DEVELOPMENT OF A RISK MODEL FOR ORAL SQUAMOUS CELL CARCINOMA - A PRELIMINARY STUDY

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DEVELOPMENT OF A RISK MODEL FOR ORAL SQUAMOUS CELL CARCINOMA - A PRELIMINARY STUDY

Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is a disease that has a global presence. According to the latest Global cancer incidence, mortality and prevalence (GLOBOCAN) data from 2018; there were 354,864 new cases of cancers of the lip and oral cavity with 177,384 deaths in the same year. The prognostic factors for OSCC include a variety of socio-demographic, clinical, and histopathological related factors. Over the years, there have been several risk models developed utilizing these various prognostic factors. However, previous studies have shown that these existing risks models are limited in their prognostic determination of patients with OSCC. **Aim:** To identify the socio-demographic, clinical and histopathological characteristics that may be utilized for the development of a preliminary risk model in OSCC. **Materials and Methods:** This study was conducted in a retrospective manner on patients who have been diagnosed with OSCC within the Malaysian Oral Cancer Database and Tissue Bank System (MOCDTBS). A total of 73 patients that met the inclusion criteria were utilized in this study. Socio-demographic and clinical data of the OSCC cases were obtained from the MOCDTBS. Histopathological data was retrieved from the archived hematoxylin and eosin stained slides from the MOCDTBS. Histopathological scoring was also calculated based on previous risk models. A univariate and multivariate analysis were then utilized to determine the association of these various socio-demographic, clinical, and histopathological characteristics with lymph node metastasis, local recurrence and overall survival. Characteristics that showed statistical significance ($p<0.05$); indicating prognostic potential, were identified. **Results:** Characteristics with prognostic potential in OSCC included extracapsular spread (ECS), depth of tumour

invasion (DOI), tumour budding, sarcolemmal spread, bone invasion, and tumour associated tissue eosinophilia (TATE). These factors were then included in a preliminary risk model for OSCC. **Conclusion:** There are significant associations between ECS, DOI, tumour budding, sarcolemmal spread, bone invasion and TATE with patient outcomes in OSCC, which when integrated within a preliminary risk model would potentially aid in the prognostic determination of these patients. However, it is recommended to use a larger sample size for further validation of these results.

Keywords: Oral squamous cell carcinoma, risk model, overall survival, lymph node metastasis, local recurrence.

MENJANA MODEL RISIKO UNTUK KARSINOMA SEL SKUAMUS MULUT – SUATU KAJIAN AWAL

Abstrak

Pengenalan: Karsinoma sel skuamus mulut (OSCC) adalah penyakit yang wujud secara global. Menurut data terkini Global Cancer Insidence, Mortality and (GLOBOCAN) untuk 2018; terdapat 354,864 kes baharu kanser bibir dan rongga mulut dengan 177,384 kematian dalam tahun yang sama. Faktor prognostik bagi OSCC termasuk faktor pelbagai berkaitan sosiodemografi, klinikal dan histopatologi. Sejak beberapa tahun, terdapat beberapa model risiko telah dibangunkan menggunakan faktor prognostik pelbagai ini. Walau bagaimanapun, kajian lepas menunjukkan bahawa model risiko sedia ada ini terhad dalam menentukan prognostik pesakit dengan OSCC. **Matlamat:** Untuk menjana model risiko untuk OSCC menggunakan ciri-ciri sosiodemografi, klinikal dan histopatologi. **Bahan dan Kaedah**: Kajian telah dijalankan secara retrospektif atas pesakit yang telah didiagnos dengan OSCC melalui pangkalan data Malaysian Oral Cancer Database and Tissue Bank System (MOCDTBS). Sejumlah 73 pesakit yang menepati ciri inklusif telah digunakan dalam kajian ini. Data sosiodemografi dan klinikal daripada kes OSCC telah diperoleh daripada MOCDTBS. Data histopatologi telah didapati daripada slaid pewarna hematoksilin dan eosin yang disimpan di MOCDTBS. Skor hispatologi juga dikira berdasarkan model risiko lepas. Analisis univariat dan multivariate kemudiannya digunakan untuk menentukan hubungan faktor-faktor sosodemografi, klinikal dan histopatologi dengan metastasis nodus limfa, perulangan tempatan dan survival keseluruhan. Faktor-faktor yang menunjukkan signifikan dari segi statistik ($p<0.05$); menunjukkan potensi prognostik, kemudiannya disepadukan ke dalam model risiko awal. **Dapatan:** Di antara faktor-faktor yang mempunyai potensi prognostik untuk OSCC termasuk penyebaran

ekstrakapsular (ECS), kedalaman serangan tumor (DOI), pertunasan tumor, penyebaran sarcolemmal, serangan tulang dan eosinofilia tisu berkaitan tumor (TATE). Faktor-faktor ini kemudiannya dimasukkan ke dalam model risiko awal untuk OSCC. **Kesimpulan:** Terdapat hubungan signifikan di antara ECS, DOI, pertunasan tumor, serangan tulang dan TATE dengan hasil pesakit untuk OSCC, apabila disepadukan dengan model risiko mungkin berpotensi membantu dalam menentukan prognosis pesakit-pesakit ini.

Kata kunci: Karsinoma sel skuamos mulut, model risiko, survival keseluruhan, metastasis nodus limfa, perulangan tempatan.

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OC	:	Oral cancer
OSCC	:	Oral squamous cell carcinoma
GLOBOCAN	:	Global cancer incidence, mortality and prevalence
MNCR	:	Malaysian National Cancer Registry
HNSCC	:	Head and neck squamous cell carcinoma
QOL	:	Quality of life
ECS	:	Extracapsular spread
POI	:	Pattern of invasion
wPOI	:	Worst pattern of invasion
DOI	:	Depth of tumour invasion
AJCC	:	American Joint Committee on Cancer
PNI	:	Perineural invasion
LVI	:	Lymphovascular invasion
LHR	:	Lymphocytic host response
TIL	:	Tumour infiltrative lymphocytes
HPV	:	Human Papilloma virus
TATE	:	Tumour associated tissue eosinophilia
UICC	:	International Union Against Cancer
ENE	:	Extranodal extension
SCC	:	Squamous cell carcinoma
TNM	:	Tumour, node, metastasis
cTNM	:	Clinical tumour, node, metastasis
pTNM	:	Pathological tumour, node, metastasis
DFS	:	Disease-free-survival

DSS	:	Disease-specific-survival
WHO	:	World Health Organization
MOCDTBS	:	Malaysian Oral Cancer Database and Tissue Bank System
OCRCC	:	Oral Cancer Research and Coordinating Centre

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Research Background

Oral cancer (OC) is a disease that has a global presence. According to the latest Global cancer incidence, mortality and prevalence (GLOBOCAN) data from 2018; there were 354,864 new cases of cancers of the lip and oral cavity (accounting for 2% of new cancer cases) with 177,384 deaths (accounting for 1.9% of cancer related deaths) in the same year. Cancer of the lip and oral cavity are the 18th most common from a total of 36 cancers (Bray et al., 2018). When compared with GLOBOCAN data from 2012 which reported an estimated 300,400 new cases of OC, with 145,500 deaths in the same year, (Torre et al., 2015) an increase in the incidence and death rate of OC is clear.

A variety of cancers may occur within the oral cavity, however oral squamous cell carcinoma (OSCC) accounts for more than 90% of OC (Neville & Day, 2002). Cancers of the lip and oral cavity are highly frequent in South Asian countries such as India and Sri Lanka, as well as the Pacific Islands (with Papua New Guinea having the highest incidence rate worldwide in both sexes) (Bray et al., 2018). OC is the leading cause of cancer death among men in India and Sri Lanka (Bray et al., 2018). In Malaysia, according to the Malaysian National Cancer Registry (MNCR) report (2007-2011), there were 1,863 reported cases of OC. OC is predominant in the Malaysian Indian population, registered as the 4th most common in females and 8th most common in males (Zainal & Nor Saleha, 2011).

It has been established that OC is a multifactorial disease, mostly related to habitual or lifestyle behaviour. These habits include tobacco use; either smoking or chewing, betel quid chewing, alcohol consumption, and low fruits and vegetables consumption (Zain, 2001). Other suggestive etiologic agents of OC include radiation and excessive exposure to sunlight; especially for lip cancer (Warnakulasuriya, 2009). It must also be

emphasized that carcinogenity is dose dependent and is magnified by multiple exposures (Petti, 2009).

Mainstream treatment modalities for OC have not changed much over the years, consisting primarily of surgery and radiotherapy with chemotherapy in some cases. The primary goal in the treatment of OC is to eliminate the cancer, preserve and restore form and function of the oral tissues, reduce the side effects of treatment and prevent the recurrence of the cancer (Shah & Gil, 2009). In recent years, the utilization of immunotherapy for head and neck squamous cell carcinoma (HNSCC) has shown promise in providing a better prognosis for patients, specifically with the use of monoclonal anti-bodies (Rancoule et al., 2016).

Worldwide, the 5-year survival rate for cancers of the oral cavity, tongue and oropharynx is 50% while the 5-year survival rate for cancers of the lip is significantly better at 90% (Warnakulasuriya, 2009). Meanwhile, the age adjusted death rate for OC for males is estimated at 3-4 per 100,000 and for females estimated at 1.5-2 per 100,000 (Warnakulasuriya, 2009). For patients who successfully undergo treatment for OC, the rather devastating side effects of treatment such as difficulty in eating, drinking, speaking, as well as problems with appearance; negatively affect the patient's quality of life (QOL).

With regards to OC, the prognostic factors encompass the patient related factors, tumour related factors and histopathological related factors. In the context of patient factors (Lo, Kao, Chi, Wong, & Chang, 2003); there was no significant correlation between 5-year survival rate and patient age or gender. A higher mortality rate was seen in patients that smoke or drink alcohol (de Cássia Braga Ribeiro et al, 2003). Betel quid chewing has also been shown to result in a poorer prognosis for patients undergoing treatment (Kao, Chi, Wong, & Chang, 2003). Patients that are from a lower socio-

economic status and education background have a less favourable prognosis compared to patients from a higher socio-economic status and education background (Leite & Koifman, 1998). Other factors such as comorbid conditions that include heart disease, pulmonary disease and renal disease negatively affect the prognosis of OC as well (Piccirillo et al, 2002).

Tumour related prognostic factors are closely related to the Tumour, Node, Metastasis (TNM) staging system, with a higher TNM staging indicating a poor prognosis. A study reported a higher 5-year survival (73.3%) of cases with TNM stage I and stage II compared to cases with TNM stage III and IV (41.8%) (Dissanayaka et al., 2012). A multivariate analysis also showed TNM staging to be a significant individual prognostic indicator of survival (Dissanayaka et al., 2012). When the anatomical site of OC is studied, more posteriorly located tumours in the oral cavity have a poorer prognosis compared to those more anteriorly located (Scully & Bagan, 2009). OC of the lip has a better prognosis, with a 5-year survival rate of 89.6% compared to other sites of the oral cavity (56%) (Carvalho et al, 2005). It must also be emphasized that cervical lymph node metastasis is widely accepted as an important prognostic factor for patients with OSCC (Shingaki et al., 2003).

Histopathological prognostic factors that have previously been established as prognostic indicators include extracapsular spread (ECS), histological differentiation, perineural invasion and angiogenesis (Massano et al, 2006). Other significant histological prognostic factors that have been mentioned include bone invasion, vascular invasion and pattern of invasion (POI) (Woolgar & Triantafyllou, 2009).

There exist multiple histological grading systems developed throughout the years, namely: Broder's classification (1920), Anneroth's multifactorial grading system (1987), Bryne's deep invasive margin model (1989), Bryne's deep invasive margin

model (1992), Brandwein-Gensler's histological risk model (2005), and the Tumour budding and depth of invasion grading (2015) (Almangush et al., 2015). These grading systems or risk models also included potential parameters such as depth of invasion (DOI), lymphocytic infiltration, number of mitosis, degree of keratinization and extent of nuclear pleomorphism.

In recent years, these various risk models have been studied with regards to their prognostic potential for patients with OSCC. The results have been mixed, with many of these risk models showing limited prognostic potential. However, the more recently developed risk models, which include Brandwein-Gensler's histological risk model and the Tumour budding and depth of invasion grading has shown some promise as a prognostic determinant for OSCC (de Castro Ribeiro Lindenblatt et al., 2012a; Santosa et al., 2014). In light of these findings, it proves that if well designed, a risk model does have potential in determining risk and prognosis of OSCC.

1.2 Aim and Objectives

1.2.1 Aim

To identify the socio-demographic, clinical and histopathological characteristics that may be utilized for the development of a preliminary risk model in OSCC.

1.2.2 Specific Objectives

i. To determine the socio-demographic, clinical and histopathological characteristics that serve as good prognostic indicators of lymph node metastasis.
ii. To determine the socio-demographic, clinical and histopathological characteristics that serve as good prognostic indicators of local recurrence.
iii. To determine the socio-demographic, clinical and histopathological characteristics that serve as good prognostic indicators of overall survival.

iv. To evaluate the prognostic potential of currently existing risk models for OSCC.

v. To develop a preliminary risk model for OSCC by selecting socio-demographic, clinical and histopathological characteristics that are good prognostic indicators.

1.3 Research Hypothesis

There are certain socio-demographic, clinical and histopathological characteristics that have significant prognostic potential in cases of OSCC.

CHAPTER 2: LITERATURE REVIEW

2.1 Socio-Demographic and Clinical Factors of OSCC

2.1.1 Demographics & Risk habits

According to the latest GLOBOCAN report in 2018, cancers of the lip and oral cavity for males, there were a total of 246,420 cases with a cumulative risk of 0.66% while for females there were a total of 108,444 cases with a cumulative risk of 0.26%. From these cases, a total of 119,693 cases resulted in death for males (cumulative risk of 0.32%) and a total of 57,691 cases resulted in death for females (cumulative risk of 0.14%). Based on this data, it appears that cancers of the lip and oral cavity are more common in males with higher risk of death. A possible explanation for higher rates in men could be due to the heavier indulgence in risk habits such as tobacco and alcohol use in males compared to females (Warnakulasuriya, 2009).

The risk of developing oral cancer appears to increase as a person ages, with most cases occurring above 50 years of age (Warnakulasuriya, 2009). However, in more recent studies, there appears to be a trend towards an increase in cases of OSCC of the tongue occurring in younger patients; below 45 years of age (Ng, Iyer, Tan, & Edgren, 2017).

Race also appears to have a role in OSCC, with Indians having a higher incidence of OSCC in the Malaysian population (Zainal & Nor Saleha, 2011). Similarly, in the United States, it was shown that Blacks had a lower survival rate of oral and oropharyngeal cancers compared to other racial groups (Osazuwa-Peters, Massa, Christopher, Walker, & Varvares, 2016).

There appears to be an increased risk of OSCC for patients having first degree relatives with head and neck cancer history (Negri et al., 2009; Radoi et al., 2013). The risk of OSCC increases with the number of affected relatives (Garavello et al., 2008;

Radoi et al., 2013)However, no significant association was seen between risk of OSCC with non-head and neck cancers (Radoi et al., 2013)

Use of tobacco products, alcohol intake, and betel quid usage are all established risk factors for OSCC (Warnakulasuriya, 2009). These factors have the potential to act synergistically if more than one risk factor is present in a single patient, with heavy drinkers and smokers having an increased risk of developing OSCC. Heavy drinkers and smokers have 38 times the risk of developing OSCC compared to those that abstain from both of these products (Warnakulasuriya, 2009). Similar results are also seen when smokeless tobacco products are used with alcohol which significantly increases the risk of developing OSCC (Azhar et al., 2018). The carcinogenic components within these products such as tobacco specific nitrosamines which are found in tobacco products or methylating agents which are found in betel quid cause DNA mutation and damage, predisposing patients to OSCC development.

2.1.2 Site

Location or site of the tumour is an important prognosticator of OSCC. Within the oral cavity, the various sites such as the buccal mucosa, tongue, lips and palate each have their own variations in tissue histology, blood and lymphatic supply. These variations have an influence on the prognosis of OC. Common sites for OSCC include the tongue, buccal mucosa and floor of mouth; with the tongue being the most common site of OC (De Camargo Cancela et al., 2010; Müller, Pan, Li, & Chi, 2008). It has been established that the 5-year survival rate of more posteriorly located tumours is less compared to those that are more anteriorly found in the mouth. This is most likely due to the tumour site influence on nodal metastasis. Nodal metastasis was reported in 59-64% tumour cases involving the tongue, retromolar and oropharynx area, compared to tumours of the buccal mucosa, where nodal metastasis was reported in only 22% of

cases (Woolgar, 2006) which can be explained by the rich lymphatic and blood supply found at the floor of the mouth (in close relation to the tongue) compared to the buccal mucosa. Similarly, another study showed that increased cases of advanced stages of carcinoma were more commonly found in intraoral sites compared to the lips (extraoral) (Santosa et al., 2014). Another reason that more anteriorly placed tumours have better prognosis compared to posterior located ones could be that they are more readily visible for early detection, allowing prompt and early intervention of these cases. It is also worth noting that tumours occurring on the buccal mucosa tend to be well differentiated, whereas those involving the tongue, palate and floor of mouth tend to be poorly differentiated (Rai & Ahmed, 2016).

2.1.3 TNM Staging

Pierre Denoix developed the TNM classification of tumours between 1943 and 1952. Since then, over the years there have been further modifications and amendments, with both the International Union Against Cancer (UICC) and AJCC adapting the system. The TNM classification denotes the size and extent of the tumour (T), the presence or absence of tumour in regional lymph nodes (N) and the presence or absence of tumour beyond regional lymph nodes/ distant metastasis (M). Based on these T, N and M values, a stage grouping is provided ranging from stage I to IV. Currently the latest edition of TNM, the 8th edition was published in 2016. Compared to the 7th edition, the changes included were the incorporation of the DOI within the T component and extranodal extension (ENE) of tumour metastasis of lymph nodes within the N component. Incorporation of both DOI and ENE in the 8th edition resulted in the upstaging of 10.0% of OSCC. The most significant upstaging was seen in stages II and III due to DOI up staging deeply invasive tumors as well as severe ENE upstaging other tumours to stage IVB. The 8th edition staging system provides marginally improved prognostication with a mild increase in the survival concordance index from 0.699 in

the 7th edition to 0.704 in the 8th edition, which is supported by changes in the Kaplan-Meier survival curves (Cramer, Reddy, Ferris, Duvvuri, & Samant, 2018).

The two types of TNM widely used in practice are the clinical TNM (cTNM) and the pathological TNM (pTNM). Over the years, it has been shown that in early stage OSCC, cTNM values have very limited prognostic abilities (Yuen et al., 2002) cTNM values have also been compared to pTNM values in a study involving 392 OC patients, showing that pTNM had a higher prognostic superiority when compared to cTNM (Hellmich et al., 2016) However, it is worth noting that stage grouping agreement between cTNM and pTNM was 86.6% (from 245 cases) (Jerjes et al., 2012). Similar results were seen in another study with 82.5% (from 252 cases) of stage grouping agreement between cTNM and pTNM (Choi et al., 2017). In both studies, the cases which showed difference in stage grouping were due to the underestimation during cTNM grading of tumour size and nodal involvement.

2.2 Histopathological Factors of OSCC

2.2.1 Depth of Invasion

The lack of precise definition, measurement technique and consensus has led to the usage of the two terms depth of invasion (DOI) and tumour thickness interchangeably by pathologist. However, DOI and tumour thickness are separate entities with DOI considered the extent of invasion below the epithelial basement membrane and tumour thickness on the other hand, refers to the entire tumour mass (Moore, Kuhns, & Greenberg, 1986). It has been reported that there was better correlation with patient survival when measured from the line of a ‘‘basement membrane constructed through the tumor’’ (i.e., DOI) compared with the entire thickness of the exophytic tumor (tumour thickness) (Moore et al., 1986).

Higher DOI was linked to perineural invasion (Rahima, Shingaki, Nagata, & Saito, 2004), lymphovascular invasion (Masood, Farquhar, Vanleer, Patel, & Hackman, 2018), local recurrence (Ghazi, Ghazi, Shafiee, & Fayyazi, 2018; Rahima et al., 2004), lymph node involvement (Ghazi et al., 2018) and poor survival (Ghazi et al., 2018; Rahima et al., 2004) in OSCC. The possible explanation for the correlation of DOI and lymph node involvement could be due to the higher possibility of tumour invasion into the lymphatic vessels as the tumour invades deeper into the tissue and comes in close proximity to larger lymphatic vessels, thereby facilitating the metastasis process. Increasing DOI, particularly > 10mm, there is also a significantly increased risk of occult nodal metastasis (53%) and decrease in 5-year survival to 45% (Faisal et al., 2018). It is also worth noting that in OSCC of the tongue with DOI< 4mm had a better 5-year overall survival (68.8 vs. 41.6%, p=0.012), disease-specific survival (67.1 vs. 41.1%, p=0.026) and local recurrence-free survival (89.5 vs. 65.4%, p = 0.035) compared to patients that had a DOI≥ 4mm (Tan, Chia, Tan, Soo, & Iyer, 2013). Considering these pertinent prognostic implications of DOI, it has recently been included in the T staging criteria for the American Joint Committee on Cancer (AJCC) 8th edition (TNM staging) of oral cavity cancers, whereby T1 is <5mm, T2 is between >5mm and <10mm, and T3 is >10mm.

2.2.2 Perineural Invasion

Among the various histological parameters used to predict the outcome of OSCC, perineural invasion (PNI) is in wide use as an indicator of aggressive behavior. PNI in head and neck cancer was first recognized by Cruveilheir in 1835, however even till today; the exact mechanism of PNI is still poorly understood. The incidence of PNI in OSCC varies from 25.7% to 40.5% (Rahima et al., 2004; Varsha et al., 2015). Historically, PNI was defined as tumor cell invasion in, around and through the nerves (Anneroth, Batsakis, & Luna, 1987). However, the most widely accepted and referenced

description of PNI is the presence of tumors seen in close proximity to a nerve that involves one-third (33%) of its circumference or the presence of tumor cells within any of the three layers of the nerve sheath (Liebig, Ayala, Wilks, Berger, & Albo, 2009). It is also defined as the presence of malignant cells in the perineural space with total or near-total circumferential involvement of the nerve in tangential histopathological sections (Dunn, Morgan, & Beer, 2009). These definitions however are lacking, as they do not differentiate between 'perineural' invasion (presence of tumor cells in and around the perineural space without infiltration of the nerve fascicle) and 'intraneural' invasion (penetration of tumor cells within the nerve itself), differences that could affect prognosis. Another important feature that may affect prognosis which requires investigation is the location of the PNI, either occurring at the tumour center or at the tumour invasive front.

PNI is one of the independent prognostic factors that are associated with an increased risk of lymph nodes metastasis (53.6% with PNI and 15.1% without PNI) (Rahima et al., 2004). The same study also showed that there is a correlation between PNI and regional recurrence of OSCC (Rahima et al., 2004). There was an increased disease specific mortality for patients with PNI (54%) when compared to without PNI (25%) (Johnson et al., 1998). Similarly, the 5-year survival rates for patients with PNI were 56.6% when compared to without PNI (94.6%) (Rahima et al., 2004). Similar results showed a reduced overall survival for patients with PNI (Nair et al., 2018). It is also worth noting that prognosis worsens when major or large nerves (>1mm) are involved compared to smaller nerves (≤1mm) (Woolgar, 2006). Although PNI represents a third mode of tumor metastasis besides lymphatic and blood vessel invasion, it has not been well studied. More work, especially in understanding the underlying mechanisms of this phenomenon will therefore be required.

2.2.3 Lymphovascular Invasion

Tumour invasion of blood and lymphatic vessels has long been suggested as an important pathological element in OSCC. The first studies to link vascular invasion to aggressive tumour characteristics of SCC of the head and neck was by Poleksic in 1978. Lymphovascular invasion (LVI) is defined as the presence of aggregates of tumour cells within endothelial lined channels or invasion of the media of a vessel with ulceration of the intima (Woolgar, 2006). The presence of LVI indicates that the tumour cells are invading the vascular compartment, which provides a route for these cells to potentially metastasize to other distant sites of the body. The incidence of LVI in OSCC varies from 15% to 35% (Jones et al., 2009; Liu et al., 2017; Rahima et al., 2004). LVI has been demonstrated to be a good prognostic tool. LVI correlates with higher cervical metastasis rate (Close, Brown, Vuitch, Reisch, & Schaefer, 1989; Sutton, Brown, Rogers, Vaughan, & Woolgar, 2003), associated with recurrence (Sutton et al., 2003) and with low/poor survival rate (Jones et al., 2009; Liu et al., 2017). However, other studies have shown dissimilar results regarding LVI where it has not demonstrated to be an independent predictor of overall survival (Adel et al., 2015; Jardim, Francisco, Gondak, Damascena, & Kowalski, 2015). Therefore, more studies are required to ascertain the prognostic value of LVI in OSCC, especially in terms of patient survival.

2.2.4 Lymphocytic Host Response

The lymphocytic host response (LHR) was first utilized by (Brandwein-Gensler et al 2005) in their histological risk model for OSCC as one of the studied prognostic indicators. In this model, LHR was histologically quantified as the density of lymphocytes at the tumour interface. It was classified as strong, intermediate or weak. LHR was investigated as a potential prognostic factor due to the concept of immune surveillance and adaptive immunity which have protective impact factors for cancer patients possibly due to the anti-tumour effects of inflammatory cells mediated by

cytokines secretion induced by the response of these various inflammatory cells to tumour stimulation (Chatzistamou et al., 2010)

An inverse correlation between LHR and local recurrence was reported as an independent prognostic factor. The study showed that a weak LHR at the tumour interface is strongly associated with local recurrence (p=0.005) and death (p=0.001) (Brandwein-Gensler et al., 2005). Similar more recent study showed similar trends, with LHR having a significant association with locoregional recurrence (p=0.0297) and disease specific survival (p= 0.0269) (Li et al., 2013). It has also been shown that tumours with a moderate or weak LHR showed acceleration in disease when compared to tumours with a strong LHR (Kolokythas, Park, Schlieve, Pytynia, & Cox, 2015). It has also been demonstrated that most cases with a strong LHR did not have a recurrence episode (Lundqvist, Stenlund, Laurell, & Nylander, 2012).

2.2.5 Tumour Infiltrative Lymphocytes

The initial mention and description of tumour infiltrative lymphocytes (TIL) appeared in literature over 40 years ago. Over the years, there has been emerging evidence to support the positive role of TIL in OSCC prognosis. TIL are considered an immune biomarker which indicates the ability of the immune system to eliminate tumour cells. Therefore, cases with higher TIL levels are better able to mount an immune response and disrupt tumour invasion and provide a better prognosis for patient survival. To date, no meta–analysis of the prognostic value of TIL in OSCC has been carried out, however such studies have been carried out for both lung cancer and breast cancer; showing that patients with higher TIL values had more favourable outcomes. In relation to this, patients with higher TIL levels were found to have a better prognosis with more favourable outcomes in disease specific survival and disease-free survival (Xu, Wang, Yuan, Feng, & Han, 2017). Similar results were also produced in a 2016

study; showing better overall survival, relapse free survival, and disease specific survival (Nguyen et al., 2016). A high TIL level, (specifically CD3 or CD8 expression) was considered an independent factor for favourable overall survival, local progression-free survival, and distant metastases-free survival (Balermpas et al., 2016). It has also been shown that a correlation is present between lymph node extracapsular spread and TIL, whereby patients with higher TIL levels showed less possibility of lymph node extracapsular spread compared to those with lower TIL levels (Xu et al., 2017). It is also worth noting that in cases of Human Papilloma Virus (HPV) positive tumours, patients with high TIL levels showed improved survival compared to patients with low levels of TIL (King, Ottensmeier, & Thomas, 2014). However at this juncture, as existing studies lack standardization, have small sample sizes and have rather short follow up periods; definitive evidence on the effect of TIL on OSCC prognosis is still debatable and deemed inconclusive.

2.2.6 Tumour Associated Tissue Eosinophilia

Tumour associated tissue eosinophilia (TATE) was first described in 1896 by Przewoski as seen in carcinoma of the cervix. TATE was characterized by the presence of eosinophils as a component of peritumoral and intratumoral inflammatory infiltrate. Since then, over the years, the presence and prognostic implications of TATE in malignancies of various sites including the colon, skin, nasopharynx, esophagus, and oral cavity have been studied. Eosinophils are hypothesized to have tumouricidal activity associated with the release of cytotoxic proteins and facilitate penetration of tumour killing cytokines into tumour cells. However, it also appears that eosinophils may promote tumour angiogenesis by producing angiogenic factors. (Pereira et al 2011). With regards to the presence of TATE and OSCC, there appears to be mixed results with some research showing a favourable prognosis, whilst others indicate an unfavourable prognosis. In some studies, there even appears to be no influence of TATE

on patients' outcome. TATE had a good prognostic role with higher eosinophilic counts seen in non-metastatic OSCC compared to metastatic OSCC (Jain et al., 2014). Intense TATE were associated with 72% of 5-year disease free cumulative survival whereas only 44% were associated with moderate TATE and 32% with absent or mild TATE (Landman et al., 2003). However another study demonstrated that patients with high TATE counts had a significantly lower survival than those with lower TATE counts (Alrawi et al., 2005). On the other hand, another study concluded that TATE had no correlation with prognostic parameter in OSCC (Tadbir, Ashraf, & Sardari, 2009). Therefore, considering these mixed results in relation with regards to TATE and OSCC, further studies are required to further elucidate the exact nature of TATE and its effect on patient's prognosis.

2.2.7 Pattern of Invasion

Pattern of invasion (POI) refers to the nature in which the tumour cells invade the underlying tissue at the tumour-host interface or also known as the tumour invasive front. The concept of pattern of invasion (type I to IV) was initially described by Anneroth in 1987. Type I and II POI were further sub-classified as cohesive type of POI whereas type III and IV were sub-classified as non-cohesive type of POI. Over the years, POI has been a commonly reported feature in histological reports, modified and included in various risk models for OSCC.

POI showed prognostic implications in relation to lymph node metastasis with higher levels of POI having greater tendency for lymph node metastasis (De Silva, Siriwardena, Samaranayaka, Abeyasinghe, & Tilakaratne, 2018). POI was also demonstrated to have significant association with local recurrence and overall survival, with the higher levels of POI having worse prognosis (Dissanayaka et al., 2012). The

POI reflects cellular cohesion and is the single most important feature as well as having predictive value in the clinical setting (Woolgar, 2006).

In 2005, Brandwein-Gensler introduced the concept of worst pattern of invasion (wPOI) by including a type V category into the existing POI classification, which consisted of type I to IV. Furthermore, type I, II and III were grouped as non-aggressive POI whereas type IV and V were grouped as aggressive POI. A number of studies on wPOI demonstrated that it was associated with local recurrence and overall survival (A. Almangush et al., 2015; Brandwein-Gensler et al., 2005; Li et al., 2013). wPOI was also shown to be significantly predictive for disease specific survival (Li et al., 2013).

2.2.8 Tumour Budding

Tumour budding has been demonstrated to be a good prognostic marker for colorectal carcinoma (Lugli, Karamitopoulou, & Zlobec, 2012; Prall, 2007), esophageal carcinoma (Brown et al., 2010) and breast carcinoma (Liang et al., 2013). The presence of tumour budding represents two malignant features: cellular discohesion and active invasion; therefore, the presence of tumor buds has been considered to be characteristic of an aggressive cancer (Wang et al., 2011).

Among the earliest to study tumour budding within the oral cavity was Wang in 2011; where prognosis of tumour budding in SCC of the tongue was studied. In this study, the 5-year survival was significantly reduced in patients exhibiting high-intensity tumor budding (≥5 tumour buds) compared with patients with low-intensity budding (<5 tumour buds), with the difference in survival rates based on a Kaplan-Meier analysis being statistically significant (p<0.001). Tumour budding was also shown to be strongly associated with lymph node metastasis (p=0.001) and overall survival (p=0.002) (Hong et al., 2018).

Subsequently, tumour budding was utilized in the 'BD model'; a novel prognostic model for early stage OSCC consisting of tumour budding and depth of tumour invasion (Almangush et al., 2015). Following this, a meta-analysis of tumour budding in OSCC showed that tumour budding was significantly associated with lymph node metastasis, disease free survival and overall survival (Almangush et al., 2018)

Considering the simplicity and reproducibility of the application of the tumour budding index without the need for additional cost demanding techniques, it shows great promising benefits as a prognostic indicator for patients with OSCC.

2.2.9 Mitosis

Reporting of mitotic index in histopathological reports of OSCC is commonly done over the years as a standard prognosticator factor of OSCC. Mitotic index has been demonstrated to be a prognostic factor for cancer; for instance in hepatocellular carcinoma (Ha, Choi, Lee, & Park, 2016) and cutaneous melanoma (Azzola et al., 2003)

Among the earliest risk models for OSCC to include the mitotic index was Anneroths multifactorial grading system in 1987 and Brynes deep invasive margin model in 1989. In both grading systems, cases with larger mitotic counts were given a poor prognosis. In later studies, the mitotic index in OSCC was significantly associated with recurrence and death (p=0.008) (Natarajan, Mahajan, Boaz, & George, 2012). A higher mitotic index was also noted in older patients (>50 years) as well as a correlation between higher mitotic index and lymph node metastasis was also demonstrated (Siriwardena et al., 2007).

2.2.10 Differentiation

The degree of differentiation in OSCC is often categorized into three; well, moderate and poor. The presence or lack thereof of certain cellular or architectural features within the tumour determines the overall grading given.

A Kaplan-Meier analysis showed that poorly differentiated tumours exhibited lower survival rates (28%) compared to moderately differentiated (54%) and well differentiated (81%) (Padma, Kalaivani, Sundaresan, & Sathish, 2017). In another study, the 5-year survival rate for well differentiated (94.7%), moderately differentiated (57.1%) and poorly differentiated (25.0%); showed a worse prognosis from well to poorly differentiated tumours (Geum et al., 2013).

A significant association between recurrence and lymph node metastasis with degree of differentiation was demonstrated (Padma et al., 2017). Similarly, an association between lymph node metastasis and degree of tumour differentiation was also demonstrated (Haksever et al., 2012). A higher incidence of extracapsular spread was also significantly associated (p<0.001) with degree of tumour differentiation (Siriwardena et al., 2018). However, in contrast, one study has demonstrated no significant correlation between the degree of tumour differentiation and prognosis of OSCC (Oliveira et al., 2008).

2.2.11 Keratinization

The degree of keratinization is an often-reported histological feature in cases of OSCC of the head and neck region, over the years it has shown to have potential as a prognostic factor for OSCC. Among recent studies, it has been demonstrated that the 5-year disease-free-survival (DFS) was significantly decreased for low keratinized cases of OSCC (52.9%) compared to highly keratinized cases of OSCC (93.2%) (p=0.0008) (Wolfer, Elstner, & Schultze-Mosgau, 2018). Similarly, the 5-year disease-specific-

survival (DSS) was reduced to 66.1% (p=0.0136) for low keratinized cases of OSCC (Wolfer et al., 2018). A multivariate analysis also demonstrated that degree of keratinization (p=0.002) is an independent, significant prognostic factor for recurrence in OSCC with more recurrences being observed amongst patients with low keratinized OSCC (p=0.0008) (Wolfer et al., 2018).

Another study showed that there was a significant association between the degree of keratinization and lymph node metastasis, with cases of OSCC that showed minimal keratinization having significantly higher lymph node metastasis compared with cases of OSCC that showed a high degree of keratinization (Dissanayaka et al., 2012). Keratinization also significantly affected the possibility of distant metastasis with cases of low keratinization showing higher rates of distant metastasis compared to cases that are highly keratinized (Yasui, Okada, Mataga, & Katagiri, 2010).

2.2.12 Extracapsular Spread

The first description of extracapsular spread (ECS) in cervical lymph nodes was by Willis in 1930. ECS is defined as expansion of the tumour beyond the lymph node capsule. A carcinoma that is able to infiltrate and spread beyond the lymph node capsule, exhibits characteristics of a more aggressive disease rather than as a feature of late presentation (Matsumoto et al., 2017). In recent years, the prognostic implications of ECS has been evaluated by numerous studies, the majority of which has demonstrated the significance of ECS as a prognostic factor for OSCC, resulting in its inclusion in the 8th edition of the AJCC TNM staging.

The presence of ECS indicates a poor prognosis, with the 5-year disease-free survival, disease-specific survival, and overall survival rates at 30.6%, 28.3%, and 14.3 % respectively in the ECS positive group, compared to 61.9%, 61.9% and 48.2% respectively in the ECS negative group (Suton, Salaric, Granic, Mueller, & Luksic,

2017). Similar results were also seen in an earlier study, where the 5-year adjusted survival for patients without metastatic nodes was at 85.5%, for patients with neck node metastasis without ECS it was at 62.5% and for patients with neck node metastasis with ECS it was at 29.9% (de Juan et al., 2013).

The incidence of local (p=0.011) and regional (p=0.008) recurrence was significantly greater in ECS positive cases compared to ECS negative cases. The time to recurrence was also found to be significantly shorter in ECS positive cases (Shaw et al., 2010; Suton et al., 2017). The presence of ECS also appears to double the incidence of local recurrence and distant metastasis in OSCC patients (Shaw et al 2010).

2.2.13 Tumour Thickness

The size of the primary tumour affects both the choice and outcome of treatment as it is important in determining the surgeon's ability to achieve tumour free margins and in patients requiring radiotherapy, the dose required for effective treatment. An optical micrometer was most often used to measure maximum tumor thickness, with various measurement techniques, depending on the chosen starting point of the mucosal surface, the tumor surface, or the ulcer base (Pentenero, Gandolfo, & Carrozzo, 2005).

A meta-analysis of 16 studies with a total of 1136 pooled patients demonstrated a positive correlation between tumour thickness and lymph node metastasis, with increased tumour thickness having increased risk of lymph node metastasis (Huang, Hwang, Lockwood, Goldstein, & O'Sullivan, 2009). Over the years, a number of studies demonstrated similar positive correlation between tumour thickness and lymph node metastasis (Jang et al 2016, Khan et al 2017, Punhani et al 2017).

It is worth noting that a tumour thickness greater than 7mm is predictive of a higher incidence of lymph node metastasis (odds ratio= 8.7, p=0.002) where as a tumour

thickness greater than 10mm is predictive of worse disease free survival (hazard ratio=12.2, p=0.003) (Matos et al., 2014).

A multivariate analysis showed that tumour thickness had direct influence on survival. Patients with tumour thickness of ≤3mm had a 5-year survival of 85.7%, (p<0.05) significantly greater than the rates of 58.3% and 57% for patients with tumour thickness of 4–7mm and >7mm, respectively (Gonzalez-Moles, Esteban, Rodriguez-Archilla, Ruiz-Avila, & Gonzalez-Moles, 2002).

2.2.14 Margins

In patients with OSCC, proper surgical resection of tumours with clear surgical margins is an integral component of treatment. Although there is some debate on what actually constitutes a "clear" margin, most centers adhere to the guidelines issued by the UK Royal College of Pathologist in 1998; with a clear margin being >5mm, close being 1-5mm and involved being <1mm.

Patients with positive surgical margins had a 2.5-fold increased risk of death at 5 years and those with a close margin (≤3mm) had a 1.5-fold increased risk of death at 5 years when compared to patients with margins more than 3mm (Nason, Binahmed, Pathak, Abdoh, & Sándor, 2009). Interesting to note was that each 1mm increase of clear surgical margin decreased the risk of death at 5 years by 8% (Nason et al., 2009). At 5 years, only 11% of patients with involved margins were alive, compared to 47% with close margins, and 78% with clear margins (Woolgar, 2006). The relative risk of local recurrence in patients with an involved margin was 7.89 and in patients with a close margin (<5mm) it was 3.79 (Kurita et al., 2010).

The presence of moderate or severe epithelial dysplasia at the surgical margin reduces the diseases free survival, whereas, mild epithelial dysplasia had no similar

effect (Sopka et al., 2013). A multivariate analysis of cases with mild or moderate epithelial dysplasia at the surgical margins revealed that moderate epithelial dysplasia at the margin was a significant independent factor for survival (Gokavarapu et al., 2017). Local recurrence was only observed in cases with severe epithelial dysplasia at the margin compared to mild or moderate epithelial dysplasia (Kurita et al., 2010).

2.2.15 Bone Invasion

In patients with OSCC, extensive tumours may invade the maxilla, mandible or other hard tissue of the orofacial region due to the close proximity with these structures. In previous studies related to bone invasion, bone invasion was present in 62% of cases (Shaw et al., 2004) and in 32% of cases (Fives et al., 2017) of patients with OSCC.

Previous studies on bone invasion showed that bone invasion was associated with recurrence and disease specific survival (Shaw et al., 2004). More recent studies also demonstrated that patients having tumours with bone involvement had significantly worse overall survival (p=0.0005) and increased incidence of local recurrence (p=0.004) (Fives et al., 2017). In TNM staging (7^{th} and 8^{th} edition), the poor prognosis of cases with bone invasion is evident, as these cases are upstaged to T4 in the TNM staging when bone involvement is present. These factors would therefore indicate that cases of OSCC with the presence of bone invasion would show a poorer prognosis.

2.3 Risk models

Over the years, there have been a number of risk models for OSCC, which were primarily based on various histological parameters. Examples of these risk models include: Broder's classification (1920), Anneroth's multifactorial grading system (1987), Bryne's deep invasive margin model (1989), Bryne's deep invasive margin model (1992), Brandwein-Gensler's histological risk model (2005), and the Tumour budding and depth invasion grading (2015).

Broders classification was developed in 1920 and was even utilized by the World Health Organization (WHO) as a grading system for OSCC for a time. However, research has demonstrated a lack of correlation between Broder's classification and patients prognosis (Anneroth et al., 1987), one of the main reasons being that OSCC tends to exhibit a heterogeneous cell population with differences in the degree of differentiation, resulting in these cells having different potential for invasion and metastasis. Similarly, in more recent studies involving Broder's classification, no prognostic value was demonstrated by the classification (Bhargava, Saigal, & Chalishazar, 2010).

This resulted in the development of other more significant histological risk models such as Anneroth's system which utilized a multifactorial histological risk model. Research has shown that Anneroth's system has a positive predictive significance for lymph node metastasis (Okada, 2010). However, Anneroth's system was claimed to have less reproducibility compared to other later developed histological risk models, such as Brynes model (Larsen, Johansen, SØrensen, & Krogdahl, 2009). There have also been studies showing both Broders classification and Anneroths's system of not having any significant predictive value for outcome measures such as regional metastasis, local recurrence and 5-year survival and neither system was superior to the other (Weijers, Snow, Dick Bezemer, & Van Der Waal, 2009).

Bryne subsequently introduced a histological grading system which was applied specifically to the tumour invasive front. Bryne's model (1989) is predictive of overall survival in patients with OSCC showing significant prognostic indication (Dissanayake, 2017; Sawairz et al., 2003). Similarly, Bryne's model (1992), which omitted the mitosis parameter also showed high prognostic value for patients with OSCC (Kurokawa et al., 2005). Other studies also showed that Bryne's model showed significant relation with

lymph node metastasis, while Broder's classification and Anneroth's system failed to show any relation with metastasis (Neena, Siddharth A, Keyuri, Munira, & Doshi, 2011; Yazdi I., 2013)

This was later followed by Brandwein-Gensler whom introduced a histological risk model in 2005, which was also applied to the invasive front as Bryne's model, however with some differences in the parameters evaluated. Brandwein-Gensler also introduced the worst pattern of invasion, which included type V pattern of invasion. An initial Kaplan-Meier survival analysis demonstrated that the Brandwein-Gensler model was predictive of both overall survival and local recurrence (Brandwein-Gensler et al., 2005). Further research demonstrated the significance of the Brandwein-Gensler model in predicting outcome for patients with low-stage (stage I & II) OSCC (Li et al., 2013) as well as regional lymph node metastasis (p=0.017) (Santosa et al., 2014)

In 2012, a study was conducted comparing Broder's classification, Anneroth's multifactorial grading system, Bryne's deep invasive margin model (1992) and Brandwein-Gensler's model. Among the four, Brandwein-Gensler's model showed the best statistical results, demonstrating associations with overall survival (p=0.015), DSS (p=0.029) and DFS (p=0.037). Therefore suggesting that Brandwein-Gensler's model is the best method to predict OSCC behavior and prognosis (de Castro Ribeiro Lindenblatt et al., 2012). However, there was another study that did not identify any significant correlation between the Brandwein-Gensler model and disease progression or outcomes (Kolokythas et al., 2015).

The tumour budding and depth invasion grading was developed by Almangush in 2015. In a multivariate analysis, a strong relationship was demonstrated for the model in relation with loco-regional recurrence (p=0.033) and with DSS (p<0.001) (Almangush et al., 2015). In a separate study, the tumour budding and depth invasion grading in a

univariate analysis, was significantly correlated with DSS (p=0.009) and DFS (p=0.005) where as Bryne's deep invasive margin model (1992), Broder's classification and Anneroth's multifactorial grading system did not show similar results (Sawazaki-Calone et al., 2015)

CHAPTER 3: MATERIALS AND METHODS

3.1 Ethics Approval

Ethics approval was obtained from the Medical Ethics Committee, Faculty of Dentistry, University of Malaya, with reference number: DFOS1713/0036(P).

3.2 Study Design and Sample Population

The study was conducted in a retrospective manner from patients whom have been diagnosed with OSCC within the Malaysian Oral Cancer Database and Tissue Bank System (MOCDTBS), maintained by the Oral Cancer Research and Coordinating Centre (OCRCC), Faculty of Dentistry, University of Malaya.

3.3 Sample Size Calculation

Power and sample size calculation software was used to calculate the optimum sample size for this study. A sample size calculation with a power of 80% and alpha 0.05 indicated that a sample size of 152 was required for this study. However, due to certain limitations, this number of cases was not able to be met in this study.

3.4 Sampling Method

Convenience sampling method was employed, where patients that qualified the predefined criteria (**Table 3.1)** were included in the study.

Table 3.1: Inclusion and exclusion criteria for sample selection.

Inclusion criteria	Exclusion criteria
Patients diagnosed histopathogically with OSCC.	Patients diagnosed histopathogically with non-OSCC.
Patients who had OSCC that had been surgically removed.	Patients who had other treatments besides surgical removal of OSCC.
Patients with adequate records (e.g. history, examination and investigations done, treatment undergone).	Patients with inadequate records.

3.5 Sample Collection

A total of 110 cases of patients with OSCC were obtained from MOCDTBS. From this, 37 cases were removed due to exclusion criteria; either due to patient having undergone neoadjuvant therapy or due to missing or incomplete data. Subsequently, 73 cases that met the inclusion criteria were utilized in this study.

3.6 Data Collection

Socio-demographic and clinical data regarding the OSCC cases were obtained from the MOCDTBS. Histopathological data was retrieved from the archived hematoxylin and eosin stained slides for each individual OSCC case from the MOCDTBS by two observers (one trainee and one oral pathologist) (RK and AR). Any discrepancy between the two was discussed with a 3rd observer (oral pathologist) (ZZ). Histopathological scoring was also calculated based on previous risk models.

3.6.1 Socio-Demographic Data

Patients' socio-demographic data such as: i) age, ii) race, iii) gender, iv) presence of risk factors (e.g. smoking tobacco, drinking alcohol, chewing betel quid), v) previous history & family history of cancer were collected.

3.6.2 Clinical Data

Patients' clinical data such as i) site of tumour, ii) size of tumour (cT), iii) nodal metastasis (cN), iv) distant metastasis (cM), v) cTNM according to AJCC 8th edition, vi) treatment received, vii) date of diagnosis viii) survival status, ix) date of decease / date of last visit, x) recurrence (re-emergence of OSCC at the same site after > 6 months of removal).

3.6.3 Histopathological Data

Histopathological data were collected according to various histopathological characteristics as given below.

3.6.3.1 Degree of keratinization

The degree of keratinization was recorded semi-quantitatively base on the percentage of tumor that was keratinized. It was sub-classified into the categories as in **Appendix** A(i) (adapted from Anneroth's multifactorial grading system 1987; Bryne's deep invasive margin model 1989). **(Figures 3.1 to 3.4)**

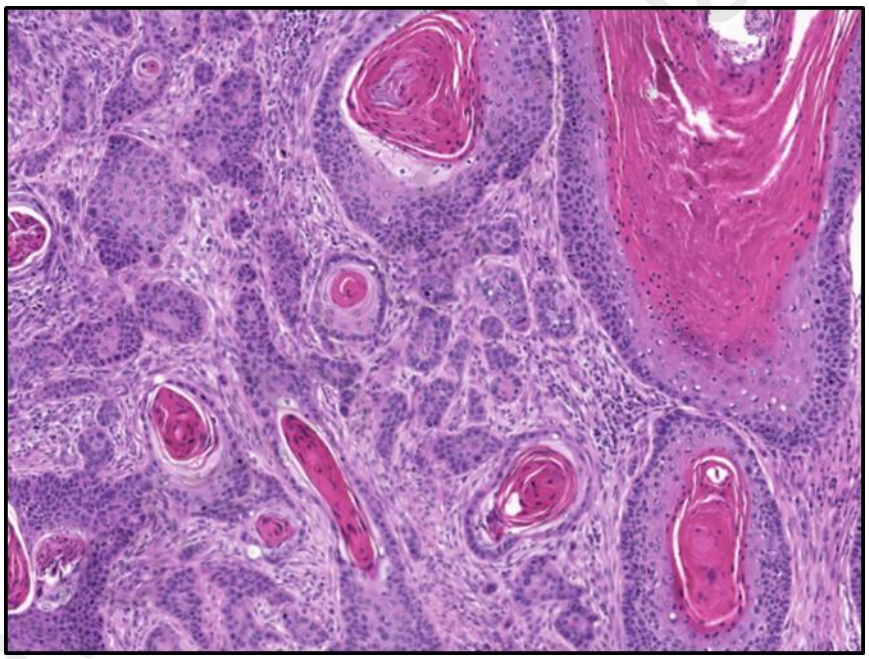


Figure 3.1: Photomicrograph showing highly keratinized OSCC (Original magnification 100x; Stain H&E)

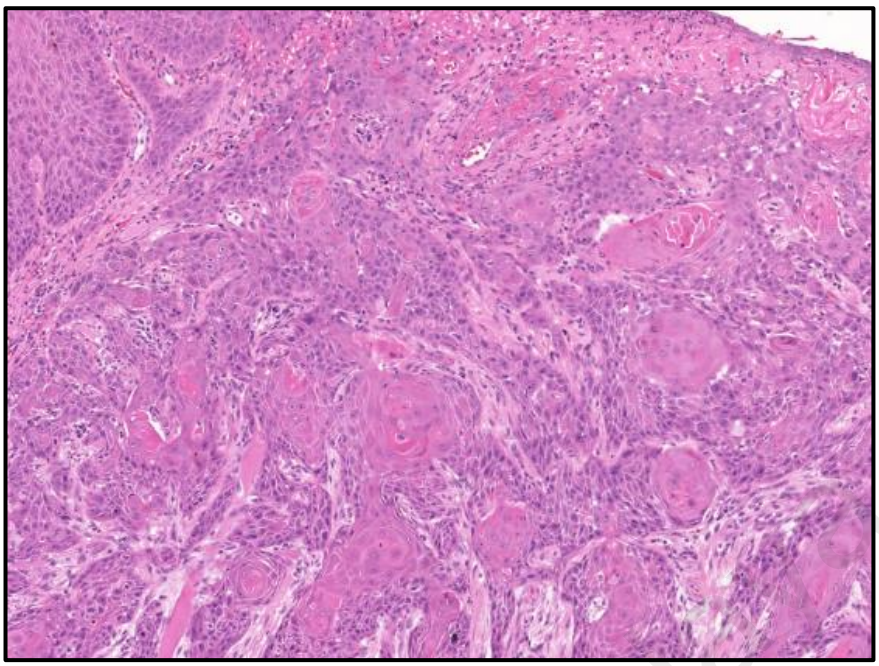


Figure 3.2: Photomicrograph showing moderately keratinized OSCC (Original magnification 100x; Stain H&E)

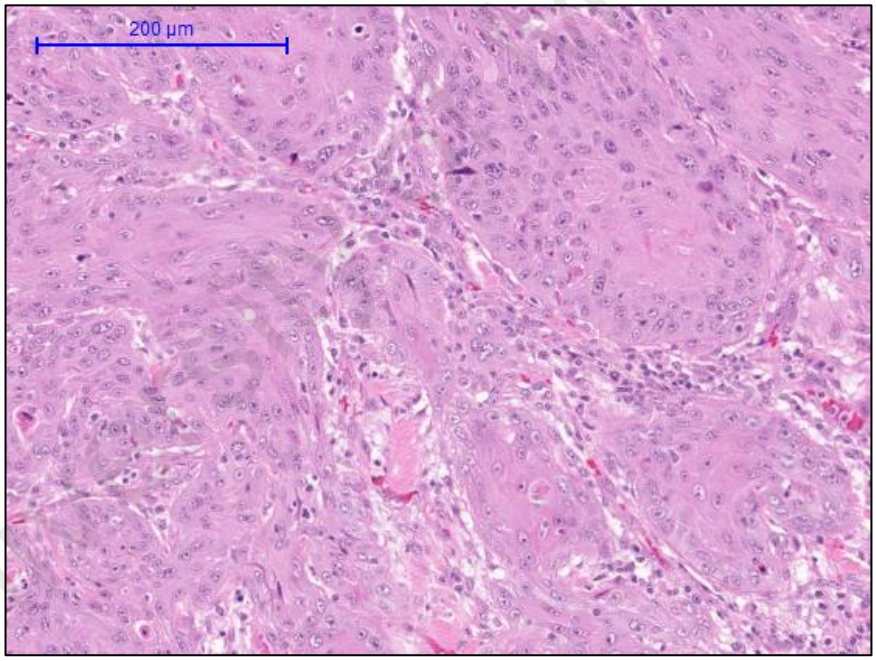


Figure 3.3: Photomicrograph showing minimally keratinized OSCC (Original magnification 100x; Stain H&E)

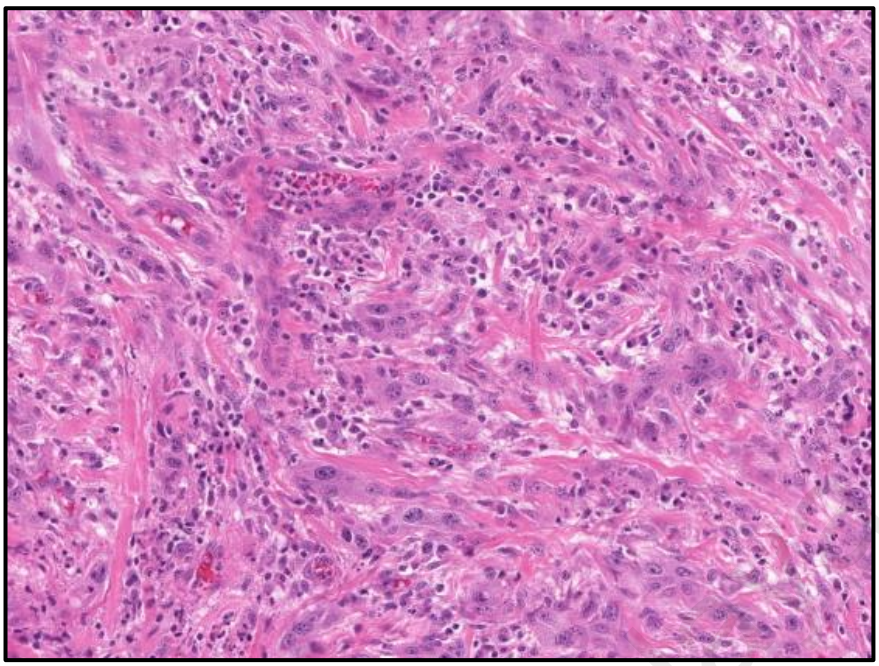


Figure 3.4: Photomicrograph showing OSCC with no keratinization (Original magnification 100x; Stain H&E)

3.6.3.2 Nuclear pleomorphism

The extent of nuclear pleomorphism was recorded semi-quantitatively as percentage of tumor cells that were mature. It was sub-classified into the categories as in **Appendix A(ii)** (adapted from Bryne's deep invasive margin model 1989).

3.6.3.3 Extent of differentiation

The extent of differentiation of the tumour was recorded semi-quantitatively based on percentage of undifferentiated cells. It was sub-classified into the categories as in **Appendix A(iii)** (adapted from Broder's classification 1920). **(Figures 3.5 to 3.7)**

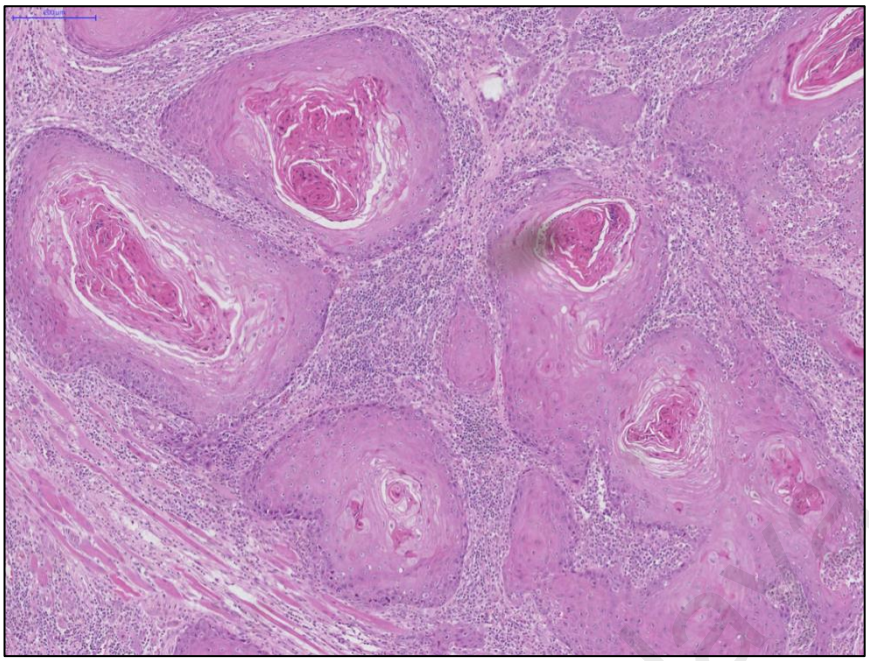


Figure 3.5: Photomicrograph showing well differentiated OSCC (Original magnification 100x; Stain H&E)

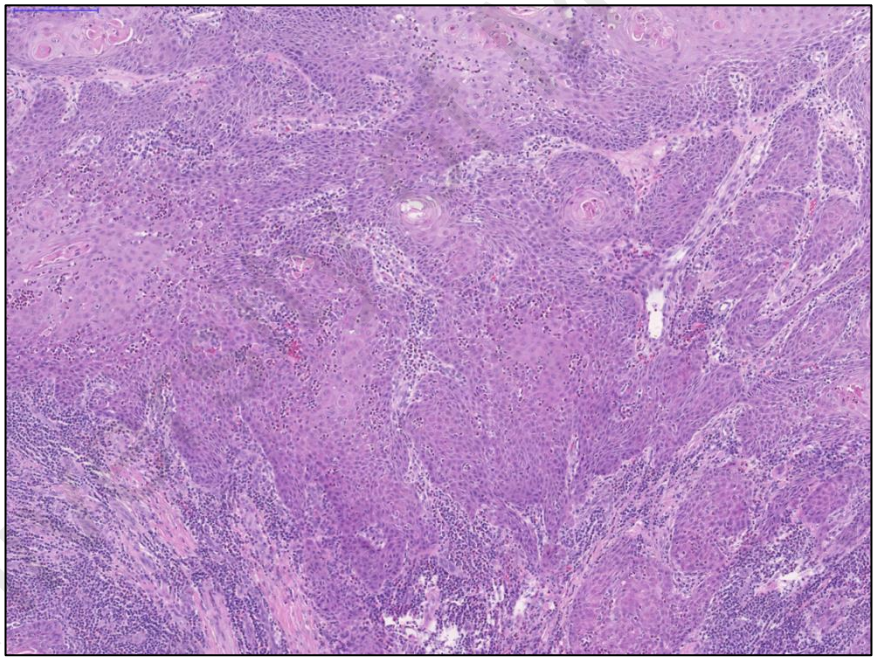


Figure 3.6: Photomicrograph showing moderately differentiated OSCC (Original magnification 100x; Stain H&E)

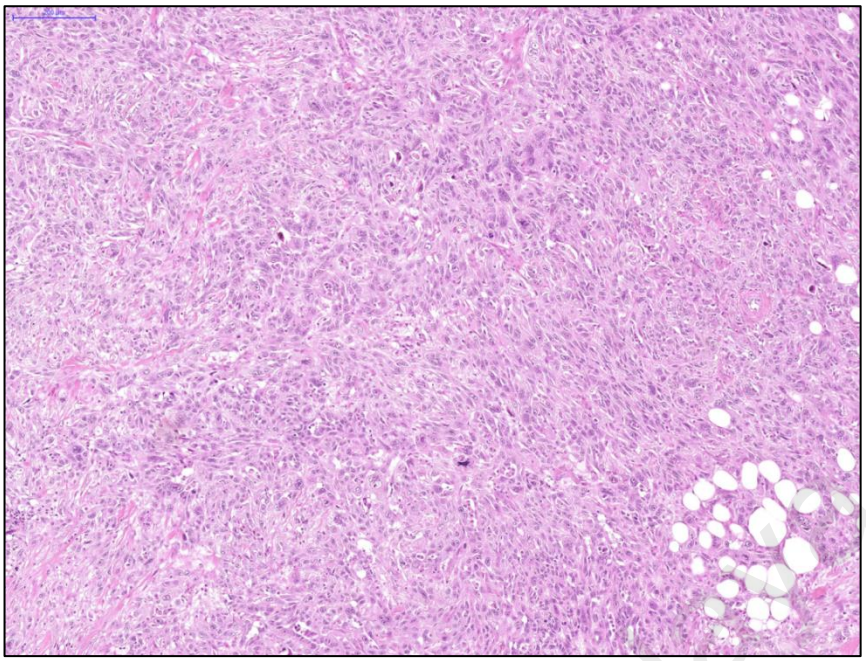


Figure 3.7: Photomicrograph showing poorly differentiated OSCC (Original magnification 100x; Stain H&E)

3.6.3.4 Number of mitosis

The number of mitosis in 10 high power fields (hpf) (Magnification 400x) was recorded at the invasive front of the tumour. It was sub-classified into the grades as in **Appendix A (iv)** (adapted from Anneroth's multifactorial grading system 1987).

3.6.3.5 Depth of tumour invasion (DOI)

It was measured from the basement membrane of the overlying epithelium to the deepest point of invasion of the underlying tissue. The measurements were given in mm **(Appendix A(v))**.

3.6.3.6 Pathological tumour size (pT) AJCC 7th edition

It was measured in millimeters and was sub-classified into the categories of pathological tumour size (pT) as in **Appendix A(vi)**

3.6.3.7 Pathological tumour size (pT) AJCC 8th edition

It was measured in millimeters and was sub-classified into the categories of pathological tumour size (pT) as in **Appendix A(vii)**

3.6.3.8 Pattern of invasion (POI)

The POI was recorded as Type I, II, III or IV based on the criteria given in **Appendix A(viii)** (adapted from Bryne's deep invasive margin model 1989). **(Figures 3.8 to 3.11)**

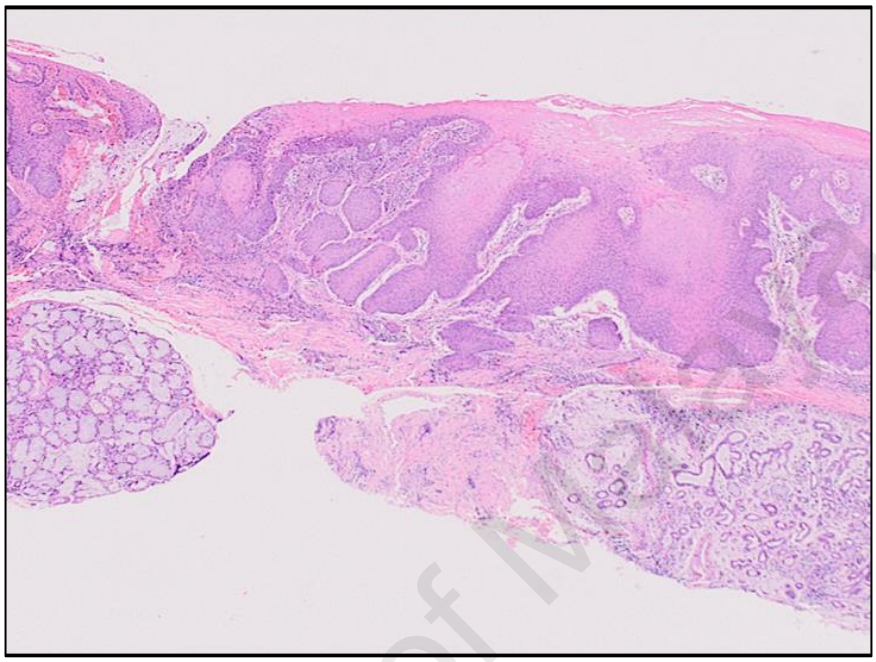


Figure 3.8: Photomicrograph showing Type I pattern of invasion (Original magnification 40x; Stain H&E)

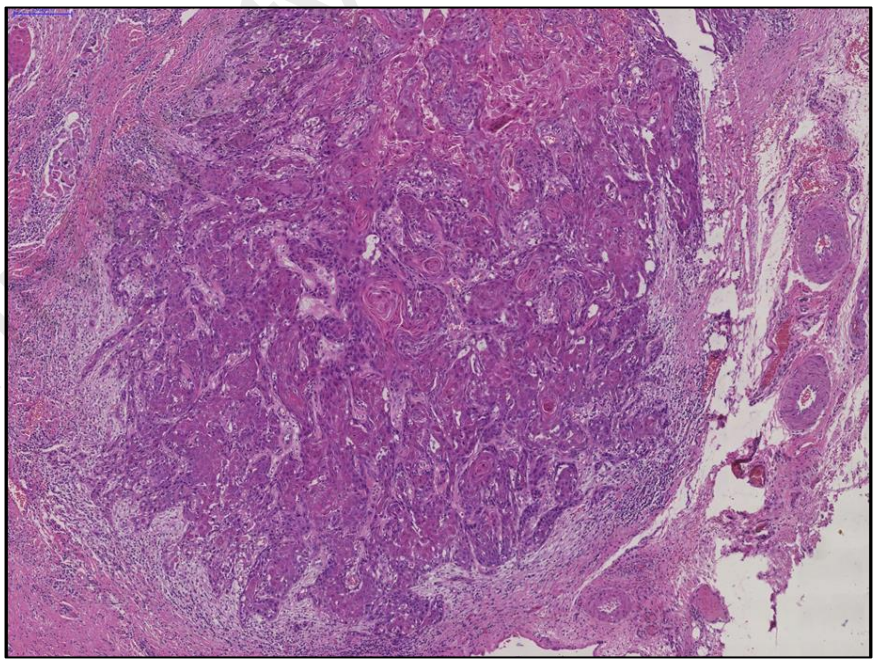


Figure 3.9: Photomicrograph showing Type II pattern of invasion (Original magnification 40x; Stain H&E)

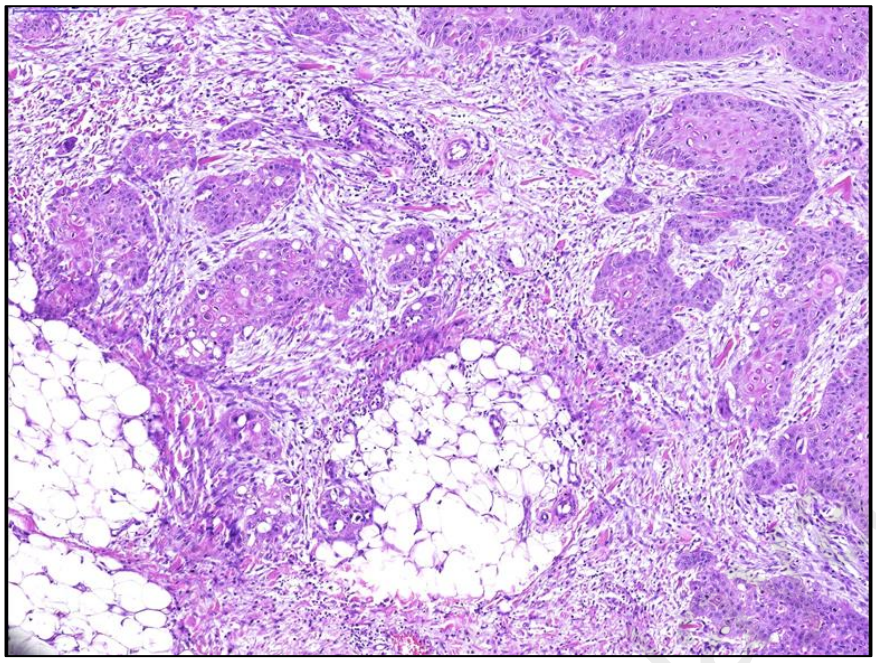


Figure 3.10: Photomicrograph showing Type III pattern of invasion (Original magnification 40x; Stain H&E)

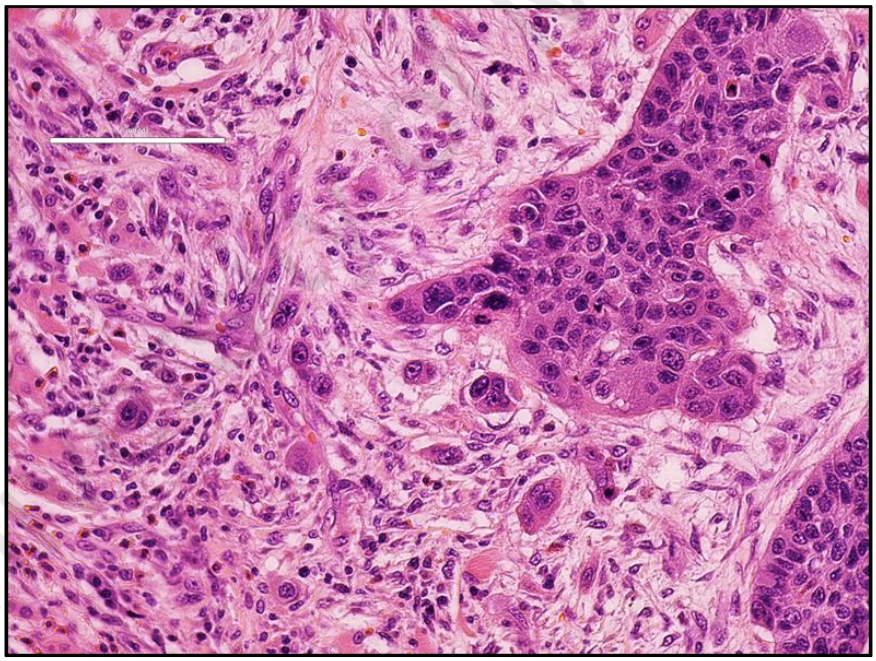


Figure 3.11: Photomicrograph showing Type IV pattern of invasion (Original magnification 100x; Stain H&E)

3.6.3.9 Cohesive and non-cohesive POI:

Type I and II POI were sub-classified as cohesive type of POI whereas type III and IV were sub-classified as non-cohesive type of POI **(Appendix A(ix)).**

3.6.3.10 Worst pattern of invasion (wPOI)

Type I and II POI remained the same whereas type III and IV may be sub-classified as type V (adapted form Brandwein-Gensler's histological risk model 2005) **(Appendix A(x))**.(**Figure 3.12**). Furthermore, type I, II and III were grouped as non-aggressive POI whereas type IV and V were grouped as aggressive POI.

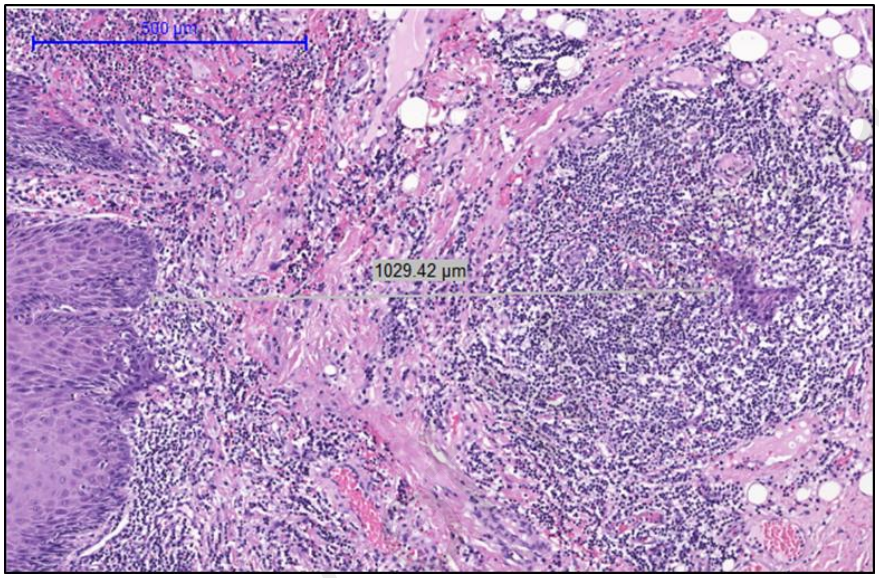


Figure 3.12: Photomicrograph showing Type V pattern of invasion with tumour satellite more than 1mm from main tumour (Original magnification 40x; Stain H&E)

3.6.3.11 Tumour budding grading

The number of tumour buds at the invasive front were counted and sub-classified as low activity and high activity (adapted from tumour budding and depth invasion grading classification 2015) (**Appendix A(xi)**). **(Figures 3.13 to 3.14)**

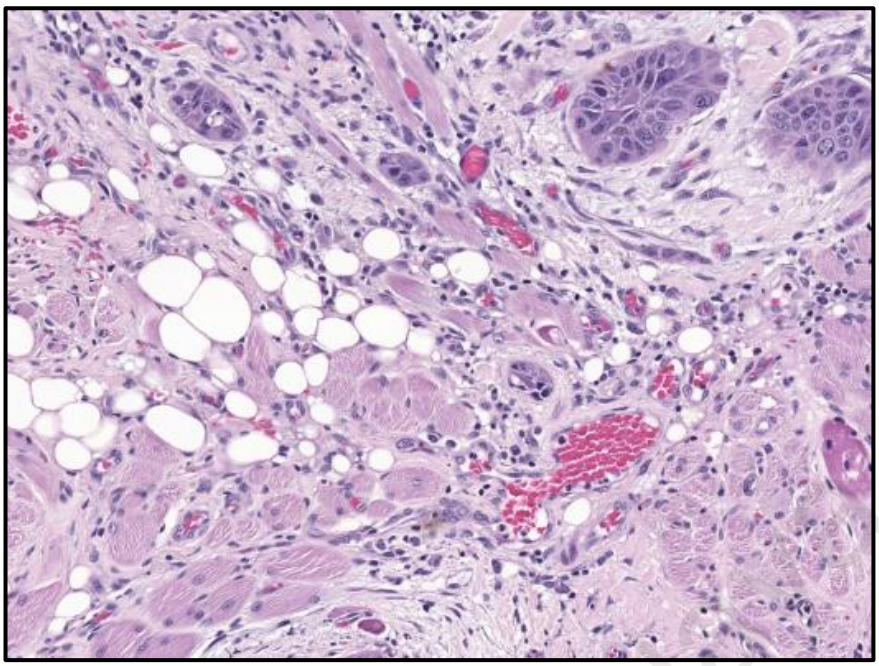


Figure 3.13: Photomicrograph showing low activity of tumour budding (Original magnification 200x; Stain H&E)

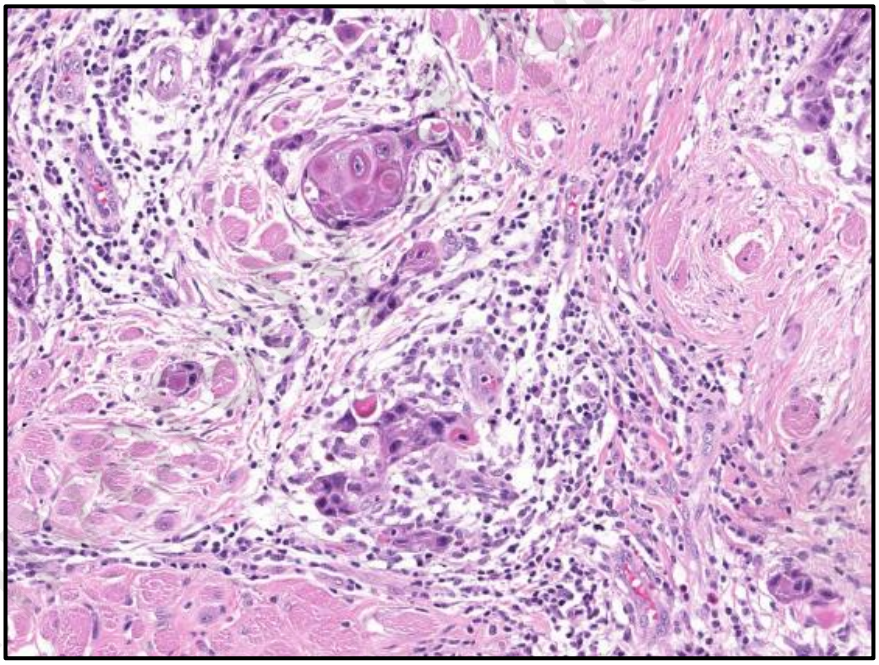


Figure 3.14: Photomicrograph showing high activity of tumour budding (Original magnification 200x; Stain H&E)

3.6.3.12 Perineural invasion (PNI)

The presence or absence of infiltration of perineural space by tumour cells was recorded. **(Figure 3.15)**

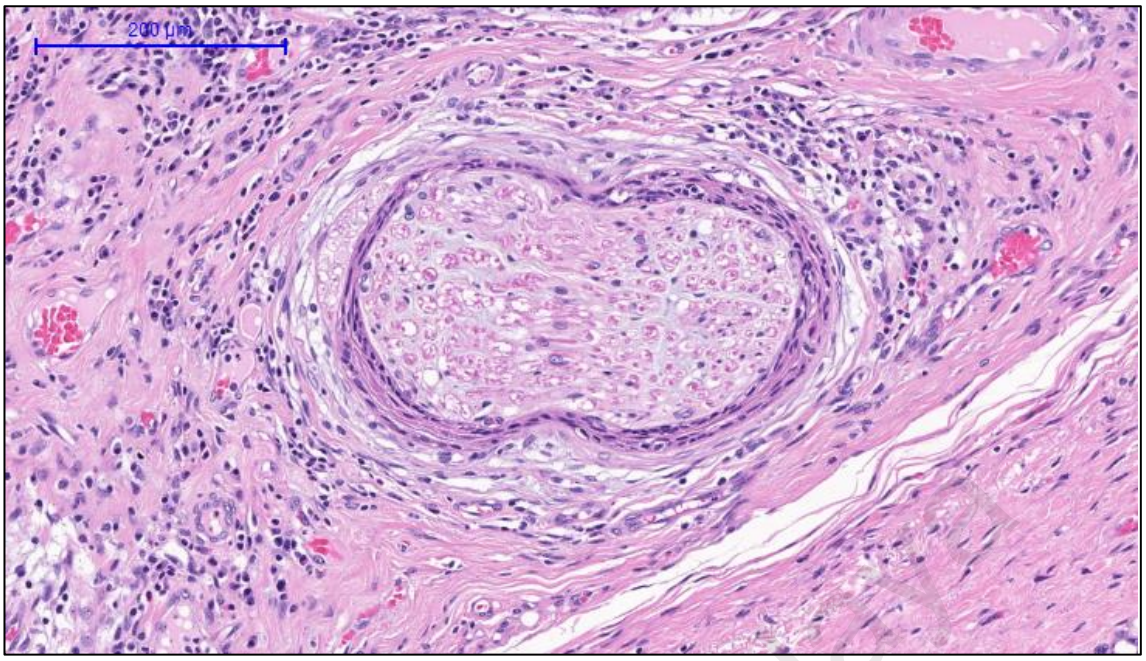


Figure 3.15: Photomicrograph showing perineural invasion (Original magnification 100x; Stain H&E)

3.6.3.13 Intraneural invasion

The presence or absence of infiltration of intraneural space by tumour cells was recorded. **(Figure 3.16)**

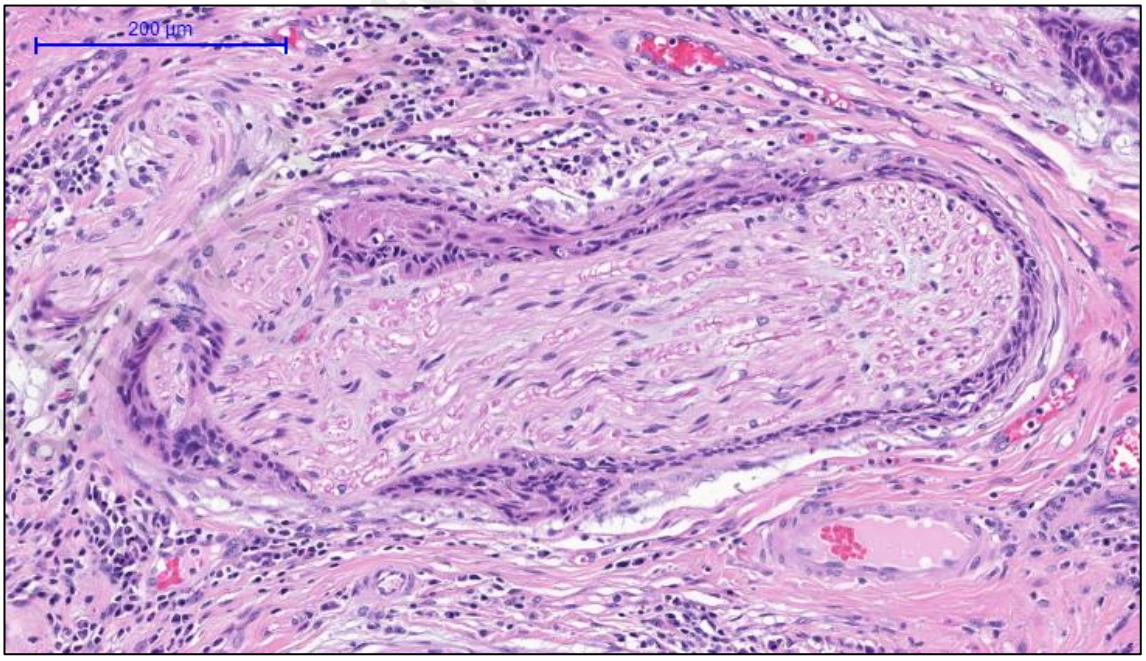


Figure 3.16: Photomicrograph showing intraneural invasion (Original magnification 100x; Stain H&E)

3.6.3.14 Lympho-vascular invasion (LVI)

The presence or absence of infiltration of lympho-vascular vessels by tumour cells was recorded. **(Figure 3.17)**

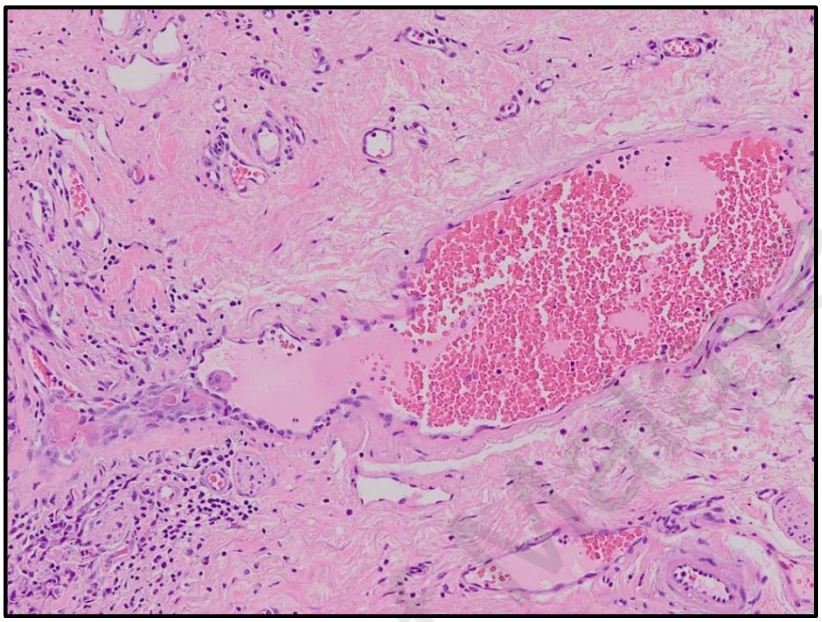


Figure 3.17: Photomicrograph showing lymphovascular invasion (Original magnification 100x; Stain H&E)

3.6.3.15 Bone invasion

The presence or absence of infiltration of bone by tumour cells was recorded (**Figure 3.18**).

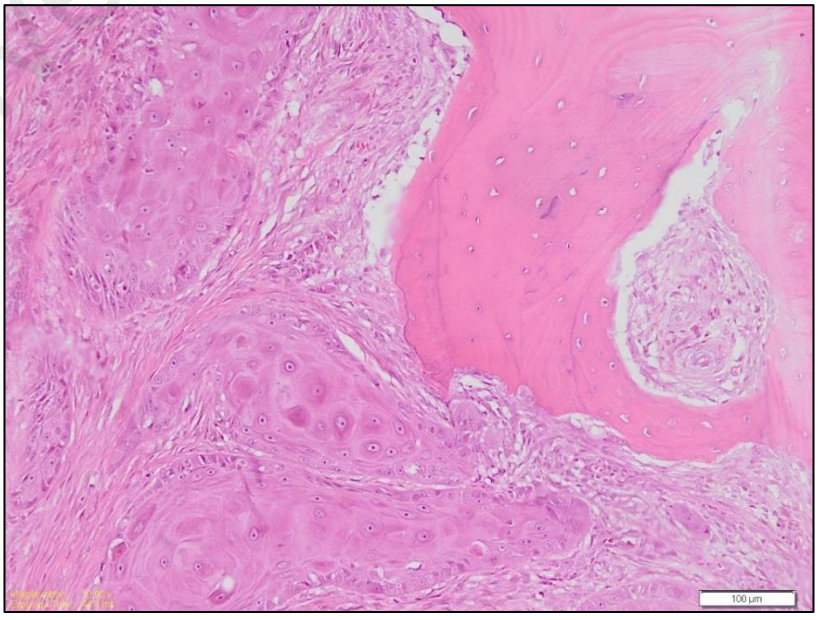


Figure 3.18: Photomicrograph showing bone invasion (Original magnification 100x; Stain H&E)

3.6.3.16 Pathological Nodal metastasis (pN) AJCC 7th edition

The presence or absence of nodal metastasis was recorded and sub-classified as in **Appendix A(xv).**

3.6.3.17 Pathological Nodal metastasis (pN) AJCC 8th edition

The presence or absence of nodal metastasis was recorded and sub-classified as in **Appendix A(xvii).**

3.6.3.18 Extracapsular spread (ECS)

The absence or presence of infiltration of tumour cells beyond the capsule of involved lymph nodes was recorded **(Appendix A(xvi)).**

3.6.3.19 Pathological distant metastasis (pM)

The absence or presence of distant metastasis to other organs for the primary OSCC was recorded based on the data obtained from MOCDTBS **(Appendix A(xviii)).**

3.6.3.20 Pathological TNM staging (pTNM) 7th edition

The pTNM staging was recorded according to AJCC 7th edition **(Appendix A(xix)).**

3.6.3.21 Pathological TNM staging (pTNM) 8th edition

The pTNM staging was recorded according to AJCC 8th edition **(Appendix A(xx)).**

3.6.3.22 Surgical margins

The surgical margins of the tumour were recorded as either clear, close or involved as given in **Appendix A(xxi).**

3.6.3.23 Dysplasia at margins

If dysplasia was present at the surgical margins it was recorded as mild, moderate, severe, or carcinoma in situ, as given in **Appendix A(xxii). (Figures 3.19 to 3.21)**

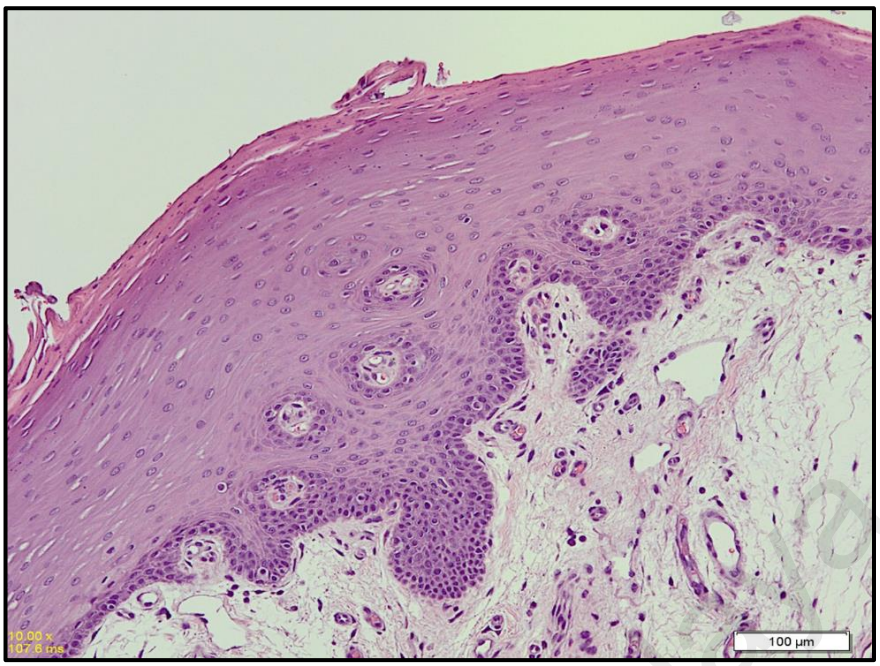


Figure 3.19: Photomicrograph showing mild epithelial dysplasia at margin (Original magnification 100x; Stain H&E)

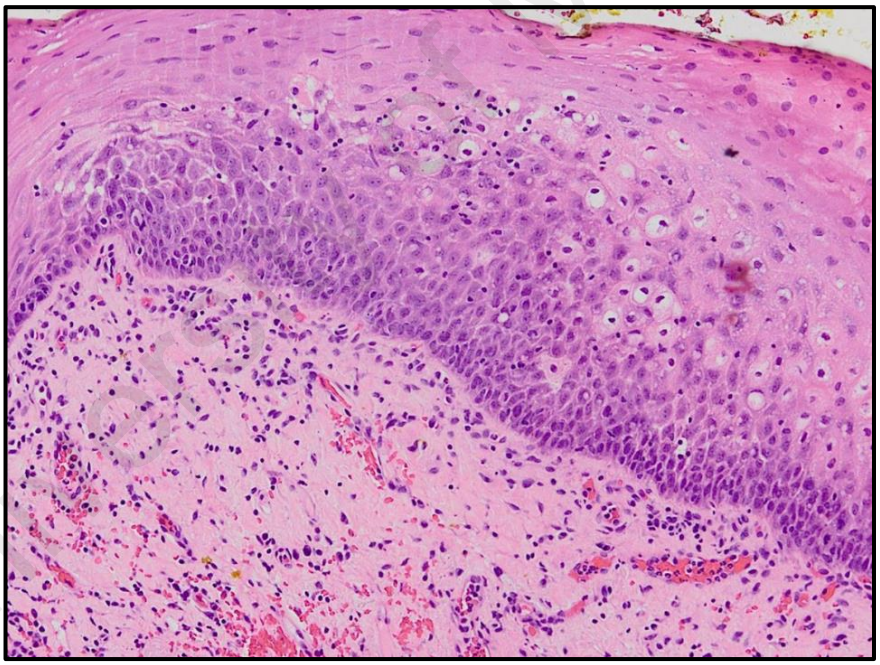


Figure 3.20: Photomicrograph showing moderate epithelial dysplasia at margin (Original magnification 100x; Stain H&E)

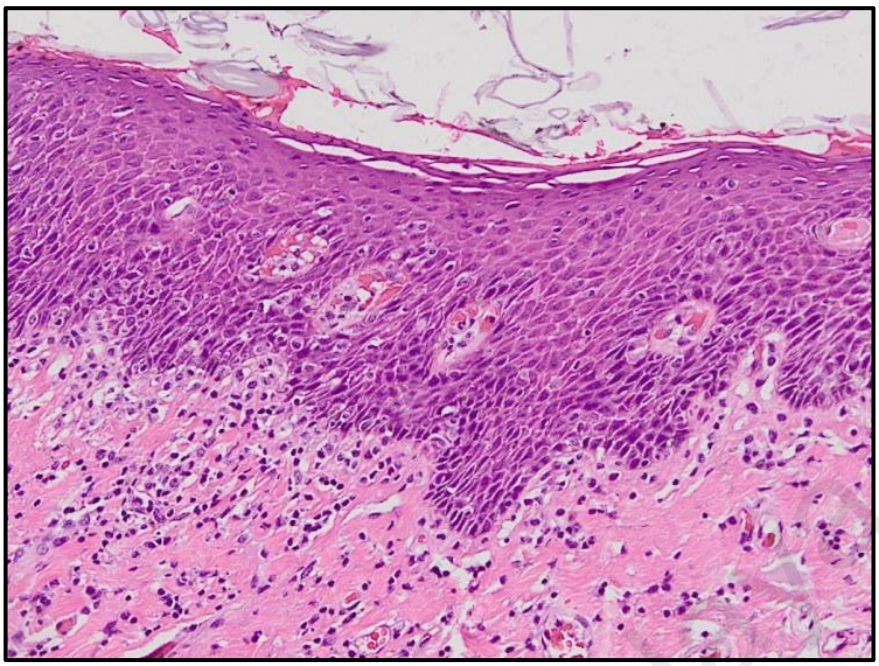


Figure 3.21: Photomicrograph showing severe epithelial dysplasia at margin (Original magnification 100x; Stain H&E)

3.6.3.24 Connective tissue

The connective tissue underlying the tumor invasive front was assessed and recorded as given in **Appendix A (xxiii).**

3.6.3.25 Muscle bundles

The skeletal muscle bundles underlying the tumor invasive front was assessed and recorded as given in **Appendix A(xxiv). (Figure 3.22)**

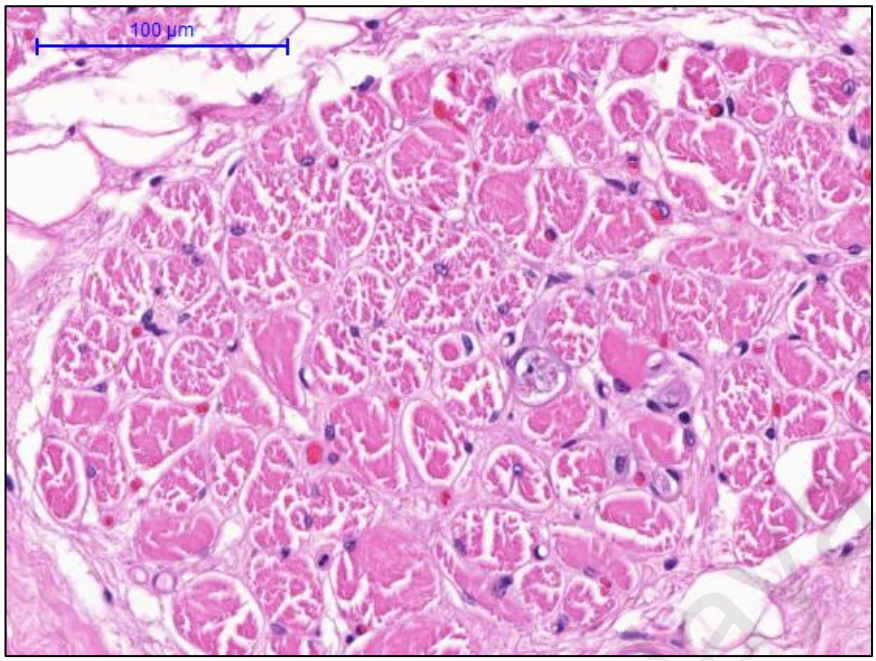


Figure 3.22: Photomicrograph showing skeletal muscle degeneration (Original magnification 200x; Stain H&E)

3.6.3.26 Sarcolemmal spread

The presence or absence of sarcolemmal spread at the invasive front was determined. **(Figure 3.23)**

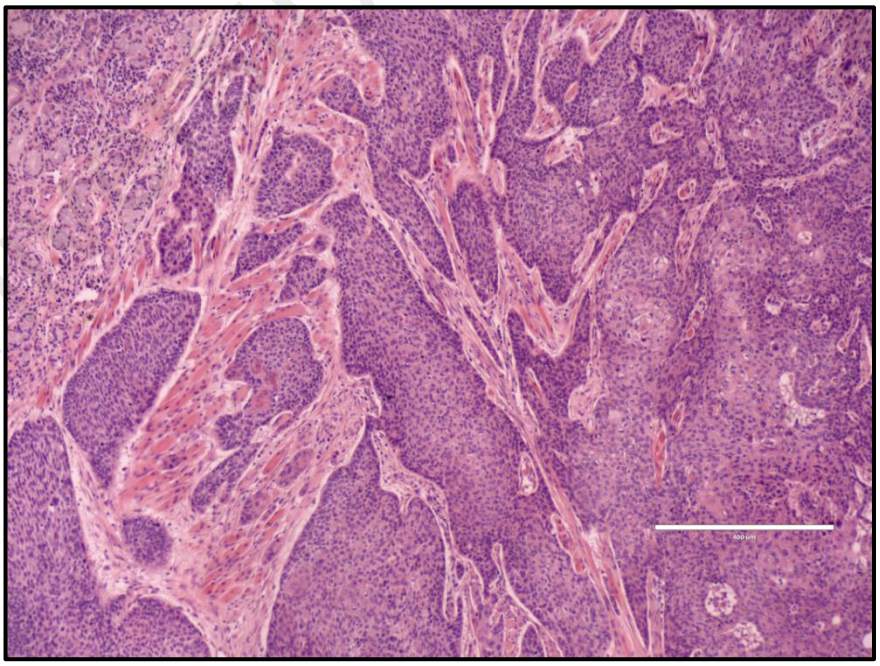


Figure 3.23: Photomicrograph showing sarcolemmal spread (Original magnification 100x; Stain H&E)

3.6.3.27 Salivary glands

The salivary glands adjacent to or within the tumor was assessed and recorded as given in **Appendix A(xxv). (Figures 3.24 to 3.27)**

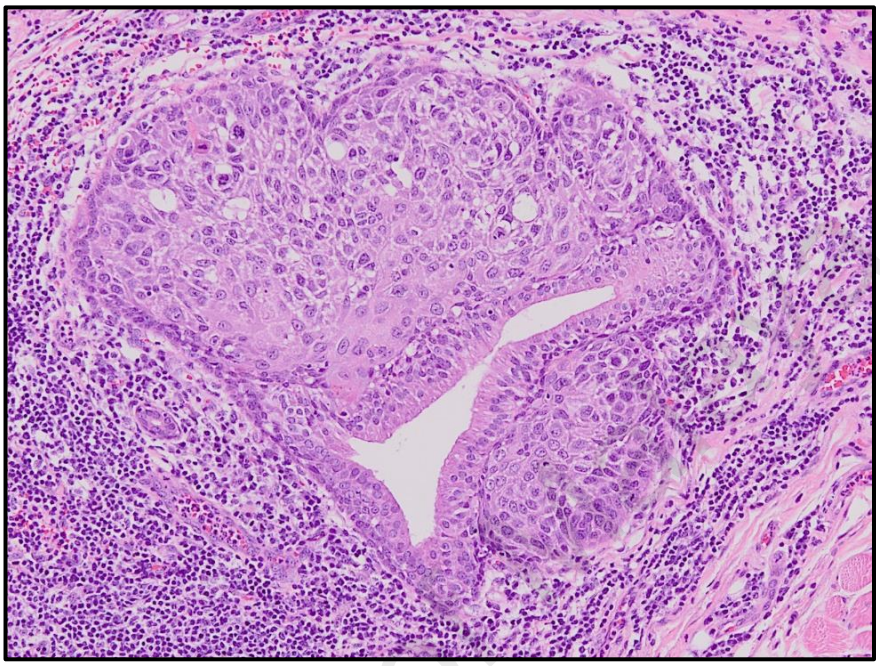


Figure 3.24: Photomicrograph showing dysplasia of salivary duct (Original magnification 100x; Stain H&E)

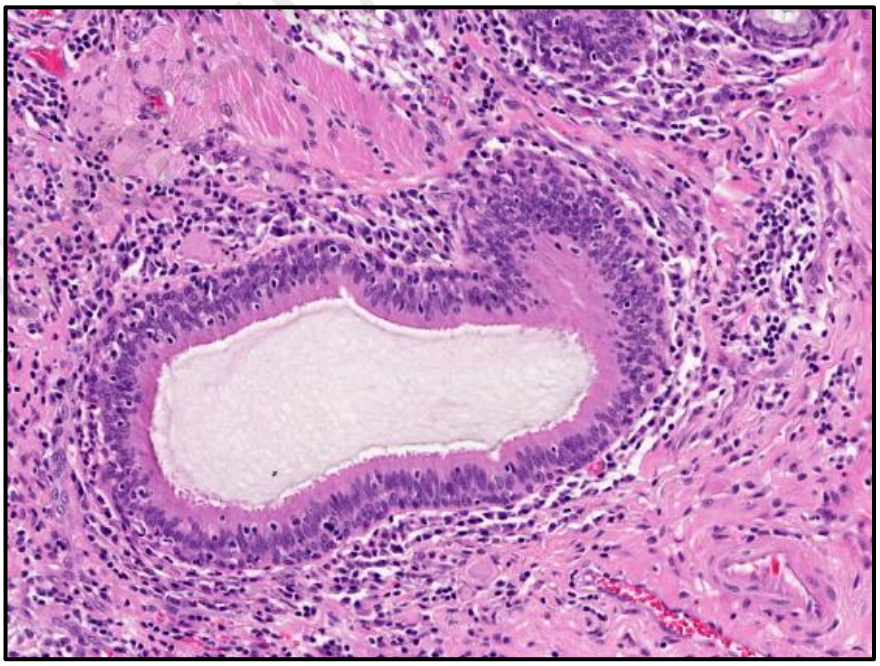


Figure 3.25: Photomicrograph showing squamous metaplasia of salivary duct (Original magnification 100x; Stain H&E)

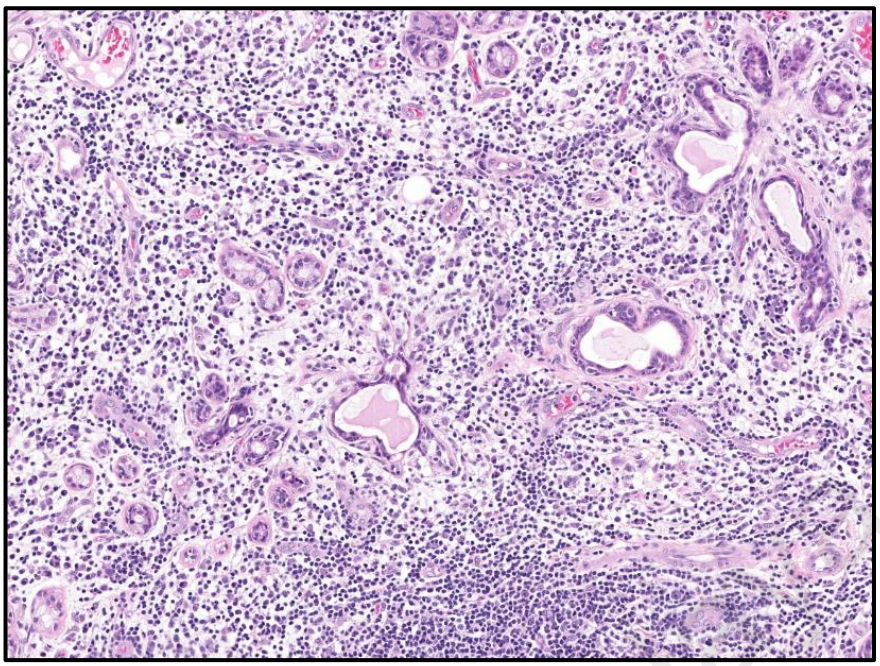


Figure 3.26: Photomicrograph showing acinar degeneration of salivary gland (Original magnification 100x; Stain H&E)

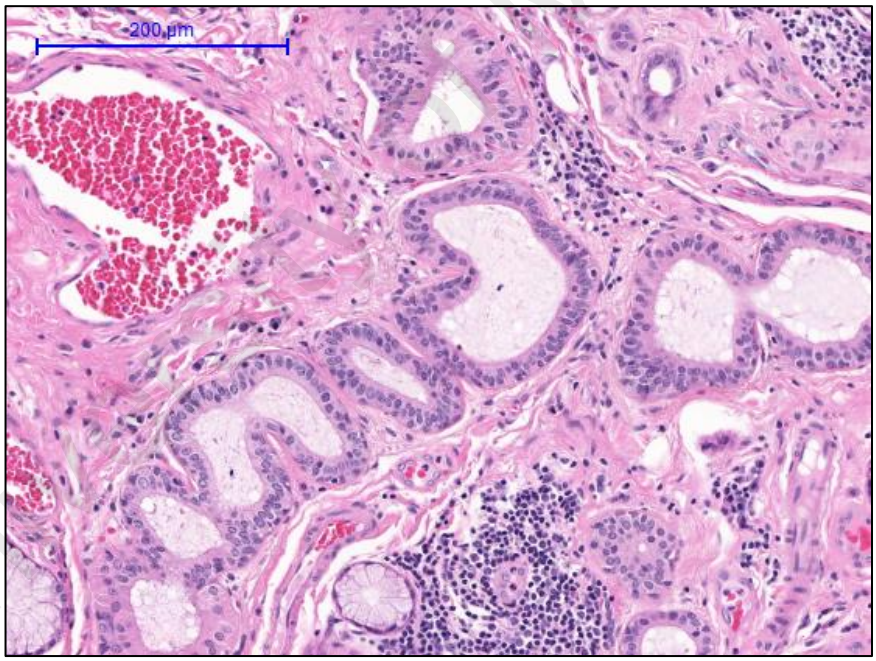


Figure 3.27: Photomicrograph showing hyperplastic and ectatic ducts (Original magnification 100x; Stain H&E)

3.6.3.28 Lymphocytic host response (LHR)

The LHR was assessed and recorded at the invasive front of the tumor at low power magnification (40x). It was sub-classified into strong, intermediate and weak as given in **Appendix A(xxvi)** (adapted and modified from Maleki et al 2011). **(Figures 3.28 to 3.30)**

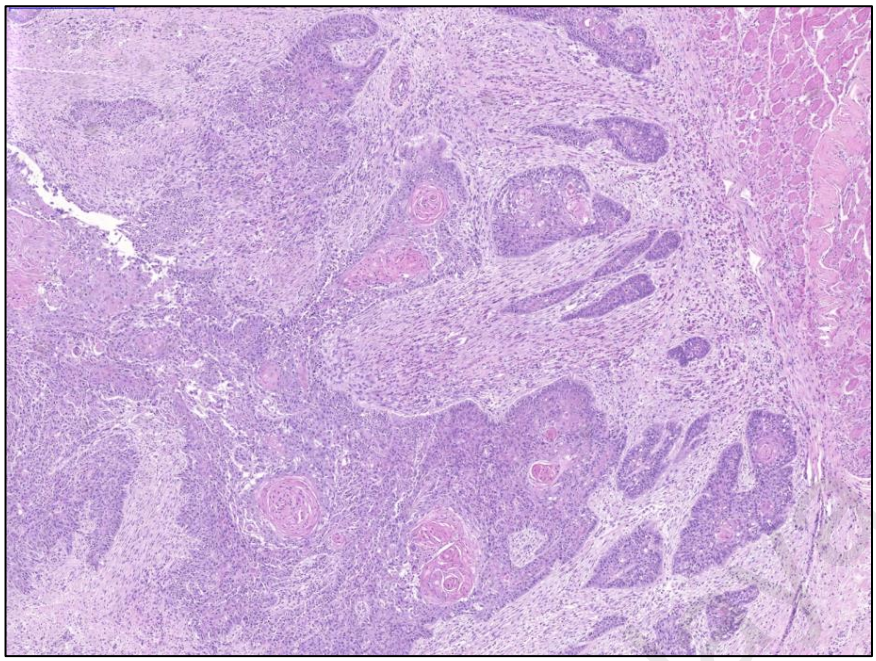


Figure 3.28: Photomicrograph showing weak LHR
(Original magnification 40x; Stain H&E)

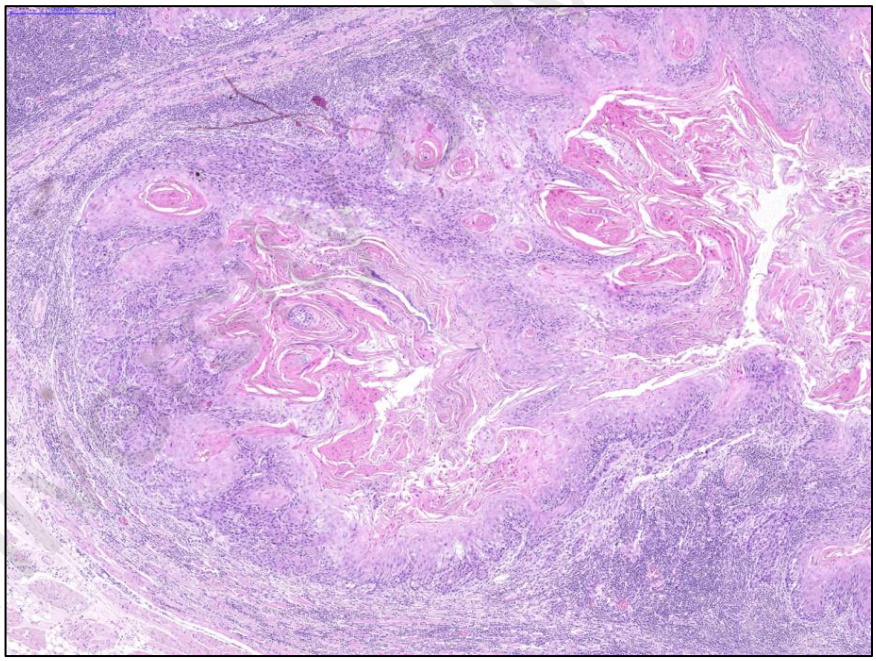


Figure 3.29: Photomicrograph showing intermediate LHR
(Original magnification 40x; Stain H&E)

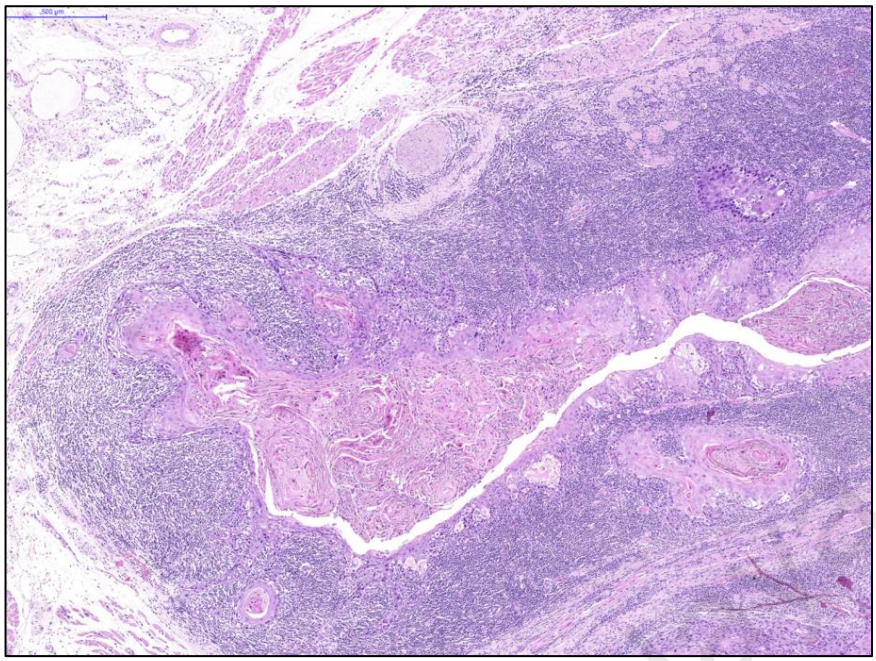


Figure 3.30: Photomicrograph showing strong LHR (Original magnification 40x; Stain H&E)

3.6.3.29 Tumour infiltrative lymphocytes (TIL)

The TILs were assessed and recorded from the tumour stroma at low power magnification (40x). It was subdivided into high, moderate and low as given in **Appendix A(xxvii)** (adapted and modified from Xu et al 2017).

3.6.3.30 Tumour associated tissue eosinophilia (TATE)

The presence or absence of eosinophilia at the invasive front was determined. **(Figure 3.31)**

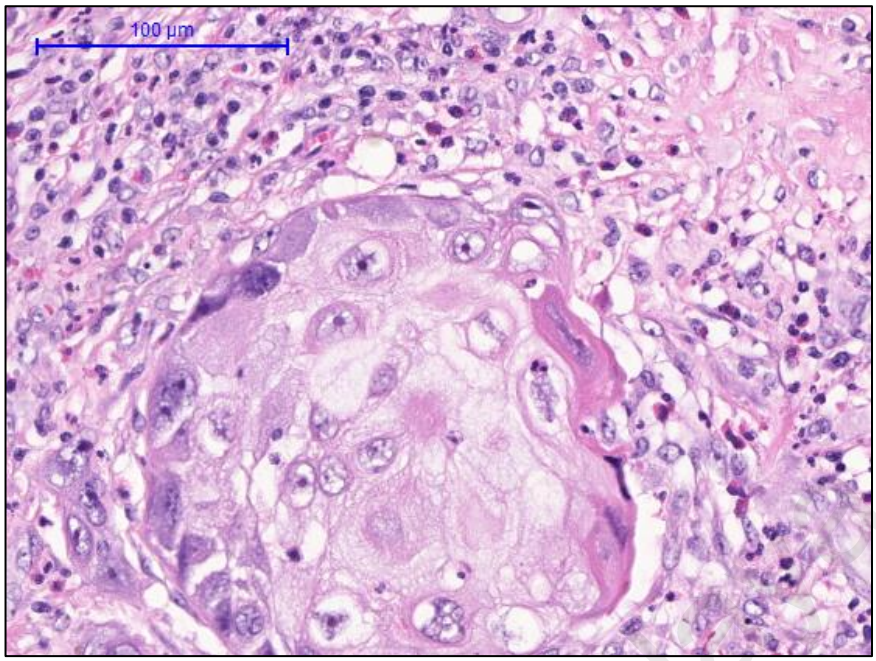


Figure 3.31: Photomicrograph showing TATE at invasive front (Original magnification 200x; Stain H&E)

3.6.4 Risk Model Scoring

Scoring of risk models based on histological grading systems according to Broder's classification, Anneroth's multifactorial grading system, Bryne's deep invasive margin model (1989), Bryne's deep invasive margin model (1992), Brandwein-Gensler's histological risk model and the Tumour budding and depth invasion grading were conducted. These grading systems may be seen within the appendix section **(Appendix B)**

3.7 Data Verification

All data extracted from the MOCDTBS were collected by researcher (RK) and verified by two observers; one oral pathology trainee (RK) and one oral pathologist (AR). Data verification was conducted to ensure accuracy of existing data within the database by comparing the existing reports and histopathological slides. Any discrepancy between the two was discussed with a 3rd observer (oral pathologist) (ZZ). The verified data was used for statistical analysis.

3.8 Statistical analysis

All statistical analysis was performed using the statistical software package SPSS 23.0 for Windows (SPSS Inc., Chicago, IL).

A univariate analysis (Fisher Exact test) was used for categorical characteristics to determine the correlation between the various characteristics of socio-demographic, clinical, and histopathological of OSCC with local recurrence and lymph node metastasis. Characteristics that were significant on univariate analysis were subsequently entered into a multivariate analysis (Binary logistic regression). The *p*-value <0.05 was considered statistically significant.

A univariate analysis (Kaplan-Meier) was used to determine the associations between individual characteristics of socio-demographic, clinical and histopathological with overall survival for patients with OSCC. The period for time-to-event was defined as the interval between the date of diagnosis and the date of death or date of last follow-up visit (for censored observation). Characteristics that were significant on univariate analysis were subsequently entered into a multivariate analysis (Cox regression). The *p*-value <0.05 was considered statistically significant.

CHAPTER 4: RESULTS

4.1 Descriptive Analysis

Seventy-three patients who satisfied the predetermined inclusion criteria were included in this study. The socio-demographic data of the samples are summarized in **Figure 4.1**.

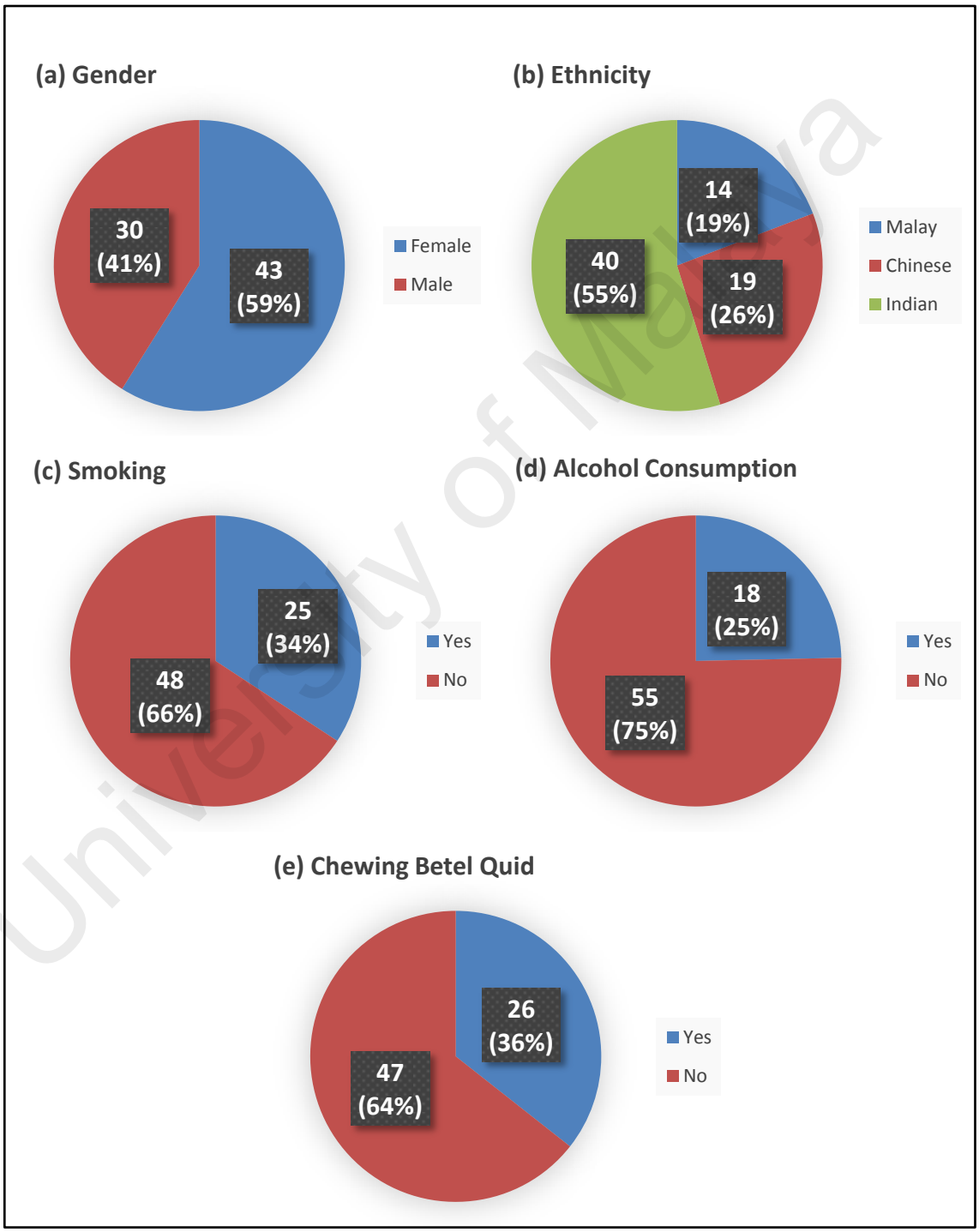


Figure 4.1: Pie diagram showing the distribution of socio-demographic data (a) gender, (b) ethnicity, (c) smoking status, (d) alcohol consumption, and (e) chewing betel quid.

The distribution of the various socio-demographic, clinical, and histopathological characteristics studied within this population is shown in **Table 4.1**, **Table 4.2**, and **Table 4.3.**

Table 4.1: Distribution of socio-demographic characteristics

Characteristics		Frequency	%
Age	≥60	42	57.5
	<60	31	42.5
Gender	Female	43	58.9
	Male	30	41.1
Ethnicity	Malay	14	19.2
	Chinese	19	26.0
	Indian	40	54.8
Smoking	Yes	25	34.2
	No	48	65.8
Alcohol Consumption	Yes	18	24.7
	No	55	75.3
Chewing Betel quid	Yes	26	35.6
	No	47	64.4
Family history of cancer	Yes	7	9.6
	No	66	90.4
Previous history of cancer	Yes	8	11.0
	No	65	89.0

Table 4.2: Distribution of clinical characteristics

Characteristics		Frequency	%
Site	Buccal mucosa	22	30.1
	Tongue	29	39.7
	Palate	7	9.6
	Floor of mouth	2	2.7
	Alveolar process	13	17.8
cTNM	Stage I	11	15.1
	Stage II	19	26.0
	Stage III	11	15.1
	Stage IV	32	43.8

Table 4.3: Distribution of histopathological characteristics

Characteristics		Frequency	%
Degree of keratinization	No keratin	2	2.7
	Minimal	20	27.4
	Moderate	36	49.3
	High	15	20.5
Extent of differentiation	Well	11	15.1
	Moderate	59	80.8
	Poor	3	4.1
Extent of pleomorphism	Mild	11	15.1
	Moderate	59	80.8
	Abundant	3	4.1

Table 4.3: Continued

Characteristics		Frequency	%
Grading of mitosis per 10 HPF	Grade 1	53	72.6
	Grade 2	17	23.3
	Grade 3	1	1.4
	Grade 4	2	2.7
pTNM 7th edition	Stage I	8	11.0
	Stage II	22	30.1
	Stage III	6	8.2
	Stage IVa	35	47.9
	Stage IVB	2	2.7
pTNM 8th edition	Stage I	7	9.6
	Stage II	20	27.4
	Stage III	8	11.0
	Stage IVa	21	28.8
	Stage IVB	17	23.3
Pattern of invasion	Type I	3	4.1
	Type II	11	15.1
	Type III	15	20.5
	Type IV	54	74.0
Pattern of invasion - Cohesiveness	Cohesive	4	5.5
	Non-cohesive	69	94.5
Worst pattern of invasion	Type I	3	4.1
	Type II	1	1.4
	Type III	15	20.5
	Type IV	44	60.3
	Type V	10	13.7
Pattern of invasion – Aggressiveness	Non-aggressive	19	26.0
	Aggressive	54	74.0
Perineural invasion	Present	22	30.1
	Absent	51	69.9
Intraneural invasion	Present	6	8.2
	Absent	67	91.8
Lymphovascular invasion	Present	9	12.3
	Absent	64	87.7
Surgical margins	Clear	18	24.7
	Close	23	31.5
	Involved	32	43.8
	None	53	72.6
Dysplasia at margins	Mild	6	8.2
	Moderate	8	11.0
	Severe	6	8.2
Lymphocytic host response	Weak	10	13.7
	Intermediate	45	61.6
	Strong	18	24.7
Tumour associated tissue eosinophilia	Present	55	75.3
	Absent	18	24.7
Dysplasia at margins	Mild	6	8.2
	Moderate	8	11.0
	Severe	6	8.2

Table 4.3: Continued

Characteristics		Frequency	%
Connective tissue	Fibrous	62	84.9
	Dense collagenous	10	13.7
	Myxomatous	1	1.4
Skeletal muscle bundles	Dense	20	27.4
	Degenerated	53	72.6
Sarcolemmal spread	Present	49	67.1
	Absent	24	32.9
Salivary gland acini	Normal	14	19.2
	Degeneration with inflammation	57	78.1
	Degeneration without inflammation	2	2.7
Salivary gland ducts	Normal	12	16.4
	Ductal degeneration	5	6.8
	Ectatic ducts	11	15.1
	Hyperplasia of ducts	29	39.7
	Squamous metaplasia	10	13.7
	Mucous metaplasia	4	5.5
	Tumour infiltrating duct	2	2.7
Depth of invasion	≤5	21	28.8
	5<x≤10	26	35.6
	>10	26	35.6
Tumour infiltrative lymphocytes	Low	11	15.1
	Moderate	38	52.1
	High	24	32.9
Tumour budding grading	None	19	26.0
	Low	35	47.9
	High	19	26.0
Bone invasion	Present	18	24.7
	Absent	55	75.3

4.1.1 Socio-Demographic and Clinical Data

The mean age of the study population was 61.4 ±13.2 with more than half; 42 (57.5%) being 60 years old and above. The majority of patients were female 43 (58.9%). With regards to ethnicity, more than half of the study population was of Indian ethnicity (55%). Within this study population, the most common site of OSCC was the tongue at 29 (39.7%) cases, followed by the buccal mucosa with 22 (30.1%) cases, the alveolar process at 13 (17.8) cases and finally the palate and floor of mouth at 7 (9.6%) cases and 2 (2.7%) cases respectively. With regards to high-risk habits associated with OSCC (Figures 4.3, 4.4, and 4.5); 25 (34%) patients smoked, 18 (25%) patients drank

alcohol, and 26 (36%) patients chewed betel quid. Among the study population, 7 (9.6%) patients had a family history of cancer while 8 (11.0%) patients had previous history of cancer.

4.1.2 Histopathological Data

Histopathogically, almost half of the study sample, 36 (49.3%) had a moderate degree of keratinization. Majority of the study sample had a moderate extent of differentiation, 59 (80.8%). With regards to pTNM staging (8^{th} edition); 7 (9.6%) were stage I, 20 (27.4%) were stage II, 8 (11.0%) were stage III, 21 (28.8%) were stage IVa and 17 (23.3%) were stage IVb. For POI, the majority of the sample had type IV pattern of invasion; 54 (74%), followed by type III pattern of invasion; 15 (20.5%), type II; with 11 (15.1%) and least of all, type I; with 3 (4.1%). When lymphocytic host response was studied, 45 (61.6%) of the sample exhibited an intermediate response, while 18 (24.7%) had a strong response and 10 (13.7%) had a weak response. For DOI; 21 (28.8%) were less than 5 mm in depth, 26 (35.6%) were between 5 and 10 mm in depth, and 26 (35.6%) were more than 10 mm in depth. Other studied histological characteristics and their frequencies are listed in **Table 4.3**.

4.2 Association between Different Characteristics with Lymph Node Metastasis

The socio-demographic, clinical, and histopathological characteristics of the study sample were statistically analyzed with Fisher Exact test to determine the association between these characteristics with lymph node metastasis.

No significant association was seen between the socio-demographic characteristics and lymph node metastasis (**Table 4.4)**. However, a significant association was observed with cTNM staging and lymph node metastasis (p<0.001) (**Table 4.5**).

Table 4.4: Association between socio-demographic characteristics and lymph node metastasis (n=73).

Characteristics		Presence of lymph node metastasis n(%)	Absence of lymph node metastasis n(%)	*p*-value
Age	≥60	18 (24.7)	24 (32.9)	0.812
	<60	12 (16.4)	19 (26.0)	
Gender	Female	18 (24.7)	25 (34.2)	1.000
	Male	12 (16.4)	18 (24.7)	
Ethnicity	Malay	7 (9.6)	7 (9.6)	0.590
	Chinese	6 (8.2)	13 (17.8)	
	Indian	17 (23.3)	23 (31.5)	
Smoking	Yes	7 (9.6)	18 (24.7)	0.134
	No	23 (31.5)	25 (34.2)	
Alcohol Consumption	Yes	8 (11.0)	10 (13.7)	0.787
	No	22 (30.1)	33 (45.2)	
Chewing Betel quid	Yes	12 (16.4)	14 (19.2)	0.621
	No	18 (24.7)	29 (39.7)	
Family history of cancer	Yes	4 (5.5)	3 (4.1)	0.435
	No	26 (35.6)	40 (54.8)	
Previous history of cancer	Yes	1 (1.4)	7 (9.6)	0.130
	No	29 (39.7)	36 (49.3)	

Table 4.5: Association between clinical characteristics and lymph node metastasis (n=73).

Characteristics		Presence of lymph node metastasis n(%)	Absence of lymph node metastasis n(%)	*p*-value
Site	Buccal mucosa	8 (11.0)	14 (19.2)	0.809
	Tongue	12 (16.4)	17 (23.3)	
	Palate	2 (2.7)	5 (6.8)	
	Floor of mouth	1 (1.4)	1 (1.4)	
	Alveolar process	7 (9.6)	6 (8.2)	
cTNM	Stage I	0	11 (15.1)	**<0.001***
	Stage II	4 (5.5)	15 (20.5)	
	Stage III	8 (11.0)	3 (4.1)	
	Stage IV	18 (24.7)	14 (19.2)	

*Fisher Exact test showing statistically significant association with lymph node metastasis (p<0.05)

There were also significant associations observed between a number of histopathological characteristics such as pTNM staging 7th edition (p<0.001), pTNM staging 8th edition (p<0.001), TATE (p=0.026), sarcolemmal spread (p=0.022) and DOI

(p=0.024) with lymph node metastasis (Table 4.6). Patients with higher cTNM and pTNM staging showed increased incidence of lymph node metastasis. The presence of histopathological features such as TATE and sarcolemmal spread within a sample also increased the incidence of lymph node metastasis. An increased DOI; >10mm had an increased incidence of lymph node metastasis compared to cases with ≤5 mm depth of tumour invasion.

Table 4.6: Association between histopathological characteristics and lymph node metastasis (n=73).

Characteristics		**Presence of lymph node metastasis n(%)**	**Absence of lymph node metastasis n(%)**	***p*-value**
Degree of keratinization	No keratin	0	2 (2.7)	0.224
	Minimal	6 (8.2)	14 (19.2)	
	Moderate	15 (20.5)	21 (28.8)	
	High	9 (12.3)	6 (8.2)	
Extent of differentiation	Well	2 (2.7)	9 (12.3)	0.174
	Moderate	26 (35.6)	33 (45.2)	
	Poor	2 (2.7)	1 (1.4)	
Extent of pleomorphism	Mild	2 (2.7)	9 (12.3)	0.174
	Moderate	26 (35.6)	33 (45.2)	
	Abundant	2 (2.7)	1 (1.4)	
Grading of mitosis per 10 HPF	Grade 1	23 (31.5)	30 (41.1)	0.428
	Grade 2	5 (6.8)	12 (16.4)	
	Grade 3	1 (1.4)	0	
	Grade 4	1 (1.4)	1 (1.4)	
pTNM 7th edition	Stage I	0	8 (11.0)	**<0.001***
	Stage II	0	22 (30.1)	
	Stage III	5 (6.8)	1 (1.4)	
	Stage IVa	25 (34.2)	10 (13.7)	
	Stage IVB	0	2 (2.7)	
pTNM 8th edition	Stage I	0	7 (9.6)	**<0.001***
	Stage II	0	20 (27.4)	
	Stage III	4 (5.5)	4 (5.5)	
	Stage IVa	11 (15.1)	10 (13.7)	
	Stage IVB	15 (20.5)	2 (2.7)	

Table 4.6: Continued

Characteristics		Presence of lymph node metastasis n(%)	Absence of lymph node metastasis n(%)	*p*-value
Pattern of invasion	Type I	0	3 (4.1)	0.324
	Type II	0	1 (1.4)	
	Type III	5 (6.8)	10 (13.7)	
	Type IV	25 (34.2)	29 (39.7)	
Pattern of invasion - Cohesiveness	Cohesive	0	4 (5.5)	0.139
	Non-cohesive	30 (41.1)	39 (53.4)	
Worst pattern of invasion	Type I	0	3 (4.1)	0.517
	Type II	0	1 (1.4)	
	Type III	5 (6.8)	10 (13.7)	
	Type IV	20 (27.4)	24 (32.9)	
	Type V	5 (6.8)	5 (6.8)	
Pattern of invasion – Aggressiveness	Non-aggressive	5 (6.8)	14 (19.2)	0.177
	Aggressive	25 (34.2)	29 (39.7)	
Perineural invasion	Present	13 (17.8)	9 (12.3)	0.068
	Absent	17 (23.3)	34 (46.6)	
Intraneural invasion	Present	5 (6.8)	1 (1.4)	0.075
	Absent	25 (34.2)	42 (57.5)	
Lymphovascular invasion	Present	6 (8.2)	3 (4.1)	0.148
	Absent	24 (32.9)	40 (54.8)	
Surgical margins	Clear	5 (6.8)	13 (17.8)	0.303
	Close	9 (12.3)	14 (19.2)	
	Involved	16 (21.9)	16 (21.9)	
Dysplasia at margins	None	26 (35.6)	27 (37.0)	0.068
	Mild	0	6 (8.2)	
	Moderate	3 (4.1)	5 (6.8)	
	Severe	1 (1.4)	5 (6.8)	
Lymphocytic host response	Weak	2 (2.7)	8 (11.0)	0.202
	Intermediate	22 (30.1)	23 (31.5)	
	Strong	6 (8.2)	12 (16.4)	
Tumour associated tissue eosinophilia	Present	27 (37)	28 (38.4)	**0.026***
	Absent	3 (4.1)	15 (20.5)	
Connective tissue	Fibrous	26 (35.6)	36 (49.3)	0.478
	Dense collagenous	3 (4.1)	7 (9.6)	
	Myxomatous	1 (1.4)	0	
Skeletal muscle bundles	Dense	5 (6.8)	15 (20.5)	0.112
	Degenerated	25 (83.3)	28 (65.1)	
Sarcolemmal spread	Present	25 (34.2)	24 (32.9)	**0.022***
	Absent	5 (6.8)	19 (26.0)	

Table 4.6: Continued

Characteristics		Presence of lymph node metastasis n(%)	Absence of lymph node metastasis n(%)	*p*-value
Salivary gland acini	Normal	3 (4.1)	11 (15.1)	0.115
	Degeneration with inflammation	27 (37.0)	30 (41.1)	
	Degeneration without inflammation	0	2 (2.7)	
Salivary gland ducts	Normal	2 (2.7)	10 (13.7)	0.212
	Ductal degeneration	3 (4.1)	2 (2.7)	
	Ectatic ducts	5 (6.8)	6 (8.2)	
	Hyperplasia of ducts	16 (21.9)	13 (17.8)	
	Squamous metaplasia	3 (4.1)	7 (9.6)	
	Mucous metaplasia	1 (1.4)	3 (4.1)	
	Tumour infiltrating duct	0	2 (2.7)	
Depth of invasion	≤5	5 (6.8)	16 (21.9)	**0.024***
	5<x≤10	9 (12.3)	17 (23.3)	
	>10	16 (21.9)	10 (13.7)	
Tumour infiltrative lymphocytes	Low	3 (4.1)	8 (11.0)	0.446
	Moderate	18 (24.7)	20 (27.4)	
	High	9 (12.3)	15 (20.5)	
Tumour budding grading	None	5 (6.8)	14 (19.2)	0.323
	Low	17 (23.3)	18 (24.7)	
	High	8 (11.0)	11 (15.1)	
Bone invasion	Present	8 (11.0)	10 (13.7)	0.787
	Absent	22 (30.1)	33 (45.2)	

*Fisher Exact test showing statistically significant association with lymph node metastasis (p<0.05)

When the six risk models: Broder's classification, Anneroth's classifciation, Bryne's model (1989), Bryne's model (1992), Brandwein-Gensler's model, and the Tumour budding and depth invasion grading were assessed for correlation with lymph node metastasis (**Table 4.7**), it was demonstrated that none of the risk models studied had a significant association with lymph node metastasis.

Table 4.7: Association between different risk models and lymph node metastasis (n=73).

Risk model		Presence of lymph node metastasis n (%)	Absence of lymph node metastasis n (%)	*p*-value
Broders Classification	Grade I	2 (2.7)	8 (11.0)	0.381
	Grade II	26 (35.6)	33 (45.2)	
	Grade III	2 (2.7)	2 (2.7)	
	Grade IV	0	0	
Anneroths Classification	Grade I	0	0	0.252
	Grade II	27 (37.0)	35 (47.9)	
	Grade III	3 (4.1)	8 (11.0)	
	Grade IV	0	0	
Brynes Model (1989)	Good	8 (11.0)	19 (26.0)	0.147
	Moderate	22 (30.1)	24 (32.9)	
	Poor	0	0	
Brynes Model (1992)	Good	4 (5.5)	12 (16.4)	0.195
	Moderate	26 (35.6)	30 (41.1)	
	Poor	0	1 (1.4)	
Brandwein-Gensler Model	Good	0	3 (4.1)	0.313
	Intermediate	17 (23.3)	26 (35.6)	
	Poor	13 (17.8)	14 (19.2)	
Tumour Budding & Depth Invasion Grading	Low risk	2 (2.7)	9 (12.3)	0.319
	Intermediate risk	20 (27.4)	25 (34.2)	
	High risk	8 (11.0)	9 (12.3)	

4.3 Association between Different Characteristics with Local Recurrences

Fisher Exact test was utilized to determine the correlation between socio-demographic, clinical, and histopathological characteristics with local recurrence (**Table 4.8**, **Table 4.9**, and **Table 4.10**). For socio-demographic characteristics, significant association was observed with previous history of cancer ($p<0.001$) (**Table 4.8**), whereby patients having previous history of cancer experienced higher incidence for local recurrence. For clinical characteristics, no significant association was demonstrated with local recurrence (**Table 4.9**). No significant association was observed between any histopathological variable and local recurrence as well (**Table 4.10**).

Table 4.8: Association between socio-demographic characteristics and of recurrence (n=73).

Characteristics		Presence of recurrence n(%)	Absence of recurrence n(%)	*p*-value
Age	≥60	3 (4.5)	36 (53.7)	0.635
	<60	1 (1.5)	27 (40.3)	
Gender	Female	2 (3.0)	37 (55.2)	1.000
	Male	2 (3.0)	26 (38.8)	
Ethnicity	Malay	0	12 (17.9)	0.812
	Chinese	1 (1.5)	17 (25.4)	
	Indian	3 (4.5)	34 (50.7)	
Smoking	Yes	1 (1.5)	23 (34.3)	1.000
	No	3 (4.5)	40 (59.7)	
Drinking Alcohol	Yes	0	16 (23.9)	0.565
	No	4 (6.0)	47 (70.1)	
Chewing Betel quid	Yes	2 (3.0)	21 (31.3)	0.603
	No	2 (3.0)	42 (62.7)	
Family history of cancer	Yes	0	7 (10.4)	1.000
	No	4 (6.0)	56 (83.6)	
Previous history of cancer	Yes	4 (6.0)	3 (4.5)	**<0.001***
	No	0	60 (89.6)	

*Fisher Exact test showing statistically significant association with presence of recurrence ($p<0.05$)

Table 4.9: Association between clinical characteristics and recurrence (n=73).

Characteristics		Presence of recurrence n(%)	Absence of recurrence n(%)	*p*-value
Site	Buccal mucosa	0	21 (31.3)	0.094
	Tongue	1 (1.5)	26 (38.8)	
	Palate	0	5 (7.5)	
	Floor of mouth	0	2 (3.0)	
	Alveolar process	3 (4.5)	9 (13.4)	
cTNM	Stage I	1 (1.5)	10 (10.3)	1.000
	Stage II	1 (1.5)	17 (25.4)	
	Stage III	0	10 (14.9)	
	Stage IV	2 (3.0)	26 (38.8)	

*Fisher Exact test showing statistically significant association with presence of recurrence ($p<0.05$)

Table 4.10: Association between histopathological characteristics and recurrence (n=73).

Characteristics		Presence of recurrence n (%)	Absence of recurrence n (%)	*p*-value
Degree of keratinization	No keratin	0	2 (3.0)	0.207
	Minimal	3 (4.5)	15 (22.4)	
	Moderate	1 (1.5)	33 (49.3)	
	High	0	13 (19.4)	
Extent of differentiation	Well	0	10 (14.9)	1.000
	Moderate	4 (6.0)	50 (74.6)	
	Poor	0	3 (4.5)	
Extent of pleomorphism	Mild	0	10 (14.9)	1.000
	Moderate	4 (6.0)	50 (74.6)	
	Abundant	0	3 (4.5)	
Grading of mitosis per 10 HPF	Grade 1	3 (4.5)	46 (68.7)	1.000
	Grade 2	1 (1.5)	16 (23.9)	
	Grade 3	0	1 (1.5)	
	Grade 4	0	0	
pTNM 7th edition	Stage I	1 (1.5)	7 (10.4)	0.767
	Stage II	1 (1.5)	19 (28.4)	
	Stage III	0	6 (9.0)	
	Stage IVa	2 (3.0)	29 (43.3)	
	Stage IVB	0	2 (3.0)	
pTNM 8th edition	Stage I	0	7 (10.4)	0.729
	Stage II	2 (3.0)	17 (25.4)	
	Stage III	0	7 (10.4)	
	Stage IVa	2 (3.0)	18 (26.9)	
	Stage IVB	0	14 (20.9)	
Pattern of invasion	Type I	0	3 (4.5)	0.668
	Type II	0	1 (1.5)	
	Type III	0	13 (19.4)	
	Type IV	4 (6.0)	46 (68.7)	
Pattern of invasion - Cohesiveness	Cohesive	0	4 (6.0)	1.000
	Non-cohesive	4 (6.0)	59 (88.1)	
Worst pattern of invasion	Type I	0	3 (4.5)	0.700
	Type II	0	1 (1.5)	
	Type III	0	13 (19.4)	
	Type IV	3 (4.5)	37 (55.2)	
	Type V	1 (1.5)	9 (13.4)	
Pattern of invasion – Aggressiveness	Non-aggressive	0	17 (25.4)	0.565
	Aggressive	4 (6.0)	46 (68.7)	
Perineural invasion	Present	0	20 (29.9)	0.309
	Absent	4 (6.0)	43 (64.2)	

Table 4.10: Continued

Characteristics		Presence of recurrence n (%)	Absence of recurrence n (%)	*p*-value
Intraneural invasion	Present	0	5 (7.5)	1.000
	Absent	4 (6.0)	58 (86.6)	
Lymphovascular invasion	Present	0	8 (11.9)	1.000
	Absent	4 (6.0)	55 (82.1)	
Surgical margins	Clear	1 (1.5)	17 (25.4)	1.000
	Close	1 (1.5)	20 (29.9)	
	Involved	2 (3.0)	26 (38.8)	
Dysplasia at margins	None	2 (3.0)	46 (68.7)	0.144
	Mild	0	6 (9.0)	
	Moderate	2 (3.0)	6 (9.0)	
	Severe	0	5 (7.5)	
Lymphocytic host response	Weak	1 (1.5)	7 (10.4)	0.294
	Intermediate	3 (4.5)	39 (58.2)	
	Strong	0	17 (25.4)	
Tumour associated tissue eosinophilia	Present	3 (4.5)	48 (71.6)	1.000
	Absent	1 (1.5)	15 (22.4)	
Connective tissue	Fibrous	2 (3.0)	56 (83.6)	0.084
	Dense collagenous	2 (3.0)	7 (10.4)	
	Myxomatous	0	0	
Skeletal muscle bundles	Dense	2 (3.0)	16 (23.9)	0.291
	Degenerated	2 (3.0)	47 (70.1)	
Sarcolemmal spread	Present	2 (3.0)	43 (64.2)	0.593
	Absent	2 (3.0)	20 (29.9)	
Salivary gland acini	Normal	1(1.5)	12 (17.9)	1.000
	Degeneration with inflammation	3 (4.5)	50 (74.6)	
	Degeneration without inflammation	0	1 (1.5)	
Salivary gland ducts	Normal	1 (1.5)	10 (14.9)	0.384
	Ductal degeneration	1 (1.5)	2 (3.0)	
	Ectatic ducts	0	10 (14.9)	
	Hyperplasia of ducts	2 (3.0)	26 (38.8)	
	Squamous metaplasia	0	10 (14.9)	
	Mucous metaplasia	0	4 (6.0)	
	Tumour infiltrating duct	0	1 (1.5)	
Depth of invasion	≤5	0	21 (31.3)	0.315
	5<x≤10	3 (4.5)	21 (31.3)	
	>10	1 (1.5)	21 (31.3)	

Table 4.10: Continued

Characteristics		Presence of recurrence n (%)	Absence of recurrence n (%)	*p*-value
Tumour infiltrative lymphocytes	Low	1 (1.5)	7 (10.4)	0.578
	Moderate	2 (3.0)	34 (50.7)	
	High	1 (1.5)	22 (32.8)	
Tumour budding grading	None	0	17 (25.4)	0.360
	Low	2 (3.0)	30 (44.8)	
	High	2 (3.0)	16 (23.9)	
Bone invasion	Present	2 (3.0)	14 (20.9)	0.239
	Absent	2 (3.0)	49 (73.1)	
Lymph node metastasis	Yes	0	26 (38.8)	0.152
	No	4 (6.0)	63 (94.0)	
Extracapsular spread	Present	0	14 (20.9)	0.572
	Absent	4 (6.0)	49 (73.1)	
Skip metastasis	Present	0	10 (14.9)	1.000
	Absent	4 (6.0)	53 (79.1)	

When the six risk models: Broder's classification, Anneroth's classifciation, Bryne's model (1989), Bryne's model (1992), Brandwein-Gensler's model, and the Tumour budding and depth invasion grading were assessed for correlation with recurrence (**Table 4.11**), it was demonstrated that only Anneroth's classification had a significant association with recurrence (p=0.004) while the other risk models did not show a significant relation with recurrence.

Table 4.11: Association between different risk models and recurrence (n=73).

Risk model		Presence of recurrence n(%)	Absence of recurrence n(%)	*p*-value
Broders Classification	Grade I	0	9 (13.4)	1.000
	Grade II	4 (6.0)	50 (74.6)	
	Grade III	0	4 (6.0)	
	Grade IV	0	0	
Anneroths Classification	Grade I	0	0	**0.004***
	Grade II	1 (1.5)	58 (86.6)	
	Grade III	3 (4.5)	5 (7.5)	
	Grade IV	0	0	
Brynes Model (1989)	Good	0	24 (35.8)	0.288
	Moderate	4 (6.0)	39 (58.2)	
	Poor	0	0	

Table 4.11: Continued

Risk model		Presence of recurrence n(%)	Absence of recurrence n(%)	*p*-value
Brynes Model (1992)	Good	0	15 (22.4)	0.592
	Moderate	4 (6.0)	47 (70.1)	
	Poor	0	1 (1.5)	
Brandwein-Gensler Model	Good	0	3 (4.5)	0.691
	Intermediate	2 (3.0)	38 (56.7)	
	Poor	2 (3.0)	22 (32.8)	
Tumour Budding & Depth Invasion Grading	Low risk	0	11 (16.4)	0.330
	Intermediate risk	2 (3.0)	39 (58.2)	
	High risk	2 (3.0)	13 (19.4)	

*Fisher Exact test showing statistically significant association with presence of recurrence ($p<0.05$).

4.4 Association between Different Characteristics with Overall Survival

The mean follow-up for this cohort was 30 months and the average 5-year survival rate was 66%. Throughout the course of this study, 21 patients were lost to follow up. A univariate logistic regression analysis was utilized to determine associations between the socio-demographic, clinical, histopathological characteristics, and established risk models with overall survival (**Table 4.12, 4.13, 4.14, and 4.15**). A significant association was seen between overall survival with bone invasion ($p=0.016$), ECS ($p=0.010$), DOI ($p=0.031$) and number of tumour budding ($p=0.028$). Among the six risk models studied, the Tumour budding and depth invasion grading demonstrated a significant association with overall survival ($p=0.035$)

Table 4.12: Association between socio-demographic characteristics and overall survival.

Characteristics	Overall survival (*p*-value)
Age	0.998
Gender	0.443
Ethnicity	0.808
Smoking	0.345
Alcohol	0.354
Betel quid	0.359
Family history of cancer	0.275
Previous history of cancer	0.897

Table 4.13: Association between clinical characteristics and overall survival.

Variable	Overall survival (*p*-value)
Site	0.688
cTNM	0.742

Table 4.14: Association between histopathological characteristics and overall survival.

Variable	Overall survival (*p*-value)
Degree of keratinization	0.214
Extent of differentiation	0.198
Extent of pleomorphism	0.198
Grading of mitosis	0.657
pTNM (7^{th} edition)	0.188
pTNM (8^{th} edition)	0.168
Pattern of invasion	0.318
Pattern of invasion - Cohesiveness	0.234
Worst pattern of invasion	0.415
Pattern of invasion - Aggressiveness	0.069
Perineural invasion	0.353
Intraneural invasion	0.571
Lymphovascular invasion	0.720
Surgical margins	0.626
Dysplasia at margins	0.070
Extracapsular spread	**0.010***
Skip metastasis	0.071
Lymphocytic host response	0.757
Tumour associated host response	0.253
Connective tissue	0.184
Skeletal muscle bundles	0.364
Sarcolemmal spread	0.300
Salivary gland acini	0.886
Salivary gland ducts	0.917
Lymph node metastasis	0.422
Depth of invasion	**0.031***
Tumour infiltrative lymphocytes	0.409
Tumour budding grading	**0.028***
Bone invasion	**0.016***

*Kaplan-Meier showing statistically significant overall survival ($p<0.05$).

Table 4.15: Association between different risk models and overall survival.

Risk Model	Overall survival (*p*-value)
Broders risk model	0.505
Anneroths risk model	0.832
Brynes (1989) risk model	0.054
Brynes (1992) risk model	0.713
Brandwein-Gensler risk model	0.406
Tumour budding and depth invasion risk model	**0.035***

*Kaplan-Meier showing statistically significant overall survival ($p<0.05$).

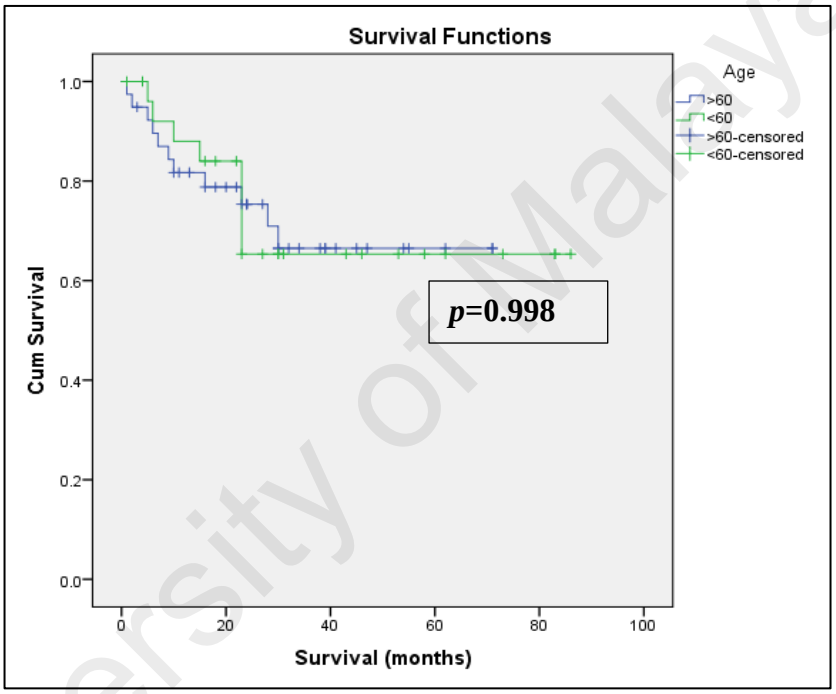


Figure 4.2: Kaplan-Meier Curve for association between age and overall survival.

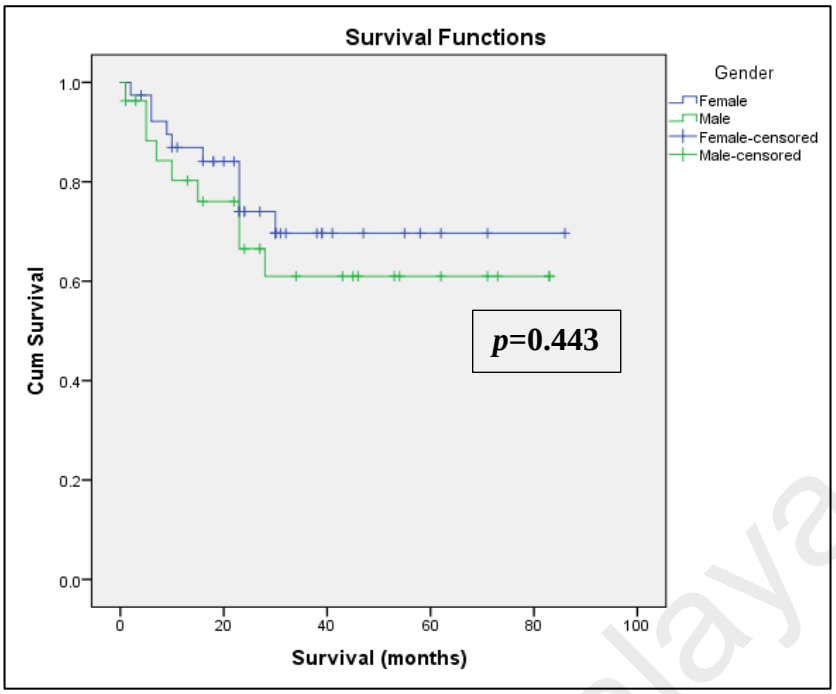


Figure 4.3: Kaplan-Meier Curve for association between gender and overall survival.

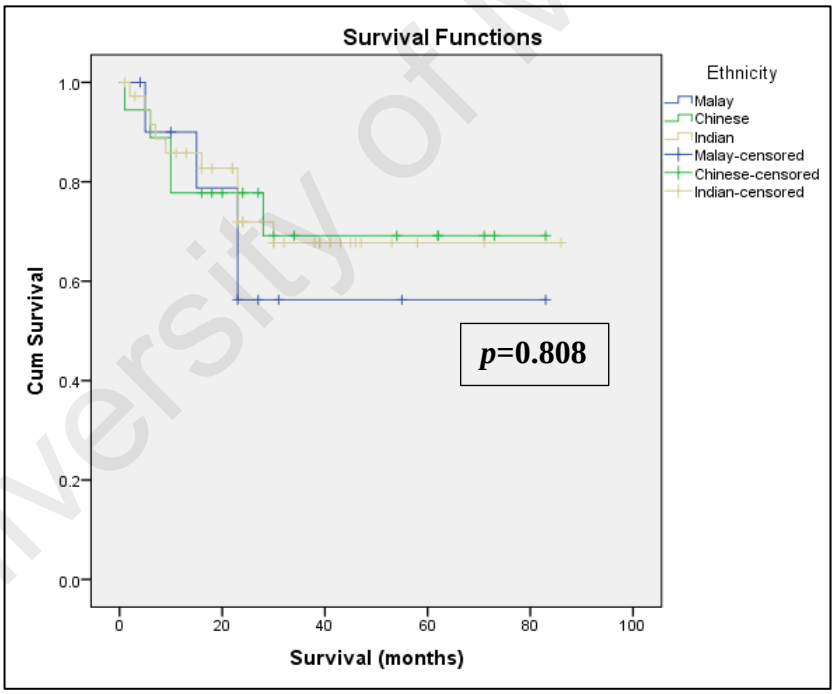


Figure 4.4: Kaplan-Meier Curve for association between ethnicity and overall survival.

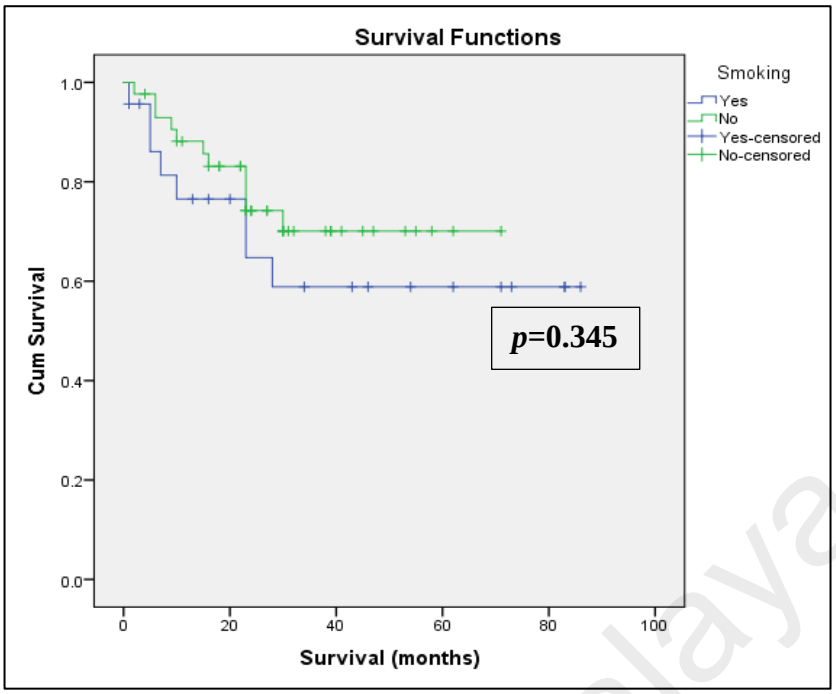


Figure 4.5: Kaplan-Meier Curve for association between smoking status and overall survival.

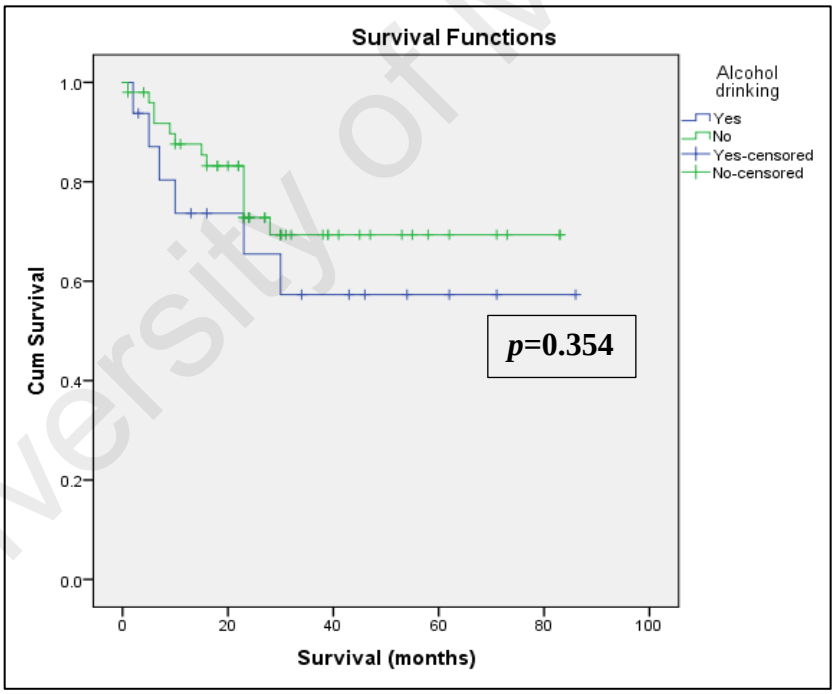


Figure 4.6: Kaplan-Meier Curve for association between alcohol consumption and overall survival.

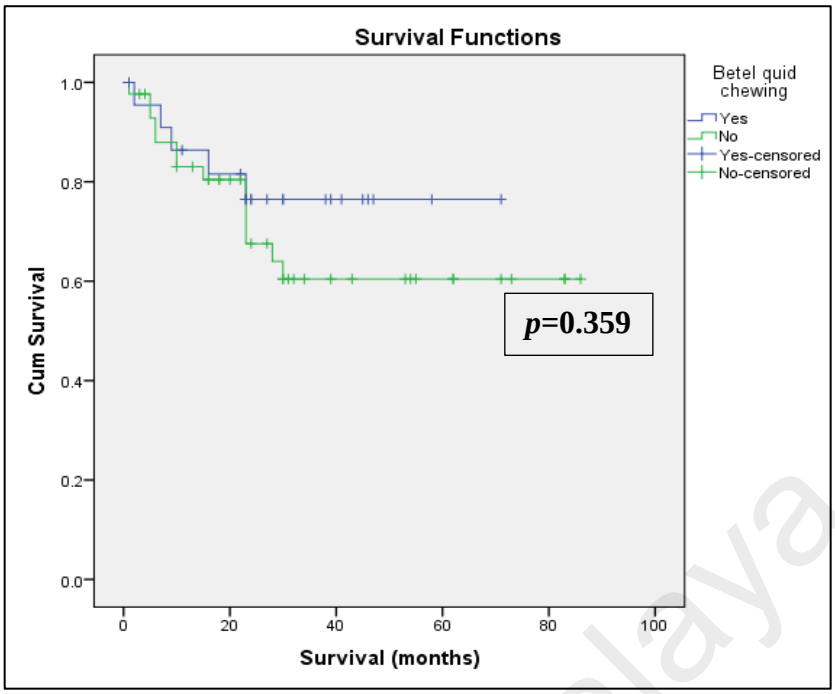


Figure 4.7: Kaplan-Meier Curve for association between betel quid use and overall survival.

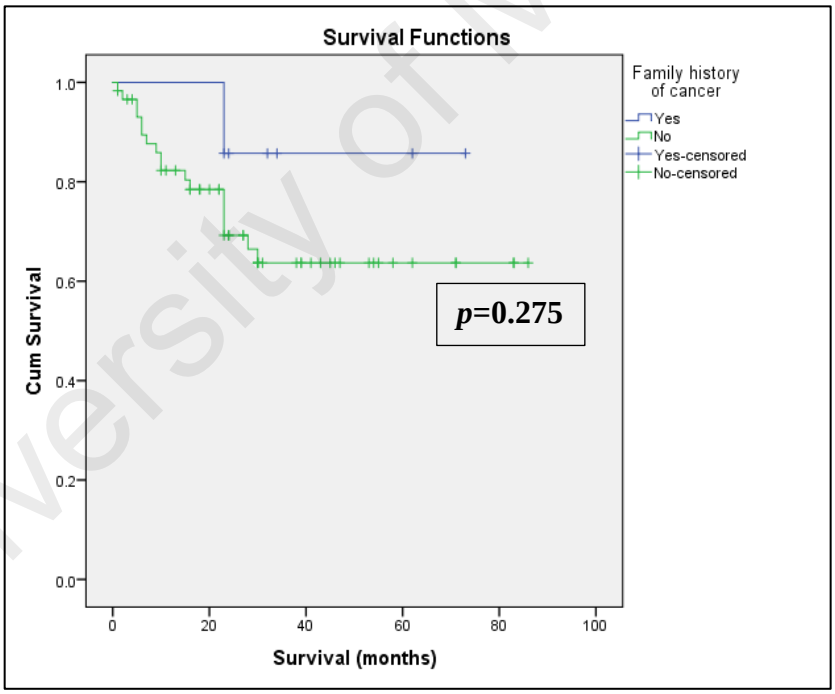


Figure 4.8: Kaplan-Meier Curve for association between family history of cancer and overall survival.

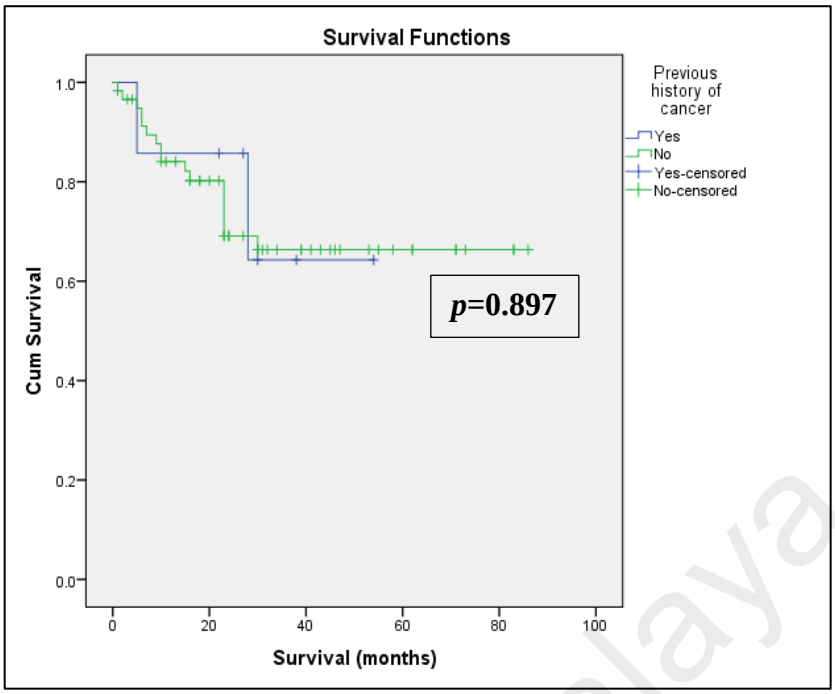


Figure 4.9: Kaplan-Meier Curve for association between previous history of cancer and overall survival.

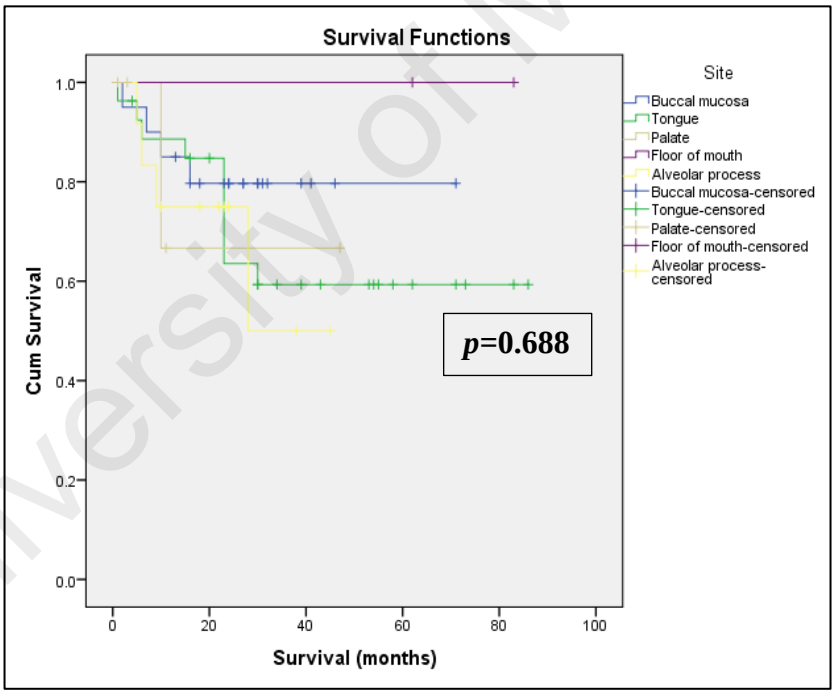


Figure 4.10: Kaplan-Meier Curve for association between site of cancer and overall survival.

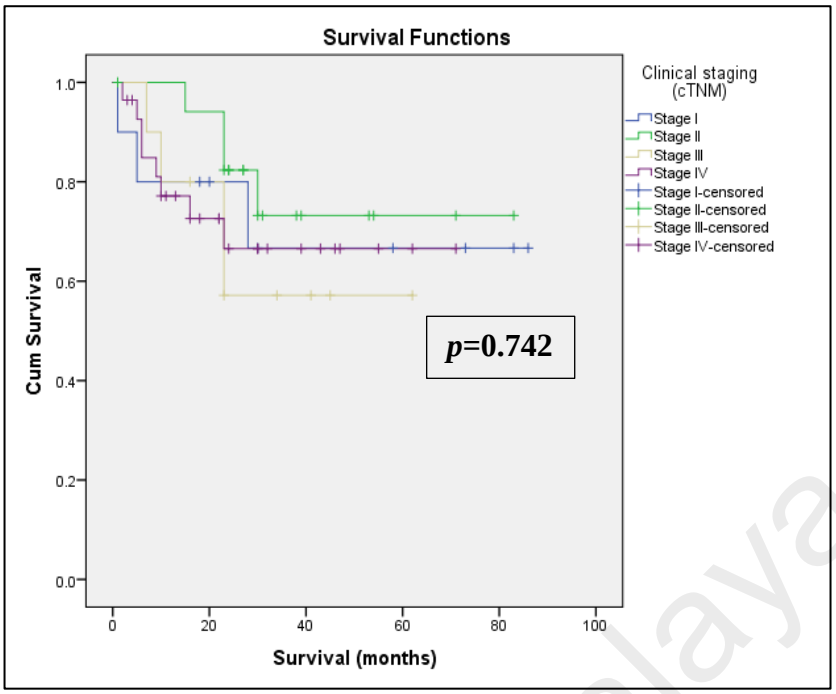


Figure 4.11: Kaplan-Meier Curve for association between cTNM and overall survival.

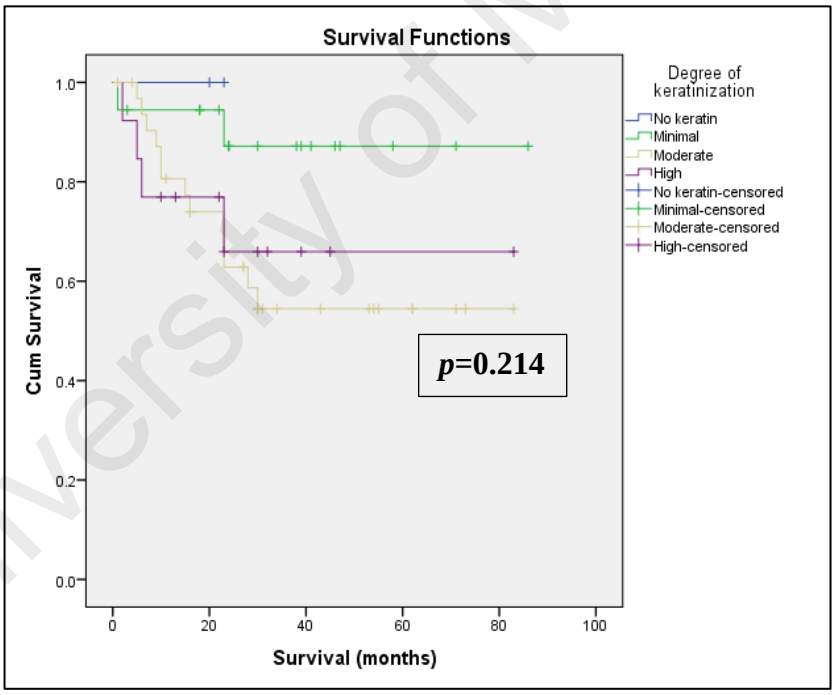


Figure 4.12: Kaplan-Meier Curve for association between degree of keratinization and overall survival.

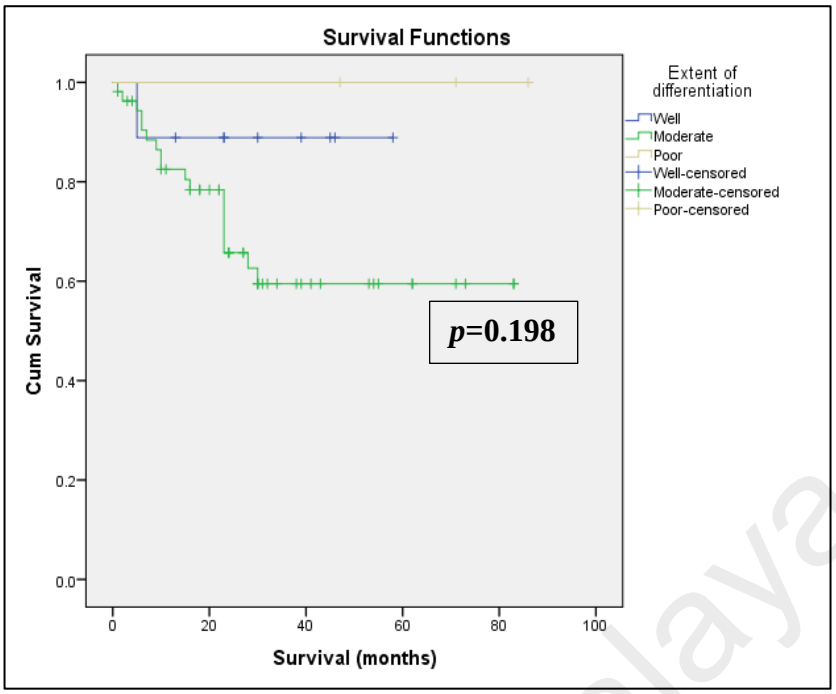


Figure 4.13: Kaplan-Meier Curve for association between extent of differentiation and overall survival.

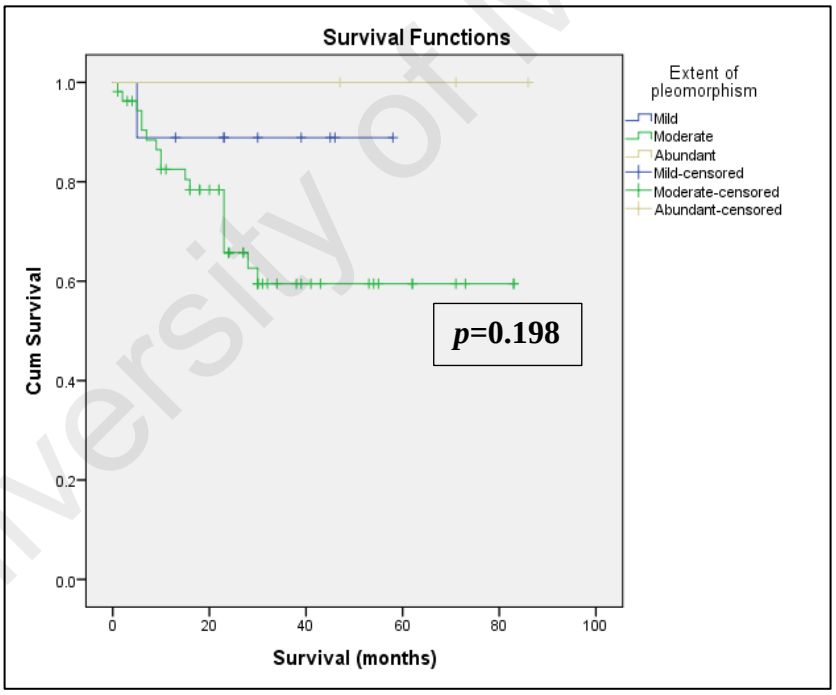


Figure 4.14: Kaplan-Meier Curve for association between extent of pleomorphism and overall survival.

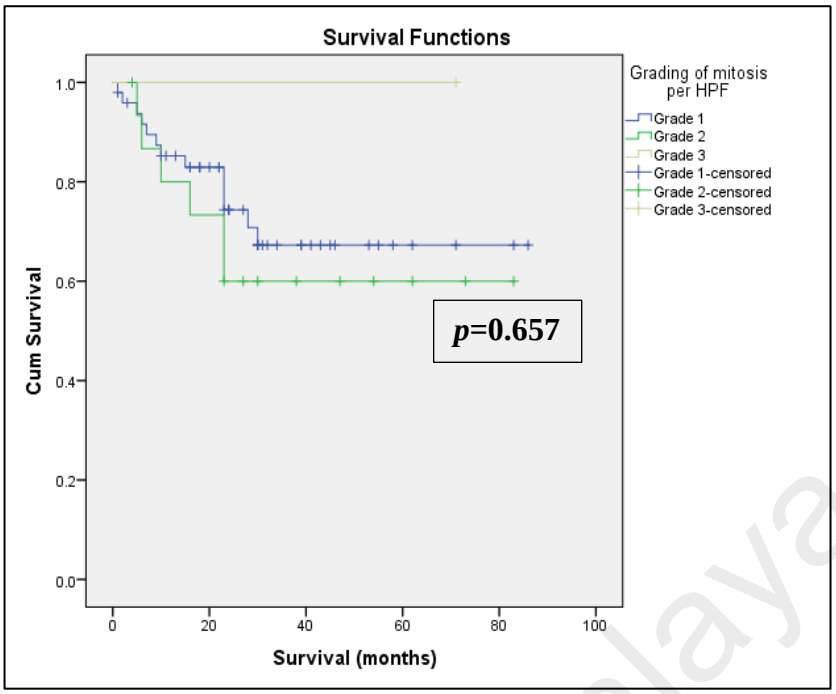


Figure 4.15: Kaplan-Meier Curve for association between grade of mitosis and overall survival.

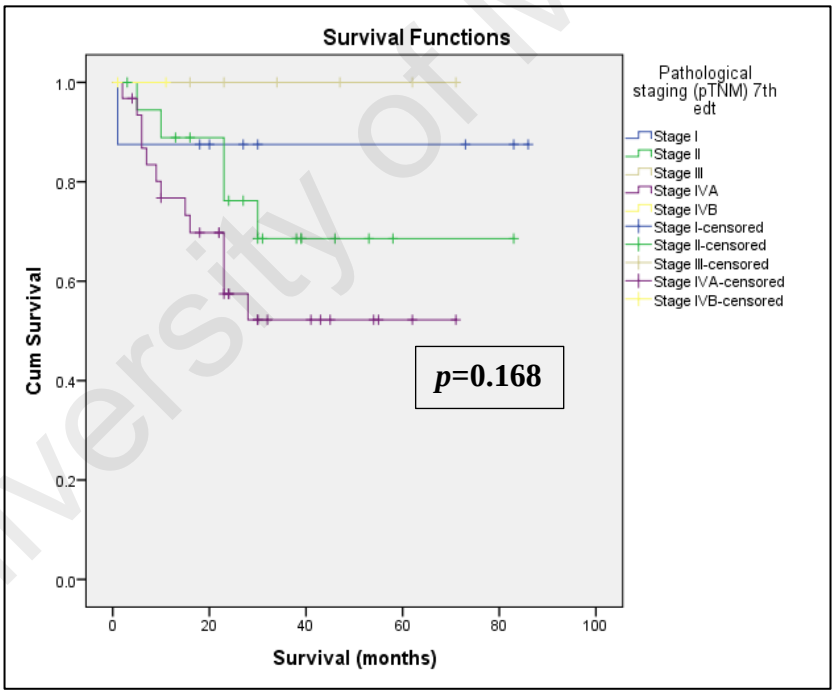


Figure 4.16: Kaplan-Meier Curve for association between pTNM (7th edt) and overall survival.

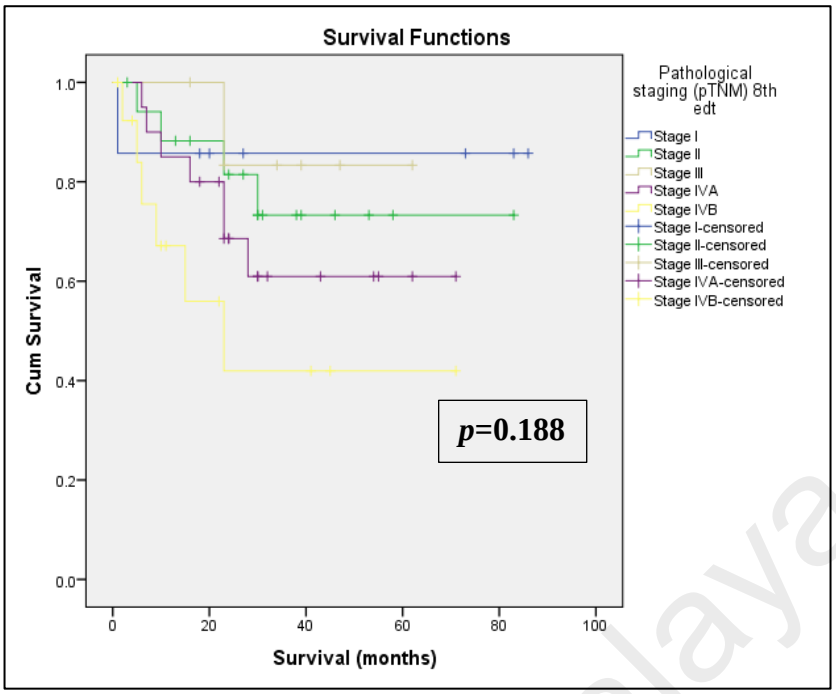


Figure 4.17: Kaplan-Meier Curve for association between pTNM (8th edt) and overall survival

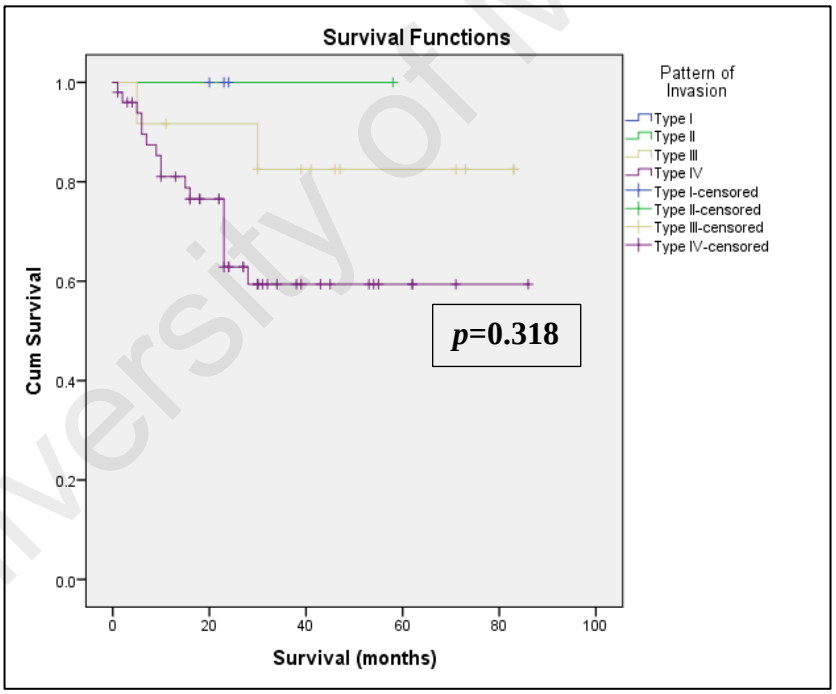


Figure 4.18: Kaplan-Meier Curve for association between POI and overall survival.

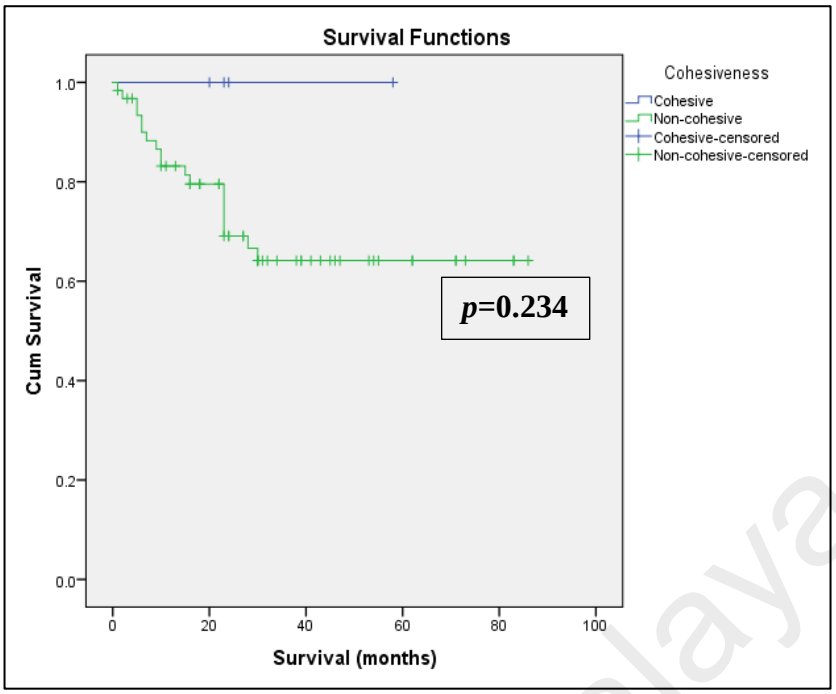


Figure 4.19: Kaplan-Meier Curve for association between POI-cohesiveness and overall survival

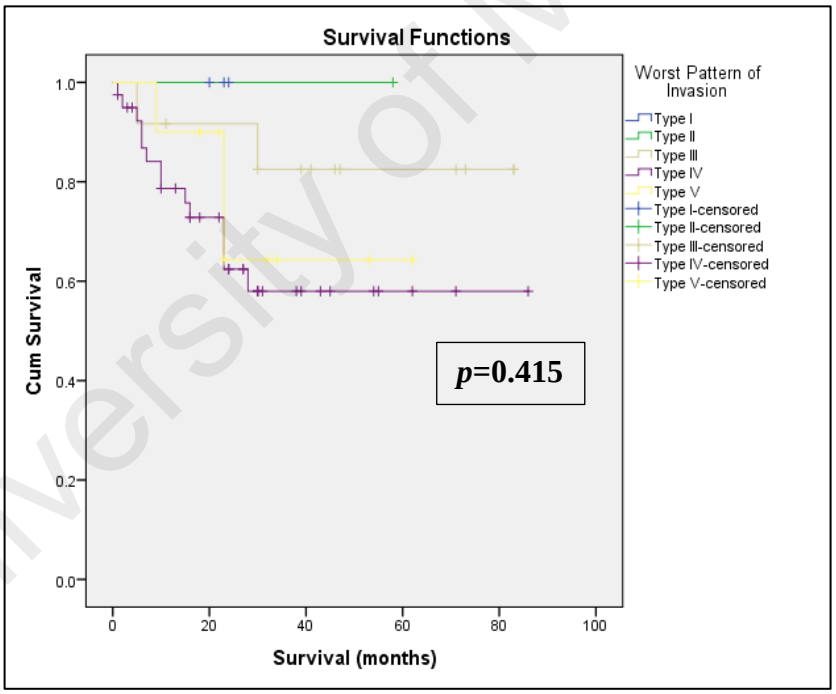


Figure 4.20: Kaplan-Meier Curve for association between wPOI and overall survival.

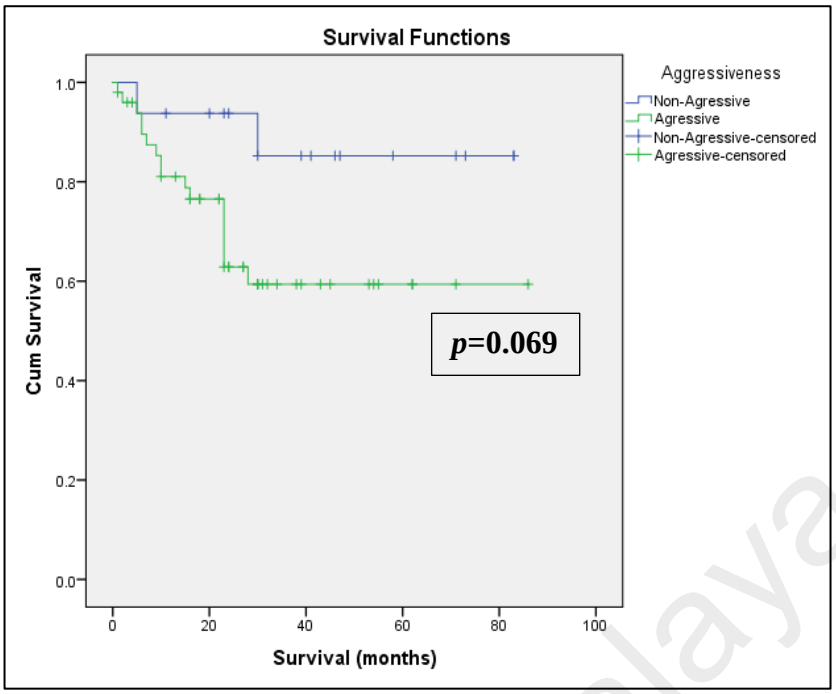


Figure 4.21: Kaplan-Meier Curve for association between POI-aggressiveness and overall survival.

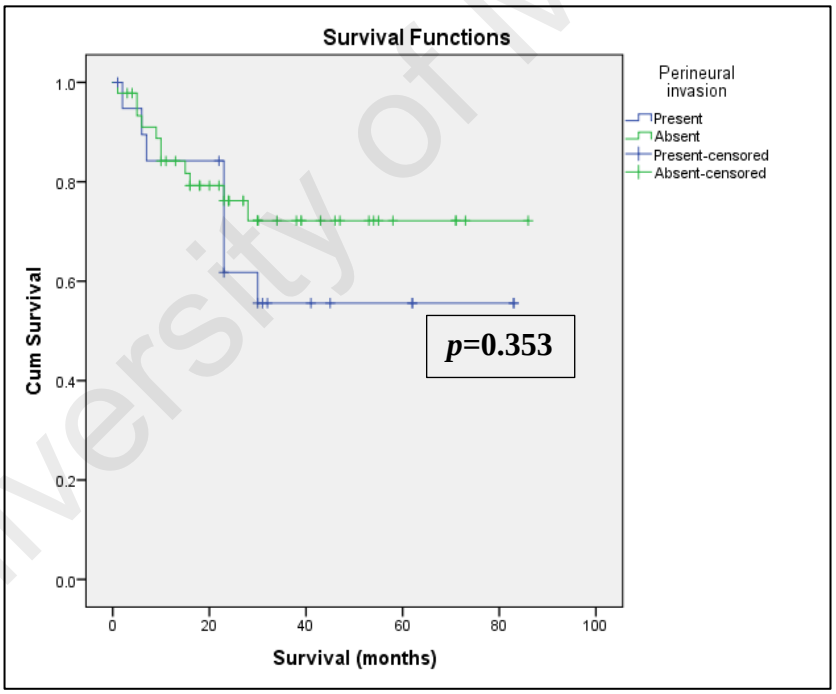


Figure 4.22: Kaplan-Meier Curve for association between PNI and overall survival.

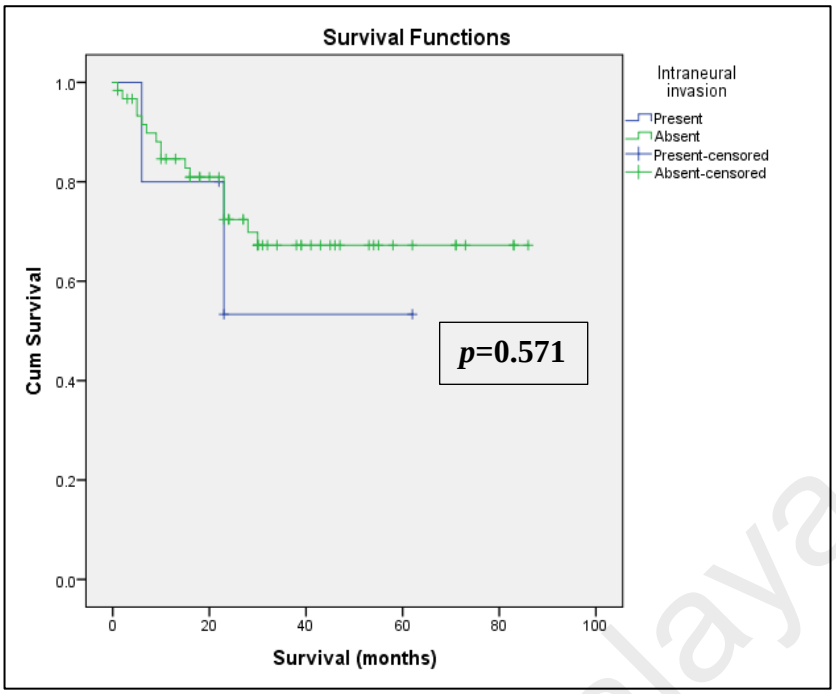


Figure 4.23: Kaplan-Meier Curve for association between intraneural invasion and overall survival.

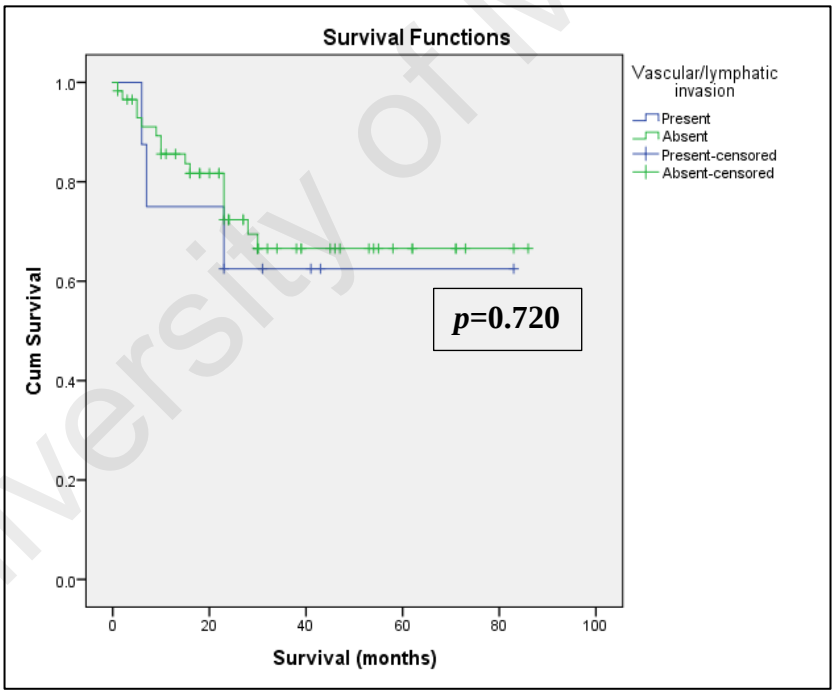


Figure 4.24: Kaplan-Meier Curve for association between LVI and overall survival.

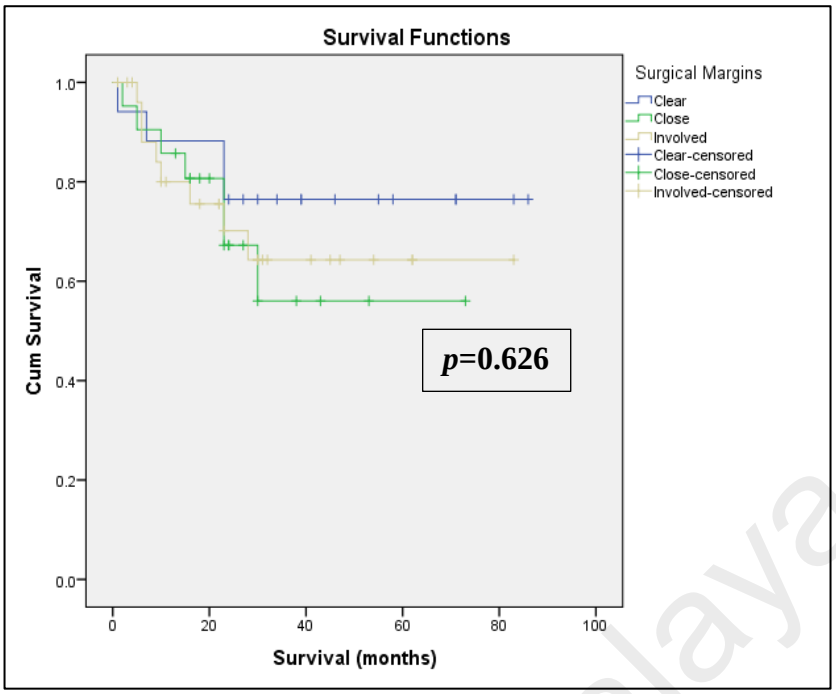


Figure 4.25: Kaplan-Meier Curve for association between surgical margins and overall survival.

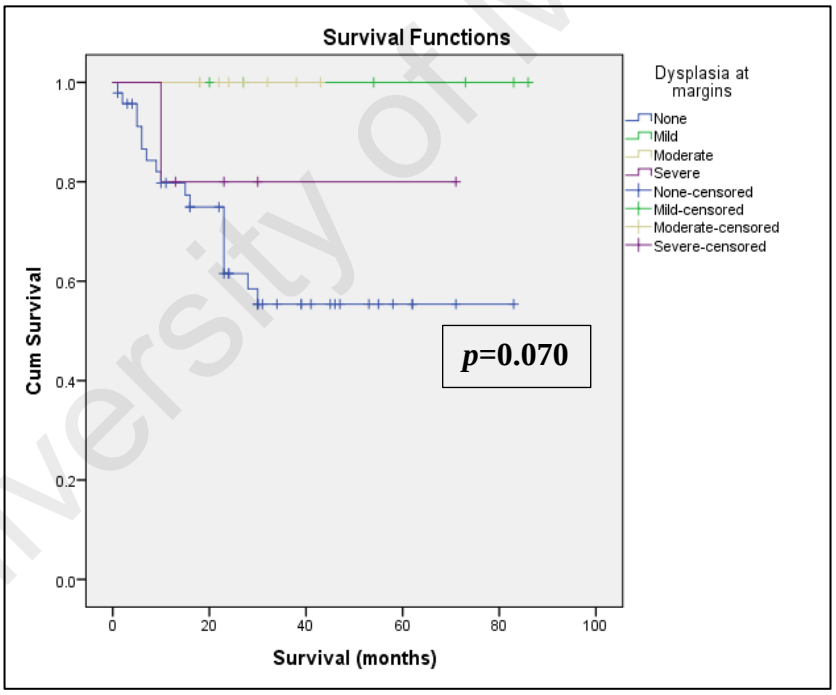


Figure 4.26: Kaplan-Meier Curve for association between dysplasia at margin and overall survival.

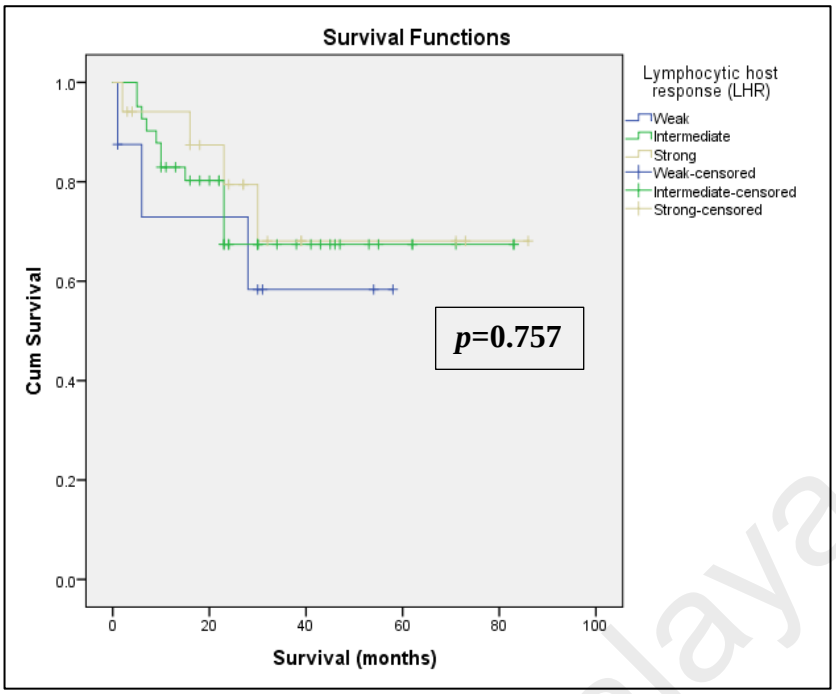


Figure 4.27: Kaplan-Meier Curve for association between LHR and overall survival.

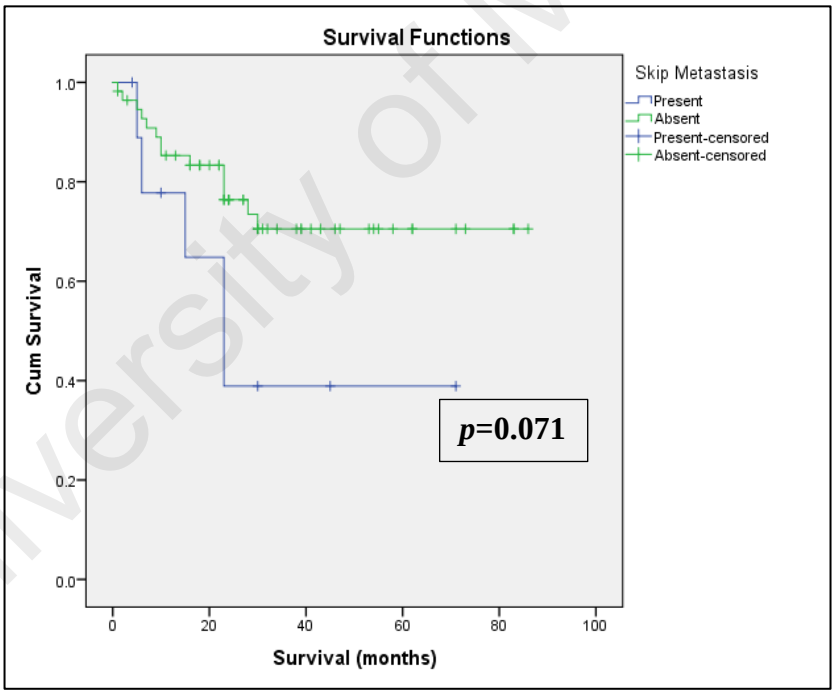


Figure 4.28: Kaplan-Meier Curve for association between skip metastasis and overall survival.

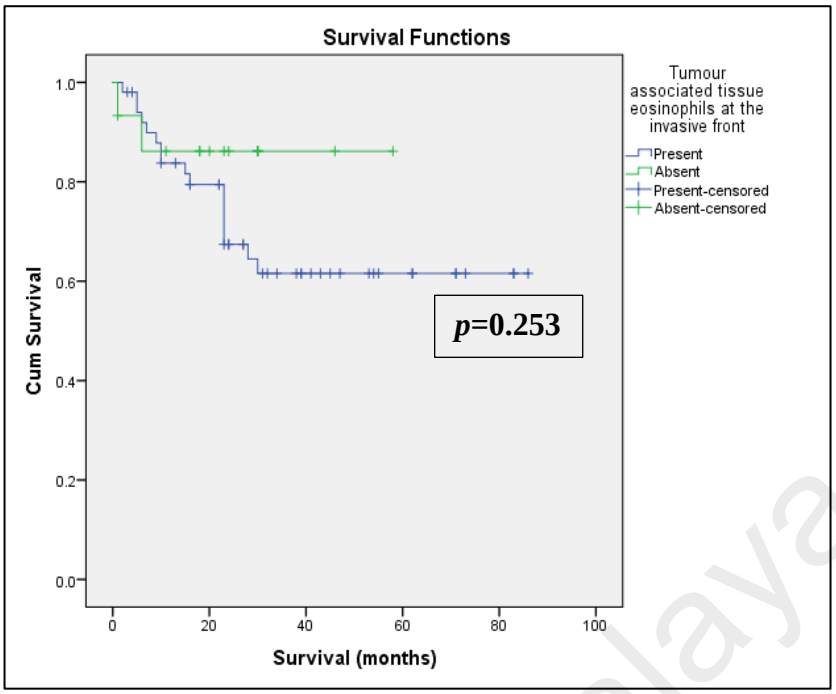
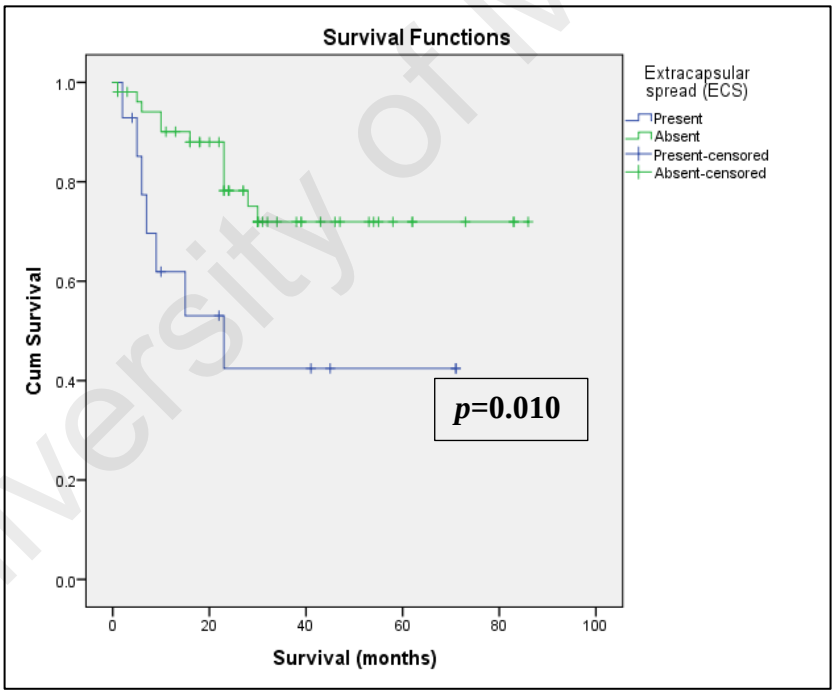


Figure 4.29: Kaplan-Meier Curve for association between TATE and overall survival.



*Kaplan-Meier showing statistically significant overall survival ($p<0.05$)

Figure 4.30: Kaplan-Meier Curve for association between ECS and overall survival.

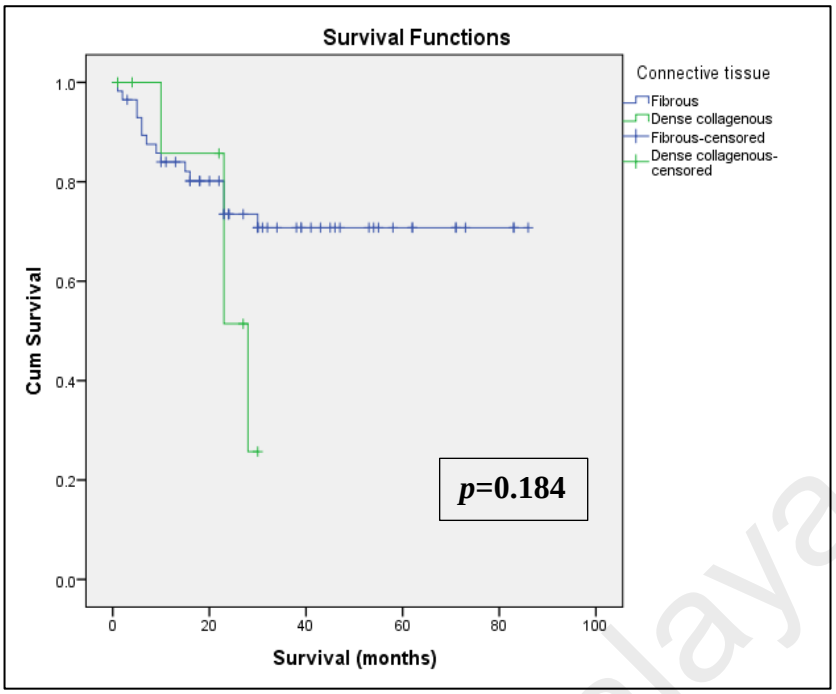


Figure 4.31: Kaplan-Meier Curve for association between type of connective tissue and overall survival.

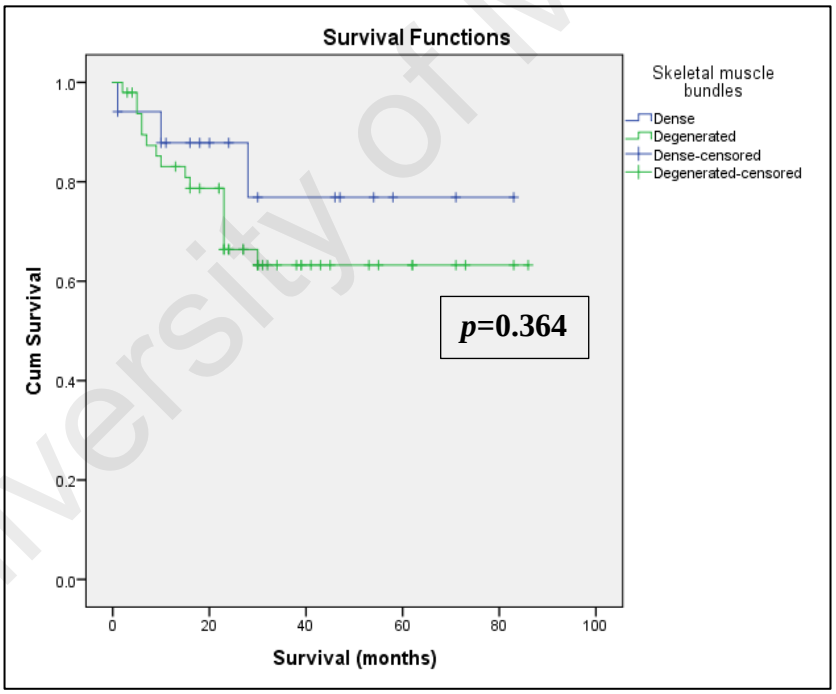


Figure 4.32: Kaplan-Meier Curve for association between condition of skeletal muscle bundles and overall survival.

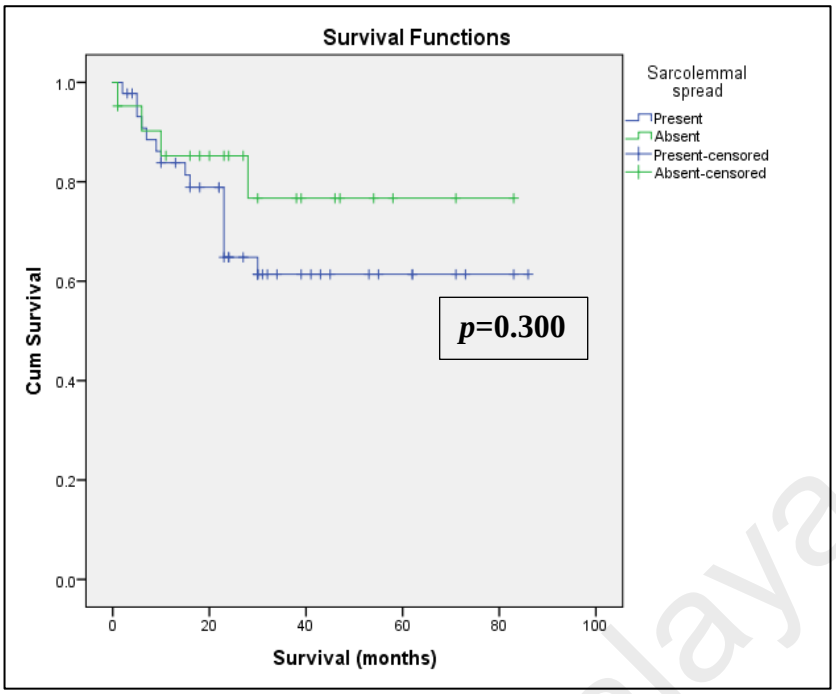


Figure 4.33: Kaplan-Meier Curve for association between sarcolemmal spread and overall survival.

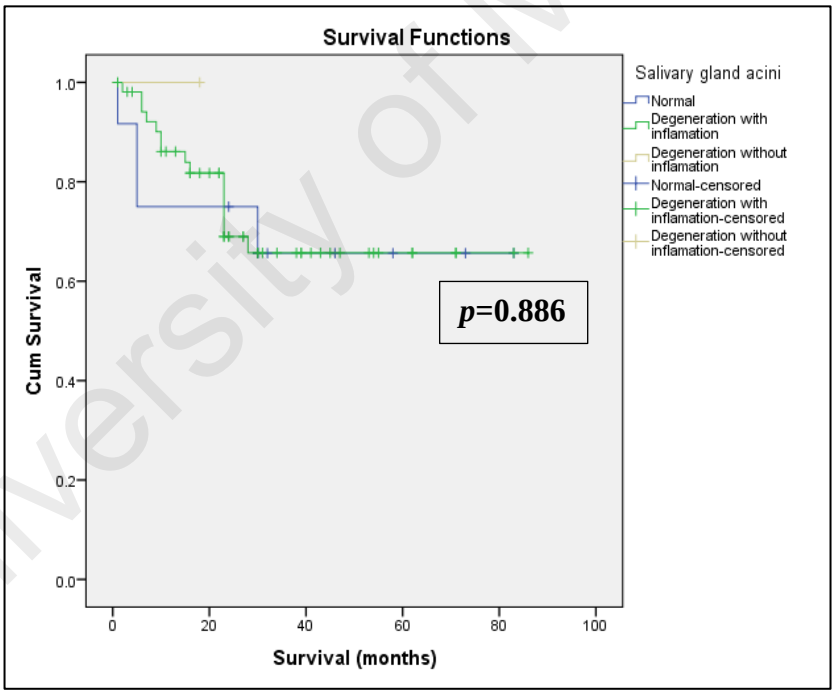


Figure 4.34: Kaplan-Meier Curve for association between status of salivary gland acini and overall survival.

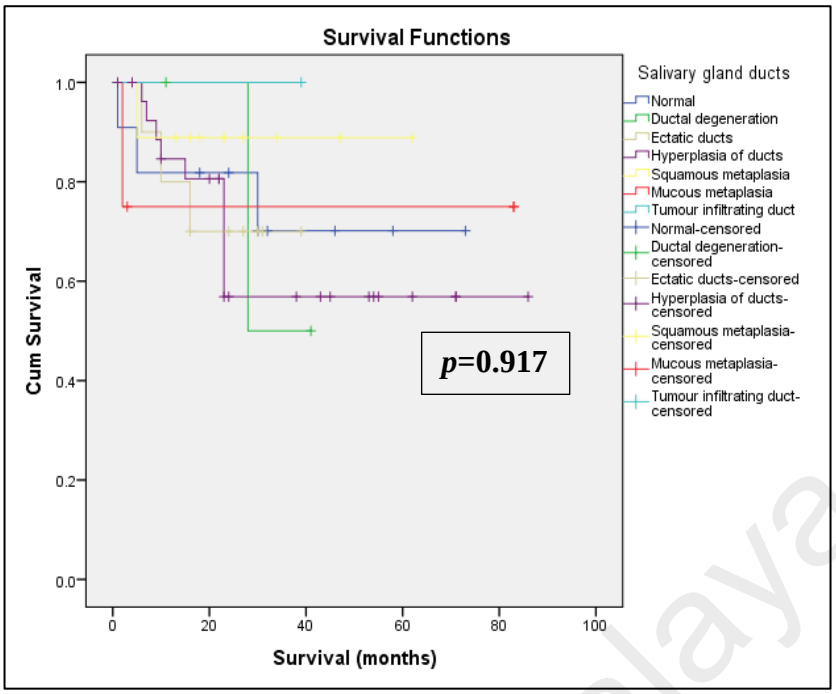


Figure 4.35: Kaplan-Meier Curve for association between status of salivary gland ducts and overall survival

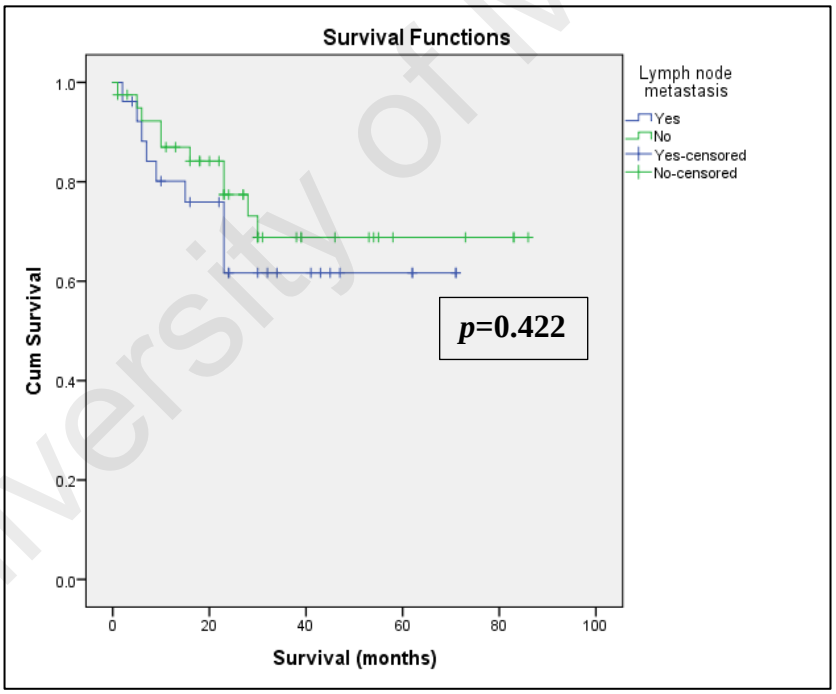
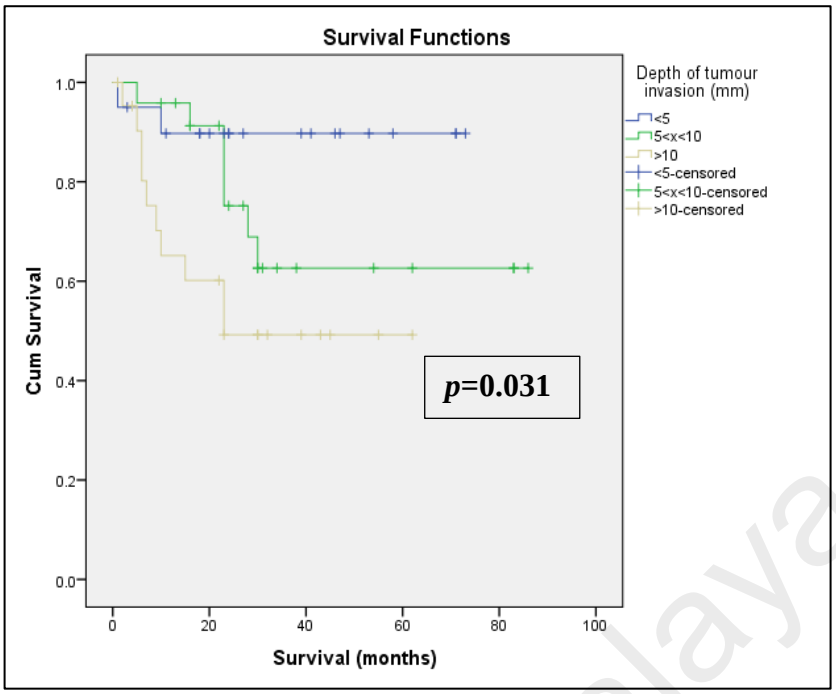


Figure 4.36: Kaplan-Meier Curve for association between lymph node metastasis and overall survival.



*Kaplan-Meier showing statistically significant overall survival (p<0.05)

Figure 4.37: Kaplan-Meier Curve for association between DOI and overall survival.

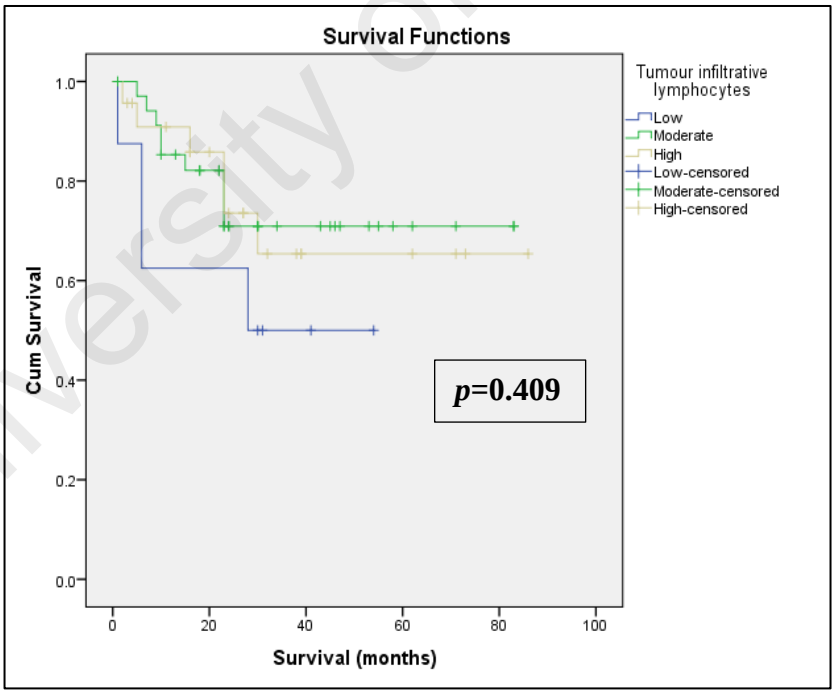
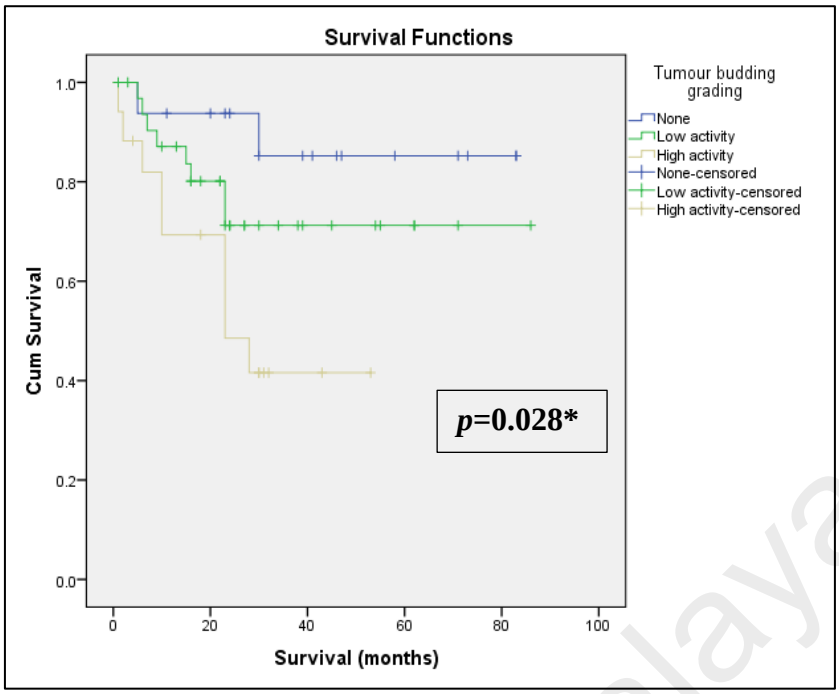
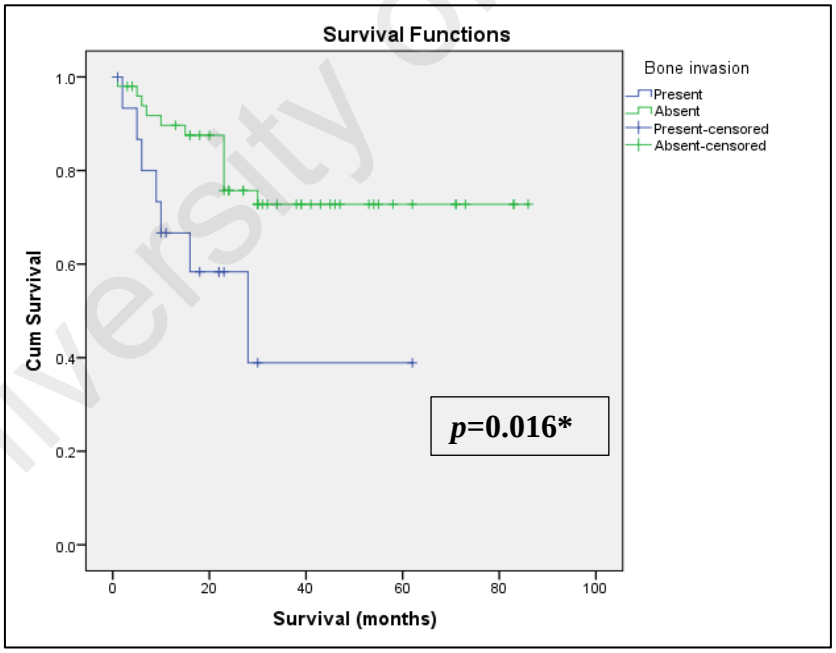


Figure 4.38: Kaplan-Meier Curve for association between TIL and overall survival.



*Kaplan-Meier showing statistically significant overall survival (p<0.05)

Figure 4.39: Kaplan-Meier Curve for association between tumour budding grading and overall survival.



*Kaplan-Meier showing statistically significant overall survival (p<0.05)

Figure 4.40: Kaplan-Meier Curve for association between bone invasion and overall survival.

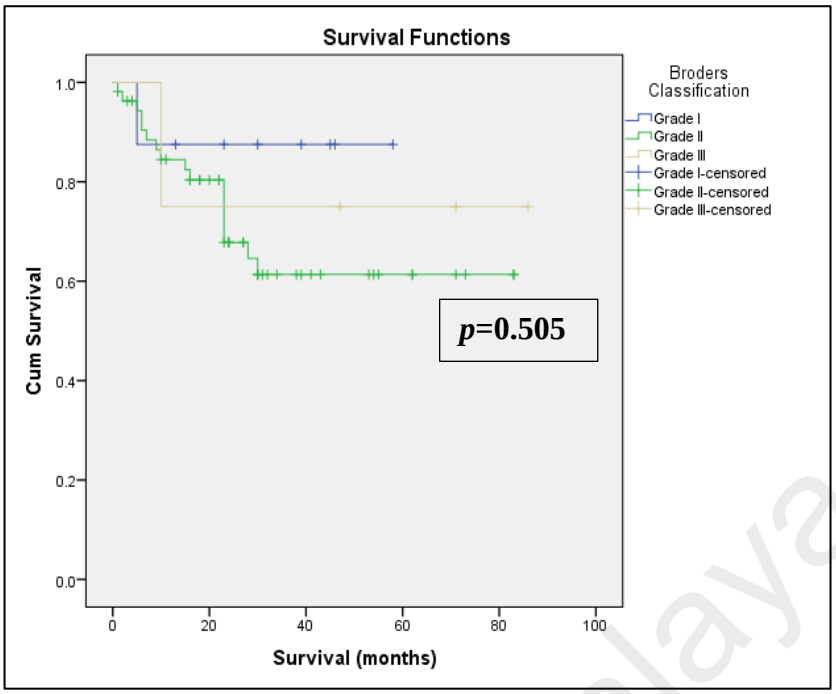


Figure 4.41: Kaplan-Meier Curve for association between Broders classification and overall survival.

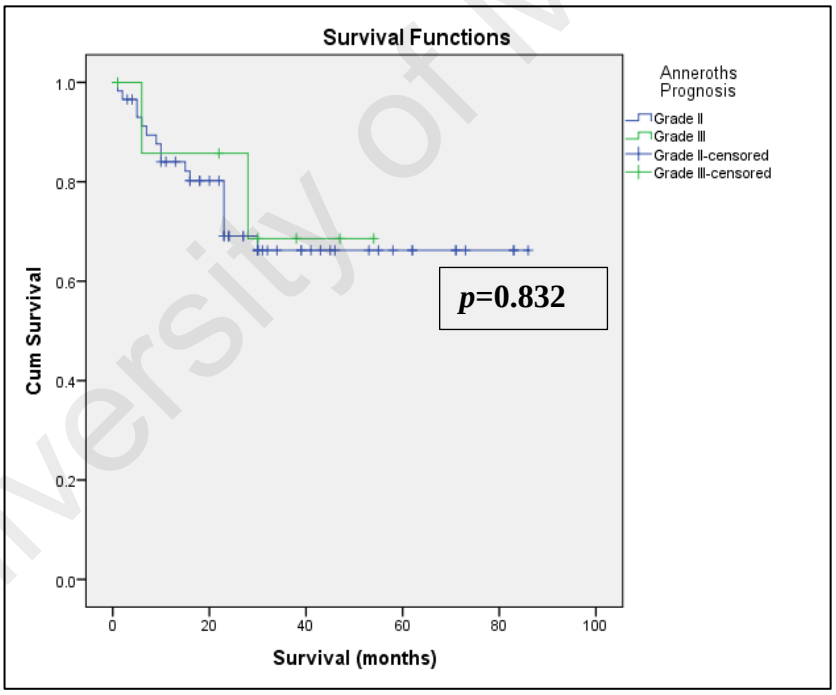


Figure 4.42: Kaplan-Meier Curve for association between Anneroths classification and overall survival.

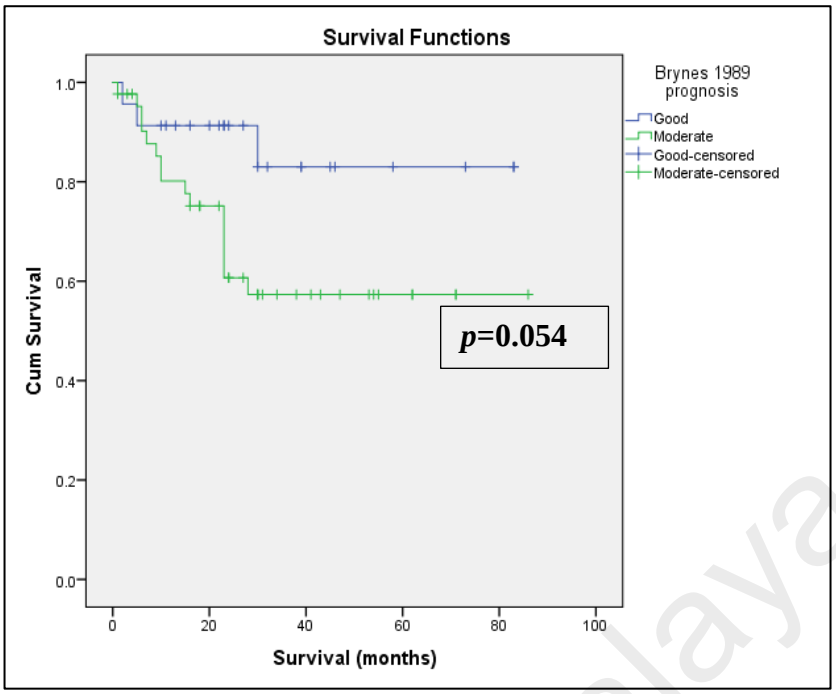


Figure 4.43: Kaplan-Meier Curve for association between Brynes model (1989) and overall survival.

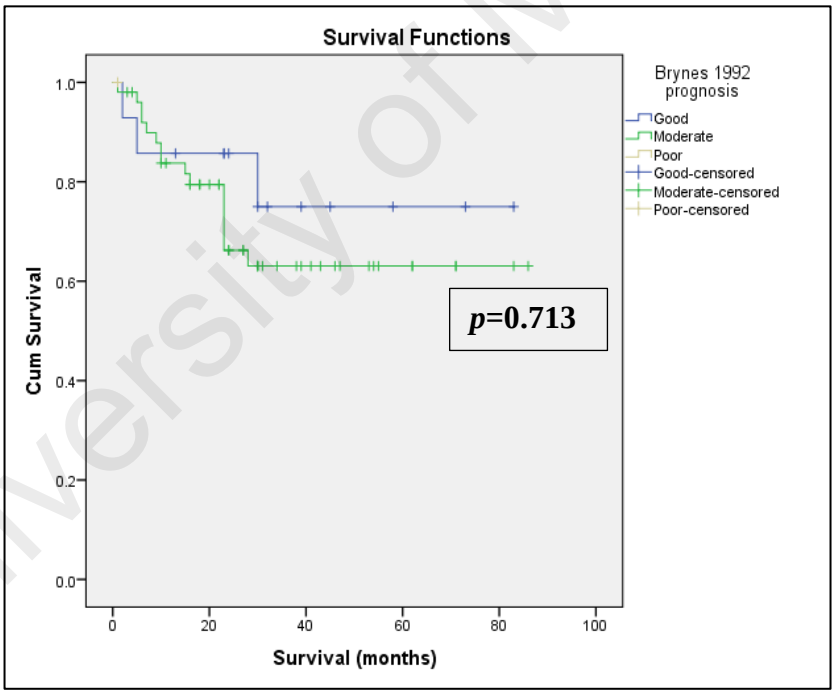
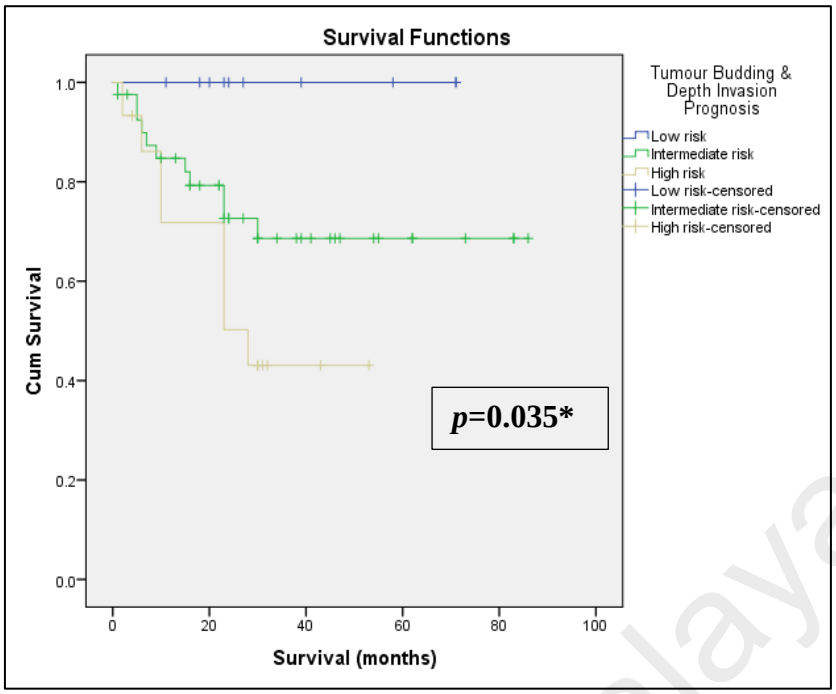


Figure 4.44: Kaplan-Meier Curve for association between Brynes model (1992) and overall survival.



*Kaplan-Meier showing statistically significant overall survival (p<0.05)

Figure 4.45: Kaplan-Meier Curve for association between tumour budding and depth of invasion grading and overall survival.

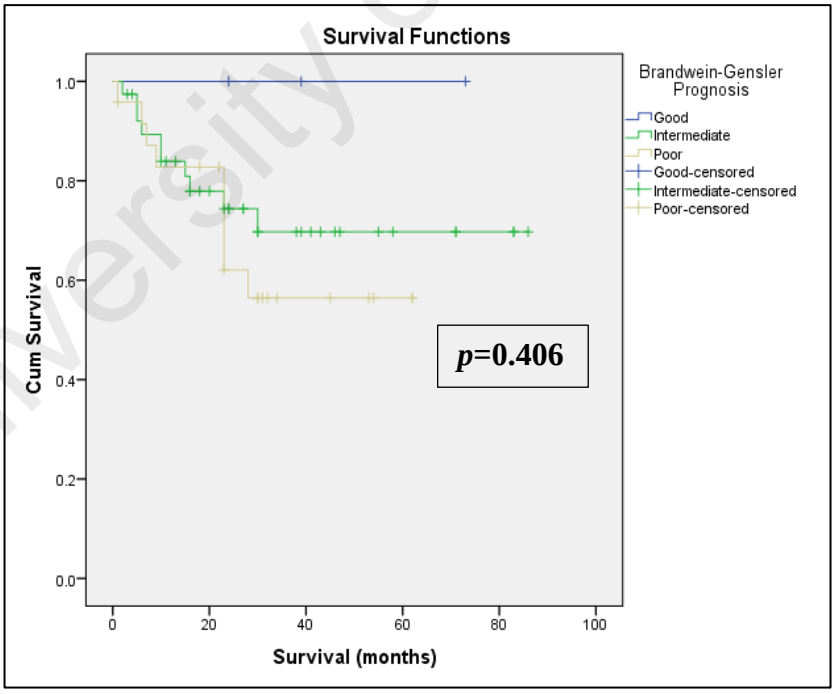


Figure 4.46: Kaplan-Meier Curve for association between Brandwein-Gensler model and overall survival

4.5 A Multivariate Analysis for Significant Characteristics

A multivariate analysis utilizing Cox regression (overall survival) (**Table 4.16**) and Binary logistic regression (lymph node metastasis and recurrence) (**Table 4.17** and **Table 4.18**) determined that only ECS had an independent association with overall survival (p=0.003, HR= 5.702, CI= 1.778-18.286).

Table 4.16: Cox regression showing characteristics, associated *p*-values, hazard ratio and confidence interval for overall survival.

Characteristics	*p*-value	Hazard ratio	95% Confidence Interval
ECS	**0.003***	5.702	1.778-18.286
DOI (mm)(1)	0.284	2.717	0.437-16.894
DOI (mm)(2)	0.208	3.149	0.527-18.799
Tumour budding grading (1)	0.999	0.999	0.166-6.008
Tumour budding grading (2)	0.139	3.885	0.643-23.461
Bone invasion	0.133	2.118	0.796-5.632

*Cox regression showing statistically significant association with overall survival (p<0.05).

Table 4.17: Binary logistic regression showing characteristics, associated *p*-values, hazard ratio and confidence interval for lymph node metastasis.

Characteristics	*p*-value	Hazard ratio	95% Confidence Interval
Depth of tumour invasion(1)	0.830	1.167	0.284-4.789
Depth of tumour invasion(2)	0.080	0.287	0.071-1.162
Tumour associated tissue eosinophils at the invasive front(1)	0.091	0.261	0.055-1.240
Sarcolemmal spread(1)	0.240	0.453	0.121-1.697

Table 4.18: Binary logistic regression showing characteristics, associated *p*-values, odds ratio and confidence interval for local recurrence.

Characteristics	*p*-value	Hazard ratio	95% Confidence Interval
Site (1)	0.998	.000	.000
Site (2)	0.999	.000	.000
Site (3)	1.000	.000	.000
Site (4)	0.998	.000	.000
Previous history of cancer(1)	0.997	.000	.000

4.6 Development of a Preliminary Risk Model

From the data collected and statistical analysis carried out, a suggested preliminary modified risk model may be developed:

Features	Score		
	0	1	2
ECS	Absent	Present	
DOI	≤5mm	5<x≤10mm	>10mm
Tumour budding	Absent	Low (<5)	High (≥5)
Bone invasion	Absent	Present	
TATE	Absent	Present	
Sarcolemmal spread	Absent	Present	

Risk Scoring:

Low	Intermediate	High
0-2	3-5	6-8

Figure 4.47: Preliminary risk model for OSCC.

CHAPTER 5: DISCUSSION

This preliminary retrospective study examined various socio-demographic, clinical, and histopathological characteristics of OSCC patients to determine their prognostic significance, so that they may be utilized for the development of a risk model in OSCC.

Similar to the latest national cancer report conducted in Malaysia (MNRC report 2007-2011), the highest percentages of OSCC cases in this study were Indian (55%), and for gender, OSCC was more commonly seen in females (59%). A majority of cases (57.5%) were seen in patients ≥60 years old, this was similar to previous research findings (Warnakulasuriya, 2009), where there was increased incidence of OSCC in patients >50 years old. The most common site of OSCC occurrence in this study was the tongue; with 29 patients (39.7%) which was similar to previous research showing that the tongue was the most common site of OSCC (De Camargo Cancela et al., 2010; Suton et al, 2017). This was followed closely by the buccal mucosa with 22 patients (30.1%) and the alveolar process with 13 patients (17.8%).

5.1 Lymph Node Metastasis

In this study, among the various socio-demographic, clinical, and histopathological characteristics studied, it was shown that cTNM staging, pTNM staging (7^{th} and 8^{th} edition), TATE, sarcolemmal spread, and DOI had an association with increased incidence of lymph node metastasis.

With regards to sarcolemmal spread; the majority of cases with sarcolemmal spread had lymph node metastasis (51.0%) when compared to those without sarcolemmal spread (20.8%) Thus sarcolemmal spread was statistically associated with lymph node metastasis, indicating that cases having sarcolemmal spread were more aggressive in nature and had increased incidence of lymph node metastasis. Based on previous literature, there were limited studies regarding sarcolemmal spread of OSCC in relation

to prognosis. However one study showed that the positive predictive value of muscle invasion in relation to lymph node metastasis was 23.3% with a likehood ratio of 1.36 (Chandler, Vance, Budnick, & Muller, 2011). Taking this into consideration, similar to nerve invasion and vascular invasion, muscle invasion could also be a significant possible route for lymph node metastasis, as the invasion of OSCC into the underlying musculature would indicate a later stage of OSCC, and in turn increase the possibility of lymph node metastasis.

In this study, DOI was statistically associated with lymph node metastasis (p=0.024), indicating that cases having an increased DOI had an increased frequency of lymph node metastasis. Similar results were also seen in previous research; whereby lymph node metastasis was seen in 23% of tumors with DOI ≤5mm, 34% in tumors with DOI between 6 and 10 mm, and 53% in tumors with > 10 mm (Abu Bakar et al., 2018). This would indicate a poor prognosis for patients with tumours that penetrate deeper into the underlying tissue.

When assessing TATE in this study, majority of cases with TATE (49.1%) had lymph node metastasis when compared to those without TATE (16.7%) having lymph node metastasis. Moreover, presence of TATE was statistically associated with lymph node metastasis (p=0.026).Based on these results, it would appear that the presence of TATE at the invasive front would indicate a poor prognosis for the patient by having an increased incidence of lymph node metastasis. These results are similar to a previous study where 37.1% of cases with intense TATE presented with lymph node metastasis whereas only 8.3% of cases with absent or mild TATE presented with lymph node metastasis (Oliveira et al., 2008). These results however appear to be in contrast with another study, which demonstrated that higher TATE counts were seen in non-metastatic OSCC compared to lower TATE counts seen in cases of OSCC with

metastasis (Jain et al, 2014). These mixed results would therefore warrant further studies on TATE and its prognostic potential in OSCC.

When cTNM staging and its association with lymph node metastasis was examined in this study, cTNM staging showed significant association with lymph node metastasis (p<0.001). Advance cTNM staging was associated with increase in frequency of lymph node metastasis. Similar results were seen when pTNM staging (7^{th} and 8^{th} edition) and its association with lymph node metastasis were examined, demonstrating significant association with lymph node metastasis (p<0.001). Advance pTNM staging were associated with increase in frequency of lymph node metastasis. However when considering the nature of the TNM staging, where the status of the lymph node metastasis (presence or absence and number involved) are already taken into consideration in the staging, such a strong association is to be expected.

Although only a small number of characteristics studied showed significant association with lymph node metastasis, there were a number of other characteristics that were inclined to show potential for association with lymph node metastasis. These characteristics include PNI, intraneural invasion, LVI, degree of keratinization, extent of differentiation, POI, and surgical margins. It is speculated if the study sample size were to be increased, there is a possibility that some of these characteristics may show statistical significance with lymph node metastasis.

When the six risk models: Broder's classification, Anneroth's classification, Bryne's model (1989), Bryne's model (1992), Brandwein-Gensler's model, and the Tumour budding and depth of invasion grading were assessed for correlation with lymph node metastasis in this study, it was demonstrated that none of these risk models had a significant association with lymph node metastasis. Although some of these risk models; namely Broder's, Brandwein-Gensler's, and the Tumour budding and depth of

invasion grading showed a trend of increased incidence of lymph node metastasis in cases that had higher grading (poor prognosis) compared to those with lower grading (good prognosis). These risk models may have not showed significant association with lymph node metastasis statistically, due to the modest sample size. Therefore, it is speculated, if the study sample size were to be increased, there is a possibility that some these risk models may show statistical significance with lymph node metastasis.

5.2 Local Recurrence

In this study, local recurrence developed in 4 patients (6%) during the follow up period. When the various socio-demographic, clinical and histopathological characteristics were studied for association with local recurrence of OSCC; only patients with previous history of cancer showed significant association ($p<0.001$).

Regarding previous history of cancer, 7 patients had some form of cancer (e.g. colon, thyroid, breast) prior to being included in this study and of these 7 patients; 4 patients (57.1%) had local recurrence of OSCC during the follow up period. It would suggest based on these results that some patients have a predisposition to develop cancer and recurrence; either genetically or related to some other underlying factor. However, due to the small number of patients having recurrence within this study, such associations would need to be further examined with a larger sample size.

Although only a small number of characteristics studied showed significant association with local recurrence, there were a number of other characteristics that were inclined to show potential for association with local recurrence. These characteristics include site, tumour budding and LHR. Again, it is speculated, if the study sample size were to be increased, there is a possibility that some of these characteristics may show statistical significance with recurrence

When the six risk models: Broder's classification, Anneroth's classification, Bryne's model (1989), Bryne's model (1992), Brandwein-Gensler's model, and the Tumour budding and depth of invasion grading were assessed for correlation with local recurrence using Fisher Exact test, the results showed that only Anneroth's classification was associated with increased incidence of local recurrence (p=0.004). However, this may not best represent the study population, as within this study sample, there was a lack of Grade I (low grade) and Grade IV (high grade) cases, as all cases within this study sample were either Grade II or Grade III only (intermediate grades). Similarly, as discussed, the small number of patients with recurrence may also prove to be an issue. These results were in contrast to another study which demonstrated that Anneroth's classification had no significant predictive value in association to local recurrence (Weijers et al, 2009).

When cTNM and pTNM (7^{th} and 8^{th} edition) were assessed in this study, there was no significant association with local recurrence. A larger sample size would therefore be required to further explore if there is any possible correlation between TNM staging and local recurrence in oral cancer.

5.3 Overall Survival

In this study, among the various socio-demographic, clinical, and histopathological characteristics studied, it was shown by univariate analysis that a significant association was observed between overall survival (OS) with bone invasion, ECS, DOI, and number of tumour budding.

With regards to bone invasion, the 5-year OS rate for cases with presence of bone invasion was 39% while the 5-year OS rate for those without bone invasion was 73%. A univariate analysis (Kaplan-Meier) demonstrated a significant association between OS with bone invasion (p=0.016). These results were similar to a previous study showing

that medullary bone invasion resulted in worse OS (Fives et al, 2016), but was contradictory to another study demonstrating that bone invasion was not a predictor for OS (Okura et al, 2016).

When ECS was studied, the 5-year OS rate for cases with presence of ECS was 42% while the 5-year OS rate for those without ECS was 72%. A univariate analysis (Kaplan-Meier) demonstrated a significant association between OS with ECS (p=0.010). Similar results were also demonstrated in another study, where cases with ECS had a 5-year OS rate of 14.3% and those without ECS had a 5-year OS rate of 48.2% (Suton et al, 2017). The results of this study would therefore imply that the presence of ECS of lymph nodes could serve as a prognostic indicator in OSCC.

For DOI, the 5-year OS rate for cases with DOI ≤5mm was 90%, for DOI 5<x≤10mm it was 63% and for DOI > 10mm it was 49%. A univariate analysis (Kaplan-Meier) demonstrated a significant association between OS with DOI (p=0.031). The results seen in this study were similar to previous research whereby a DOI<4mm had a 5 year survival of 68.8% and a DOI≥4mm had a 5 year survival of 41.6% (Tan et al, 2013). This would indicate that patients having tumours that have extended deeper into the underlying tissue, would have a worse prognosis compared to those with more superficial tumours.

When the number of tumour budding was studied at the invasive front, the 5-year OS rate for cases with no tumour budding was 85%, for cases with low activity of tumour budding it was 71% and for cases with high activity of tumour budding it was 42%. A univariate analysis (Kaplan-Meier) demonstrated a significant association between OS with number of tumour budding (p=0.028). Previous studies also had similar findings, where by a significant association was demonstrated between tumour budding and overall survival; p<0.001 (Wang et al, 2011) and p=0.002 (Hong et al, 2018). The

results of this study would therefore imply that number of tumour budding could serve as a prognostic indicator in OSCC.

When the six risk models: Broder's classification, Anneroth's classification, Bryne's model (1989), Bryne's model (1992), Brandwein-Gensler's model, and the Tumour budding and depth of invasion grading were assessed for correlation with OS, the results showed that only the Tumour budding and depth of invasion grading was associated with OS (p=0.035). Cases classified as low risk had a 5-year OS rate of 98%, for intermediate risk it was 69% and for high risk it was 43%. These results are similar to previous studies assessing the significance of the Tumour budding and depth of invasion grading in relation to patient survival, with low risk cases having better survival rates than high risk cases (Almangush et al 2015, Sawazaki-Calone et al 2015).

Interestingly, in this study, both cTNM and pTNM (7^{th} and 8^{th} edition) failed to show any significant association with overall survival of patients, although the prevailing pattern showed that patients with stage I and II appeared to have better overall survival compared to stage III and IV. A similar pattern of overall survival was also seen in previous research (Dissanayaka et al 2012).

5.4 Development of a Preliminary Risk Model

The development of a preliminary risk model (**Figure 4.47**) within this study utilized characteristics such bone invasion, ECS, DOI, tumour budding, sarcolemmal spread and TATE. These factors were significant on a univariate analysis, however upon further testing in a multivariate analysis for association with lymph node metastasis, neither of these characteristics were significant and independent predictors of lymph node metastasis. Testing in a multivariate analysis for association with overall survival however did demonstrate that ECS was an independent predictor of overall survival (p=0.003). However, as this a preliminary study with a modest sample size, all these

characteristics mentioned above were taken into consideration when formulating the preliminary risk model.

For local recurrence, the only characteristic which showed significance was patients' past history of cancer. However, considering that only a very small number of patients experienced local recurrence within this study; this characteristic was not included within the current preliminary risk model. Nonetheless, this characteristic does show promise as a possible prognosticator and should be explored further in the future with a larger sample size.

Using pre-existing risk models as a guide, the risk scoring was then set as; a total score of 0-2 was considered as low risk, score of 3-5 as intermediate risk, and score of 6-8 as high risk.

When the preliminary risk model developed within this study was compared to pre-existing risk models and TNM staging, there were some common prognostic characteristics such as DOI, ECS, bone invasion and tumour budding which were also present. However, within this preliminary risk model, there were two characteristics which have not been included in any of the pre-existing risk models; which are TATE and sarcolemmal spread. The actual significance of these two factors however will require further evaluation of the risk model with a larger sample size.

Considering the preliminary nature of this study with a modest sample size, the risk model developed here will most definitely require modifications and improvements with further expansion of this study.

5.5 Limitations

Due to the relatively modest sample size (n=73) of this study, in part caused by selection of data that only fulfilled inclusion criteria from the database, the results may not fully represent the study population in question.

Due to only a very small number of patients having local recurrence within the study sample, any significant associations between the different characteristics examined and local recurrence may be deemed weak.

5.6 Clinical Implications

By utilizing this preliminary risk model in patients with OSCC and determining the prognosis of these patients (low, intermediate, or high risk), clinicians will be better equipped in deciding on suitable treatment plans for their patients.

5.7 Recommendations

A future study should be carried out which includes multiple centres from where data and samples can be obtained, so that a larger sample size may be evaluated.

As this is only a preliminary study, validation of the preliminary risk model developed within this study should be carried out on a separate study sample and modifications to this preliminary risk model should be done as required based on the results of these further studies.

CHAPTER 6: CONCLUSION

To conclude, there were significant associations between certain clinical and histopathological characteristics and patient outcomes in OSCC. The histopathological characteristics include ECS, DOI, bone invasion, TATE, tumour budding, and sarcolemmal spread. When these significant characteristics are integrated within a framework of a preliminary risk model for OSCC, they should be able to aid in better assessing the prognosis of patients with OSCC, thus in turn helping clinicians in the aspect of treatment planning of these patients.

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