CLINICO-PATHOLOGICAL FEATURES OF ORAL SQUAMOUS CELL CARCINOMA IN RELATION TO CONVENTIONAL PROGNOSTIC PARAMETERS AND NUCLEOLAR ORGANIZER REGIONS

BY

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the University of Dar es Salaam a dissertation entitled: Clinico-pathological features of oral squamous cell carcinoma in relation to conventional prognostic parameters and nucleolar organizer regions, in partial fulfillment of the requirement for the degree of Master of Dentistry in Oral Surgery of the University of Dar es Salaam.

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DECLARATION

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DEDICATION

DEDICATED TO ALMIGHT GOD WHO MADE ME TO LIVE AND ACHIEVE FURTHER STUDIES, TO MY WIFE RUTH AND MY BELOVED DAUGHTER AMEZIA, TO MY FRIEND MARY ISAACK AND TO MY PARENTS MR. AND MRS. A.M. EGURUKI.

ABSTRACT

Oral squamous cell carcinoma (OSCC) is an aggressive tumor that accounts for over 90% of oral tumors, and which has high mortality rate. The aim of carrying this study was to establish the clinico-pathological features of oral squamous cell carcinoma in patients reporting at Muhimbili National Hospital and relate to traditional clinical staging and see if silver stained nucleolar organizer regions can provide additional information on biological behaviour of OSCC and therefore be applied as prognostic marker in order to aid treatment plan of an individual patient.

This study was performed on 111 patients with OSCC. TNM clinical staging was done as recommended by UICC. Two slides were prepared from paraffin-embedded tissue specimen, one for H&E stain which was reviewed for histological diagnosis and grading, and the other slide for silver staining following the procedure of Ploton et al (1986) and modified by Vuhahula et al (1995). Thereafter the counting was done in randomly selected 100 nuclei.

The results of this study showed that there were more males affected (56.8%) than females (43.2%) with male to female ratio of 1.3:1. The older age group (≥ 40 years {89.2%}) was more affected than the younger age group (<40 years {10.8%}). Majority of patients sought treatment when the tumors were already in advanced stage (88.3%) (Stage III and IV), only 11.7% reported with stage II tumors and none with stage I. Most of patients (94.6%) reported after the 4th month from the beginning of the symptoms with mean duration of 12.5 ± 7.82 (SD) months. More than eighty percent of

tumors (81.1%) measured more than 4 cm, with mean size of 5.74 ±1.84 (SD) cm, and majority (60.4%) had regional lymph nodes involvement ranging from N_1 to N_3. Distant metastasis to the lungs was encountered in one patient. This was detected by plain chest radiograph. Histologically, majority of the tumors (77.5%) were well differentiated, 14.4% were moderately differentiated and 8.1% were poorly differentiated.

Mean AgNOR count of these 111 cases ranged from 3.79 – 14.90. Cut off point at 4.6 was established as it was the value which separated the distribution of AgNOR counts into two groups when the scatter diagram of clinical stage and AgNOR count was plotted. Majority of tumors displayed high AgNOR count. The mean AgNOR count of each tumor was related to size, duration, anatomical location, lymph node involvement, histological grade and clinical stage.

Strong correlation was established between AgNOR count and lymph nodes involvement ($p=0.000005$). Furthermore, a positive trend towards increased AgNOR count with advanced stage was observed with statistical significant difference established between stage II and IV ($p=0.034874$). A trend towards increased AgNOR count was also observed in poorly differentiated tumors than in well and moderately differentiated tumors, but it was not statistically significant. There was no association established between AgNOR count with other parameters such as age, sex, size, anatomical location and duration of OSCC.

This study has characterized the oral squamous cell carcinoma of patients attending at MNH. These patients present late with advanced tumors (stage III and IV), lymph node involvement and large tumors. Assessment of OSCC by AgNOR has shown potential

ability of this technique for estimation of biological behavior of OSCC and therefore it may be performed prior to treatment planning of OSCC.

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ABBREVIATIONS

ACC	Adenoid Cystic Carcinoma
AgNOR	Silver stained Argyrophilic Nucleolar Organizer Regions
AIDS	Acquired Immunodeficiency Syndrome
AJCC	American Joint Committee of Cancer
DDS	Doctor of Dental Surgery
H&E	Haematoxylin & Eosin
HIV	Human Immunodeficiency Virus
MNH	Muhimbili National Hospital
MSc	Master of Science
MUCHS	Muhimbili University College of Health Sciences
NOR	Nucleolar Organizer Regions
ORCI	Ocean Road Cancer Institute
OSCC	Oral Squamous Cell Carcinoma
PhD	Doctor of Philosophy
rRNA	Ribosomal Ribonucleic Acid
SCC	Squamous Cell Carcinoma
TNM	Tumor, Nodes and Metastases
UICC	International Union Against Cancer

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INTRODUCTION AND REVIEW OF LITERATURE

1. EPIDEMIOLOGY

Oral squamous cell carcinoma (OSCC) is the commonest malignant tumor of the oral cavity worldwide (Linaena and Maria 2000, Walker et al. 2003, Winston et al. 2003), accounting over 90% of oral cancers (Daftary et al. 1992, de Rosa 1999). It constitutes 3-4% of all cancers and about 2% of all cancer deaths in the world (Worrall 1995, Winston et al. 2003). The incidence of oral squamous cell carcinoma is increasing and the survival rate continues to decrease despite improvement in diagnostic methods and treatment (Linaena and Maria 2000). Many new cases of oral cancer are reported annually, two thirds of which occur in developing countries (Walker et al. 2003). In United States nearly 30,000 new cases of OSCC are diagnosed annually (Boring et al. 1994). In Canada approximately 3,000 new cases of oral squamous cell carcinoma are diagnosed each year (Hawkins et al. 1999). In the European Union the crude incidence of squamous cell carcinoma of the head and neck (SCCHN) has been reported to be 48.9 cases/100,000/year (ESMO 2002). In a 5-year study in Turkey about 88% of patients with cancer of the lip, oral cavity and oropharynxy had squamous cell carcinoma (SCC) (Erisen et al. 1996). In Africa, OSCC is the commonest oral tumor and carries a high mortality rate (Ogunbodede et al. 1997). In Tanzania it is the commonest oral neoplasm and constitutes about 92.7% among oral tumors (Mwakyoma et al. 1998).

2. ETIOLOGY

The etiology of OSCC is not certain (Cawson et al. 1996). Several environmental factors that are strongly supported by epidemiological studies have been implicated in the development of this neoplasm (Shafer et al. 1983, Daftary et al. 1992, Walker et al. 2003). These factors include tobacco, alcohol, sunlight, and viruses such as human papilloma virus (HPV). The strong association of OSSC with tobacco and alcohol has been documented by several studies (Idris et al. 1995, Neville and Day 2002). Tobacco chewing, smoking, paan and betel quid chewing are significantly common habits in patients with oral carcinoma (Daftary et al. 1992, Idris et al. 1995, Pacella-Norman et al. 2002). These habits have been suggested to explain why certain sites are more frequently involved than others (Daftary et al. 1992, Idris et al. 1995, Adewole 2002). Other factors such as syphilis, nutritional deficiencies, immune suppression, poor oral hygiene, trauma and dental irritation are risk factors in the etiology of OSCC but have been less strongly associated with the neoplasm (Shafer et al. 1983, Cawson et al. 1996).

3. CLINICAL FEATURES

3.1 AGE

Oral squamous cell carcinoma like most other malignant tumors affects individuals in the older age group, most of the patients being over the age of 40 years (Daftary et al.1992, Cawsons et al. 1996). In United States over 95 % of patients were over 40 years of age (Silverman 1998), whereas in Brazil 85.6 % of all OSCC cases were above

40 years (Gervasio et al. 2001). The variations in the age distribution with regard to race have been observed in different studies (Onyango et al. 1995, Neville and Day 2002). Africans and Asians have been found to have lower peak age of 50-60 years in contrast to peak age of 60-70 years in western countries (Daftary et al. 1992). Similar observation was made by Neville and Day (2002) in a study among Whites and African Americans in US population where they found a peak age of 60-70 and 50-60 years respectively. Mean age of Tanzanian patients documented by Mwakyoma and coworkers was 52.4 years, with peak age group between 40 and 50 years. These findings correlate with those from other African countries such as Kenya (Onyango et al. 1995), Nigeria (Arotiba et al. 1999), and Congo (Kayembe and Kalengai 1999) in which the mean age have been reported to range from 49.0 to 53.7 years.

3.2 SEX

Oral squamous cell carcinoma predominantly affects men. In the U.S, oral carcinoma presents 3% of all malignancies in men and 2% in females (Neville and Day 2002). In India OSCC constitutes 17% of all cancers in males and 10.8% in females (Lype et al. 2001). In Tanzania male to female ratio was 1.4:1 as reported by Mwakyoma et al. (1998). This ratio coincides with worldwide ratio which ranges from 1.3:1 - 2:1(Odukoya et al. 1986, Maroo 1992, Arotiba et al. 1999, Neville and Day 2002). In Brazil very high male to female ratio of 4.8:1 has been reported by Gervasio and co-workers (2001).

3.3 ANATOMICAL LOCATION

Epidemiological studies have shown that anatomical distribution of OSCC differs geographically and between Africans and non-Africans, with certain sites found to be more frequently involved than others (Daftary et al. 1992). Tongue, lip and floor of the mouth are the most frequent sites of squamous cell carcinoma in the oral cavity documented elsewhere (Cawsons et al. 1996). Different tobacco habits have been suggested to explain the variation in anatomical sites and why certain sites are more frequently involved than others (Daftary et al. 1992, Idris et al. 1995, Adewole 2002).

3.3.1 Tongue

The tongue is the most common site documented worldwide, followed by the floor of the mouth. In U.S and Canada the tongue was reported as the commonest site constituting 40% of all sites affected by SCC in the oral cavity proper (Chen et al. 1990, Neville and Day 2002). In Brazil, the tongue was the most frequent site, accounting 44% of all cases (Gervasio et al. 2001). In Tanzania the tongue was as well found to be the most commonly affected site, and accounted for 24.6% of all sites (Mwakyoma et al. 1998). Majority of tongue SCC affect the middle third of the lateral margin and extend early in the course of the disease into the ventral aspect and floor of the mouth. Approximately 25% affect the posterior third of the tongue, 20% the anterior third and only 4 % the dorsum (Cawson et al. 1996).

3.3.2 Gingiva and alveolar ridge

Cancer in this location is generally seen among people who chew tobacco and practice snuff dipping (Daftary et al. 1992, Cawson et al. 1996). The incidence of OSCC in this location varies in different countries. In Nigeria and Kenya mandibular gingiva has been reported as the commonest site (Odukoya et al. 1986, Maroo 1992). However, in other studies authors from different regions of Nigeria have found variable results. For example, Ogunbodede et al. (1997) found that mandibular alveolar ridge and gingiva were the commonest sites in Ife Ife, but were not the commonest site in Lagos (Adewole 2002) and Ibadan (Arotiba et al. 1999). Studies done in Tanzania and U.S.A, reported that the gingiva was the least affected site by SCC (Mwakyoma et al. 1998, Neville and Day 2002). On the other hand mandibular gingiva is affected three times more than the maxillary gingiva (Shafer et al. 1983). In the dentate patients, gingival SCC is likely to be clinically misdiagnosed by the dentists because the clinical signs closely mimic dental sepsis and periodontal disease, even when radiographs show bone destruction. Loosening of teeth due to early spread of tumor is common (Cawson et al. 1996).

3.3.3 Lip

Most studies have come up with comparable findings on SCC of the lip, whereby a striking predilection for the lower lip was found (Cawson et al. 1996, Neville and Day 2002). Lower lip used to be the commonly affected site in light-skinned individuals and the least in black skinned individuals (Shafer 1983). The incidence of OSCC in light-

skinned individuals has been reported by some authors to decline in recent decades because more people now hold indoor activities therefore getting less sun exposure which contain ultra-violet light known to cause lip cancer (Daftary et al. 1992, Bast et al. 2000).

3.3.4 Floor of mouth

Oral squamous cell carcinoma of the floor of the mouth is fairly common in some countries, and this site is documented as second commonly affected after the tongue (Shafer et al. 1983). Study done by Adewole in Lagos, Nigeria, documented floor of the mouth as the most affected site by OSCC (Adewole 2002). Gervasio et al. (2001) in Brazil, reported floor of the mouth as the second affected site accounting for 16% of cases. However, the site has been observed by Mwakyoma and co-workers to be among the least affected site in Tanzania and accounted for 1.3% of all sites (Mwakyoma et al. 1998). Most tumors in this site affect the anterior segment of the floor of the mouth to one side of the midline. Although arising in an anatomically distinct area, namely the U-shaped area between the lower alveolar ridge and the ventral surface of the tongue, SCC in this site quickly involves the tongue and lingual aspect of the mandible (Cawson et al. 1996).

3.3.5 Buccal mucosa

Buccal and labial mucosa involvement is frequently encountered in population groups who habitually keep a mixture of tobacco-lime in the labial and buccal grooves (Daftary

et al. 1992) or where tobacco and/or betel-tobacco chewing prevails. In Taiwan Lo and co-workers documented buccal mucosa to be the most affected site, followed by gingiva and tongue (Lo et al. 2003). Chattopadhyay (1989) in India reported buccal mucosa was the commonest site similar to Taiwan. In contrast, SCC in this location is least common in USA (Neville and Day 2002). Lower buccal mucosa is more commonly involved than the upper mucosa because of the habit of tobacco and/or betel-tobacco chewing or placing tobacco in the grooves for a long time (Idris et al. 1995, Lo et al. 2003). In most cases the advanced lesion in this site is predominantly ulcerated and infiltrative which can later involve the overlying skin of the cheek resulting into multiple sinuses and fistulas (Cawson et al. 1996).

3.3.6 Palate

In most countries SCC of the palate is rather uncommon (Daftary et al. 1992), except in Ibadan, Nigeria where Arotiba et al (2001) documented the palate as the commonest encountered site. In a study conducted in Tanzania by Mwakyoma and co-workers, the palate was reported as the third site and constituted 12.9% of all sites affected by OSCC (Mwakyoma et al. 1998). In areas where reverse smoking is practiced, palatal cancer is very common comprising 38-48% of all oral cancers (Daftary et al. 1992). The cancer that primarily originates in the soft palate is uncommon (Shafer et al. 1983).

3.3.7 Oropharynxy

In the oropharyngeal region, OSCC often arise on the lateral soft palate and tonsilar regions but may also extend from the base of the tongue. Unfortunately, such tumors are typically larger and more advanced at the time of discovery than those that are more anteriorly located (Neville et al 2002). Management of oral squamous cell carcinoma of this region is usually difficult since surgical excision is often unsatisfactory. It is usually difficult to excise the whole lesion in this region without cutting through it, because it is difficult to ascertain its limits. Likewise, the assessment of lymph nodes involvement is hampered by their location in the lateral pharyngeal wall just below the palatine tonsil, therefore presenting prognostic complications (Ackerman and Regatos 1985).

4. GROSS APPEARANCE

Oral squamous cell carcinoma manifests in different clinical presentations depending on the stage of diagnosis. Early lesions often present as painless white lumps, nodules, white patches (leukoplakia), red patches (erythroplakia), or mixed red and white patches (erythroleukoplakia). With time, superficial ulceration of mucosal surface may develop, which in advanced stage, may appear as a discrete ulcer with a raised indurated border or a firm slightly raised plaque. Squamous cell carcinoma of the oral mucosa may appear also as an exophytic mass with a fungating or papillary surface. Other OSCC have endophytic growth pattern characterized by a depressed, ulcerated surface with a raised, rolled out border (Cawson et al. 1996, Neville and Day 2002).

5. SIGNS AND SYMPTOMS

Early OSCC manifests itself in a variety of ways, depending upon anatomical location but it is typically painless initially. Non healing ulcer, or progressive swelling or enlargement is observed in early presentation. Other signs and symptoms include sudden tooth ache or mobility without apparent cause, and unusual oral bleeding. Late presenting signs and symptoms often include dysphagia, trismus, difficulty in breathing and speech, paresthesia, and dysesthesia of the tongue. Large advanced lesions may present with pain during swallowing (odysnophagia), airway obstruction, pain referred to the ear (otalgia) and cervical lymphadenopathy (Cawson et al. 1996, Donald 1996).

6. DIAGNOSIS

Toluidine blue test and acridine binding methods have been suggested as screening methods commonly used for cancer of the cervix but also have been tried in oral cancer and accepted in screening of oral premalignant lesions. Toluidine blue test is an in vivo staining method for delineation of dysplasia and carcinoma in situ. This method consists of painting the lesion with 1 % aqueous solution of toluidine blue and then, after 10 seconds, decolorizing it with 1 % acetic acid solution. If the dye is retained, malignancy or dysplasia is suspected. The efficacy of this technique depends upon the amount of DNA present which relates to the number and size of superficially stained nuclei in the tissues to which the toluidine blue is applied. Because of the high degree of false-negatives results this method is not reliable for diagnosis (Daftary et al. 1992, Bast et al.

Exfoliative cytology (cell scraping) has been advocated as useful diagnostic method, probably because of its simplicity. However, false negative results have been observed in 31-62 % of cases, therefore for this reason this method is not recommended, however, it can still be used if there is delay to biopsy or contraindication to biopsy (Daftary et al. 1992).

Considering the accessibility of the oral cavity for biopsy, the simplicity of the procedure and the high degree of reliability, biopsy is the ideal material specimen for definite diagnosis of OSCC. However, it is essential that the specimen must be adequate in size and depth and should include part of adjacent normal mucosa. Biopsies are obtained using surgical scalpels, biopsy punch, typically performed under local anaesthesia, either excisional for small lesions or incisional biopsy for large ones. Useful adjuncts often include routine dental radiographs and other plain films such as chest radiographs, and imaging with computed tomography (CT) or magnetic resonance imaging (MRI). A diagnostic imaging evaluation either computed tomography (CT) or magnetic resonance imaging (MRI) are used to assess the extent of local and regional tumor spread, the depth of invasion, and the extent of lymphadenopathy. Computed tomography is superior in detecting early bone invasion and lymph node metastasis, but MRI is preferred for assessing the extent of soft tissue involvement and for providing a three dimensional display of the tumor.

7. HISTOPATHOLOGY

The microscopical characteristics of OSCC consist of irregular nests, columns and strands of malignant epithelial cells that infiltrate the sub-epithelial connective tissue stroma. The tumor cells may resemble any or all the layers of stratified squamous epithelium. Histologically, OSCC have been graded as well, moderately, or poorly differentiated. The two main features on which grading is based are cellular proliferation and differentiation. Rapid abnormal proliferation is characterized by hyperchromatism, increased mitotic activity, cellular pleomorphism and nuclear atypia. Differentiation of tissue is assessed by the presence or absence of epithelial bridges and ability to produce keratin. An advanced degree of differentiation, such as closeness to the structural characteristics of the tumor to the parent tissue, is regarded as a feature of favorable prognosis, while lack of differentiation is considered to spell poor prognosis (Daftary et al. 1992, Evans et al. 1982, Bast et al. 2000). However, the grading of OSCC, based on the differentiation and maturation of the tumor cell population alone is of limited value as a basis for choice of treatment as well as for prediction of the outcome of the disease (Yazdi and Khalili 1993). Lack of correlation between Broders grades and the prognosis of OSCC is explained by the fact that SCC usually exhibits a heterogenous cell population with probable differences in invasiveness and metastatic behavior. Moreover, it has been documented that classification based on histological features has shown frequent lack of inter-observer agreement, which limits the clinical value of subjective histological classifications (Bryne et al. 1991).

8. ORAL SQUAMOUS CELL CARCINOMA VARIANTS

Variants of OSCC include verrucous carcinoma, which is a low-grade malignancy with good prognosis, spindle cell carcinoma also known as carcinosarcoma (sarcomatoid squamous carcinoma) most frequently encountered on the lip, basaloid squamous cell carcinoma and adenoid squamous cell carcinoma (Cawson et al. 1996). Verrucous carcinoma is characterized by marked hyperkeratosis, acanthosis, parakeratin crypts and large pushing bulbous rete ridges said to resemble elephant foot. Verrucous carcinoma does not have dysplastic histological features and habitually do not metastasize (Daftary et al. 1992).

9. DIFFERENTIAL DIAGNOSIS

There are few other lesions that may clinically mimic OSCC. The true nature of those lesions can be decided only on biopsy.

9.1 Leukoplakia

Leukoplakia appears as white patchy lesion in various locations of the oral cavity. White patches usually on the edges of the tongue which are frequently diagnosed as leukoplakia may prove on histological examination to be hyperkeratosis, epithelial dysplasia of different severity, carcinoma in situ, or invasive OSCC (Daftary et al. 1992). Leukoplakia of the floor of the mouth causes a whitish patch on the mucous membrane. There is no certainty as to the neoplastic nature of such clinical finding until it is verified by histological analysis. There is little evidence that a great number

of these lesions when proved to be benign may actually undergo malignant transformation. On the other hand, many carcinomas of the oral cavity which are often multicentric have a whitish appearance. Leukoplakia of the buccal mucosa is frequently found particularly around the commissures of the lips usually in the form of an isolated patch of raised whitish mucous membrane. Leukoplakia of the lower gingiva may be from incipient infiltrating SCC or carcinoma in situ or areas of hyperkeratosis that may or may not be associated with cancer. A white patch of the lower lip usually diagnosed as leukoplakia may be a result of SCC or hyperkeratosis (Cawson et al. 1996, Bast et al. 2000).

9.2 Tuberculous ulcer

Tuberculous ulcer usually occurs on the dorsum of the tongue and of the lip, but may also occur on the tip of the tongue and other parts of the mouth. The ulcers are usually angular or stellate with overhanging edges (undermined) and pale floor. Nevertheless, they can be ragged and irregular. They are painless in their early stages, later on may become painful, and they are secondary to pulmonary tuberculosis. In the early stage the ulcers mimic early stage OSCC (Cawson et al. 1996).

9.3 Syphilitic chancre

Primary syphilitic chancre usually occurs on the lip, tip of the tongue and rarely on other oral sites. Initially it occurs as exophytic indurated lesion about 6 to 7 mm in

diameter, whose surface breaks down after few days leaving a rounded ulcer with a raised edges that may resemble OSCC (Cawson et al. 1996).

9.4 Verrucous hyperplasia

Verrucous hyperplasia usually mimics verrucous carcinoma and develops into verrucous carcinoma or SCC. Verrucous hyperplasia may be associated with SCC or dysplasia in the oral cavity. It is commonly found on the mandibular gingival, tongue and buccal mucosa (Daftary et al. 1992).

9.5 Verruciform xanthoma

Verruciform xanthoma is a rare benign condition of the oral cavity which occurs as a solitary reddish or hyperkeratotic lesion. It is commonly seen on the gingiva and alveolar mucosa, but any oral mucosa may be involved. It may clinically be very similar to papilloma, verrucous carcinoma, condylomata accuminatum or OSCC, (Shafer et al. 1983). Biopsy may be the only means by which to distinguish one from the other.

9.6 Keratoacanthoma

Keratoacanthoma of the oral cavity is only a rare condition but may clinically resemble SCC. It presents as solitary, rapidly growing lesion than SCC, firm dome-shaped, flesh coloured to slightly pink lesion. Despite its rapid growth, keratoacanthoma is a benign tumor which involutes spontaneously even if untreated. Histological appearance of keratoacanthoma depends on the stage of evolution. In a well developed tumor, the

lesion is cup shaped, and consists of islands and cords of pale pink ground glass keratinocytes extending into upper dermis. The centre of the cup is filled with keratin. Hyperchromatic nuclei and atypical mitoses are often seen (Bast et al. 2000).

9.8 Verrucous carcinoma

Verrucous carcinoma appears clinically as a benign condition because of its lack of ulceration, secondary infection and symptoms. In additional repeated biopsies it may show nothing but hyperkeratinization, hyperplasia, and chronic inflammation. In spite of these features, the lesion is locally invasive and destructive although it seldom metastasizes. Usually it occurs on the buccal mucosa, and gingiva (Shafer et al. 1983).

The clinician's challenge is to differentiate cancerous lesions from these many lesions which resembling OSCC. Most of these lesions are benign, but have appearances that mimic malignant lesions. On the other hand, early stages of OSCC may be mistaken for benign changes. Any oral lesions that does not regress spontaneously or respond to the usual therapeutic measures should be considered potentially malignant until histologically proved otherwise. A period of 10 days is considered an appropriate period of time to evaluate the response of a lesion to therapy before obtaining a definitive diagnosis. A definitive diagnosis requires a histological confirmation of the biopsed lesion.

10. TREATMENT AND PROGNOSIS

With exception of lip cancer, the prognosis of OSCC in intra-oral location is usually poor. In most instances, the poor prognosis is contributed by the late diagnosis and therefore late initiation of treatment (Daftary et al. 1992). The choice of treatment modality of OSCC depends on the histological grade and the clinical stage of the tumor. The location of the tumor and age of the patient are also important factors in the treatment plan of OSCC. The variety of accepted modalities of treatment available for consideration includes surgery, chemotherapy and radiation therapy either alone or in combination (Bast et al. 2000). In case of a well differentiated tumor in early stages (I and II) with no evidence of metastases the goal is complete eradication of the tumor. However, in case of a poorly differentiated, recurrent tumor, or presence of lymph node metastases, palliative treatment is usually considered, if complete eradication is not possible. For localized disease such as stages I and II (T_1N_0 and T_2N_0) SCC which is confined to the oral mucosa such as of the lip, floor of mouth, tongue, alveolar ridge, retromolar trigone, hard palate or buccal mucosa, patients are treated with curative intent, usually involving surgery and/or radiation therapy, and both offer an equal chance of cure (Worrall 1995, Bast et al. 2000). However, about 20-40% of these patients have been observed to develop a local recurrence of the initial tumor. Most centres advocate surgery of such lesions (Donald 1996). Squamous cell carcinoma of the oropharynxy and base of the tongue has shown no definitive advantage for surgery over radiotherapy (Donald 1996). Surgery has the disadvantage of causing velopharyngeal incompetence, loss of tongue musculature, loss of tongue mobility, and

loss of part of mandible. In many centres treatment consist of radiotherapy to the primary site, with or without subsequent neck dissection. Squamous cell carcinoma of the base of tongue frequently metastasizes to the submandibular, jugulodigastric, deep cervical lymph nodes and has poorly differentiated histology. Since SCC of the base of tongue strongly responds to irradiation, it is usually treated by radiotherapy. Nevertheless, large tumors are seldom eradicated by radiotherapy alone. Where radiotherapy is the sole treatment modality, local control rates is 10% for tumors which have invaded adjacent tissues (Worrall 1995). In stage III and IV disease, it has been observed that local failure rates increase from 39% to 73% where surgical margins are less than 5mm (Worrall 1995). With current surgical techniques it is possible to resect most tumors in the head and neck region. However, because of the advanced age, weak general health, mutilating effect of the tumor and unfavorable anatomical location most of the advanced neoplasms should not be resected. Such kind of patients can not be cured in total and therefore, are treated with palliative intent, usually involving chemotherapy and/or radiotherapy.

For stage III and IV tumors, the sole treatment offered at MNH and ORCI is palliative radiotherapy or chemotherapy. Chemotherapy is not curative but has continually been used in attempt to improve survival, to reduce the incidence of distant metastases, and to serve as an adjunct to radiotherapy for organ preservation. However, how much chemotherapy actually contributes to achieving these goals remains controversial (Donald 1996). Chemotherapy has been applied as induction (neoadjuvant therapy), concurrently with radiotherapy and as post-treatment adjuvant therapy. Neoadjuvant

therapy has been widely studied in recent years and a number of drug regimes have been used (Donald 1996). OSCC responds well to chemotherapy with combinations of Cisplatin and 5-Flourouracil (5-FU) giving best results of 93% response rate and 54% complete remission rate (Worrall 1995). The combination of Cisplatin and Fluorouracil (5-FU) has achieved considerable popularity because of high rates of response with acceptable rates of toxicity. In patients previously not treated by chemotherapy, response rates of 60-90% have been reported (Donald 1996).

11. CLINICAL STAGING AND ITS RELATION TO PROGNOSIS

Using the TNM classification system of clinical staging, where T represents size of the tumor, N indicates the status of the regional lymph nodes, and M indicates the presence or absence of distant metastasis, four stages of OSCC are known. The addition of numbers to these three components (TNM) indicates the extent of the tumor, thus: T_0, T_1, T_2, T_3, T_4, N_0, N_1, N_2, N_3, and M_0, M_1. T_1 indicates small tumor with good prognosis, whereas, T_4 stands for a tumor above 4 cm and/or which has involved adjacent tissues. TNM deals with the anatomical extent of tumor before treatment. There is no doubt that for most solid tumors, anatomic extent is one of the most important guidelines to treatment and the strongest predictor of outcome (Hermanek et al. 1996). The prognosis of carcinoma is reflected by the tumor stage. Stages I ($T_1N_0M_0$) and II ($T_2N_0M_0$) carcinomas are known as early stage tumors since they are relatively small localized neoplasms without lymph nodes or distant metastases. Stage III ($T_3N_0M_0$ and/or N_1) is an advanced stage with a tumor size of above 4 cm, with or

without ipsilateral lymph node involvement but without distant metastasis. Stage IV (T_4 any N/M and/or any T/M, $N_{2/3}$, or M_1 any T/N) is more advanced stage with large tumor, which has involved adjacent tissues and/or metastasized either into regional nodes or distant tissues (UICC 1997). Survival rate of these patients depends on regional spread and distant metastasis status. Presence of metastatic lymph nodes drastically reduces the survival rate. The five year survival rate of patients with localized disease is 81.9%, however, this drops to 46% with regional spread and 21% for those with distant metastases (Neville et al. 2002). Five year survival rates of OSCC as reported by Lo and co-workers are 75%, 65.6%, 49%, and 30% for stages I, II, III, and IV respectively (Lo et al. 2003).

12. PROGNOSTIC INDICATORS AND ITS LIMITATIONS

The TNM system has been proved to be a useful instrument but has limitations that make it not adequate to accurately determine the prognosis of an individual patient with OSCC. T-stage alone is not suggestive for an occult nodal metastasis. This has been proved in some cases of early stage of OSCC, (T_1 or T_2) where no palpable nodes were clinically found but nodal metastasis was found during surgical excision of the lesions (Russolo et al. 2002). A disparity between pathological and clinical lymph node staging has been reported by Greenberg and co-workers (2003). They found 34% had occult lymph node metastases in clinically N_0 patients and 19% had extra capsular spread. Due to this, pathological lymph node staging have been recommended to be useful in decision making to identify high risk patients who may benefit from additional adjuvant

therapy (Greenberg et al. 2003). However, when there is metastasis to internal organs it is usually difficult to detect. Such metastasis can be visualized by x-rays and other expensive diagnostic tools such as CT scan and MRI.

Over or under classification of many tumors is another limiting factor of clinical TNM system. Over classification of some tumors is a result of definition of T_4 (Horward et al. 1999). Too many tumors are classified as T_4, because in the oral cavity even small neoplasm often infiltrate adjacent structures such as the rim of alveolar bone or muscles of the floor of mouth therefore, irrespective of small size once the tumor has infiltrated these adjacent structures will be categorized as T_4. Underreporting is also seen in current criteria of N_3, which has resulted in reporting too few lymph node involvement, since appearance of neck nodes bigger than 6 cm in diameter is rare (Horward et al. 1999). Several studies have shown nodal assessment to be a better prognostic factor affecting survival rate than tumor size (Hibbert et al. 1983, Tankere et al. 2000, Ioona et al. 2002, Greenberg et al. 2003, Woolgar et al. 2003). It has been found that despite survival depending on clinical staging, the tumor size did not affect survival in those patients who on presentation had palpable nodes (Hibbert et al. 1983). The most significant factor determining survival was the palpable nodes on presentation, which were more likely present in patients with large tumors than in those with small tumors (Hibbert et al. 1983). In spite of nodal status being the most significant factor in determining survival, Ioona et al (2002) were able to identify 60-70% of patients with stage I SCC of the tongue who had micro metastasis using sentinel lymph node biopsy. Such lymph nodes could not be detected by clinical examination and/or imaging

techniques (Ioona et al. 2002). Sentinel lymph node biopsy was documented to be more effective in identifying such micro metastasis (Ioona et al. 2002). This method was therefore suggested as a valuable staging technique in T_1 and T_2 oral cancer with uninvolved neck lymph nodes in patients whose lymphatic drainage of the neck has never been altered by previous surgery or radiotherapy. The identification of the sentinel lymph node was done using blue dye and gamma rays both of which are expensive and delicate to handle. Moreover, the independent prognostic factor and clinical relevance of histopathological features of occult metastases of OSCC as detected by sentinel node biopsy is still unclear (Stoeckli et al. 2002).

Despite the fact that prognostic evaluation for OSCC is mainly based on clinical TNM classification, this staging system is not sufficient for optimal prognostication and should be supplemented by other reliable methods (Yazdi and Khalili 1993). Other studies have recommended combination of tumor thickness, selection of appropriate treatment (O-Charoenrat et al. 2003), age and sex of the patient, shape of the tumor (Dequanter et al. 2001, O-charoenrat et al. 2003) and invasive tumor front (Bankfalve and Piffko 2000), in addition to clinical staging to be more prognostic. However, some of these clinical variables such as age and sex are difficult to substantiate in daily clinical practice (Baatenburg de Jong et al. 2001), while others supply addition information to TNM classification but cannot be of independent prognostic value (Ribeiro et al. 2000).

Survival rate of OSCC has also been found to be related to the anatomical location. Survival by specific anatomical site ranged from a low of 23% for unspecified site or ill defined site of oropharynxy to as high as 91% for lip SCC. Prognosis of buccal mucosa is better than for elsewhere within the oral cavity (Cawson et al. 1996). This may be due to several factors such as late bone involvement in this site and unlike other sites, fewer tumors are typical SCC. Usually this is a common site for verrucous carcinoma which has favorable prognosis (Cawson et al. 1996). Anatomical site can not be of independent prognostic value, moreover, late reporting with advanced stage OSCC override the importance of anatomical site as reliable prognostic indicator.

The discovery of microscopic approach that can define cellular and/or nuclear alterations associated with degree of malignancy can benefit the prognostic process hence influence the treatment plan of an individual patient. Example of such methods is cytochemistry assessment of DNA content by means of flow cytometry, which has been reported to be a helpful prognostic tool in the evaluation of OSCC (Wang 1990, Garib et al. 2002), but the procedure of analysis is rather complicated and expensive. Immunohistochemistry is even more useful when antibodies against nuclear proteins working on routinely processed paraffin-embedded tissue are used. Such nuclear antigens exemplified by Ki-67 and PCNA are used in measuring cell proliferation and hence detect the biological behaviour of tumor (Helga et al. 1998, Helga et al. 1999, Vuhahula et al. 2000). However, the cost involved in these methods is unaffordable in most of laboratories in the developing countries such as Tanzania. On the other hand, silver stained nucleolar organizer regions (AgNOR) is a relatively new method which

has been applied extensively in normal, hyperplastic and malignant tissue of several organs and a difference in quantity and/or quality of AgNOR has been demonstrated between high and low grade tumors and between benign and malignant counterparts of tumors of various origin. AgNOR method has an advantage over immunohistochemistry because the one step AgNOR method is simple, cheap, and can be applied to routinely-processed paraffin-embedded tissue section (Vuhahula et al. 1995, Derenzini 2000, Remmerbach et al. 2003).

13. Silver nucleolar organizer regions (AgNOR)

Nucleolar organizer regions (NOR) are non-histone proteins associated with loops of ribosomal DNA localized on the short arms of accrocentric chromosomes 13, 14, 15, 21, and 22. They are transcribed by the RNA polymerase 1 to ribosomal RNA and are assembled in the ribosomes. They are of vital importance in the ultimate synthesis of protein. AgNORs are NOR-associated acid proteins that are selectively stained by silver methods (Hernandez-verdum et al. 1980). AgNOR staining technique was initially defined by Thiebaut et al (1984). Since then there have been more effective staining methods (Vuhahula et al. 1995). AgNOR staining technique is simple, inexpensive procedure that can be performed at room temperature on paraffin embedded tissue specimens and can be visualized by an optical microscope (Vuhahula et al. 1995). The AgNOR number has been observed to be related to variables such as cell proliferation rate, cell ploidy, cell transcription activities, and malignancy potential

(Derenzini 2000). The amount of AgNOR proteins is proportional to the proliferative activity of the cells (de Carmo and Silva 1998).

Various studies have validated the diagnostic and prognostic usefulness of argyrophilic nucleolar organizer regions (AgNOR) in certain neoplasm with varying degree of success (Vuhahula et al. 1995, de Rosa et al. 1999, Linaena and Maria 2000). Several reports have shown that enumeration of AgNORs provides clinical information that is of diagnostic and prognostic importance (Vuhahula et al.1995, de Rosa et al. 1999, Derenzini 2000, Remmerbach et al. 2003). Some authors have demonstrated a difference in the AgNOR count between well differentiated and poorly differentiated oral tumors (Epivatians 1994, Chattopadhyay et al. 1994, de Rosa et al. 1999). Higher AgNOR counts have been found in poorly differentiated malignant tumors or in neoplasms of more advanced stages. For example in stage I and II lung neoplasm, high counts were associated with shortened survival (Oyama et al. 1993). Similarly, high AgNOR counts have been found to exhibit a potential in identification of some aggressive salivary gland neoplasms such as adenoid cystic carcinomas (ACC) that cannot be detected by histology alone (Vuhahula et al. 1995). In that study by Vuhahula and co-wokers (1995), all cases with unfavorable outcome were found to have high count regardless of their histological grades. This suggested that the metabolic alterations associated with malignancy level were partly portrayed by the AgNOR count irrespective of the histological appearance. The size and count of AgNOR have been documented to be a sensitive parameter for differentiating between potentially malignant lesions and SCC of the lower lip, and constitutes a useful marker for the

prognostic evaluation of potentially malignant, as well as malignant lesions of the lip (de Rosa et al. 1999). AgNOR's discriminative value has also been shown between human normal oral mucosa, verrucous carcinoma and OSCC (Linaena and Maria 2000). The prognostic implication of AgNOR expression in OSCC has also been studied and was found to discriminate poor prognostic group from patients with favourable outcome (Sano et al. 1991). Although NOR scores have been proved to correlate with the proliferative activity of the cell (de Carmo and Silva 1998, Derenzini 2000) and the fact that it can be used in predicting tumor behavior as a prognostic index which reflects tumor proliferation, its usefulness in African OSCC, which usually present at a very advanced stage has not been documented in the literature. This study was undertaken to determine whether AgNOR counts correlate with clinical staging and histopathological grading, and other prognostic parameters of OSCC in patients attending at Muhimbili National Hospital (MNH), and whether AgNOR count can assist the prognostic assessment in a way not revealed by conventional methods namely clinical staging and histological grading.

This study is expected to illustrate whether AgNOR count is useful as a new parameter in assessing the biological behavior of OSCC. If found beneficial, its practical application would be useful in planning the treatment of an individual patient with OSCC attending at MNH, Tanzania.

RATIONALE OF THE STUDY

Worldwide an escalation in the incidence of OSCC and its attributed mortality has been observed (Llewellyn et al. 2001, Bettendorf et al. 2004). Oral cancer of which 90% is OSCC is expected to become a public health problem in the future due to increased exposure to predisposing factors such as tobacco use and alcohol consumption (Bankfalvi and Piffko 2000). A steady increase in number of patients reporting with OSCC has been observed in the department of Oral Surgery and Oral Pathology of Muhimbili National Hospital (MNH). A further increase in number of patients with OSCC is expected following increased exposure to predisposing factors namely use of tobacco and alcoholic consumption (Kilonzo and Kaaya 1994, Nikander et al. 1991, Bovet et al. 2002). Despite the fact that OSCC is the most prevalent oral tumor at MNH, only one study (Mwakyoma et al. 1995) has described the demographic data of this neoplasm. More data on this malignancy is needed to understand its presentation in our set up. Important clinico-pathological characteristics of prognostic importance such as size of lesion at presentation, duration of lesion at diagnosis from time of initial symptoms, nodal status, histological grading and clinical stage of tumor have not yet been tackled. One of the aims of this study is to assess these parameters in order to clinically characterize OSCC in Tanzania. The information gathered will assist in knowing the severity of the disease as patients present in the hospital and thence be the basis of disease prevention and policy making at all levels.

Muhimbili National Hospital where the study was conducted is the only centre in the country with Oral and Maxillofacial Surgeons. It receives patients from almost all up-

country hospitals. In the same hospital histopathological diagnosis and surgical management are carried out. In cases where radiotherapy or chemotherapy are the chosen modalities of management, patients are referred to Ocean Road Cancer Institute (ORCI) a nearby oncology centre. In both centres TNM staging is the main determinant of treatment and prognosis. Despite the usefulness of TNM staging, it has presented some limitations that make it inadequate to accurately determine the prognosis of each patient with OSCC. Under and over-treatment have been reported due to current definition of T_4 and N_3. Moreover, micro-metastasis and extra nodal spread which go undetected in clinically N_0, T_1 and T_2 tumors have been documented. On the other hand, there are some OSCC that are classified as stage IV based on tumor size alone (T_4NxMx). Such staging does not give sufficient information that can assist in the planning of the treatment. Therefore, another method that is relatively cheap and affordable is needed to assist in assessing the prognosis of an individual patient with OSCC. AgNOR has been reported to correlate with biological behavior of several tumors and the prognostic validity of AgNOR method has been compared with already established prognostic parameters of other tumors. It is relatively cheaper as compared to other methods such as immunohistochemistry and other special staining methods, and can be done to every patient with OSCC. Other advantages of AgNOR method is that the method is simple and can be used in paraffin embedded blocks of a usually small biopsy material submitted for histopathological diagnosis. AgNOR can also be used where thickness of the neoplasm cannot be assessed. Therefore, another aim of this study was to evaluate whether AgNOR count can be applicable in assessing the

biological behaviour of OSCC and therefore, be applied to plan treatment of an individual patient with OSCC.

GOALS AND OBJECTIVES

Broad Objective

To establish the clinico-pathological characteristics of oral squamous cell carcinoma of patients attending at MNH and relate with AgNOR count.

Specific objectives

1. To determine the age, sex and anatomical distribution of SCC in the oral cavity.
2. To determine the size in greatest dimension (cm) and duration of OSCC from initial symptoms to presentation in hospital.
3. To determine the clinical stage of patient presenting with OSCC using TNM classification.
4. To determine the histological grade of each OSCC.
5. To determine the AgNOR count in each case of OSCC in relation to size, duration, anatomical site, lymph node involvement, histological grade and clinical stage.

MATERIAL AND METHODS:

Study area

The study was conducted in the Department of Oral Surgery and Oral Pathology of the School of Dentistry, and Department of Histopathology and Morbid Anatomy of the School of Medicine, MUCHS. Histopathological diagnosis was done in the histopathology laboratory. Retrieval of retrospective data was done by archiving pathological reports, slides, and paraffin blocks in the same laboratory. Clinical data was obtained from Medical Records Department at MNH and Ocean Road Cancer Institute (ORCI). In this institution (MNH) a total of 247 patients with OSCC were histologically diagnosed in a period of 5 years, from 2000-2004.

Study Population and sample size

The study population included all patients with OSCC, who attended Oral Surgery and Oral Pathology department between 2000 and 2004, and available histological specimens at the histopathology laboratory. The recruitment of cases was retrospective (Jan 2000-Dec 2003) and prospective (Jan-Dec 2004) in nature, collecting both archival and fresh specimens.

Inclusion criteria

All patients with diagnosis of OSCC with availability of both tissue blocks and medical records, together with staging done by experienced oral and maxillofacial surgeons and/or experienced oncologist before initiation of therapy.

Exclusion criteria

Absence of patients' medical records or absence of paraffin blocks, and any patient with a tumor not staged by experienced Oral and Maxillofacial Surgeon and/or experienced Oncologist before surgery, and/or before radiation therapy.

Study design

This study was retrospective/prospective cross-sectional descriptive study.

METHODS

Clinical staging

Prospective patients

Thorough clinical examination of each patient with OSCC was done in cooperation with experienced Oral and Maxillofacial Surgeon. It involved appearance of the lesion, anatomical site, size of the primary tumor (at the greatest diameter in centimeter), lymph node status and distant metastasis as assessed by plain x-ray of the chest and abdomen. Staging was done according to TNM classification system of OSCC as recommended by IUCC (UICC 1997).

Retrospective patients

Such information was retrieved from patient's files. The information was filled in the structured questionnaire (see appendix I). The clinical information of only those cases

seen and staged by experienced Oral and Maxillofacial Surgeons and/or experienced Oncologists was filled in the questionnaire.

Histological diagnosis and grading

Prospective specimens were registered in the laboratory registry. The tissues were fixed in 10% formalin and embedded in paraffin wax. For retrospective cases archival tissue blocks were retrieved from the Department of Histopathology and Morbid Anatomy. In each case for both retrospective and prospective, two new sections were made for routine haematoxylin and eosin staining to confirm the diagnosis of OSCC and for histological grading. The other section was for AgNOR staining. The grading was classified depending on the degree of differentiation following the Broder's criteria as recommended by WHO criteria (Wahi et al. 1971) as follows;

Grade x (G_x) -Grade of differentiation can not be assessed,

Grade 1 (G_1) -Well differentiated SCC

Grade 2 (G_2) -Moderately differentiated SCC

Grade 3 (G_3) -Poorly differentiated SCC

The three histological grades of OSCC were based mainly upon the degree of differentiation and keratinization. With a three-grade system for squamous cell carcinoma, grade 1 was generally well differentiated as shown by the presence of keratinization, intercellular bridges, and epithelial (keratin) pearls. Grade 3 was highly anaplastic and showed frequent or atypical mitoses, while grade 2 was an intermediate between these structural extremes.

Routine hematoxylin and eosin (H&E) staining

This was performed on paraffin-embedded tissues for morphological diagnosis to confirm the diagnosis of OSCC and for histological grading. 5 µm thick sections were sectioned from formalin fixed and paraffin embedded tissue specimens and mounted on a glass slide. The sections were de-waxed by placing the slides on the hot plate and microwave oven at 60°C for 50 minutes (Kaaya et al. 1992), and then cleared in xylene. Thereafter rehydration was done by treating the tissues with absolute alcohol, followed by 95% alcohol and then 70% alcohol to water. The sections were stained by routine H &E.

AgNOR staining

In both prospective and archival specimens, silver staining was employed to all biopsy specimens of OSCC prior to chemotherapy or radiotherapy. A 5 µm thick sections were sectioned from formalin fixed and paraffin embedded tissue specimens and subjected to AgNOR staining, using the method as recommended by Ploton et al (1986) and modified by Vuhahula et al (1995) as follows;

-Deparaffinized in xylene, 3 changes, 10 minutes each

-Rehydrated through ethanol (100%, 95%, 90%, and 80%) 3 minutes each

-Rinsed in deionized distilled water for 10 minutes

-Post fixed in ethanol /acetic acid solution (3:1) for 30 minutes

-Reacted with AgNOR solution for 40 minutes. Solution A + Solution B in ratio of 1:2. (Solution A was prepared by mixing 2% gelatin and 1% aqueous formic acid and solution B was composed of 50% aqueous silver nitrate)

-Rinsed in deionized distilled water for 10 minutes

-Treated with 2% gold Chloride for gold toning for 10 minutes

-Treated with 5 times diluted photographing solution (Fuji Fix®, Tokyo Japan) for 10 minutes

-Rinsed in deionised distilled water with Mayer's hematoxylin for 10 seconds

Dehydrated, clear and mount

AgNOR counting

In each case AgNOR dots were counted in 100 randomly selected nuclei under x100 oil immersion lens. In AgNOR enumeration, the method by Vuhahula et al. (1995) was applied. Each AgNOR dot was counted as a unit whether seen separately within or outside nucleoli. Undiscernible nucleolar clusters were counted as a single dot. Continuous focusing through several planes was done to ensure an accurate count within each cell examined. In cases where the nuclei of tumor cell overlapped the AgNOR dots of these nuclei were not included. The overall mean value of each case was calculated from the different values in 100 randomly selected nuclei.

Intra-observer variability

Five (5) cases were selected. AgNOR dots were counted in 100 randomly selected nuclei. Recounting of the AgNOR dots was done. Mean value was compared statistically to see if there was variation.

Inter-observer variability

Five (5) cases were selected. AgNOR dots were counted in 100 randomly selected nuclei by the first observer (the author). Recounting of the AgNOR dots was done by another observer (experienced consultant pathologist). Mean value was compared statistically to see if there was variation.

Statistical analysis

Mean AgNOR count per nuclei was calculated and assessed. P-value cut off point was 0.05 (5%). Non-parametric method was used. Independent t-test was used to compare between different parameters. Pearson correlation test was used for inter and intra-observer variability.

Data collection and management

Data were collected in a structured questionnaire (Appendix I), and analyzed using EPI INFO 6.

Perceived limitations

-Sub optimal record keeping made the retrieval of past information for retrospective cases incomplete and therefore only 111 cases were studied instead of 247 cases which were reported between 2000 - 2004.

-No proper system of patients follows up and therefore the actual outcome of patients in comparison to prognostic parameters could not be assessed.

-Rehabilitation of Central Pathology Laboratory at MNH that was in progress during the study period made the retrieval of some patients' files and tissue blocks very difficult and time consuming.

RESULTS

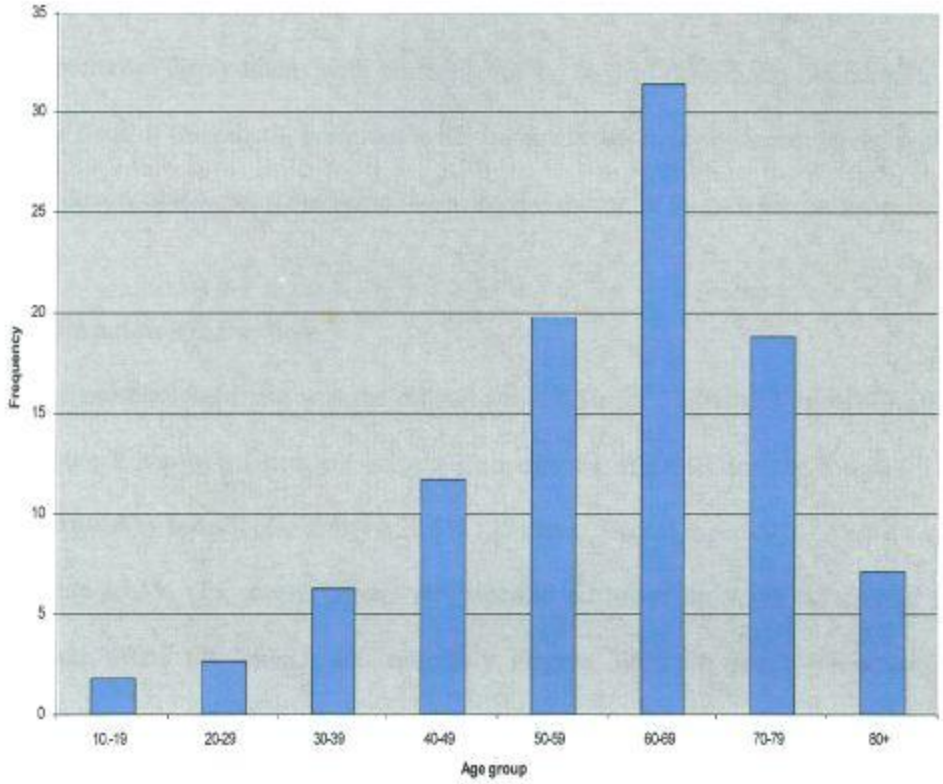
1.0 Clinical Features

Between year 2000 and 2004 a total of 29,863 biopsies were submitted at Muhimbili National Hospital (MNH), Department of Histopathology. Among these, 247 had histological diagnosis of OSCC. Out of the 247 patients diagnosed, 111 (44.9%) were recruited for this study, whereas 136 (55.1%) were excluded due to insufficient information. Among those recruited in the study, 50 patients (45.0%) were prospectively seen at the clinic during the study period, while 61 patients (55.0%) were retrospective cases retrieved from the files in the Medical Records.

1.1 Age and sex distribution

The study group consisted of 63 (56.8%) males and 48 (43.2%) females, with male to female ratio of 1.3:1. The ages at the time of diagnosis ranged from 13 to 85 years with mean age of 58.6 years ±15.01 SD years. Peak age was at 60-69 years (31.5%), followed by 50-59 years (19.8%). The mean age for female patients was slightly lower (56.29±15.31 SD years, median 57.50 years), than that of male patients (60.49±14.65 SD years, median 62.00 years). Figure 1 (pg 38) shows a sharp increase of patients as the age advances reaching a peak of 60-69 years and there after there is a steady decline of patients as age advances to 80-89 years. Patients aged 40 years and above accounted for 89.2%.

Figure 1. Age distribution of OSCC patients



1.2 Signs and symptoms

Since the description of signs and symptoms were missing in the case notes of retrospective cases, only signs and symptoms of 50 prospective cases are presented. 88.0% (44 out of 50 cases) presented with infiltrative infected ulcerated lesions which were also painful. Some ulcers had characteristic indurated margins with everted edges. Centrally, the ulcers were covered with necrotic tissue and debris (Fig 2&3 pg 40). Two cases (4%) had endophytic un-ulcerated lesions and 4 cases (8.0%) had exophytic un-ulcerated lesions. Endophytic and exophytic lesions were observed as nodules or

growths with irregular borders and rough surfaces which were fixed to underlying structures. Two patients with tumors from the base of the tongue extending to involve the floor of the mouth, presented with trismus. No patient was encountered with otalgia or airway obstruction. Foul smell was noted in 49 out of 50 prospective cases.

1.3 Anatomical location

The most affected site was the tongue accounting for 25.0% (28 cases) of all OSCC. Figure 2 shows an example of gross appearance of OSCC of the tongue. This was followed by mandibular gingiva 22.5% (25 cases), buccal mucosa 16.2% (18 cases) and palate 13.5% (15 cases). Others were located on lower lip 9.9% (11 cases), floor of mouth 9.0% (10 cases), and maxillary gingiva 3.6% (4 cases). However, among females, mandibular gingiva was the most frequently involved site (37.5%, 18 cases), (Figure 3 pg 40) followed by tongue (18.8%, 9 cases) and buccal mucosa (16.7%, 8 cases), whereas in males tongue carried about one third (30.2%, 19 cases) of all tumors followed by buccal mucosa (15.0%, 10 cases) and palate (14.3%, 9 cases). Maxillary gingiva was the least affected site with only 4 cases (3.6%). These findings are summarized in Table 1 (pg 41).



Figure 2. Gross appearance of OSCC of the tongue in a female patient aged 43 years old.



Figure 3. Gross appearance of OSCC of female patient aged 70 years old. The lesion started on the anterior lower gingiva, extended to the labial mucosa causing perforation and exposure of anterior lower teeth and bone.

Table 1: Relationship between anatomical site and sex

Anatomical location	Sex				Total	
	Female		Male			
	n	%	n	%	n	%
Buccal mucosa	8	16.7	10	15.9	18	16.2
Floor of mouth	3	6.3	7	11.1	10	9.0
Lower lip	3	6.3	8	12.7	11	9.9
Mandibular gingiva	18	37.5	7	11.1	25	22.5
Maxillary gingiva	1	2.1	3	4.8	4	3.6
Palate	6	12.5	9	14.3	15	13.5
Tongue	9	18.8	19	30.2	28	25.2
Total	**48**	**43.2**	**63**	**56.8**	**111**	**100.0**

1.4 Size of tumor

The size of lesions at the time of diagnosis ranged from 2 to 12 cm in their widest dimensions with mean of 5.74±1.84 (SD) cm and median of 5.00 cm. Majority of tumors (81.1%) measured more than 4 cm at time of initial presentation; whereas only 18.9% tumors measured between 2-4 cm. The size was assessed in relation to age, sex, and different anatomical location of OSCC.

1.4.1 Size in relation to age

Sixty six percents (66.6%) of patients with less than 40 years of age had tumors of more than 4 cm; whereas in patients with 40 years and above, 82.8% had tumors with more than 4 cm in diameter. The remaining thirty three percents (33.3%) of patients less than 40 years and seventeen percents (17.2%) of patients with 40 years and above had small tumors measuring 2-4 cm in diameter. In both young and older patients majority of tumors (66.6% and 82.8% respectively) were large, measuring more than 4 cm. There was no difference statistically regarding tumor size at time of diagnosis between young and old patients ($p= 0.055520$).

1.4.2 Relationship of size with sex

When the size of tumor was related to sex, it was found that in both males and females, 81.3% and 81.0% of tumors respectively, measured more than 4 cm in the greatest diameter during initial presentation. The remaining 19.0% of males and 18.8% of females measured 2-4 cm.

1.4.3 Size in relation to different anatomical location

All lower lip tumors (11 cases) measured more than 4 cm during time of initial presentation. Similarly in other anatomical sites majority of tumors measured more than 4 cm Table 2 (pg 43). In each location, the overall mean was more than 5 cm in greatest diameter, indicating that majority of tumors presented at advanced stage. Mandibular and maxillary gingivas were the sites with the highest mean size (6.02 cm

and 7.25 cm). Statistically no relationship was found between tumor size in different anatomical location (p=0.183125).

Table 2: Anatomical location of OSCC in relation to size

Anatomical location	Size				Overall	
	2-4 cm		> 4 cm		Mean (in cm)	Median
	n	%	n	%		
Buccal mucosa	3	16.7	15	83.3	5.83	6.00
Floor of mouth	1	10.0	9	90.0	5.70	5.00
Lower lip	-	-	11	100.0	5.64	5.00
Mandibular gingiva	4	16.0	21	84.0	6.08	7.50
Maxillary gingiva	1	25.0	3	75.0	7.25	6.00
Palate	2	13.3	13	86.7	5.53	5.00
Tongue	10	35.7	18	64.3	5.32	5.00
Total	**21**	**18.9**	**90**	**81.1**	**5.74**	**5.00**

1.5 Duration of lesion

The duration of lesion between the beginning of symptoms and the diagnosis was assessed and then related to anatomical location and size of the tumor. With this group of 111 patients, duration ranged from 1-60 months with mean of 12.5±7.82 (SD) and median of 12.00 months. Majority of tumors (58.5%) presented between 4-12 months,

while 31.5% presented between 13-24 months. Few tumors presented in 1-3 months (5.4%) and 25-36 months (3.6%), and one tumor presented with 60 months duration Tables 3 (pg 45).

1.5.1 Duration of tumor in relation to anatomical location

Majority of tumors of the tongue (75.0%), mandibular gingiva (64.0%), floor of mouth (60.0%), buccal mucosa (55.6%), and palate (53.3%) presented when they were between 4-12 months from beginning of symptoms. Most tumors of the lower lip presented when they were 13-24 months (45.5%) and 4-12 months (36.4%). Three out of four maxillary gingival tumors (75.0%) presented when they were 13-24 months duration from beginning of symptoms. No tumor from the lower lip or mandibular gingiva was reported within 3 months duration. The only tumor presented at 60 months was from the lower lip in a male patient aged 38 years. The lesion was ulcerated, and measured 6 cm in the greatest dimension (Table 3 pg 45). There was no correlation between anatomical location of the tumor and duration from beginning of symptoms to the time of diagnosis ($p=0.151524$).

Table 3: Relationship between anatomical location of OSCC and duration

Anatomical location	Duration in months										Total	
	1-3		4-12		13-24		25-36		37-60			
	n	%	n	%	n	%	n	%	n	%	n	%
Buccal mucosa	1	5.6	10	55.6	6	33.3	1	5.6	-	-	18	16.2
Floor of mouth	2	20.0	6	60.0	2	20.0	-	-	-	-	10	9.0
Lower lip	-	-	4	36.4	5	45.5	1	9.1	1	9.1	11	9.9
Mandibular gingiva	-	-	16	64.0	9	36.0	-	-	-	-	25	22.5
Maxillary gingiva	1	25.0	-	-	3	75.0	-	-	-	-	4	3.6
Palate	1	6.7	8	53.3	5	33.3	1	6.7	-	-	15	13.5
Tongue	1	3.6	21	75.0	5	17.9	1	3.6	-	-	28	25.2
Total	**6**	**5.4**	**65**	**58.6**	**35**	**31.5**	**4**	**3.6**	**1**	**0.9**	**111**	**100.0**

1.5.2 Duration of OSCC in relation to size

Majority (85.7%) of small tumors (2-4 cm) presented between 4 and 12 month, whereas only 52.2% of large tumors (> 4 cm) presented in the same duration. 4.8% (1 out of 21 cases) of small tumors and 5.6% (5 out of 90 cases) of large tumors presented between 1 and 3 months. 42.2% (38 out of 90 cases) of large tumors presented beyond 13 months from time of initial symptoms in comparison to 9.6% (2 out of 21 cases). Therefore, positive correlation between size of the tumor and duration of tumor at time of initial diagnosis from first symptoms was observed ($p=0.049779$). The longer duration from initial symptoms to diagnosis, the more likelihood of presenting a large tumor.

1.6 Lymph node involvement

Clinically palpable lymph nodes were encountered in 60.4% (67 cases) of study participants. Among these 24.3% had N_1, 30.6% had N_2 and 5.4% had N_3. The involved lymph nodes were cervical, sub-mandibular, and sub-mental nodes. Palpable cervical and sub-mandibular lymph nodes were encountered in tumors of the tongue, floor of mouth, mandibular gingiva, maxillary gingiva, buccal mucosa, palate and some advanced tumors of the lower lip. Few sub-mental nodes were encountered in tumors of the lower lip and floor of mouth. However, histological confirmation of nodal metastasis to rule out confounding factors such as concurrently underlying chronic diseases or reactive lymphadenopathy was not done in this study series.

1.6.1 Lymph node involvement in relation to size of primary tumor

Relationship between lymph node involvement and tumor size revealed that, about two thirds (61.9%) of patients who presented with small tumors (2-4 cm) had no palpable lymph nodes. On the other hand two thirds (65.6%) of patients who presented with large tumors, which measured more than 4 cm had palpable lymph nodes. There was a positive correlation between tumor size and lymph nodes involvement ($p=0.020530$). Therefore the larger the tumor the more likelihood of lymph node involvement (Fig 4.pg 48).

1.6.2 Lymph node involvement in relation to anatomical location

Most (75.0%) of patients with tumors of the tongue had palpable lymph nodes, followed by tumors of floor of mouth (70.0%), buccal mucosa (66.7%), and mandibular gingiva (64.0%). One third of patients with tumors of the palate (33.3%) and lower lip (34.4%) had palpable lymph nodes. Two patients out of four with SCC of maxillary gingiva had N_3. In most sites except the palate and lower lip, over 60% of patients had palpable lymph nodes. Table 4 (pg 49) shows lymph node involvement in different anatomical sites.

Figure 4. Lymph node involvements in relation to size of tumor

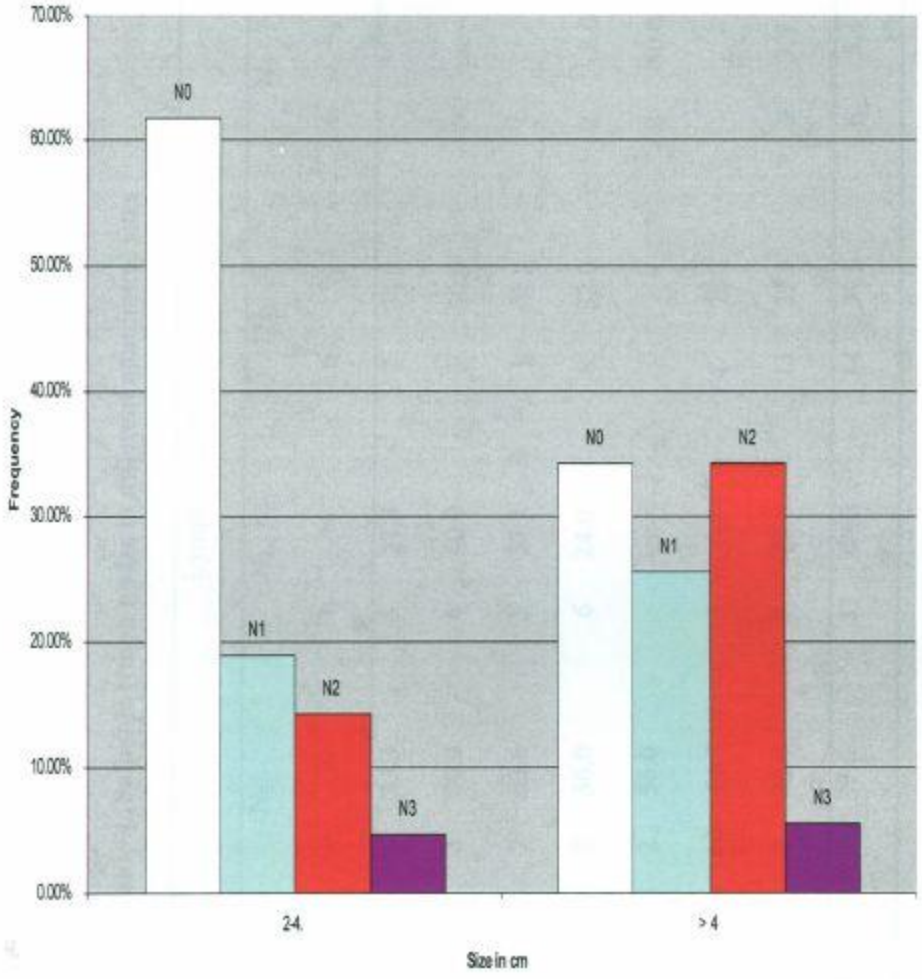


Table 4: Palpable lymph nodes in different anatomical sites

Anatomical location	Total	Lymph node involvement								Sub-Total	
		N_0		N_1		N_2		N_3		(N_1+N_2+N_3)	
	N	n	%	n	%	n	%	n	%	n	%
Buccal mucosa	18	**6**	**33.3**	5	27.8	7	38.9	-	-	**12**	**66.7**
Floor of mouth	10	**3**	**30.0**	4	40.0	3	30.0	-	-	**7**	**70.0**
Lower lip	11	**7**	**63.6**	3	27.3	1	9.1	-	-	**4**	**36.4**
Mandibular gingiva	25	**9**	**36.0**	6	24.0	8	32.0	2	8.0	**16**	**64.0**
Maxillary gingiva	4	**2**	**50.0**	-	-	-	-	2	50.0	**2**	**50.0**
Palate	15	**10**	**66.7**	1	6.7	4	26.7	-	-	**5**	**33.3**
Tongue	28	**7**	**25.0**	8	28.6	11	39.3	2	7.1	**21**	**75.0**
Total	**111**	**44**	**39.6**	**27**	**24.3**	**34**	**31.5**	**6**	**5.4**	**67**	**60.4**

1.6.3 Lymph node involvement in relation to duration

Majority (57.66%, 64 cases) of tumors that presented with lymph node involvement were those diagnosed between 4-24 months duration from the initial symptoms. Only one tumor out of six tumors presenting between 1-3 months had palpable lymph nodes. Generally, palpable lymph nodes were frequently encountered in late presenting tumors than the early ones as summarized in Table 3 (pg 31). However, the difference in lymph node involvement between tumor presenting early (1-3 months) and those presenting late (after 4 months) was not statistically significant ($p=0.068665$).

1.7 Distant metastasis

Only one case had confirmed distant metastasis to the lungs (stage IV). This was encountered in a male patient aged 60 years. The lesion had duration of 18 months, and was located on maxillary gingiva. Histologically, the primary tumor was graded as poorly differentiated tumor with nodal involvement (N_3) to the cervical and submandibular regions. The patient had medical history of cigarette smoking and alcohol drinking. The duration of such habits was not available.

Table 5: Relationship between lymph node involvement and duration of tumor

Duration in months	Total	Lymph node involvement								**Sub-Total**	
		N_0		N_1		N_2		N_3		**(N_1+N_2+N_3)**	
	N	**n**	**%**	n	%	n	%	n	%	**n**	**%**
1-3	6	**5**	**83.3**	-	-	1	16.7	-	-	**1**	**16.7**
4-12	65	**25**	**38.5**	20	30.8	19	29.2	1	1.5	**40**	**61.5**
13-24	35	**11**	**31.4**	7	20.0	13	38.2	4	11.4	**24**	**69.6**
25-36	4	**3**	**75.0**	-	-	-	-	1	25.0	**1**	**25.0**
37-48	-	-	-	-	-	-	-	-	-	**-**	**-**
49-60	1	-	-	-	-	1	100.0	-	-	**1**	**100.0**
Total	**111**	**44**	**39.6**	**27**	**24.3**	**34**	**31.5**	**6**	**5.4**	**67**	**60.4**

1.8 Clinical stage

According to TNM classification, tumors were classified into stage I, II, III, and IV. Out of 111 OSCC patients, 82.0% had stage IV tumors, 6.3% had stage III and 11.7% had stage II tumors. No patient was found with stage I tumor in this series. Majority of patients (88.3%) presented with advanced tumors (stage III and IV). Clinical stage of the tumors was compared to sex, site, size of patients and histological grade of OSCC.

1.8.1 Clinical stage in relation to sex

84.1% (53 cases) of males had stage IV tumors, 4.8% (7 cases) had stage III, and 11.1% (13 cases) stage II, whereas among females 79.2% (38 cases) had stage IV, 8.3% (4 cases) stage III, and 12.5% (6 cases) stage II tumors. This implies that in both sexes majority had advanced OSCC (stage III and IV).

1.8.2 Clinical stage of OSCC in relation to different anatomical location

Majority of patients with tumors of the lower lip (90.9%), floor of mouth (90.0%), buccal mucosa (83.3%), tongue (82.1%) and mandibular gingiva (80.0%) presented with stage IV OSCC. No tumor of the lower lip and floor of mouth presented in stage II. Over three quarter (73.3-90.9%) of tumors in all sites were in advanced stages (stage III or IV). Statistically, there was no relationship between tumor stage and anatomical location of OSCC ($p=0.833282$). Table 6 (pg 53) summarizes these results.

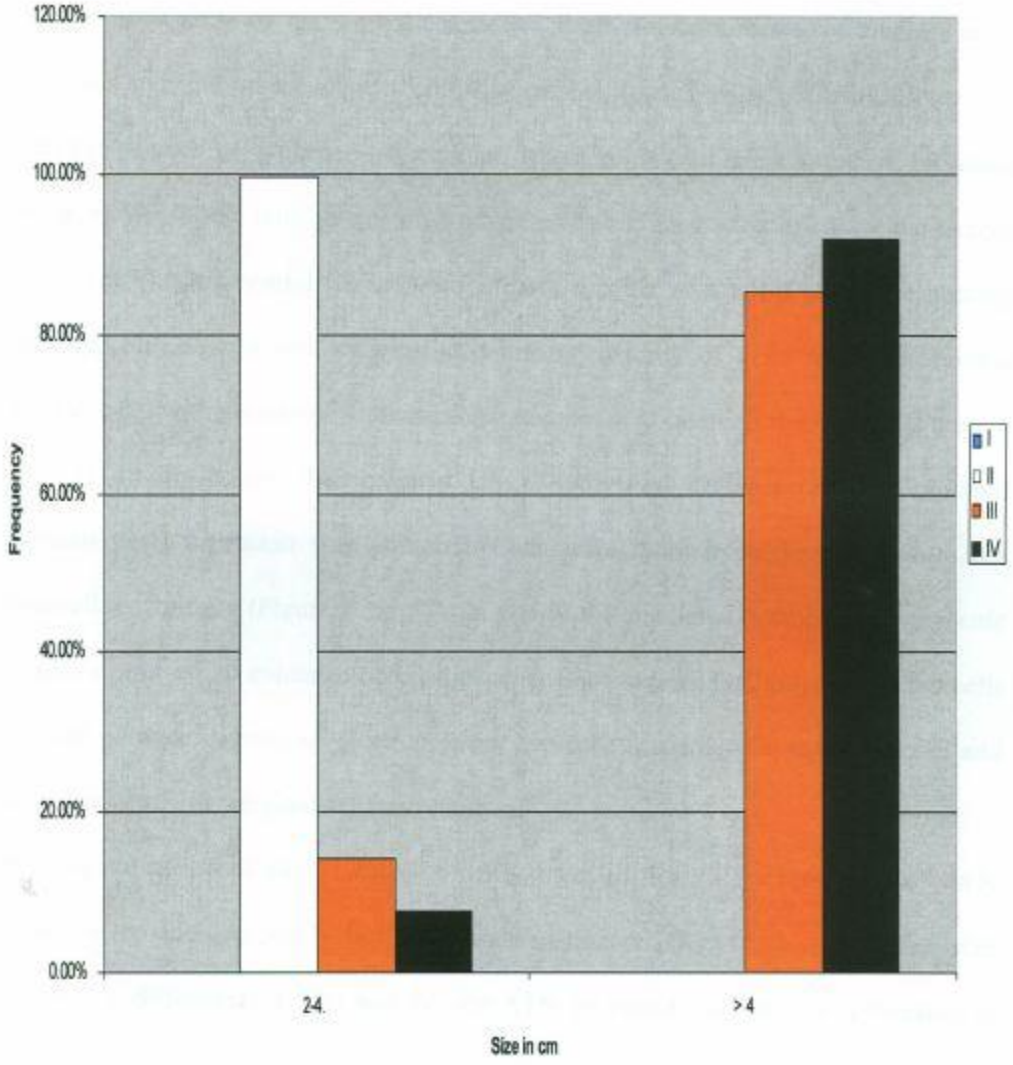
Table 6: Distribution of OSCC by clinical stage and anatomical location

Anatomical location	Stage								Total	
	I		II		III		IV			
	n	%	n	%	n	%	n	%	n	%
Buccal mucosa	-	-	3	16.7	-	-	15	83.3	18	16.2
Floor of mouth	-	-	-	-	1	10.0	9	90.0	10	9.0
Lower lip	-	-	-	-	1	9.1	10	90.9	11	9.9
Mandibular gingiva	-	-	3	12.0	2	8.0	20	80.0	25	22.5
Maxillary gingiva	-	-	1	25.0	-	-	3	75.0	4	3.6
Palate	-	-	2	13.3	2	13.3	11	73.3	15	13.5
Tongue	-	-	4	14.3	1	3.6	23	82.1	28	25.2
Total	**-**	**-**	**13**	**11.7**	**7**	**6.3**	**91**	**82.0**	**111**	**100.0**

1.8.3 Clinical stage of OSCC in relation to size

Majority of large tumors (>4 cm) were in advanced stage (stage III = {85.7%} and IV = {92.3%}) as expected. However, there were eight small tumors (≤ 4 cm) that were classified as advanced stage tumors. The one in stage III (14.3%) had $T_2N_1M_0$, was located on the floor of the mouth and had duration of 12 months. The remaining seven small tumors (7.7%) were in stage IV. Three out of seven small lesions with stage IV, had involvement of the adjacent tissues, two of which were located on the tongue, and one on the mandibular gingiva. The remaining four (4 cases) had $T_2N_2M_0$ and all were located on the tongue with duration ranging between 6 and 12 months. General comparison of tumor size with stage shows that there is a general relationship between size and stage of tumor (Figure 5 pg 55). The larger the tumor the advanced is the stage, however, few exceptions exist where small tumors were classified as advanced because of lymph node involvement and/or involvement of adjacent structures.

Figure 5. Clinical stage of OSCC in relation to size



2.0 Histological Features

Tumor tissue showing invasion of connective tissue stroma consisted of irregular nests, columns and strands of malignant epithelial cells infiltrating subepithelially. There were varying degrees of dyplasia with cellular hyperchromatism, pleomorphism, increased nuclear-cytoplasmic ratio, premature keratinization and formation of spheroidal masses of keratin (keratin pearls). There was increased number of mitoses as well, especially the atypical ones. In well differentiated tumors majority of cells resembled normal prickle cells with abundant keratinization and prominent intercellular bridges (Figure 6 pg 57). In moderately differentiated OSCC individual cell keratinization without obvious pearl formation was observed. Cells were more hyperchromatic with less intercellular bridges (Figure 7 pg 57). In poorly differentiated tumors, the neoplastic islands displayed no evidence of keratinization nor intercellular bridges, and the cells showed extreme degrees of pleomorphism, hyperchromatism with many mitoses and not infrequently tumor giant cells were present.

Histological review of all 111 cases of OSCC revealed that 77.5% (86 cases) of study sample were microscopically well differentiated tumors (G_1). 14.4% (16 cases) were moderately differentiated (G_2) and the rest 8.1% (9 cases) were poorly differentiated (G_3).

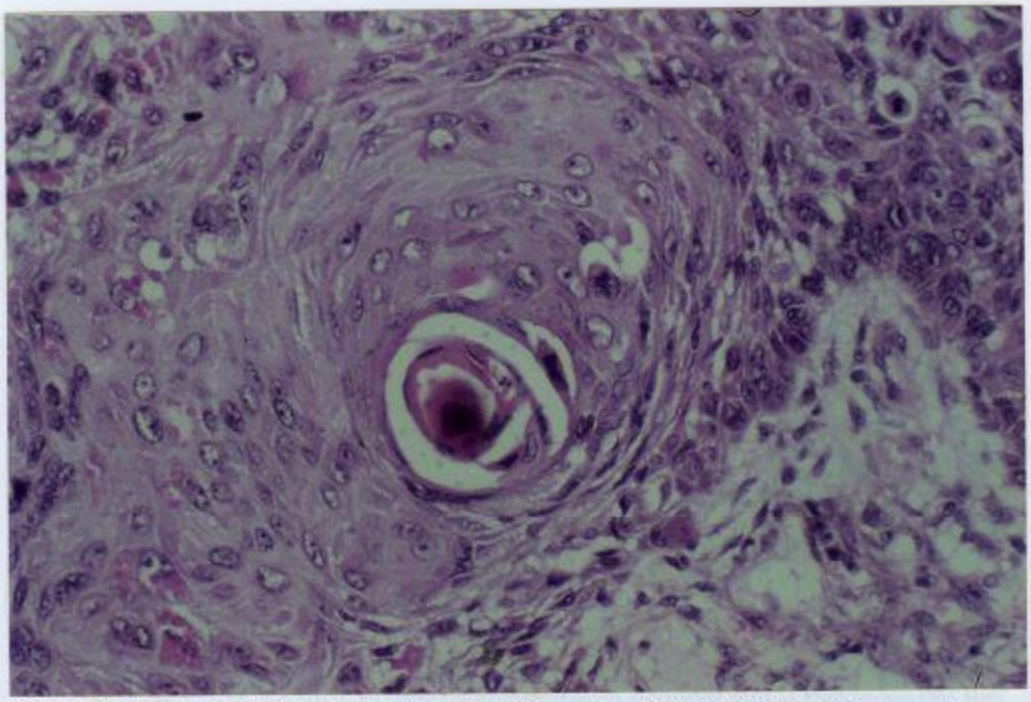


Figure 6. A microphotography of a well differentiated OSCC (G_1) with a keratin pearl formation and prominent intercellular bridges (H&E X20)

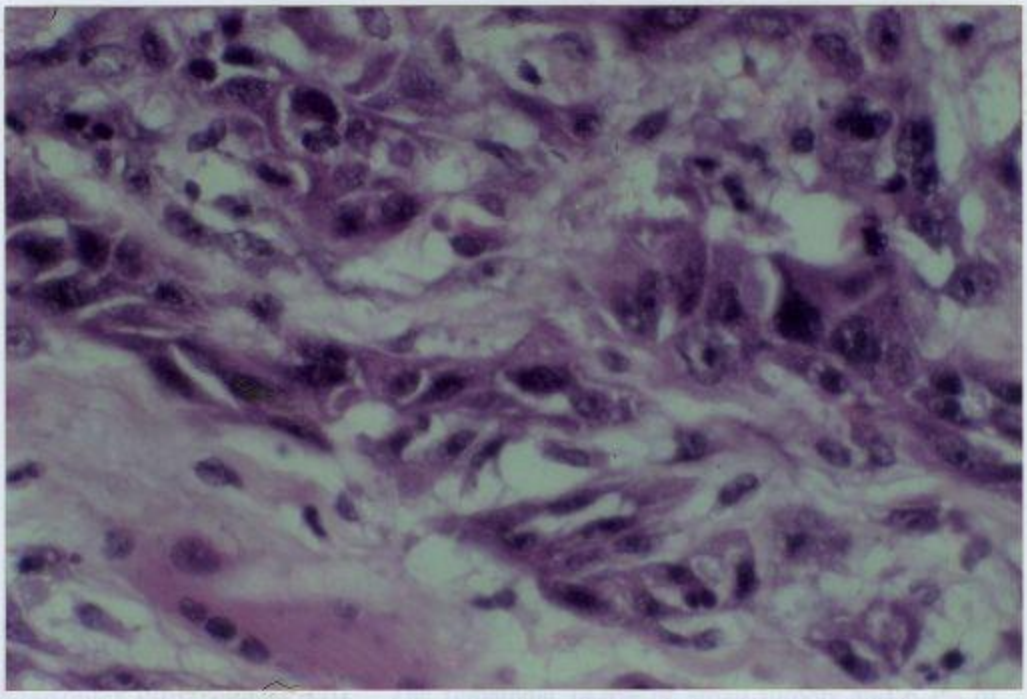


Figure 7. A microphotography of a moderately differentiated OSCC (G_2). The cells are pleomorphic with nuclear hyperchromasia and atypism. (H&E X40)

2.1 Histological grade in relation to sex

After relating histological grade to sex of OSCC patients, it was found that in both females and males, majority (87.5% and 69.8%, respectively) of tumors were well differentiated (G_1). Moderately differentiated tumors (G_2) accounted for 22.2% among males and 4.2% in females, whereas poorly differentiated tumors (G_3) comprised 7.9% of males and 8.3% of females.

2.2 Histological grade of OSCC in different anatomical location

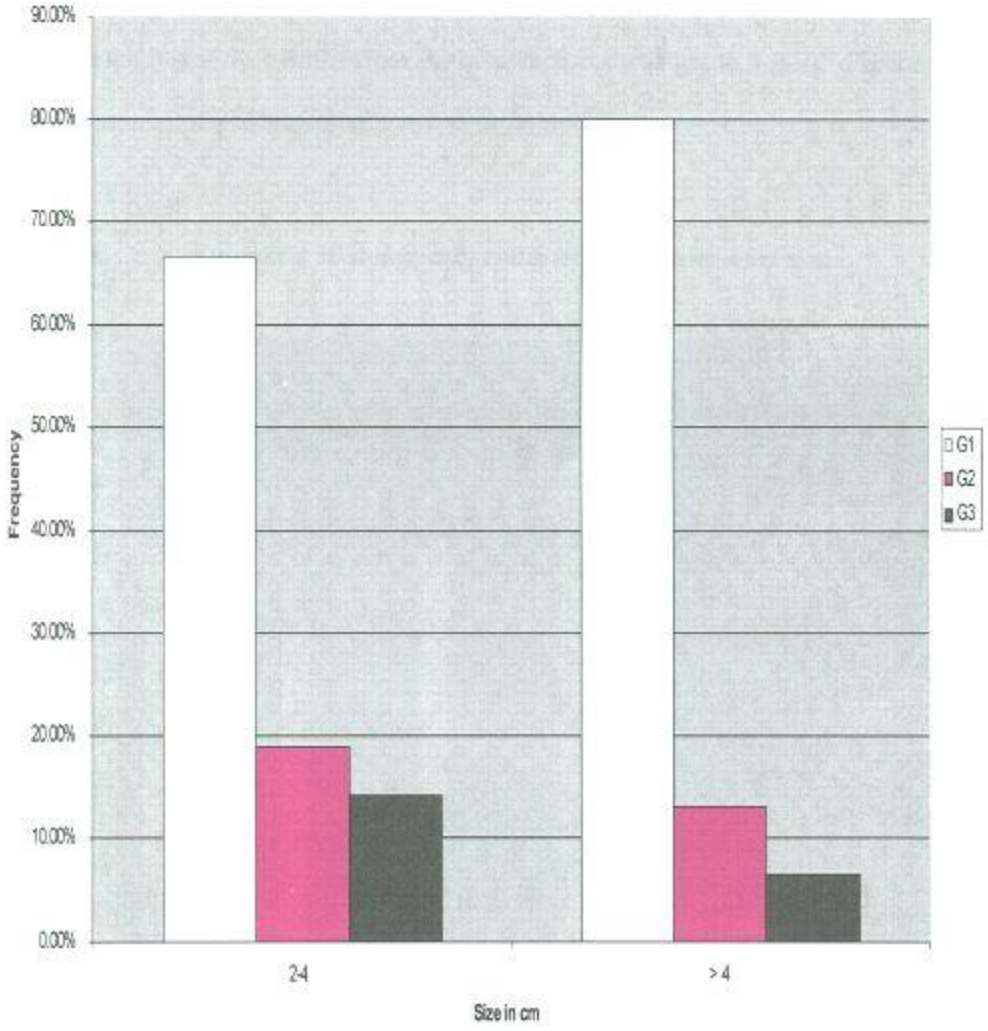
Histological grade of OSCC was related to anatomical location and it was observed that majority of the tumors located on mandibular gingiva (84.0%), buccal mucosa (83.3%), lower lip (81.8%), palate (80.0%), tongue (75.0%), floor of mouth (60.0%) as well as maxillary gingiva (50.0%) were well differentiated tumors (G_1). One tumor from each of involved anatomical site was grade 3 (G_3) except the tongue which had 3 cases classified as grade 3 (G_3). No relationship was established between anatomical location of the tumor and the histological grade of OSCC (P=0.919696).

2.3 Histological grade in relation to size

Histological grade of OSCC was assessed in relation to size; it was found that 66.7% of small tumors (2-4 cm) and 80.0% of large tumors (> 4 cm) were well differentiated OSCC (G_1), whereas 19.0% of small tumors and 13.3% of large tumors were moderately differentiated (G_2). The remaining 14.4% of small tumors and 6.7% of

large tumors were poorly differentiated (G_3) (Figure 8 below). Relationship could not be established between the size and histological grade of OSCC (P=0.368716).

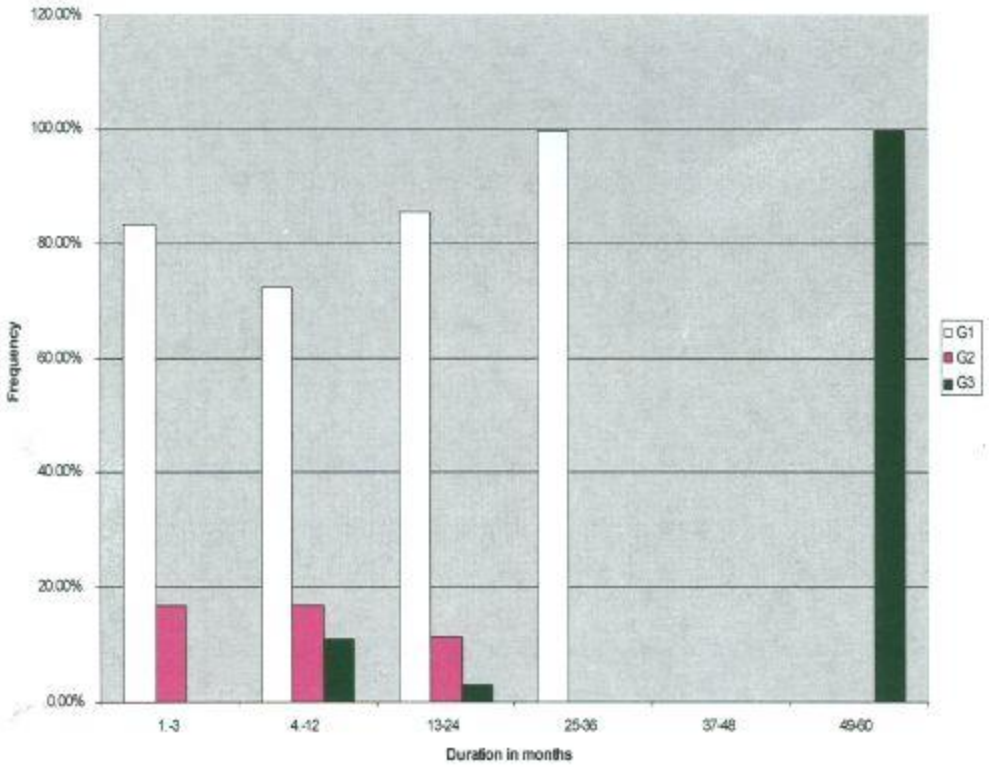
Figure 8. Histological grade and size of tumor



2.4 Relationship between histological grade of OSCC and duration

Histological grade was assessed in relation to duration of tumor at the time of diagnosis. The study revealed that majority of tumors which presented between 1-3 months (83.3%), 4-12 months (72.3%) and 13-24 months (85.7%) were grade 1 (G_1). All four tumors which presented between 25 and 36 months were also grade 1 (G_1). The one case presenting at 60 months from initial symptoms was grade 3 (G_3). Figure 9 below show histological grade in relation to duration of OSCC.

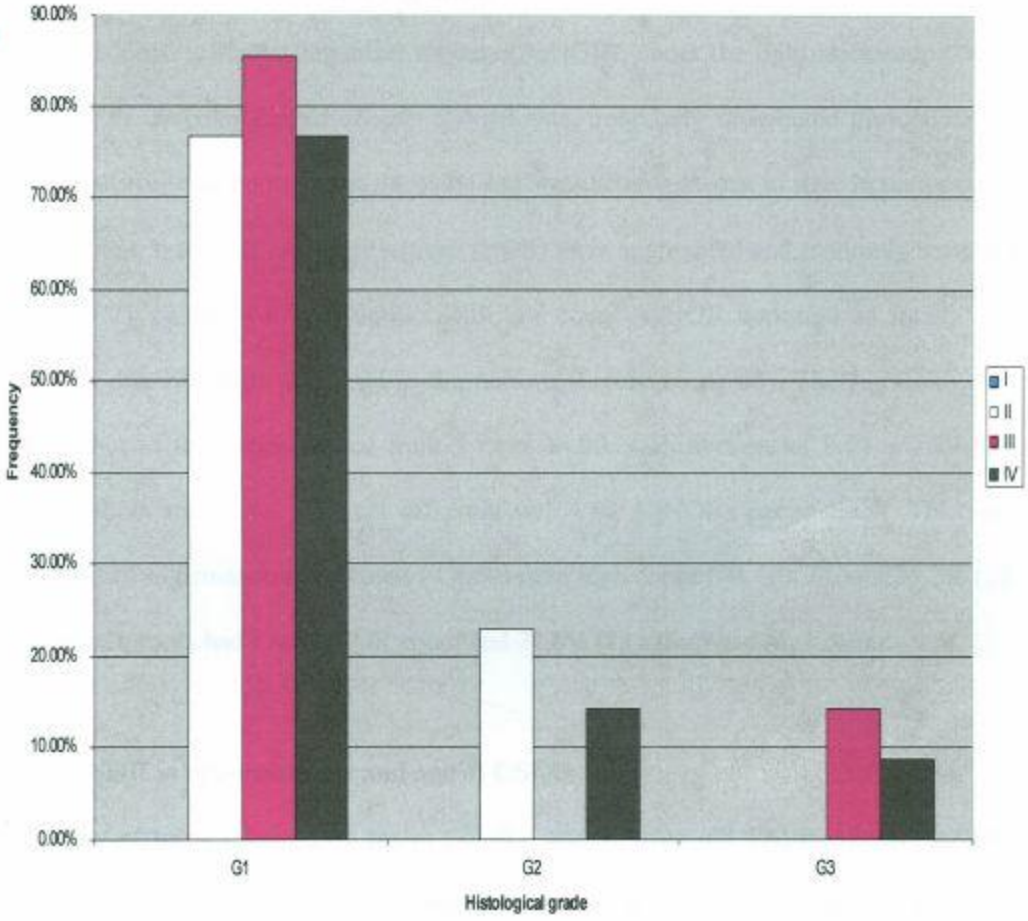
Figure 9. Histological grades of OSCC and duration



2.5 Histological grade versus clinical stage of OSCC

Figure 10 (pg 62) summarizes relationship between histological grade and clinical stage of OSCC. Grade 1 tumors were the majority in each tumor stage, 76.9% of stage II, 85.7% of stage III and 76.9% of stage IV. The remaining 23.1% of stage II tumors were in grade 2. No stage III tumor was in grade 2, while the remaining 14.3% of stage III tumors were in grade 3. Stage IV tumors were seen in all three histological grades. All grade 3 (G_3) tumors were from advanced tumor stages (stage III and IV). However, such a relation was found not statistically significant (0.526897).

Figure 10. Relationship between histological grade and clinical stage of OSCC



3.0 Silver nucleolar organizer regions staining

Silver stained nucleolar organizer regions (AgNOR), under the light microscope, were viewed as irregular shaped densely stained dots, irregularly distributed throughout the nucleus (inside and outside the nucleoli) and were heterogeneous in size. In tumors with high count, nucleolar organizer regions (NOR) were aggregated and randomly scattered (Figure 11 pg 64), while in tumors with low count AgNOR appeared as small, well defined dots distinctly dispersed in the nucleus (Figure 12 pg 65). The AgNOR count per tumor in this series ranged from 3.79 to 14.90, with average of 6.61 ± 2.79 SD. The median was 6.20. The cut off point was 4.60 AgNORs per nucleoli. This was established to demarcate low count (< 4.60) from high count (>4.60). About 25.2% (28 cases) of tumors had low AgNOR count and 74.8% (83 cases) had high count.

3.1 AgNOR in relation to sex and age of OSCC

Majority of tumors from both males (79.4%) and females (68.8%) had high AgNOR count. The remaining 31.3% of tumors in females and 20.6% of tumors in males had low count. Relationship between AgNOR count versus age of OSCC patients showed that 91.7% of patients with less than 40 years had high AgNOR count, while 72.7% of patients with 40 years and above had high AgNOR count. Only few patients in both younger (8.3%) and older age group (27.3%) had low AgNOR count. No difference in AgNOR count in relation to age and sex of patients was observed.

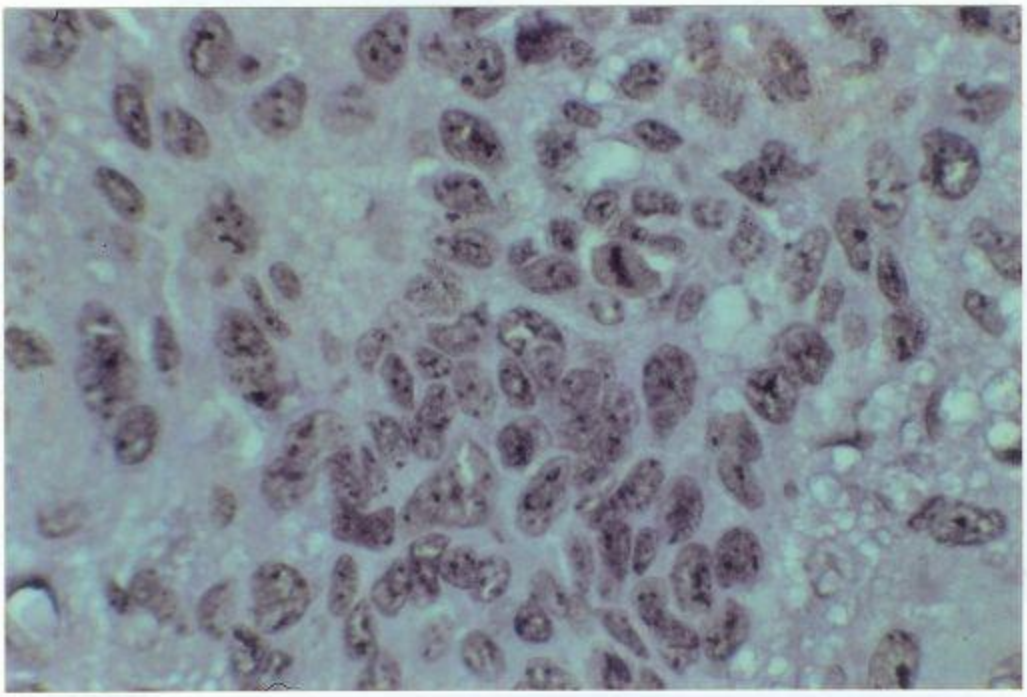


Figure 11. AgNOR staining pattern. This was a well differentiated tumor displaying high count. The total number of dots was obtained by continuous focusing to take care the dots that are in different planes of the nucleoli.

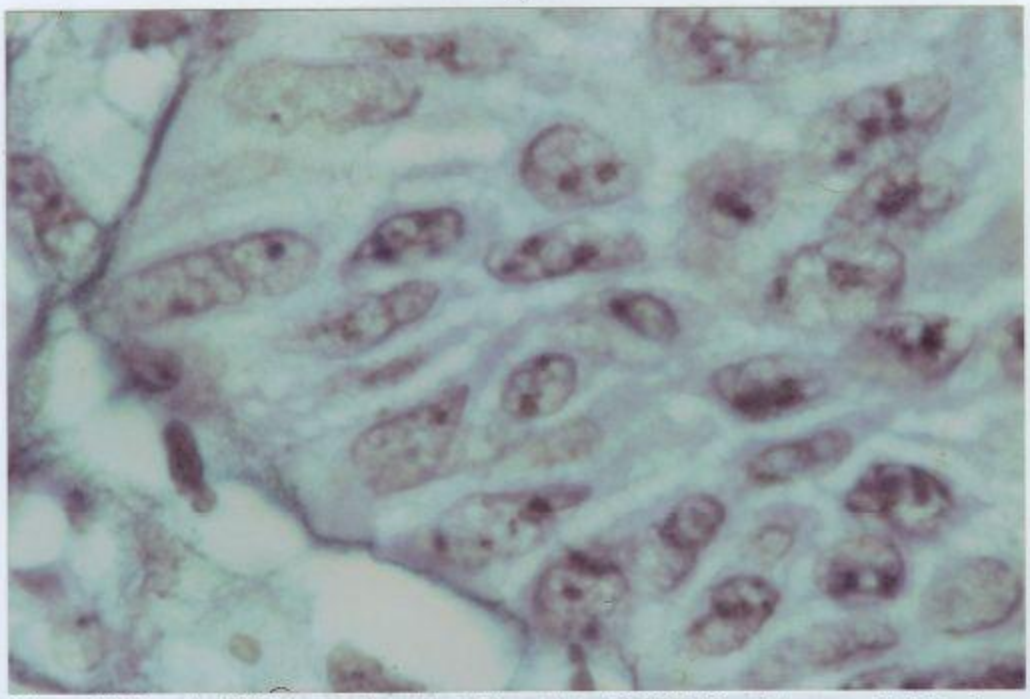


Figure 12. Low AgNOR in a moderately differentiated OSCC. The dots are well defined and relatively fewer compared to Figure 11 and 13 (AgNOR staining X100 under oil immersion).

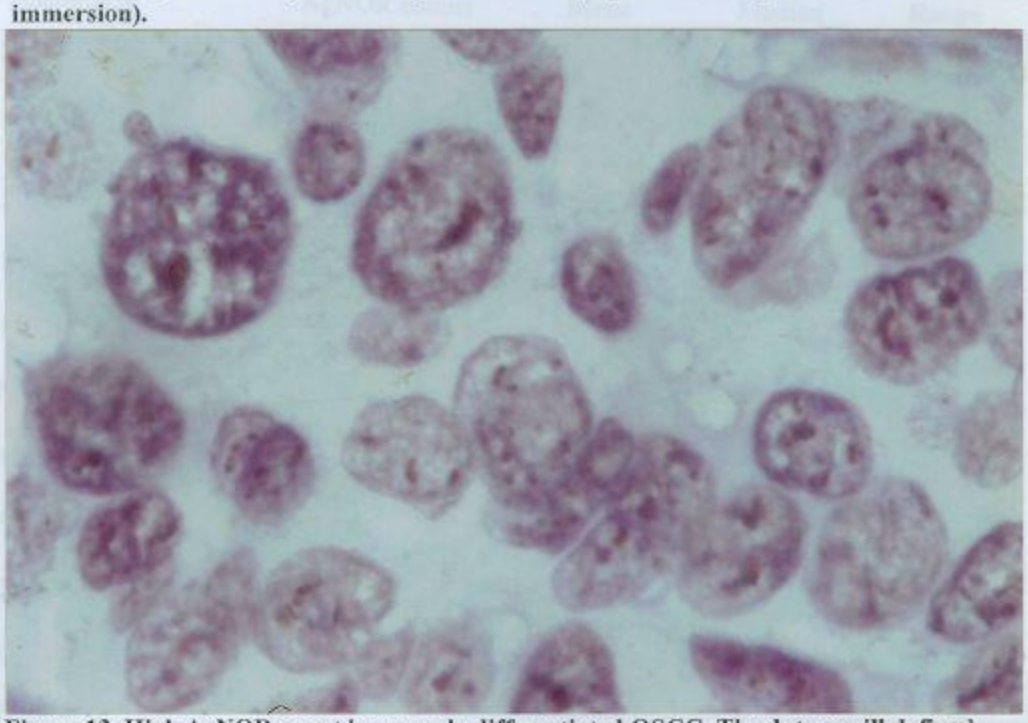


Figure 13. High AgNOR count in a poorly differentiated OSCC. The dots are ill defined and numerous than in figure 12. Continuous focusing was necessary to appreciate the many small dots within the nucleus. (AgNOR staining X100 under oil immersion).

3.2 AgNOR in relation to anatomical location

Comparison was also made between AgNOR counts and different anatomical location. Majority of tumors in mandibular gingiva (92.0%), floor of mouth (90.0%), tongue (82.1%) and buccal mucosa (61.1%) had high AgNOR count. Three (3) out of four (4) tumors of maxillary gingiva had high AgNOR count whereas about half of tumors of the lower lip (45.5%) and palate (46.7%) had low AgNOR count. AgNOR count in respect to different anatomical location is summarized in Table 7 below.

Table 7: AgNOR count in relation to anatomical location

Anatomical location	AgNOR counts				Mean AgNOR	Median AgNOR	Range AgNOR
	< 4.60		>4.60				
	n	%	n	%			
Buccal mucosa	7	38.9	11	61.1	5.75	7.29	4.27-10.11
Floor of mouth	1	10.0	9	90.0	6.99	9.00	4.61-11.33
Lower lip	5	45.5	6	54.5	5.46	5.64	4.05-7.53
Mandibular gingiva	2	8.0	23	92.0	7.69	5.84	4.80-12.21
Maxillary gingiva	1	25.0	3	75.0	9.19	6.17	4.87-14.90
Palate	7	46.7	8	53.3	5.39	6.99	3.79-8.88
Tongue	5	17.9	23	82.1	6.79	5.12	4.19-13.05
Total	**28**	**25.2**	**83**	**74.8**	**6.61**	**6.20**	**3.79-14.90**

3.3 AgNOR count in relation to duration of the disease

Table 8 below shows that there was no association between mean AgNOR count and duration of tumors from initial symptoms to the time of diagnosis. High AgNOR count was encountered in majority of early presenting as well as late presenting tumors.

Table 8: AgNOR count in relation to duration from initial symptoms to the time of diagnosis

Duration in months	AgNOR counts				Mean AgNOR	Median AgNOR	Range AgNOR
	< 4.60		> 4.60				
	n	%	n	%			
1-3	1	3.6	5	83.3	5.63	5.82	3.16-6.77
4-12	18	27.7	47	72.3	6.53	6.21	3.79-13.05
13-24	8	22.9	27	77.1	6.88	6.46	4.21-14.90
25-36	1	25.0	3	75.0	6.76	6.30	4.27-11.15
37-48	-	-	-	-	-	-	-
49-60	-	-	1	100.0	7.13	7.13	-
Total	**28**	**25.2**	**83**	**74.8**	**6.61**	**6.20**	**3.79-14.90**

3.4 AgNOR count in relation to tumor size

Table 9 (pg 68) shows the relationship of AgNOR count and size of OSCC. The mean AgNOR count of small tumors (6.34) was relatively lower than that of large tumors (6.67), however, the difference was not statistically significant (p=0.461373). Majority of both small tumors (2-4 cm) and large tumors (>4 cm) had high AgNOR count, but more large tumors had high count (77.8%) than what was found in small tumors (61.9%). However, the tendency of large tumors having relatively higher count than small tumors was not statistically significant (p=0.133277).

Table 9: AgNOR count in relation to tumor size

Size in cm	AgNOR count				Mean	Median	Range
	< 4.60		> 4.60		AgNOR	AgNOR	AgNOR
	n	%	n	%			
2-4 cm	8	38.1	13	61.9	6.34	6.05	3.82-13.05
> 4 cm	20	22.2	70	77.8	6.67	6.22	3.79-14.90
Total	**28**	**25.2**	**83**	**74.8**	**6.61**	**6.20**	**3.79-14.90**

3.5 AgNOR count versus lymph node involvement

A direct association between AgNOR count and lymph node involvement was observed. About half (52.3%) of N_0 tumors had low count, whereas majority of tumors with N_1 (96.3%), N_2 (86.2%), and all tumors with N_3 had high count. The mean, median,

and the range of AgNOR count for N_0, N_1, N_2, and N_3 are portrayed in table 10 below. The AgNOR count between tumors without (N_0) and those with palpable lymph nodes (N_1, N_2, and N_3) was highly significant (p=0.000005).

Table 10: AgNOR count in regards to lymph nodes involvement

Lymph node involvement	Total N	AgNOR counts < 4.60 n	%	AgNOR counts > 4.60 n	%	Mean AgNOR	Median AgNOR	Range AgNOR
N_0	44	23	52.3	21	47.7	4.87	4.46	3.79-13.05
N_1	27	1	3.7	26	96.3	7.58	7.26	4.90-11.33
N_2	34	4	11.8	30	88.2	7.02	6.97	4.60-11.24
N_3	6	-	-	6	100.0	11.60	12.07	7.20-14.90
Total	**111**	**28**	**25.2**	**83**	**74.8**	**6.61**	**6.20**	**3.79-14.90**

3.6 Relationship between AgNOR count and distant metastases

Out of 111, only one patient had distant metastases to the lungs. The tumor was stage IV, and the AgNOR count in this tumor was 12.03.

3.7 AgNOR count and histological grading

Majority of tumors in grade 1 (73.3%) and grade 2 (68.8%) and all grade 3 tumors (100.0%) had high AgNOR count. The highest count was found in a grade 1 tumor and

the minimum AgNOR count was in grade 2 tumors. There was statistical significant difference between AgNOR count in grade 1 tumors and those in grade 3 (p=0.049629), and between grade 2 and grade 3 (p=0.024866) but no statistical significant difference was found between grade 1 and 2 (p= 0.449024). Table 11 below summarizes the relationship between AgNOR count and histological grades of OSCC. It emphasizes that tumors of same histological grade may have different AgNOR counts and thence different biological activity.

Table 11: AgNOR count in different histological grades of OSCC

Histological grades	AgNOR counts				Mean AgNOR	Median AgNOR	Range AgNOR
	< 4.60		> 4.60				
	n	%	n	%			
G_1	23	26.7	63	73.3	6.54	6.15	3.82-14.90
G_2	5	31.3	11	68.8	6.00	5.64	3.79-11.15
G_3	-	-	9	100.0	8.34	8.43	4.03-12.03
Total	**28**	**25.2**	**83**	**74.8**	**6.61**	**6.20**	**3.79-14.90**

3.8 AgNOR count and clinical stage

Table 12 (pg 71) shows relationship between AgNOR count and clinical stage of OSCC. AgNOR count ranged from 3.79-13.05 in stage II, with mean AgNOR count of

5.20, whereas in stage III it ranged from 3.82-9.02 with mean of 5.99 and in stage IV it ranged from 4.05-14.90 with mean of 6.45. There was statistical significant difference of mean AgNOR count between stage II and IV (p=0.034874) while it was not statistically significant between stage II and III (p=0.507681), nor between stage III and IV (p=0.392240). Relatively few (46.2%) tumors under stage II had high AgNOR count, whereas majority of tumors in stage III (85.7%) and IV (78.0%) had high AgNOR count. High AgNOR count was also found in stage II tumors and some tumors with low count were classified as stage IV.

Table 12: AgNOR count in relation to different clinical stages of OSCC

Clinical stage	AgNOR count				Mean AgNOR	Median AgNOR	Range AgNOR
	< 4.60		> 4.60				
	n	%	n	%			
I	-	-	-	-	-	-	-
II	7	53.8	6	46.2	5.20	4.23	3.75-13.05
III	1	14.3	6	85.7	5.99	5.80	3.82-9.02
IV	20	22.0	71	78.0	6.85	6.45	4.05-14.90
Total	**28**	**25.2**	**83**	**74.8**	**6.61**	**6.20**	**3.75-14.90**

SUMMARY OF RESULTS

1.0 Clinical features

The study showed that OSCC affected more males (56.8%) than females (43.2%) with a male to female ratio of 1.3:1. The older age group (≥ 40 years {89.2%}) was more affected than the younger age group (< 40 years {10.8%}). However, there were 2 (1.8%) patients under 20 years who had OSCC. Majority of the patients (60.4%) reported with palpable lymph nodes ranging from N_1 to N_3. Distant metastasis was almost not encountered in this study except one patient who had distant metastases to the lung.

Majority (88.3%) of patients came with advanced stages (stage III and IV) OSCC. Very few patients (11.7%) had tumors in stage II, and no case was found in stage I disease. Furthermore, most patients sought medical care after the 4^{th} month from the beginning of symptoms (mean 12.5 ± 7.82 (SD) months) when the tumors were already large (T_3 or T_4) with mean size of 5.74 ± 1.90 (SD) cm.

2.0 Histological finding

Irrespective of age, sex, anatomical site, duration, and clinical stage, majority of tumors were microscopically well differentiated (G_1). There was no association between histological grade and other parameters such as sex, anatomical location, size, duration of the tumors nor clinical stage of OSCC.

3.0 AgNOR results

Majority of tumors displayed high number of AgNOR count. A strong correlation of AgNOR count with lymph node involvement was established. There was a positive correlation between AgNOR count and clinical stage, and AgNOR count and histological grade of the tumors. There was no significant difference in AgNOR count with regard to age, sex, size, anatomical location and duration of OSCC.

DISCUSSION

The aim of this study were three folds: 1) establishing the clinico-pathological features of OSCC patients attending at MNH and 2) relate to traditional clinical staging system and histopathological grading and 3) to see if AgNOR count can provide additional prognostic information that can aid in treatment plan of an individual patient with OSCC. It is the first study of its kind in Tanzania that was able to characterize the clinico-pathological features of OSCC in details and therefore enabling the understanding of the disease. The precise knowledge of this ailment will assist in the process of treatment planning and budgeting of each individual patient attended.

Clinical Features

This study revealed that majority of patients with OSCC (82.0%) were between the 4th and 7th decades of life with peak in the 6th decade (Figure 1 pg 38). This is in agreement with available information from other studies which indicates that OSCC is commonly seen in the 6th and 7th decades of life and is rarely seen under 40 years of age (Odukoya et al. 1986, Chattopadhyay 1989, Daftary et al. 1992, Cawson et al. 1996 Lype et al. 2001, Neville and Day 2002, Lo et al. 2003, Sasaki et al. 2005). The peak age in this study was a decade above the peak age which is reported in Africans, black Americans and in Asians (Daftary et al. 1992, Neville and Day 2002, Lo et al. 2003). The difference in peak age between this study and others (Daftary et al. 1992, Neville and Day 2002, Lo et al. 2003) may be contributed by the fact that this study had fewer cases

because it included only those patients with complete medical information and with available histological blocks. Only 10.8% were under 40 years whereas 89.2% were 40 years and above. Findings of this study conforms with the literature that OSCC is the disease of the elderly (Shafer et al. 1983, Daftary et al. 1992, Cawson et al. 1996, Bast et al. 2000), and it is rarely seen in young ages below 40 years. Results in other studies have established similar findings (Worrall 1995, Silverman et al. 1998, Lype et al. 2003, Sasaki et al. 2005,). The youngest patient in this study was 13 years old and the oldest was 85 years.

There was a slight gender bias in this study with male to female ratio of 1.3:1. This is in agreement with other studies from the African continent and elsewhere that also showed gender bias with male to female ratios ranging from 1.3:1 to 4.8:1 (Odukoya et al. 1986, Adebayo et al. 2001, Gervasio et al. 2001, Adewole et al. 2002, Neville and Day 2002). Globally high male to female ratio has been associated with high tendency of tobacco and alcohol consumption among males as compared to females (Pacella-Norman et al. 2000, Neville and Day 2002, Gepaola 2004). Analysis on the associated risk factors was beyond the scope of this study. It is recommended that such studies be carried out in order to assist in prevention strategies of this disease.

Most of the lesions in this study occurred on the tongue (25.2%). This concurs with findings from others (Chen et al. 1990, Mwakyoma et al. 1998, Neville and Day 2002, Sasaki et al. 2005). However, variation from some other studies was also observed. In Nigeria and Kenya mandibular gingiva has been documented by some authors to be the

commonest site (Odukoya et al. 1986, Ogunbodede et al. 1997, Maroo 1992). Variation of anatomical sites has been postulated to be associated with aetiological factors involved in that geographical location (Daftary et al. 1992, Cawson et. 1996). Other sites in this study were mandibular gingiva (22.5%), buccal mucosa (16.2%), palate (13.5%), lower lip (9.9%) and floor of mouth (9.0%) (Table 1). Smokeless tobacco use such as tobacco dipping and betel nut chewing has been shown elsewhere to contribute to the high rates of gingiva and buccal mucosa SCC (Daftary et al. 1992, Idrisa et al. 1995, Lype et al. 2001, Lo et al. 2003). Combined together, buccal mucosa and gingiva were the mostly affected sites and this might have been to certain extent be influenced by tobacco chewing and dipping.

OSCC is a fast growing tumor that initially starts as painless indolent lesion. As it advances in size it may present with disturbing symptoms. Foul smell is one among the symptoms that was observed in this study. Almost all patients seen at the clinic during the study (49 out 50 cases) had typical foul smell in the oral cavity. Whether the lesions were ulcerated or un-ulcerated, they presented with typical foul smell. The exact cause of this smell has not yet been established but this could be associated with the necrotic tissues and/or incapacitation to execute proper oral hygiene, and therefore resulting into foul smell in these patients. Other presenting symptom was pain which was found in 44 patients (88.0%) who had infiltrative ulcerated lesions, which were also infected. Pain in patients with OSCC is very likely to be due to secondary infection in the ulcerated tumors but also may result from invasion and destruction of the surrounding tissues by

the tumor. Pain is not only a cause of worry to patients but also a suffering and affects normal functions of the mouth. When OSCC occurs in close proximity to the teeth it may also cause erosion of supporting alveolar bone, looseness and sometimes exfoliation of teeth (Figure 3 pg 40). Pain and discomfort brought about by the advanced tumor are the major causes that prompt majority of patients to seek treatment. Such advanced tumors may cause fixation of tongue or may invade important structures like salivary glands or cause a fistula if the lip or cheek are involved (Figure 3 pg 40). Two patients with tumors involving base of the tongue and floor of the mouth presented with trismus.

Delay in reporting for diagnosis of OSCC was strongly observed in this study. Late reporting was shown by the duration of the tumors. Mean period of time that the lesion was noted before presenting for diagnosis was 12.5 ± 7.82 (SD) months with a range of 1 month to 5 years (Table 3). Majority of tumors (90.1%) were found to present between 4^{th} and 24^{th} months. In all anatomical location most tumors were reported late. This concurs with other studies showing late reporting of OSCC tumors ranging from 1-42 months and mean duration of 8.3-8.9 cm (Yazdi and Khalili 1993, Ogunbodede et al. 1997, Gervasio et al. 2001). Problems inherent to the country's cumbersome referral system, ignorance of patients and to certain extent poverty are very likely to be the main reasons for patients delay to report to referral hospital. Lack of facilities for definite diagnosis at primary health centers and district hospitals forces patients to receive non-curative symptomatic treatment for long time hence delaying proper diagnosis and

management for this disease. Such patients are referred to consultant hospitals only when treatment fails, and the disease is already advanced.

Delayed reporting for treatment in this study was also supported by the large size of tumors. Majority of the patients (81.1%) presented with large tumors as illustrated by the overall mean of 5.74 ± 1.84 (SD) cm. Both females and males and both young and old presented with large tumors. All sites even the easily accessible location such as lips presented with large tumors. The size of OSCC has been suggested to influence the prognosis (Cawson et al. 1996, Lo et al. 2003). However, some studies on survival rate of patients with OSCC have revealed that the size of tumor has influence on the outcome of the disease through lymph node involvement (Hibbert et al. 1983, Tankere et al. 2000, Ioona et al. 2002).

It has been shown that one of the effects of tumor size is the presence of metastatic disease in regional lymph nodes that is more common with large tumors than with small tumors (Hibbert et al. 1983, Tankere et al. 2000). This fact was also observed in this study. Majority (65.5%) of large tumors with more than 4 cm diameter had regional lymph node involvement and in majority (61.9%) of small tumors there was no associated lymphadenopathy. There was strong association of tumor size and lymph node involvement ($p=0.020530$). This is in agreement with the literature that the larger the tumor the more likelihood of lymph node involvement (Hibbert et al. 1983, Daftary et al. 1992). Several studies have shown that lymph node involvement is the single most important factor which determines prognosis of OSCC (Ioona et al. 2002, Greenberg et

al. 2003). Hibbert et al. (1983) and Stoeckli et al. (2003) reported that the presence of palpable nodes halves the survival rate of patients with OSCC. In the present series lymph node involvement was observed in majority of tumors of the tongue (75.0%), floor of mouth (70.0%), buccal mucosa (66.7%), and mandibular gingiva (64.0%). These sites with the exception of buccal mucosa are among the sites with unfavorable prognosis in the oral cavity proper (Bast et al. 2000), buccal mucosa has a favourable prognosis as compared to other sites in the oral cavity proper. The tumors of the palate and lower lip had relatively fewer lymph node involvements (33.3%, and 34.4%, respectively). This concurs with the literature that lymph node involvement is more likely to be encountered in majority of tumors of the tongue, floor of mouth, mandibular gingiva, and buccal mucosa ranging from 39-77% of cases (Daftary et al. 1992). Lip SCC has been reported to be slowly growing and has less tendency of lymph node metastases (2-10%) (Daftary et al. 1992, Cawson et al. 1996), a finding that was also observed in this study.

Majority of patients with lymph node involvement (60.3%) presented to hospital after 4 months from initial symptoms (Table 5). Surprisingly, there was one patient with a tumor of one month duration, but with lymph node involvement. This tumor was located on the floor of mouth and measured 5 cm in the greatest diameter. Assessment of the nodal status revealed N_2. This was a strange observation as there was a discrepancy between the clinical findings of the lesion and the reported length of duration. This might be due to the localization of the tumor that was not noticed by the

patient in its early stage or in few cases patients may have fear of telling the doctor the truth especially if they first sought treatment from traditional healers.

OSCC is an aggressive tumor with high metastatic potential (Worrall 1995). Clinically apparent distant metastases are relatively uncommon. In this study only one patient had distant metastases to the lungs out of 111 patients whose their information on distant metastasis was available. This concurs with O'Brien et al., (1971) who reported that only 4% of patients with OSCC died of distant metastases, however, during autopsy such deposits were found in about 50% of cases. With advanced diagnostic facilities such as ultrasound, CT scan and MRI imaging detection of distant metastases have improved and therefore metastasis became to be known as a major cause of deaths in OSCC (Worrall 1995). According to the literature available, lung is the commonest site of distant metastasis by OSCC (Shafer et al. 1983), although other sites may be involved such as liver, kidney, heart, and ovary (Cawson et. 1996, Kawamoto et al. 2001). Assessment of patient by several imaging modalities such as ultrasound, CT scan, MRI, and/or PET is essential to diagnose distant metastasis. In this study distant metastases were assessed using plain chest radiographs only. Assessment by plain radiographs is the most common and cheap diagnostic methods used in Tanzania, but the procedure is less sensitive and it is likely that some metastatic deposits especially those not of the lung were not detected. Lack of accessibility to the efficient and more sophisticated diagnostic facilities such as high-resolution ultrasonograph, computed tomography and magnetic resonance imaging would certainly continue to cause

underreporting of some metastatic deposits. Distant metastasis is among the factors, which have been shown to reduce the survival rate of patients with OSCC up to 21% (Neville and Day 2002, Lo et al. 2003).

Clinical stage of OSCC is the most prognostic marker used in the treatment plan of patients and in predicting the outcome of the disease (Allegra et al. 2000, Bast et al. 2000, Neville and Day 2002, Lo et al. 2003). Early stages of OSCC (I and II) are associated with good prognosis as compared with advanced stages (III and IV) which result into poor prognosis. In this study majority of tumors (88.3%) were in advanced stages (III and IV) despite of sex and anatomical location. Similar results have been reported by other studies in African continent and elsewhere. Majority of tumors in Africa and in black Americans were diagnosed at an advanced stage therefore increasing mortality rate of OSCC patients (Adebayo et al. 1986, Daftary et al. 1992, Ogunbodede et al. 1997, Kalenga and Kalengayi 1999, Neville and Day 2002,). General comparison of tumor stage with size showed that there was a direct relationship between the size and the stage of the tumor. The larger the tumor the advanced is the stage. This observation is obvious and expected, however in the present series few exceptions existed where small tumors (8 cases) were classified as advanced because of lymph node involvement and/or involvement of adjacent structures.

Recommended treatment of choice for patients with OSCC is surgery or radiotherapy with curative intent for stage I and II tumors, whereas for stage III and IV tumors the recommended treatment in our set up is radiotherapy and/or chemotherapy for palliative

intent. On the basis of treatment modalities at MNH, it can be concluded that most patients with OSCC do not fit for surgery or radiotherapy for curative intent. This is because the majority of these patients are diagnosed when the disease is at an advanced stage (III and IV) with poor prognosis and low five-year survival rate.

HISTOLOGICAL FEATURES

Histological grading is one among the prognostic parameters of oral squamous cell carcinoma (Anneroth et al. 1987, Bryne 1991, Cawson et al. 1996). It is considered virtually axiomatic that well differentiated tumors do better than poorly differentiated ones, therefore any staging procedure should logically take histological grading of OSCC into account (Cawson et al. 1996). However, the problem encountered in histological grading is that assessment of histological grade is largely subjective, and a small incisional biopsy may provide an unrepresentative area since the cell populations of tumors are frequently heterogenous (Yazdi and Khalili 1993, Cawson et al. 1996). For this reason, Broder's 1927 classification has been reported as unreliable (Yazdi and Khalili 1993), however a number of grading systems published since then testifies to the difficulties involved (Anneroth et al. 1987, Bryne 1989). In this study, lack of correlation of Broder's histological grades with other prognostic parameters was observed. Although majority of the tumors were well differentiated (77.5%) a finding in agreement with other studies (Daftary et al. 1992, Yazdi and Khalili 1993, Lype et al. 2001, Costa et al. 2002), 81.4% of the well-differentiated tumors were in stage IV. This

means that there is a possibility of a well-differentiated OSCC to behave aggressively without transforming its cells into poorly differentiated architecture. In such cases histological grading of tumor can be misleading in assessing its biological behaviour. Therefore, in attempt to assess the degree of aggressiveness, other microscopic variables such as intravascular invasion and invasive tumor front have been taken into account (Anneroth et al. 1987, Bettendorf et al. 2003). However, Anneroth and co-workers have recommended exclusion of vascular invasion because of difficulties in defining and recognizing it. Grading system which was modified from that proposed by Anneroth et al.(1987) and applied to the invasive areas of the 68 cases of OSCC strongly correlated with survival (Bryne 1989), and when compared with Broder's grading no correlation with survival was established between Broder's grading and survival. In this study all poorly differentiated tumors were in advanced stages (stage III and IV) (Fig 10), indicating that almost all poorly differentiated OSCC is likely to have poor prognosis. From the findings of this study it can be suggested that histological grading of OSCC is not specific in assessing biological aggressiveness of the tumor, therefore reliable supplementary indicators have to be applied for this purpose.

SILVER NUCLEOLAR ORGANIZER REGIONS

The AgNOR count has been correlated with biological behaviour of several tumors and therefore it has been established that high AgNOR count indicates high proliferative activity and advanced degree of malignancy (Sano et al. 1991, Vuhahula et al. 1995, de

Carmo and Silva 1998, Derenzini 2000). NOR proteins precede the synthesis of DNA. Increase of NOR proteins prior to the cell entering the mitotic cycle is related to some structural function on the nucleolar chromatin, perhaps in preparing ribosomal genes for duplication (Derenzini 2000). Silver staining binds quickly with rDNA sites active in transcription and NOR play crucial role in the nucleoli formation, directing synthesis of ribosomes and related proteins (RNA polymerase 1, C23-nucleolin, B23-protein) (Derenzini 2000). Neoplastic cells generally divide faster than the corresponding normal cells. Higher AgNOR has also been shown in cells of the breast cancer, which had mutant p53 and pRb (Derenzini et al. 2004).

In recent years, AgNOR determination has been considered to provide objective prognostic parameter to predict clinical behaviour of different neoplasms (Sano et al. 1991, Vuhahula et al. 1995, Kinoshita et al. 1996, Zergeroglu et al. 2001). Several studies on oral squamous cell carcinoma demonstrated correlation between AgNOR and other markers of cellular proliferation such as; DNA content, PCNA index and Ki 67 immunostaining (Costa Ade et al. 1999, de Rosa et al. 1999, Colleta et al. 2001). The potential prognostic value of AgNOR in oral squamous cell carcinoma has been considered for a long time and several authors since discovery of AgNOR have correlated survival rate and AgNOR count in OSCC (Sano et al. 1991, Xie et al. 1998). Other studies have provided the value of morphometric evaluation of AgNOR in the hyperplasia, papilloma, normal oral mucosa and OSCC (de Rosa et al. 1999). In this study the relationship between the survival outcome and AgNOR count was not done

because there was no information on the status of patients after diagnosis and/or treatment. Interestingly and strikingly, the present study established significant correlation between AgNOR count and lymph node involvement ($p=0.000005$). Ninety five percent of tumors with palpable lymph nodes in this study had high AgNOR count whereas only 47.7% of tumors without palpable lymph nodes had high AgNOR count. Since AgNOR count was high in almost all tumors with lymph node involvement, it is likely that the tumors with high AgNOR count but without lymph node involvement are likely to be aggressive ones as portrayed by NOR activity.

This study found a general trend towards increased AgNOR count in tumors with advanced clinical stage. The difference was statistically significant between stage II and IV ($p<0.05$). High AgNOR count was more likely to be found in stage IV. This is in consistency with other studies (Ferhan et al. 1997, Seiwerth et al. 2000). The highest count observed in stage III and IV corresponds with the expected poor prognosis. On the other hand, AgNOR count differed in tumors of the same clinical stage suggesting that tumors of the same stage may behave differently. In 46.2% of stage II, 85.7% of stage III and 78.0% of stage IV tumors had high AgNOR counts. This indicates that some stage II tumors, which are considered to have favorable prognosis, are actually biologically aggressive as assessed by AgNOR. Identifying such tumors with aggressive biological behaviour in their early stage can assist in the initial management plan and follow up of such patients.

The one tumor with metastases to the lungs had N_3 nodal involvement and high AgNOR count. AgNOR count correlated well with the biological behaviour of this tumor, thence substantiating the usefulness of AgNOR count in OSCC.

A general trend towards increased AgNOR count in association with higher histological grades, which has been reported in other tumors such as adenoid cystic carcinoma of salivary glands (Vuhahula et al. 1995) was also observed in this study. In the present study the mean AgNOR count of grade 1 tumors (6.54) and that of grade 3 tumors (8.34) was statistically significant ($p=0.049629$). This finding is in agreement with Ferhan and co-workers who reported similar observation in SCC of the larynx (Ferhan et al. 1997). However, other studies have not been able to correlate the AgNOR count and histological grade of OSCC (Sano et al. 1991, Linaena and Maria 2000). On the other hand this finding was also observed in this study. More than 73.3% of well-differentiated tumors had AgNOR count of more than 4.6. Most of these were aggressive tumors as evidenced by their large size, lymph node involvement and advanced clinical stage. Therefore, AgNOR method was able to detect all tumors with aggressive biological behaviour irrespective of histological grade. Certainly, in these cases AgNOR count was superior to histological grading.

This study has shown that about three quarters of oral squamous cell carcinoma (74.8%) had high AgNOR count, whereas 25.2% had low count. Majority of the tumors had high count irrespective of gender, age, size of lesion, duration or anatomical location of the

tumor. This indicates that OSCC as an entity is an aggressive tumor as portrayed by AgNOR assessment.

CONCLUSION

From the findings of this study it can be concluded that,

1. Oral squamous cell carcinoma is the disease of the elderly and rarely encountered in younger ages.

2. The tumor present at an advanced stage of disease as evidenced by delayed reporting (94.6%), large tumors with mean of 5.74 ± 1.84(SD) cm, lymph nodes involvement (60.4%) and advanced stage III and IV (88.3%).

3. Although most tumors are histologically well differentiated (77.5%), majority of them (88.4%) are in advanced stage. On the other hand all poorly differentiated tumors were in advanced stage (III and IV).

4. AgNOR count correlated well with some clinico-pathological prognostic parameters such as nodal status, clinical stage of disease and histologically grade 3 tumors. Therefore, AgNOR count showed a potential ability of identifying some aggressive tumors, which were well-differentiated and small tumors, which were aggressive. This indicates that AgNOR can be used as an additional prognostic indicator in OSCC.

RECOMMENDATIONS

From the results of this study it is recommended that:

1. Since the AgNOR method is simple, relatively cheap and affordable in our laboratory set-up, it is recommended that in order to precisely assess the biological behaviour of OSCC prior to treatment, AgNOR staining of an individual biopsy may be performed as a supplementary prognostic parameter for the purpose of evaluating the cellular proliferative activity. The AgNOR count will assist in portraying the biological behaviour of the tumor cells and therefore, be applied in the treatment plan of a patient with OSCC.
2. There is a need to carry studies on why patients present late in hospitals with advanced OSCC. The results of such studies will assist in the primary, secondary and tertiary management of OSCC.

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