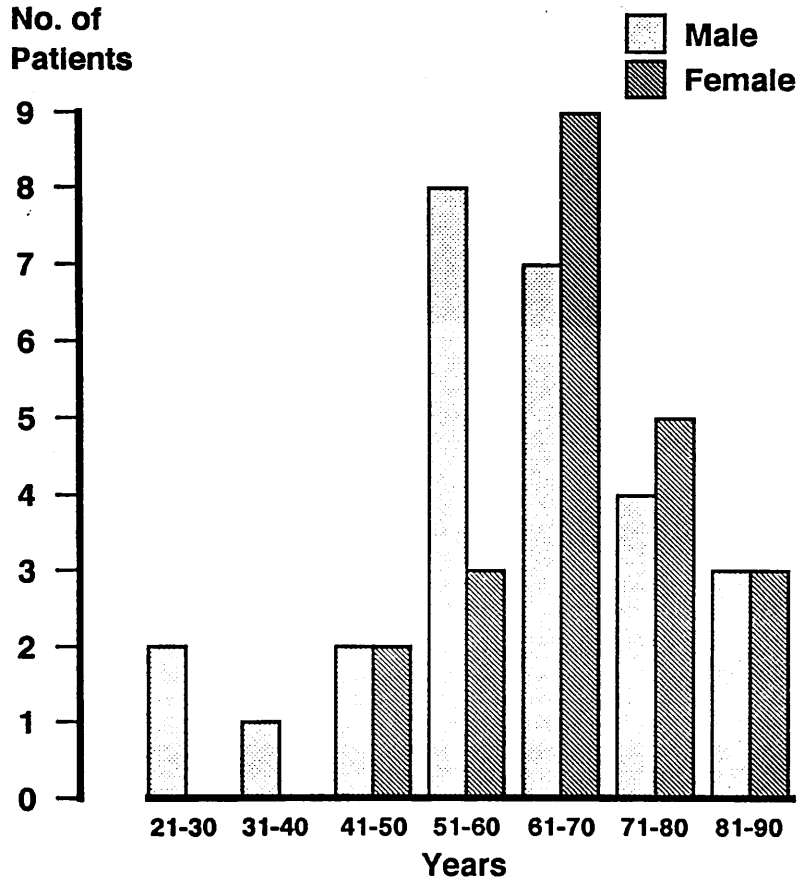


Fig. 5.1 **Age distribution (years) in patients with carcinoma of the tongue.**

Case Number	Recurrence site	Clinical TN
1	N	T2N0
2	L	T2N1
3	N	T1N1
4	L	T1N0
5	L	T4N0
6	L	T4N0
7	N	T1N0
8	N	T2N1
9	L + N	T1N0
10	N	T1N0
11	L + N	T4N1
12	L	T2N1
13	N	T1N0
14	Distant	T4N0
15	L	T4N0
16	N	T2N0
17	L	T3N1
18	L + N	T4N0
19	N	T2N1
20	N	T2N1
21	N	T2N2
Late Recurrences		
22	L	T1N0
23	N	T1N0
24	L + N	T1N0
25	L	T1N0

L = local
N = neck
L + N = local and neck

Table 5.1. Site of recurrences and the clinical tumour size (T) and nodal status (N).

Case Number	Type of Neck Surgery	Number of +ve Nodes	Extracapsular Involvement
1	RND	3	1
2	RND + SMDO	1	0
3	RND	2	1
4	*	*	*
5	RND + SMDO	0	0
6	*	*	*
7	*	*	*
8	RND	6	5
9	RND	4	4
10	*	*	*
11	RND	1	1
12	RND	9	4
13	RND	1	1
14	RND + FNDO	3	2
15	RND	4	4
16	*	*	*
17	RND + SMDO	2	0
18	RND + SMDO	3	0
19	RND	0	0
20	RND	1	0
21	RND	8	0
Late Recurrences			
22	RND	0	0
23	RND	3	3
24	*	*	*
25	RND	0	0

RND = Radical neck dissection
FND = Functional neck dissection
FNDO = Functional neck dissection opposite side
SMDO = Submandibular dissection opposite side

* Neck surgery was not performed.

Table 5.2. Type of neck surgery, number of histologically positive nodes and extracapsular involvement in patients with recurrence.

Case Number	Clinical TN	Type of Neck Surgery	Number of +ve Nodes	Extracapsular Involvement
1	T2N0	*	*	*
2	T2N1	RND	2	0
3	T1N0	RND	0	0
4	T1N0	*	*	*
5	T2N1	RND	0	0
6	T1N0	*	*	*
7	T2N0	RND + SMDO	0	0
8	T2N0	RND	0	0
9	T1N1	RND + SMDO	2	0
10	T1N2	FND + SMDO	3	1
11	T2N1	RND + SMDO	1	0
12	T1N0	RND + SMDO	0	0
13	T1N0	*	*	*
14	T1N0	*	*	*
15	T1N1	RND	2	0
16	T2N1	RND + SMDO	0	0
17	T1N0	*	*	*
18	T2N0	RND	3	2
19	T4N0	RND + SMDO	0	0
20	T1N0	*	*	*
21	T2N1	RND	3	0
22	T2N0	RND + SMDO	1	0
23	T2N0	RND + SMDO	0	0
24	T2N2	RND + SMDO	0	0

* Neck surgery was not performed.

Table 5.3. The clinical TN, type of neck surgery, number of positive nodes and extracapsular involvement in patients without recurrence.

	Case Number	AgNORs	Tumour Thickness	Surgical Margins	Tissue Type	Recurrence Site
	1	*	9	-ve		
	2	23.33	14	-ve		
	3	*	5	-ve		
	4	*	22	+ve	connective	L
	5	*	18	-ve		
	6	15.65	7	-ve		
	7	*	12	+ve	muscle	N
	8	24.94	8	-ve		
PATIENTS	9	11.50	5	-ve		
WITH	10	*	3	-ve		
RECURRENCE	11	*	27	-ve		
	12	*	22	+ve	muscle	L
	13	13.03	4	-ve		
	14	*	10	-ve		
	15	7.69	13	-ve		
	16	*	14	-ve		
	17	22.60	9	-ve		
	18	*	15	-ve		
	19	*	11	-ve		
	20	14.03	13	-ve		
	21	*	14	-ve		
	Mean	**16.60**	**12.14**			
	SD	**6.28**	**6.29**			
PATIENTS	22	*	9	-ve		
WITH LATE	23	*	1	-ve		
RECURRENCE	24	*	3	-ve		
	25	*	10	-ve		
	Mean		**5.75**			
	SD		**4.43**			
	1	7.43	6	-ve		
	2	9.85	6	-ve		
	3	*	12	-ve		
	4	*	8	-ve		
	5	*	14	-ve		
	6	*	3	-ve		
	7	*	9	-ve		
	8	9.93	2	-ve		
	9	*	3	-ve		
	10	12.49	5	-ve		
PATIENTS	11	*	5	-ve		
WITHOUT	12	11.18	5	-ve		
RECURRENCE	13	*	1	-ve		
	14	14.01	8	-ve		
	15	*	12	+ve	muscle	
	16	*	4	-ve		
	17	10.78	4	-ve		
	18	*	28	-ve		
	19	*	12	-ve		
	20	*	3	-ve		
	21	*	12	-ve		
	22	17.04	6	-ve		
	23	*	14	-ve		
	24	*	16	-ve		
	Mean	**11.59**	**8.25**			
	SD	**2.93**	**6.01**			

Recurrence site, L = local, N = neck

* AgNORs count was not evaluated

Table 5.4. AgNORs, tumour thickness and clearance of surgical margins with tissue type at positive margins in patients with and without recurrence.

Thickness (mm)	Total Number of Cases	No Recurrence	Recurrence	% Recurrence
1 - 3	6	5	1	17
4 - 6	11	8	3	27
7 - 9	7	3	4	57
10 - 12	7	4	3	43
13 - 15	8	2	6	75
> 15	6	2	4	67
	45	**24**	**21***	

* Four cases with late recurrences were excluded

Table 5.5. Number of cases according to tumour thickness grouping showing the percentage of recurrences in each group.

	Case Number	Degree of Keratinization	Nuclear Polymorphism	Number of Mitoses	Tumour Factor	Pattern of Invasion	Stage of Invasion	Lympho-plasmacytic Infiltration	Tumour-host Interaction	Total Malignancy Grade
	1	2	3	2	7	4	4	3	11	18
	2	1	1	1	3	4	4	2	10	13
	3	2	2	1	5	4	4	4	12	17
	4	4	3	2	9	4	4	4	12	21
	5	1	2	1	4	4	4	3	11	15
	6	3	3	3	9	3	4	3	10	20
	7	1	2	2	5	3	4	2	9	14
	8	2	2	2	6	3	4	3	10	16
	9	2	2	2	6	3	4	2	9	15
PATIENTS	10	3	3	2	8	4	4	3	11	19
WITH	11	2	3	3	8	4	4	3	11	19
RECURRENCE	12	4	4	3	11	3	4	3	10	21
	13	1	2	1	4	3	4	3	9	13
	14	2	2	3	7	3	4	3	10	17
	15	2	2	2	6	4	4	2	10	16
	16	3	2	1	6	4	4	3	11	17
	17	2	2	3	7	4	4	2	10	18
	18	2	2	1	5	3	4	3	10	15
	19	4	4	4	12	3	4	3	10	22
	20	1	1	2	4	2	4	1	7	11
	21	3	2	1	6	4	4	3	11	17
	Mean	**2.24**	**2.33**	**2.0**	**6.57**	**3.48**	**4.0**	**2.71**	**10.19**	**16.76**
	SD	**0.99**	**0.79**	**0.89**	**2.32**	**0.60**	**0.00**	**0.72**	**1.12**	**2.83**
PATIENTS	22	4	4	2	10	4	4	3	11	21
WITH LATE	23	1	1	1	3	1	1	1	3	6
RECURRENCE	24	3	3	4	10	4	4	2	10	20
	25	1	1	2	4	3	4	2	9	13
	Mean	**2.25**	**2.25**	**2.25**	**6.75**	**3.0**	**3.25**	**2.0**	**8.25**	**15.00**
	SD	**1.5**	**1.5**	**1.26**	**3.77**	**1.41**	**1.5**	**0.82**	**3.59**	**6.98**
	1	1	2	1	4	3	4	2	9	13
	2	2	3	2	7	4	4	2	10	17
	3	3	3	1	7	3	4	2	9	16
	4	2	3	1	6	3	4	1	8	14
	5	1	1	1	3	3	4	1	8	11
	6	1	1	1	3	1	3	1	5	8
	7	2	2	2	6	4	4	2	10	16
	8	3	4	2	9	3	3	2	8	17
	9	2	2	2	6	4	4	2	10	16
	10	2	2	2	6	3	3	3	9	15
PATIENTS	11	3	3	4	10	4	4	3	11	21
WITHOUT	12	1	2	2	5	3	4	3	10	16
RECURRENCE	13	1	1	1	3	1	1	1	3	6
	14	1	1	2	4	2	4	2	8	12
	15	2	2	4	8	2	4	3	9	17
	16	3	3	4	10	4	4	3	11	21
	17	1	2	1	4	4	4	2	10	14
	18	3	3	1	7	3	4	3	10	17
	19	4	2	1	7	4	4	4	12	19
	20	3	4	3	10	4	4	3	11	21
	21	3	4	4	11	2	4	3	9	20
	22	4	4	2	10	4	4	3	11	21
	23	2	2	2	6	2	4	2	8	14
	24	3	3	4	10	2	4	4	10	20
	Mean	**2.21**	**2.46**	**2.08**	**6.75**	**3.0**	**3.75**	**2.38**	**9.13**	**15.87**
	SD	**0.98**	**0.98**	**1.14**	**2.54**	**0.98**	**0.68**	**0.87**	**1.96**	**4.03**

Table 5.6. Scores of the malignancy grading features, tumour factor, tumour-host interaction and total malignancy grades in patients with and without recurrence.

CHAPTER 6

SQUAMOUS CELL CARCINOMA INVOLVING TONGUE PLUS FLOOR OF MOUTH - FOLLOW-UP STUDIES

6.1 INTRODUCTION

Similar criteria to those discussed in Chapter 4, Section 4.1 and Chapter 5, Section 5.1 were required for including patients in the analysis of the results of patients having carcinoma involving tongue plus floor of mouth. Out of the original 33 patients with carcinoma involving tongue plus floor of mouth, one was excluded because the required criteria described in Chapter 2, Sections 2.2.1 and 2.2.2 were not fulfilled. A further 11 patients were excluded because the required criteria described in Sections 4.1 and 5.1 were not fulfilled. Three of these patients were survivors and disease-free but were lost to follow-up within less than 24 months. The remaining eight patients died within less than 24 months of follow-up either disease-free or the condition was not known for certainty. Therefore, out of the original 33 patients with carcinoma involving tongue plus floor of mouth, 21 were eligible for the studies presented in this Chapter.

It was planned in this Chapter to give an account related to clinical follow-up and outcome of patients after treatment. The recurrence and survival will be discussed and certain clinical and structural histological features obtained from the primary tumours of these patients will be analysed in relation to outcome of the patients.

Two well defined groups were created in order to allow a meaningful comparison of the clinical and histological parameters studied in the groups. There were seven patients who developed recurrence in the follow-up period of not less than four months and within a minimum period of 24 months. This formed the first group in the analysis of the results in this Chapter. Patients who were eligible for this analyses and did not develop recurrence within a minimum follow-up period of 24 months were 14 in number and these formed the second group in the analysis. The same case number was attached to individual patients in each of the two groups during the analyses of the results.

While all the seven patients who developed recurrence had received radiotherapy, only 10 out of 14 patients in the group without recurrence were subjected to radiotherapy as part of the treatment management. Two patients who had no recurrence did not receive radiotherapy. In the remaining two patients the data concerning radiation therapy was not available.

There were five patients in the group with recurrence who died within less than 24 months. Therefore, of the origninal 32 patients with carcinoma involving tongue plus floor of mouth who were followed-up for studies presented in this Chapter, 16 had survived for over 24 months following treatment of their squamous cell carcinoma.

6.2 GENERAL OVERVIEW

6.2.1 Age and Sex

Analysis in relation to age and sex was carried out. There were only three female patients and the male patients were 18 in number. This is a female to male ratio of 1:6. The age range in the female group varied between 35 and 72. The mean was 51.6 and the median was 48. The age range in the male group varied between 34 and 78. The mean was 64.5 and the median was 65. Figure 6.1 shows the histogram of the male distribution in relation to age. It was evident that the peak was in the sixties age group. The number of female patients was too small to be usefully presented as a histogram. None of the female patients developed recurrence. By contrast, seven out of the 18 (38.9%) male patients developed recurrence. The mean age of the seven male patients who developed recurrence was 51, whereas the mean age was 73 in male patients who did not develop recurrence. This is a 22 years difference in the mean age.

6.2.2 Clinical TN in Patients with Recurrence

The data are presented in relation to whether or not the individual developed recurrence. The tumour size (T) and the clinical nodal status (N) as well as the site of recurrence are shown in Table 6.1. The recurrence developed at the primary site in three cases and in the neck in the other four. The tumours in more than half of these cases were in advanced stages at initial presentation.

6.2.3 Type of Neck Surgery in Patients with Recurrence

In only one patient in the group who developed recurrence was neck surgery not performed. The type of neck surgery in the other six patients varied from unilateral radical neck dissection to radical neck dissection plus functional neck dissection of the opposite side of the neck. Table 6.1 summarises the patterns of neck surgery in this group of patients.

6.2.4 Histological Nodal Status in Patients with Recurrence

The histological status of the nodes in patients who had neck surgery is evident in Table 6.1. This indicates the number of positive nodes as well as data on whether or not extranodal tumour spread was noted. Out of six cases with neck surgery, four had histologically proven metastases either in single or multiple nodes. In two of these cases extracapsular spread of the tumour was noted in a single node.

It was possible to evaluate the false clinical assessment through comparing data of clinical and histological assessment. There was only one case clinically which was assessed as N0 and this was histologically proven also to be tumour free. Out of the other five cases who were clinically thought to have nodal metastases, one actually showed no metastasis following histological examination. This is a 20 per cent false positive clinical assessment.

6.2.5 Clinical TN in Patients without Recurrence

Data on the tumour size (T) and the clinical nodal status (N) at the initial presentation in the 14 patients who survived for a minimum of 24 months without recurrence are shown in Table 6.1. The majority initially presented with an early tumour size of T1 or T2 category. Similarly, the same proportion of patients were assessed clinically as having nodal status of N0 category.

6.2.6 Type of Neck Surgery in Patients without Recurrence

All but two patients in this group had some sort of neck surgery as part of the treatment management. The type of neck surgery in the remaining 12 patients ranged from radical neck dissection and unilateral or bilateral functional neck dissection. Occasionally, submandibular neck dissection was carried out in patients who underwent unilateral radical or functional neck dissection. The patterns of neck surgery performed for these patients are shown in Table 6.1.

6.2.7 Histological Nodal Status in Patients without recurrence

The histological nodal status in patients who had neck surgery is evident in Table 6.1. This indicates the number of positive nodes in individual patients as well as the condition of the nodal capsule. Only three patients showed nodal metastasis, two of whom had tumour spread beyond the capsule of the nodes. Contrasting the clinical nodal status data to histological nodal status data shown in Table 6.1, it was possible to calculate the false clinical assessment. Interestingly, out of five cases thought clinically to

have nodal metastasis, four showed negative nodes during histological examination. This is a very high false positive clinical assessment. On the other hand two out of seven cases where the nodes were clinically N0 turned out histologically to have nodal metastasis. This is a 28.57 per cent false negative clinical assessment.

6.3 PREDICTION OF TUMOUR RECURRENCE

The histological parameters related to tumour thickness, microscopic surgical margins and malignancy gradings were compared between the two groups. Only five cases in the two groups were used for AgNORs count assessment. Data on AgNORs count in these five cases are shown in Table 6.2. Three cases were in the group with recurrence and another two cases were in the group without recurrence. Because of the small number of the cases, it was felt that it would be meaningless to compare data on the AgNORs counts between these cases. Therefore, statistical comparison of AgNORs count in these cases was not attempted.

6.3.1 Tumour Thickness and Prediction of Recurrence

All the 21 cases which were eligible for the studies presented in this Chapter were used for the evaluation of tumour thickness. The tumour thickness values as well as the means and standard deviations are shown in Table 6.2. There was a slight difference in the means between the groups, the mean being greater in the group of patients who developed recurrence. Statistical analysis using the Mann-Whitney U-test showed that the difference was not significant.

6.3.2 Microscopic Surgical Margins and Recurrence

The surgical margins were assessed for tumour clearance in all the cases forming the two groups of patients. The mucosal and the deep margins were studied. A margin was termed positive where the tumour was actually shown at the margin and termed negative where it was clear from the tumour. The condition of the surgical margins and the type of tissue at which the positive margin was located are shown in Table 6.2. Only two out of 21 cases showed positive margins, one of which had developed recurrence in the neck. The other case with positive margins was in the group without recurrence. The incidence of positive margins was one out of seven (14.28%) in group with recurrence and one out of 14 (7.14%) in the group without recurrence.

The type of tissue at which the positive margins were located was also evaluated. The margin in the one case of the group with recurrence was located at muscle. The margin in the other case of the group without recurrence, was located in salivary tissue.

6.3.3 Malignancy Grades and Prediction of Recurrence

All the 21 cases were also used for the evaluation of malignancy grades. Table 6.3 summarises the scores of the six histological features, tumour factor, tumour-host interaction and the total malignancy grade. The mean and the standard deviation for each of the parameters are shown also in Table 6.3.

Statistical analyses then were undertaken for the comparison of the data between the two groups. The Mann-Whitney U-test was used for all comparisons. Each of the six histological feature was

compared separately between the groups. There were slight differences in the means of the degree of keratinization, nuclear polymorphism, number of mitoses and the tumour factor. Statistical analysis showed that these differences were not significant. Similarly the features of the pattern of invasion, stage of invasion, lymphoplasmacytic infiltration and the tumour-host interaction were compared between the two groups. Again statistical analyses showed that the differences were not significant. Likewise, the total malignancy grades were also compared between the groups, and found not to differ significantly.

6.4 DISCUSSION

Brief comments are presented in this section related to certain points on carcinomas involving tongue plus floor of mouth, and more detailed discussion will follow in Chapter 8.

It is not known whether previous reports in the literature have at all dealt with studies of squamous cell carcinomas of tongue plus floor of mouth as a distinct grouping. It was felt therefore, that the closest comparable data to the data presented in this study would be those reported in studies combining together patients having squamous cell carcinoma of the tongue and patients with carcinomas of the floor of the mouth. Teichgraeber and Clairmont (1984) reported from Housten, USA, a male to female ratio of 2.9:1 with an average age of 58 years in their series of 136 patients with carcinoma of the tongue and floor of mouth. In the present study, the ratio of male to female was found to be higher and was 6:1 with an average age of 59 years. It seemed that in the present study the ratio of male to female is similar to the ratio of male to female patients with

carcinoma of floor of mouth, 7:1 (Chapter 4), by contrast, to male to female ratio of patients with carcinoma of the tongue, 1.2:1 (Chapter 5). This might indicate that tumours involving tongue plus floor of mouth initially had arisen from floor of mouth mucosa and in the later stages of growth had involved the tongue.

Similar to the data presented in Chapters 4 and 5 relating tumour size to local recurrences, it was found that none of the five cases with T1 developed local recurrence. In cases with T2 tumour 2/7 (29%) developed local recurrence and another case developed recurrence in the neck although it was T2N0. There was only one case with tumour size T3 and this developed local recurrence. None of the five cases with tumour size T4 developed local recurrence, but two of these which were T4N1 developed recurrence in the neck. Likewise neck recurrences were related to clinical nodal status and an increase in the incidence of recurrence in the neck was noted in with palpable nodes. Only one case out of 11 (9%) with N0, developed recurrence in the neck. By contrast, two of nine (22%) cases with N1 developed recurrence in the neck. There was only one case with N2 category and this developed recurrence in the neck. There was no case with N3 category in the material studied.

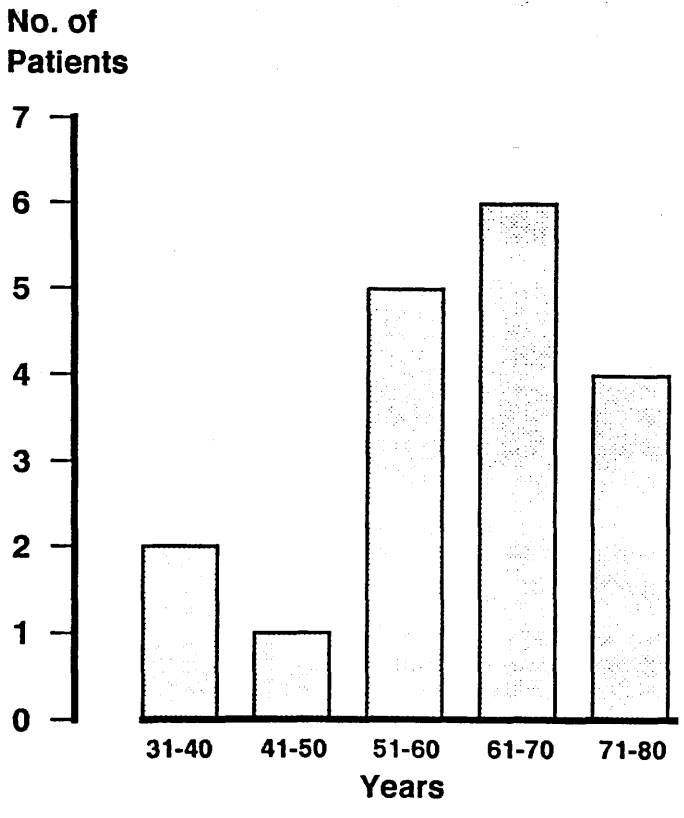


Fig. 6.1 Age distribution (years) in male patients with carcinoma involving tongue plus floor of mouth.

	Case Number	Recurrence Site	Clinical TN	Type of Neck Surgery	Number of +ve Nodes	Extracapsular Involvement
	1	L	T3N1	FND + SMDO	1	0
	2	L	T2N0	RND + FNDO	0	0
PATIENTS	3	N	T4N1	RND	4	0
WITH	4	N	T4N1	BFND	1	1
RECURRENCE	5	N	T2N0	*	*	*
	6	N	T3N2	RND + SMDO	4	1
	7	L	T2N1	RND	0	0
	1	-	T1N1	RND	0	0
	2	-	T2N0	*	*	*
	3	-	T1N0	FND + SMDO	0	0
	4	-	T1N0	*	*	*
	5	-	T2N0	FND + SHDO	3	1
PATIENTS	6	-	T4N1	RND	0	0
WITHOUT	7	-	T2N1	BFND	0	0
RECURRENCE	8	-	T4N1	RND	1	0
	9	-	T2N0	RND	1	1
	10	-	T4N1	RND	0	0
	11	-	T4N0	BFND	0	0
	12	-	T1N0	FND	0	0
	13	-	T1N0	FND + SMDO	0	0
	14	-	T3N0	RND	0	0

FND = Functional neck dissection
RND = Radical neck dissection
BFND = Bilateral functional neck dissection
SHDO = Suprahyoid dissection opposite side
SMDO = Submandibular dissection opposite side

* Neck surgery was not performed.

Table 6.1. Patient data showing site of recurrence if present, clinical TN, type of neck surgery, number of positive nodes and extracapsular involvement.

	Case Number	AgNORs	Tumour Thickness	Surgical Margins	Tissue Type	Recurrence Site
	1	9.86	17	-ve		
	2	17.95	15	-ve		
PATIENTS	3	11.75	19	+ve	muscle	neck
WITH	4	*	7	-ve		
RECURRENCE	5	*	7	-ve		
	6	*	13	-ve		
	7	*	3	-ve		
	Mean	**13.19**	**11.57**			
	SD	**4.23**	**5.97**			
	1	*	14	-ve		
	2	*	1	-ve		
	3	*	6	+ve	salivary	
	4	*	8	-ve		
	5	*	13	-ve		
PATIENTS	6	*	15	-ve		
WITHOUT	7	*	9	-ve		
RECURRENCE	8	*	14	-ve		
	9	*	14	-ve		
	10	*	4	-ve		
	11	*	16	-ve		
	12	*	12	-ve		
	13	16.66	5	-ve		
	14	17.96	8	-ve		
	Mean	**17.31**	**9.93**			
	SD	**0.92**	**4.71**			

* AgNORs count was not assessed

Table 6.2. AgNORs count, tumour thickness and condition of microscopic surgical margins and tissue type at positive margins in patients with and without recurrence.

	Case Number	Degree of Keratinization	Nuclear Polymorphism	Number of Mitoses	Tumour Factor	Pattern of Invasion	Stage of Invasion	Lympho-plasmacytic Infiltration	Tumour-host Interaction	Total Malignancy Grade
PATIENTS WITH RECURRENCE	1	3	3	2	8	4	4	4	12	20
	2	2	4	2	8	4	4	2	10	18
	3	2	2	2	6	4	4	2	10	16
	4	1	2	2	5	4	4	1	9	14
	5	3	3	3	9	3	4	2	9	18
	6	2	2	2	6	3	4	2	9	15
	7	2	2	2	6	4	4	3	11	17
	Mean	**2.14**	**2.57**	**2.14**	**6.86**	**3.71**	**4.00**	**2.29**	**10.0**	**16.86**
	SD	**0.69**	**0.79**	**0.38**	**1.46**	**0.49**	**0.0**	**0.95**	**1.15**	**2.03**
PATIENTS WITHOUT RECURRENCE	1	2	2	2	6	3	4	3	10	16
	2	2	3	2	7	3	1	2	6	13
	3	2	3	1	6	3	4	3	10	16
	4	2	2	1	5	4	4	3	11	16
	5	2	2	3	7	4	4	3	11	18
	6	4	4	3	11	2	4	3	9	20
	7	1	1	2	4	3	4	2	9	13
	8	3	4	3	10	4	4	3	11	21
	9	2	3	3	8	3	3	2	8	16
	10	2	2	2	6	4	4	3	11	17
	11	3	3	3	9	4	4	2	10	19
	12	2	2	2	6	4	4	2	10	16
	13	2	3	4	9	3	4	3	10	19
	14	3	4	4	11	3	4	3	10	21
	Mean	**2.29**	**2.71**	**2.50**	**7.50**	**3.36**	**3.71**	**2.64**	**9.71**	**17.21**
	SD	**0.73**	**0.91**	**0.94**	**2.21**	**0.63**	**0.82**	**0.50**	**1.38**	**2.58**

Table 6.3. The scores of the six histological features, tumour factor, tumour-host interaction and total malignancy grades in patients with and without recurrence.

CHAPTER 7

COMBINATION OF CASES OF SQUAMOUS CELL CARCINOMA IN THE THREE ANATOMICAL SITES - FOLLOW-UP STUDIES

7.1 INTRODUCTION

In the previous three Chapters an attempt was made to create well defined groups of patients with squamous cell carcinoma of floor of mouth, tongue and tongue plus floor of mouth respectively. The division of cases into two groups at each anatomical site was based on whether patients, following treatment, had developed recurrence at either local, neck or both sites within a minimum period of follow-up of not less than four months and not more than 24 months. Comparison of certain histological features, namely AgNORs count, microscopic surgical margins, tumour thickness and malignancy grades between the two groups in each of the three sites separately was undertaken. AgNORs count was not compared between the two groups in carcinomas involving tongue plus floor of mouth because only a few cases were eligible for analysis. It was felt that in this Chapter it would be reasonable to combine cases with recurrences in all the three sites as one group and cases without recurrences in the corresponding sites as another group. This would allow a further comparison of the structural histological features between these two major groups which it could be advantageous to assess on the larger number of cases.

7.2 PREDICTION OF TUMOUR RECURRENCE IN ALL CASES COMBINED

Following combination of the cases in the three anatomical sites together, it was found that these were 113 cases. Of these, 40

cases had developed recurrence within 24 months following treatment of their squamous carcinoma. Sixty-nine patients had not developed recurrence and were surviving and disease-free for a minimum of 24 months after treatment. In the remaining four cases which had carcinoma of the tongue, it was realized that they had developed recurrence well over 24 months of follow-up and these were termed late recurrence. These four cases were not included in the following statistical analyses. The original data on the parameters discussed in this Chapter appear in the Tables in Chapters 4, 5, and 6 and are not repeated in this Chapter.

7.2.1 AgNORs and Prediction of Recurrence

The only five cases with carcinoma involving tongue plus floor of mouth on which the AgNORs data was available were not compared between the two groups in Chapter 6. It was realized that in order to increase the number of cases for analysis of data on AgNORs, these five cases should be included while comparison was carried out between combined cases in the three anatomical sites. Overall, there were 42 cases on which the AgNORs count was assessed. Seventeen had developed recurrence and 25 survived disease-free for over 24 months. The mean AgNORs count in the group with recurrence was 15.35 and in the group without recurrence was 14.38. Statistical analysis using the Mann-Whitney U-test showed that the difference was not significant.

7.2.2 Microscopic Surgical Margins and Prediction of Recurrence

All the 109 cases eligible in the studies presented in the previous three Chapters were used for the evaluation of surgical

margins for tumour clearance. The mucosal and the deep margins in an individual case were evaluated and the case was termed positive when the tumour was actually at a margin and negative when the margin was tumour-free. Overall, there were 14 cases with positive margins. This is an incidence of 12.84 per cent. Out of these, nine were among the 40 patients who developed recurrence. This is a 22.5 per cent incidence, by contrast to only five out of 69 cases (7.25%) in the group without recurrence. The difference was statistically significant ($P < 0.025$, Chi-square test). Out of nine cases with positive margins in the group with recurrence, five had developed recurrence locally at the primary site and four at the neck. The local recurrences developed in 5/14 (35.7%) patients with positive margins and in 15/95 (15.8%) patients with negative margins. This difference in frequencies was not significant statistically (Fisher exact probability test). All the six recurrences that developed in both the local site plus the neck were in patients whose tumour resection margins were negative.

The type of tissue at the positive margins was also studied. In seven cases this was connective tissue. Four cases developed recurrence, two locally and two in the neck. No recurrence developed in the other three cases. In six cases the tissue type at the positive margin was muscle. Re-growth of the tumour occured in five cases, three of which were local and two in the neck. The type of tissue at which the positive margin was located in the remaining one case was salivary and no recurrence of tumour occured during a follow-up period of a minimum 24 months.

7.2.3 Tumour Thickness and Prediction of Recurrence

All the 109 cases eligible in the studies presented in the three previous Chapters were used for the assessment of tumour thickness. Out of these, 40 cases had developed recurrence and 69 cases survived disease-free over 24 months. The tumour thickness in all cases was measured by millimeter (mm). The mean tumour thickness in the group with recurrence was 11.25 and in the group without recurrence was 7.39. Tumours in the group with recurrence seemed therefore to be thicker than tumours in the group without recurrence. Comparison of the tumour thickness values between the groups, using the Mann-Whitney U-test, showed a highly significant difference between the groups ($P = 0.0002$).

7.2.4 Malignancy Grades and Prediction of Recurrence

Exactly the same 109 cases as used for tumour thickness evaluation were also used for the assessment of malignancy grades. The means of the scores of each of the six histological features, comprising Anneroth et al's (1987) malignancy grading system, tumour factor, tumour-host interaction and total malignancy grades were compared between the 40 cases with recurrence and the 69 cases without recurrence. There were slight differences in the means of degree of keratinization (2.2 versus 2.36), nuclear polymorphism (2.43 versus 2.64), number of mitoses (2.18 versus 2.32) and tumour factor (6.8 versus 7.32) between the groups. The Mann-Whitney U-test was used for the comparison of the values between the two groups. The results showed that in no instance was there a significant difference between groups. However, when the pattern of invasion was compared between

the two groups (3.55 versus 3.16) a statistically significant difference was demonstrated (P = 0.032). Similarly when the stage of invasion was compared between the two groups (3.98 versus 3.55), a statistically highly significant difference was demonstrated (P = 0.0005). The lymphoplasmacytic infiltration was also compared between the two groups. There was a slight difference between the groups (2.78 versus 2.52), but this was not statistically significant. When the tumour-host interaction was compared between the two groups (10.3 versus 9.23), on the other hand, statistical analysis showed a significant difference (P = 0.003). However, comparing the total malignancy grade between the groups (17.1 versus 16.55) a slight difference was noted and this was not statistically significant.

7.2.5 Histological Nodal Status and Prediction of Recurrence

Data related to neck surgery in patients with and without recurrence presented in Chapters 4, 5 and 6 were analysed. The results showed that when combining cases in all three sites together, 32 out of 40 patients who developed recurrence had neck surgery and 46 out of 69 patients without recurrence had neck surgery. Further analysis of the histological nodal status in patients who underwent neck surgery revealed that 24 out of 32 (75%) patients who developed recurrence had histologically proven nodal metastasis. By contrast, only 16 out of 46 (34%) patients who survived disease-free for a minimum of 24 months following treatment, showed metastasis in the nodes. Statistical analysis revealed that this difference was highly significant ($P < 0.0005$, Chi-square test). This indicated that positive nodes were more frequent in the group of patients with recurrence than in patients without recurrence. Further analysis was

* In Scotland the incidence of carcinoma in these intra-oral sites combined, shows the male to female ratio as 2.2:1 (data from Cancer Registry Branch, S.H.H.D, Edinburgh, 1982-1986).

carried out to study the incidence of extracapsular spread of tumour in cases with positive nodes in the two groups. Tumour beyond the nodal capsule was found in 14 out of 24 (58.3%) patients with positive nodes who had recurrence. By contrast this feature was evident in only 6 out 16 (37.5%) patients with positive nodes who survived disease-free for a minimum of 24 months after treatment. This apparent difference in the incidence of extracapsular invasion in positive nodes between the group of patients who developed recurrence and the group of patients without recurrence was not statistically significant.

7.3 DISCUSSION

When cases from all three sites were combined, the ratio of male to female was 2.8:1 with the median age 61 years. This was almost similar to 2.9:1 that was reported from Housten in the USA, by Teichgraeber and Clairmont (1984) in their series of 136 patients with carcinoma of the tongue and floor of the mouth. The majority of the female patients in the present study had tongue carcinomas.*

The overall treatment failure within 24 months follow-up was 40/109 (36.7%). This is comparable to the studies of Shaha et al. (1984) who reported a 33 per cent incidence of treatment failure in patients with carcinoma of the floor of the mouth and Callery et al. (1984) who reported a 44 per cent incidence of treatment failure in patients with carcinoma of the tongue. Increasing size of the tumours was found to be a feature directly related to increase in the incidence of local recurrences in each anatomical site separately. This trend was more strikingly apparent when cases from all the three

sites were combined together. Only 11 out of 87 (12.5%) cases with tumour size T1 or T2 developed local recurrence. By contrast, 11/22 (50%) of cases with tumour size T3 or T4 developed local recurrence. This type of relationship was also observed by Spiro and Strong (1971) in a series of patients with carcinoma of the tongue.

Failure in the neck was related to clinical nodal status (N) and showed an increasing incidence with increasing N category. Ten out of 68 (14.7%) patients with N0 category developed recurrence in the neck. This was sharply increased in N1 and N2 categories and the incidence was 10/36 (27.7%) and 2/4 (50%), respectively. Although no recurrence in the neck was evident in the only patient with N3 category, re-growth of the tumour at the local site was established. Similar trends in the relationship of clinical nodal status to tumour recurrence in the neck were observed in patients with carcinoma of the floor of mouth and in patients with carcinoma involving tongue plus floor of mouth (Chapters 4 and 6 respectively). In patients with carcinoma of the tongue (Chapter 5), no such relationship was noted.

Analysis of the factors studied in relation to prediction of tumour recurrence revealed that the surgical margins, tumour thickness, pattern of invasion, stage of invasion and total tumour-host interaction showed statistically significant differences between the group with recurrence and the group without recurrence. In the follow-up studies of the floor of the mouth carcinomas presented in Chapter 4, the local recurrences were significantly more frequent in patients whose tumour had positive resection margins. This feature was not analysed in patients with carcinoma of the tongue (Chapter 5)

and in patient with carcinoma involving tongue plus floor of the mouth (Chapter 6) because there were only a few cases with positive surgical margins in each of these two sites. A highly significant difference was noted between the two groups when tumour thickness was compared. This was also noted in carcinomas of floor of mouth (Chapter 4) and carcinomas of the tongue (Chapter 5). In carcinomas involving tongue plus floor of mouth (Chapter 6), tumour thickness did not differ significantly between the two groups. This could be due either to the small number of cases studied in this anatomical site or perhaps the predictive value of tumour thickness is limited to only tumours arising from a single intra-oral anatomical site. Although pattern of invasion and stage of invasion were found to differ significantly between the groups when all the cases in the three sites were combined, this was not evident when comparison was carried out between the groups in each site separately. It could be that the statistical results might have been influenced by the numbers of cases when the analysis was carried out on all cases combined. Total tumour-host interaction on the other hand was found to differ significantly between the groups in carcinoma of the floor of mouth and in carcinomas of the tongue but not in carcinomas involving tongue plus floor of mouth. This feature differed significantly between the combined groups of the three sites. Therefore, in diagnostic pathology, features like tumour thickness and tumour-host interaction in intra-oral carcinoma should be considered more seriously as these features might indicate the prognosis and the outcome of oral cancer patients.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

8.1 INTRODUCTION

The predictive values of the parameters studied in this thesis concerning nodal metastasis from carcinomas of floor of mouth, tongue and tongue plus floor of mouth were thoroughly discussed in Chapter 3, Section 3.10. In the present Chapter further discussions are presented concerning the predictive values of the same parameters as well as histological nodal status in relation to cure rate and outcome of patients with carcinomas arising from similar anatomical sites. Each of the parameters studied is discussed separately. The mitotic phase distribution parameters were not examined in relation to tumour recurrences and patient outcome because it was felt that highly unlikely that, following the earlier demonstration of the lack of any predictive value for nodal metastasis, there would be any relationship to recurrence.

8.2 AgNORs

While the AgNORs count had not previously been studied on oral squamous cell carcinoma in relation to patient outcome following treatment, in studies presented in Chapter 3, this parameter was found to be a good predictor for nodal metastasis in carcinoma of the floor of the mouth. The nature of AgNORs and the problems encountered during their counting and their validity in tumour pathology were discussed earlier in Chapter 3. Some data are available relating AgNORs count to prognosis in patients with cutaneous malignant

melanoma (Howat et al., 1988) and in patients with rectal adenocarcinoma (Griffiths et al., 1989). In both studies AgNORs counts were found to be of no prognostic relevance. In the present study also, the AgNORs counts failed to identify the high risk patients with carcinoma of the floor of mouth or carcinoma of the tongue who developed recurrence following treatment. The AgNORs count was not helpful in identifying patients at risk for recurrence in each site separately, or when comparison was carried out in all the three sites combined. This area might need further research by including more cases of squamous cell carcinoma especially those arising from floor of mouth since the results presented in Chapter 3, Section 3.4.2, indicated that AgNORs count was a good predictor for nodal metastasis in patients with floor of mouth carcinoma. However, the problems of determining exactly what should be counted and the subjective nature of the counting make it unlikely that this technique will find a place in routine diagnostic pathology of oral cancer.

8.3 MICROSCOPIC SURGICAL MARGINS OF THE TUMOUR

Vikram et al. (1984b) reported that failure of treatment at the local site was more frequent in patients with positive margins 4/35 (11.4%) than in patients with negative margins 2/72 (2.8%) although this difference was not statistically significant. Looser et al. (1978) reported that local rcurrences were strikingly more frequent in oral cancer patients when the surgical margins of the resected tumour were inadequate and 71 per cent of such patients developed local recurrence by contrast to only 31 per cent of patients with adequate margins. However, Looser et al. (1978) had considered surgical margins with severe dysplasia, carcinoma in situ, invasive

carcinoma or even surgical margins within a distance of 5mm from such changes as inadequate margins. This would have definitly altered the proportion of local recurrences related to inadequate margins. In the present study surgical margins were termed positive only when tumour was actually found at a margin. Nonetheless, our results also demonstrated similar trends, although not as striking as Vikram et al. (1984b) and Looser et al. (1978) studies. It was found in these studies that even the negative margins were not by any means safe for developing local recurrences. This could mean that other margins than those sampled were positive. Alternatively these results might be considered as further evidence to support Slaughter et al's (1953) theory of field cancerization. These authors proposed that oral cancers have multicentric origins. urther, they proposed that an unknown carcinogen can alter an area of epithelium giving it the potential to form multiple cancers. If one believes in this concept, the significance of negative margins become less important since the resected tumour with what appears to be adequate margins might have microscopic islands of cancer distant from the site of surgical resection. Another equally important reason is that all tumour might be removed at the primary operation but recurrence might develop in what Slaughter et al. (1953) referred to as a field of preconditioned epithelium.

8.4 TUMOUR THICKNESS

Tumour thickness as a single independent prognostic factor has recently been assessed by several workers. Urist et al. (1987) studied tumour thickness in carcinoma arising from buccal mucosa and

found that patients with tumours less than 6mm in thickness had significantly better survival rates compared with those patients with tumours greater than 6mm in thickness. In patients with carcinoma of the tongue and floor of mouth Spiro et al. (1986) reported that tumour-related death was unusual when oral tumours were thin (2mm or less). In the present study, tumour thickness was a good indicator for tumour recurrence and a highly significant difference (P = 0.0002) was noted between the group of patients who developed recurrence and the group of patients who survived disease-free for a minimum of 24 months. These findings offered further evidence that tumour thickness seemed to be a good prognostic indicator. Perhaps tumours arising from the floor of the mouth or tongue with thickness values greater than 3mm should be considered high risk for recurrence. In previous studies, various tumour thickness values have been considered as dividing lines between groups of patients with a good prognosis and patients with a poor prognosis. Tumours in the material presented in this study seemed to be thicker than tumours studied by other workers. In patients with floor of mouth carcinoma with tumour size T1 or T2 and clinical nodal status N0, Mohit-Tabatabai et al. (1986) reported that tumour thickness greater than 1.5mm should considered high risk for nodal metastasis. Tumours measuring this value, are actually very thin. However, these authors did not mention how the tumour thickness was evaluated. Sometimes oral carcinomas grow and fungate above the level of the adjacent actual epithelial surface from which the tumour has arisen. Thus tumour thickness measurement could be problematical on such occasions. One possible solution is to evaluate tumour thickness as the mean of two measurements. The first measurement should represents the distance between the actual epithelial surface

of the growth and the deepest point of the tumour within the underlying tissues. The second measurement should be the distance between the arbitrary line of the original epithelial surface and the deepest growing point of the tumour. The mean of the two measurement might well represent the tumour thickness value for predictive purposes. Ulceration is a common finding encountered in oral carcinomas. The surface of the ulcer is usually covered by necrotic tissue and fibrin. Measurement of thickness in such tumours can also be problematical. In the present study the tumour thickness was considered as the distance between the surface of the ulcer and the deepest growing point of the tumour. Whether this represents the best tumour thickness assessment for prognostic predictions is not known. It could be urgued that the measurement from the surface in such tumours should start at the base of the ulcer where the viable neoplastic cells are or alternatively in a deep ulcer the measurement might be from the level of the presumed original surface. There is a need for establishing a standard method for assessing the thickness of tumours with various patterns of growth since encouraging results have been achieved in relating this parameter to the prognosis and outcome of patients with oral cancer, in various studies. In this respect selection of homogeneous groups of patients with tumours arising from similar anatomical site is required for detailed analysis. Additionally, the criteria of how to measure the tumour thickness, especially from the surface point of the tumour should be established and clarified in order that comparison of the results between studies from different centres becomes more meaningful.

8.5 MALIGNANCY GRADINGS

In the present study the total malignancy grade did not differ significantly between the group of patients who developed recurrence and the group of patients who did not. This might indicate that Anneroth et al's (1987) malignancy grading system required more work in order to overcome the subjective nature of the evaluation of some of the features involved in the system. Furthermore, the weighting of the scores given to the features might need re-assessing. It cannot be justifiable considering, for example, the feature, number of mitoses as equally important as pattern of invasion. Increased number of mitoses might not indicate the aggressivness of the tumour. Mitosis occurs in normal, hyperplastic and benign neoplastic epithelium and strikingly so in some hyperplastic lesions. On the other hand pattern of invasion was found to have a potential predictive value for nodal metastasis in patients with floor of mouth carcinoma and also to differ significantly between the group of patients who developed recurrence and the group who did not when cases of all three sites were combined. The clinical course of diffuse invasive carcinoma of the tongue in respect to frequency of tumour recurrence and tumour-free survival was evaluated by Yamamoto et al. (1983). These authors found that such tumours were very much malignant in the sense that local recurrences were extremely frequent and very few patients had survived tumour-free for more than 16 months.

It is also not justifiable considering the feature, lymphoplasmacytic infiltration, as important as stage of invasion. Evaluation of lymphoplasmacytic infiltration can be very unreliable, particularly in ulcerated tumours where most of the inflammatory infiltrate, in fact, would not be due to immunological reaction to the tumour. By contrast, stage of invasion is often accurately evaluated on resection specimens, and thus a more reliable feature.

Recently, a new malignancy grading system has been suggested by Bryne et al. (1989). This is a modification of Anneroth et al's (1987) grading system in the sense that the histological features, excluding the stage of invasion, are evaluated on the histologically most invasive areas of tumour in biopsy material. This new system was applied on squamous cell carcinomas of buccal and maxillary alveolar mucosa and was also compared with the Broders (1920) grading system. These authors found that the new system was of prognostic value in terms of survival of the patients. However, assessment of the system on biopsy material is problematical. Most oral squamous cell carcinomas infiltrate to some depth within the underlying tissues and the biopsy specimens are unlikely to contain the deepest part of the tumour. Therefore, assessment of the features following this system might have been performed on tissue not representative of the most invasive part of the tumours. Additionally, Fisher (1975) stated that malignancy grading on resection specimens gives a better prognostic indication than the corresponding biopsy specimens because there are variations in malignancy grading scores evaluated on biopsy compared to those evaluated on resection specimens. This was presumably due to the fact that biopsy specimens are not always representative for the

evaluation of features involved in such malignancy grading systems. Stage of invasion, for example, cannot be reliably evaluated on biopsy of deeply invasive tumours. Vascular invasion, a feature which was evaluated by Fisher (1975), might not be evident on biopsy material but be clear enough on defintive resection. However, these two features were not evaluated in Bryne et al's (1989) grading system in spite of stage of invasion being one of the tumour-host interaction features which has been found to be a good indicator for patient outcome and cure rate (Shah et al., 1976 and Crissman et al. 1984). In the present study also the stage of invasion was found to be a good predictor of patient outcome when comparison was made between the two major groups of all sites combined together. A highly significant difference was noted and, overall, tumours in patients who developed recurrence were more deeply invasive than tumours in patients without recurrence.

8.6 HISTOLOGICAL NODAL STATUS

Histologically proven metastasis in the nodes and the presence of extracapsular extension of tumour put patients in a high risk group for developing tumour recurrence either locally or in the neck (Shah et al., 1976 and O'Brien et al., 1986) and for developing distant metastasis (Vikram et al., 1984a). This trend was true in the results of the present study as well (Chapter 7), at least in relation to local and neck failure. Both nodal metastasis and the spread of tumour beyond the nodal capsule were more commonly encountered in the group of patients who developed recurrence than in patients without recurrence (75% versus 34% and 58.3% versus 37.5% respectively). The frequency of positive nodes was significantly greater in patients who developed recurrence ($P < 0.0005$, Chi square test). A close follow-up for such

patients therefore, is required. Further treatment modalities might well need to be considered.

8.7 CONCLUSIONS AND FUTURE STUDIES

In the studies presented in this thesis the attempt has been made to further investigate the current view of the value in risk assessment of structural features which can be interpreted by the diagnostic pathologist. Features like AgNORs count which is a relatively new method of assessment thought to be related to tumour proliferation was found to be of predictive value for nodal metastasis in carcinomas arising from floor of mouth. Similarly pattern of invasion and tumour-host interaction were of value. Tumour thickness, as a single independent variable, was also found to be useful in predicting of nodal metastasis of floor of mouth carcinomas. These finding strongly suggest that assessment of such parameters may be a better way to select those floor of mouth cancer patients who are most likely to benefit from prophylactic treatment of the N0 neck.

In relation to patient outcome following treatment, features like pattern of invasion, stage of invasion, tumour-host interaction and tumour thickness were found useful for separating high risk patients for developing tumour recurrence. Metastasis in the nodes and involvement of nodal capsule by tumour were also features more commonly encountered in patients who developed recurrence. Assessment of these structural features may help in pointing out a risk group of patients who might be considered for further treatment modalities.

Further research is therefore indicated. The findings need to be verified in prospective studies involving a larger number of patients with carcinomas arising from similar anatomical sites and also studying carcinomas arising from other intra-oral anatomical sites.

The investigative methods employed in the work of this thesis have been restricted to those likely to be available to a routine diagnostic pathology service. Emphasis has been placed on evaluation of histological features in relation to the prediction of prognosis of oral cancer. The results suggest that the information available from such studies is limited. Although some morphological features, such as pattern of invasion, do appear to have prognostic relevance it is believed that more detailed assessment of other factors will be required to improve the prediction of prognosis. It is felt that the most fruitful possible avenues for further research are likely to be detailed cell kinetic studies involving flow cytometry. Such studies would also allow assessment of ploidy which may have prognostic relevance. Image cytometry for assessment of structural features is a further possible approach but like flow cytometry is dependent upon expensive technology not readily available in routine diagnostic services. Another alternative is the study of oncogenes and oncogene products by molecular biological techniques but it is considered that such studies are more likely to advance knowledge of the neoplastic process rather than provide practicable indicators of prognosis.

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