CLINICOPATHOLOGIC FACTORS FOR DISEASE-FREE SURVIVAL IN EARLY STAGE ORAL SQUAMOUS CELL CARCINOMA

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Submitted to the Graduate Faculty of

School of Dental Medicine in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2018

UNIVERSITY OF PITTSBURGH

SCHOOL OF DENTAL MEDICINE

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November, 30, 2018

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OBJECTIVE**:** Oral squamous cell carcinoma (OSCC) is a life-threatening disease that can cause significant morbidity and mortality. OSCC recurrence occurs frequently with the rates varying between 15% and 40% depending on the extent of the tumor. Our aim was to determine association between select clinicopathologic factors and the risk of local recurrence in early stage (T1N0) OSCC. METHODS: After approval by the University of Pittsburgh IRB (PRO17100554), 65 cases of T1N0 stage OSCC over a period of 12 years (2000-2012) were retrieved. Cancer originating from non-mucosal epithelium such as lip, cases with positive surgical margins, and HPV-related tumors were omitted from our study. Relevant clinicopathologic data collected included sex, age, oral site, history of dysplasia, histologic grade, depth of invasion, and surgical treatment modality. RESULTS: 33.8% (22/65) cases experienced locoregional recurrence with the median time to recurrence of 31 months (range: 4-119). The majority 56.9% (37/65) of the T1N0 lesions were classified as moderately-differentiated tumors; the average depth of invasion was 1.7 mm. The tongue was the most prevalent site (49.2%, 32/65) followed by the mandibular gingiva (9/65, 13.8%) and floor of mouth (8/65, 12.3%). A higher risk of recurrence was found to be associated with a previous history of dysplasia (OR 12.0, 95% CI 3.1, 45.6, P <0.001) and "low risk" oral site (P <0.05) when clustered into high and low risk sites for OSCC development. Age, sex, histologic grade, depth of invasion, and treatment modality were not found to have statistically significant associations with locoregional recurrence. CONCLUSION: Our findings suggest that patients with a history of dysplasia and with OSCC development at traditionally lower risk areas have a higher

risk of locoregional recurrence. Surprisingly, higher histologic grade, larger depth of invasion, and elective neck dissections did not appear to decrease risk of locoregional recurrence in early stage (T1N0) cancers. Understanding of the clinicopathological risk factors associated with disease-free survival will aid in improving post-treatment follow-up protocols for oral cancer patients. Dental professionals hold a unique position in their proficiency in the diagnosis of oral lesions and their ability to follow up with patients at frequent intervals.

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LIST OF ABBREVIATIONS

Abbreviation	Full name/description
AJCC	American Joint Committee on Cancer
AUC	Area under curve (ROC analysis)
CT	Chemotherapy
DOI	Depth of invasion
DFS	Disease-free survival
END	Elective neck dissection
HPV	Human papillomavirus
HNSCC	Head and neck squamous cell carcinoma
LR	Locoregional recurrence
OSCC	Oral squamous cell carcinoma
ROC	Receiver operating characteristic
RT	Radiation therapy
SCCA	Squamous cell carcinoma
SEER	Surveillance, Epidemiology, and End Results Program from National Cancer Institute
TNM	Tumor, nodes, metastasis for cancer staging: T: tumor size N: lymph nodes involved

	M: metastasis
UDHS	University Dental Health Services, University of Pittsburgh

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1.0 BACKGROUND

1.1 ORAL CANCER EPIDEMIOLOGY

Head and neck cancers include malignancies from the oral cavity, oropharynx, pharynx, and nasopharynx. The National Cancer Institute SEER (Surveillance, Epidemiology, and End Results Program) database estimates 51,540 new cases (3.0% of all cancers) for 2018 with 10,030 related deaths. The 5-year overall survival rate (2008-2014) hovers at 64.8%, which has increased over the past 40 years from a mere 52% in the 1970s. Primary oral cavity tumors accounts for approximately half of all head and neck cancers with a reported age-adjusted rate in the United States among all races of 6.1 per 100,000 (SEER, 2017).

The vast majority (80-90%) of these malignancies are histologically squamous cell carcinomas which arise from the stratified squamous epithelium of the oral mucosa. Anatomically, the oral cavity includes the gingiva, the palate at the superiorly, the wet-dry line of the lips anteriorly, the buccal mucosa laterally, and the floor of mouth inferiorly, and the portion of the tongue anterior to the circumvallate papillae. The distinction between oral cavity and oropharynx has become critical due to the association of oropharyngeal cancers with high-risk human papilloma virus (HPV) whereas oral cavity cancers are linked to tobacco and alcohol usage. For oral squamous cell carcinoma (OSCC), the tongue and floor of mouth are the two most common subsites followed by the gingiva and hard palate.

The incidence of OSCC for males has consistently outpaced females at a ratio of approximately 2.5:1 (M:F) reported in 2015. The mortality rate for males is 3x higher compared to females (2011-2015) with females being nearly 2x more likely to present with localized or regional cancer (SEER, 2017; Cronin et al, 2018). Like all cancers, mortality is strongly associated with the spread of the malignancy at time of diagnosis. The majority (~75%) of cancers are local or regional at time of diagnosis. OSCC increases with age for both males and females with a spike in incidence after the 6th decade, and a reported median age of diagnosis of between ages of 60-65 (SEER, 2017).

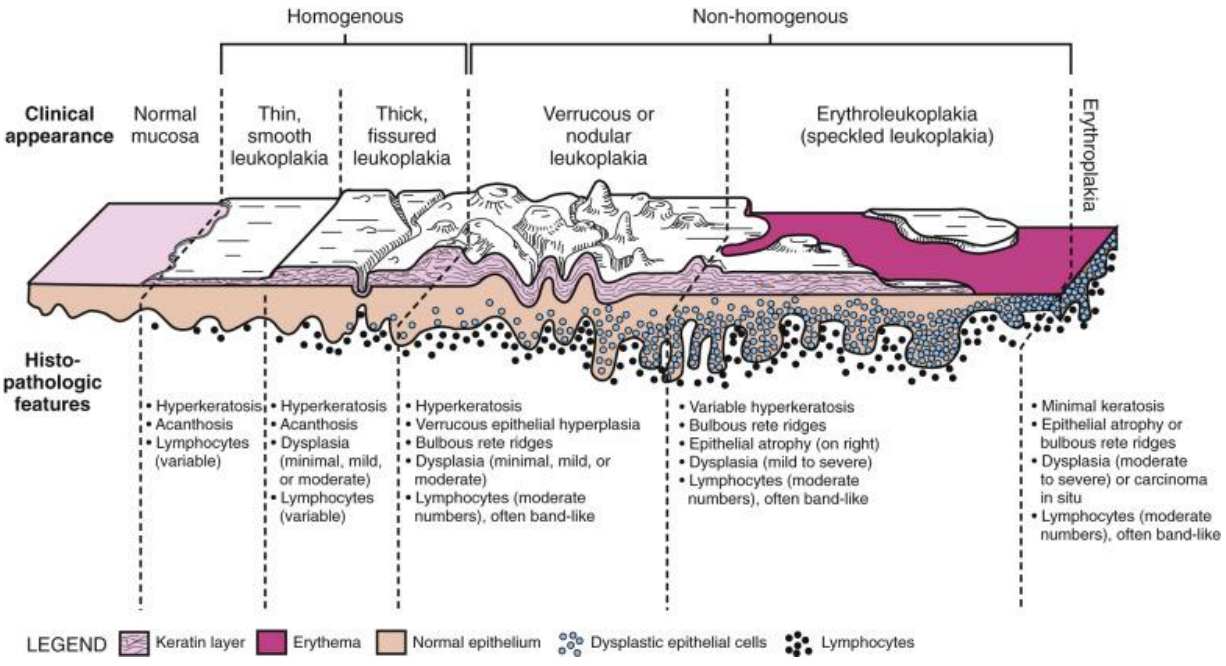
1.1.1 Premalignant oral lesions

All cases of OSCC originate from potentially malignant precursor disorders. The clinical presentation of potentially malignant lesions typically appears as white (leukoplakia) or a red-white (erythroplakia) plaque. The plaque may be smooth (homogenous) or rough (non-homogenous) in texture. Non-homogenous and erythroplakic lesions generally undergo malignant transformation at a significantly higher rate compared to homogenous leukoplakias. (Amagasa et al, 2011). Progression to malignancy occurs in a step-wise fashion from starting from epithelial dysplasia and progressing to invasive carcinoma. This phenomenon is best seen with histologic examination as the clinical appearance alone is not a reliable marker to evaluate the likelihood of progression to OSCC. Dysplastic epithelium is characterized by a host of changes including basilar hyperchromasia, disorganization of the basement membrane layer, aberrant mitotic figure, dyskeratosis, and bulbous rete ridges (Napier et al, 2008; Liu et al, 2010).

These dysplastic alterations can range from mild to moderate to severe. Previous studies show that the rate of malignant transformation is higher for moderate and severe dysplasia compared to mild dysplasia with an overall transformation rate of 3-16% for all dysplastic lesions (Schepman et al, 1998; Arduino et a, 2009; Speight et al, 2017). In addition, the site of dysplasia may also play a role in the development of OSCC. Several systematic reviews suggest that the tongue and floor of mouth have a higher rate of transformation (3-5 times higher) compared to other oral sites (Narayan and Shilpashree, 2016; Schepman et al, 1998, Ho et al, 2012). A review conducted by Warnakulasuriya et al reported the overall rate of transformation for premalignant lesions with or without dysplasia at all oral sites to be 1.36% per year. (Warnakulasuriya et al, 2007). Clinically, as the lesion progresses as to OSCC, it not only increases in size, but also develop areas of erythema and/or ulceration. The dysplasia advances to carcinoma in situ full thickness dysplasia of the epithelial without stromal invasion, and ultimately invasive carcinoma. Figure 1 and Figure 2 offer photographic and illustrative depictions, respectively, of the common clinical changes seen in epithelial dysplasia and OSCC.

Figure 1: Clinical photos of epithelial dysplasia and SCCA. (A)- Clinically evident changes appearing as a homogenous leukoplakia of the ventral tongue in epithelial dysplasia; (B) – Red, exophytic, nodular growth of the mandibular gingiva in OSCC. (C) –Conventional microscopic presentation of OSCC as infiltrative islands of squamous epithelium with prominent keratinization.

Figure 2: Illustration of the clinical and histologic progression of epithelial dysplasia to malignancy. (Bouquot & Gnepp, 1991)



1.1.2 Risk factors in development of oral squamous cell carcinoma

Numerous studies indicate that the primary risk factors for head and neck squamous cell carcinoma (HNSCC) lie in tobacco usage, alcohol consumption, and HPV positivity, all of which may act alone or in concert in the development of malignancy. Although some patients have underlying genetic causes which increases the propensity for developing OSCC, the exact genomic alterations remain unclear. The most wide-spread risk factors involve exposure to carcinogenic compounds in tobacco products and alcohol. Other strongly linked risk factors include infection with betel nut chewing, and certain inherited and immunosuppressive medical conditions. High-risk human papillomavirus (HPV) infection is strongly associated with the development of squamous cell

carcinomas in the oropharynx rather than the oral cavity. (IARC, 2004; Syrjänen et al, 2011; Turati et al, 2013; Chi et al, 2015)

Alcohol and tobacco products are the most widely-studied risk factors in OSCC. A plethora of research has shown tobacco to contain potent carcinogens which increase the risk of malignancy in a host of tissues with cancers of the upper aerodigestive system. The oral cavity in particular endures direct topical exposure to many of the noxious chemicals in tobacco products. The International Agency for Research on Cancer (IARC) found that chronic users of tobacco products have a odds ratio of developing OSCC of 1.91 -2.18, and a relative risk of 3.43 compared to non-smokers (IARC, 2004). Cessation of tobacco use decreases the risk of HNSCC development with the relative risk approaching that of non-smokers by the 10-year mark (Chi et al, 2015).

Alcohol consumption has also been shown to have a dose-dependent relationship with the development of oral cancer. Turati et al. reported the relative risk (RR) for head and neck cancer is 1.3 for 10 grams of ethanol per day versus 13.0 for 125 grams per day (Turati et al, 2013). Although alcohol is an independent risk factor for OSCC, the combination of tobacco and alcohol increased the RR significantly with the IARC reporting a RR of 15 (IARC, 2004).

Betel quid chewing is prevalent in Southeast Asia. Betel quid is a combination of substances including areca nut, fillers, and tobacco. Both the tobacco content and the areca nut contribute to the carcinogenicity of betel quid. Recent large-scale studies, meta-analyses, and systematic reviews have reported ORs for HNSCC of approximately 7 to 8 for betel quid with tobacco and 3 to 6 for betel quid without tobacco. (Chi et al, 2015)

Individuals with medical conditions with germline mutations leading to chromosomal instability and immunocompromised patients also have a higher rate of OSCC development compared to the general population. For instance, individuals with Falconi's anemia, who inherit

mutations in proteins essential for DNA repair and replication, have a 500 times increased risk of developing squamous cell carcinomas (Nalepa & Clapp, 2018). Studies have also demonstrated an increased incidence of squamous cell carcinomas in patients with HIV infections or solid organ transplantations (Grulich et al, 2007). Even though the majority of malignancies in this immunosuppressed population are cutaneous squamous cell carcinoma or basal cell carcinomas, Rabinovics and colleagues reported that 4% have head and neck cancer at various mucosal sites versus 1.5% in the general population (Rabinovics et al, 2014).

High risk human papillomavirus types (HPV-16,18,31,33) can alter apoptotic pathways and cell cycle checkpoints via the production of E6 and E7 oncoproteins. Although transcriptionally active high-risk HPV is a leading agent in the development of carcinoma in the oropharynx (tonsils, base of tongue), studies show less than 10% of OSCC contain high-risk HPV. Therefore, HPV infection is currently not considered a risk factor for OSCC (Syrjänen et al, 2011; Chi et al, 2015; Kobayashi et al, 2018).

1.1.3 Overview of cellular and genomic alterations in OSCC

Progression from premalignant lesions to carcinoma involves a series of genetic and cellular changes within the cell. Several studies have demonstrated that certain genetic alterations, via carcinogenic agents and environmental insults to the mucosa, initiate the process to malignant transformation precede histologic dysplasia. For the development of carcinoma, the abnormal cells must continue to accumulate mutations which alter the normal pathways in cell growth, division and apoptosis, and the cancerous cells must escape detection of the immune system. Certain individuals are more genetically susceptible to environmental and infectious insults as evidenced by the observation that patients with a family history of upper aerodigestive tract cancer have a 3-

4x higher risk of HNSCC (Lacko et al, 2014)). The underlying genetic susceptibilities are not well elucidated for OSCC.

Chromosomal abnormalities in particular have been found to significantly increase the risk of developing invasive carcinoma. Common genetic alterations in OSCC encompass the following genes: loss of heterozygosity (LOH) or deletion occurs at 9p (CDKN2A, NOTCH1); 3p (various tumor suppressor genes), and 17p (TP53) while amplification at 11q (CCDN1, FADD, BIRC2, YAP1) and 3q (TP63, SOX2, PIK3CA) are present. Collectively, these pathogenic alterations lead to deregulation of the cell cycle through upregulation of positive mediators (e.g. cyclin D1, c-myc) and silencing of important cell cycle checkpoint regulators (e.g. p53, p16). (Van der Riet et al, 1994; Ha et al, 2003; Loyo et al, 2013). A recent comprehensive genomic study suggested that up to 86% of HPV- tumors harbored P53 mutations, especially in tobacco related cases. Overexpression of anti-apoptotic proteins (bcl-xl, bcl-2) have also been found in a number of HNSCC cases (The Cancer Genome Atlas (TCGA), 2015).

Dysregulation of cell signaling pathways further galvanize tumor development and growth. Upregulation of cell surface growth receptors (EFGR, HER2neu) provide a major mechanism directed entry into the cell cycle and proliferation. Over half of OSCCs have been shown to overexpress EGFR and/or HER2neu. (Shin et al, 1994; Pomerantz and Grandis, 2014) In addition, up to 6-35 % of OSCC carry mutations within the protein kinases of the RAS pathway (HRAS, PIK3A, PTEN) lead to constitutively actively pathways which promote cell differentiation, division, and survival (Stransky et al, 2011).

This brief overview of the primary cellular and genetic events involved in progression from dysplasia to full malignancy underscores the complexity of the disease. In the vast majority of

cases, OSCC is driven by a combination of genomic alterations, immune dysfunction, cell cycle disturbances, and malfunctions within critical mitogenic pathways.

1.2 HPV-RELATED OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

Recently, the 4th Edition of the World Health Organization Classification of Head and Neck Tumors has portioned head and neck squamous cell carcinomas into two distinct categories: HPV positive and HPV negative (WHO, 2017). This was performed because HPV positive tumors differ greatly in pathogenesis, histological presentation, and prognosis compared to HPV negative cancer. HPV driven neoplasms typically arise in the oropharynx/base of tongue within tonsillar tissue as opposed to OSCC which arrive from oral cavity mucosa. In addition, the histopathologic appearance of HPV-SCC is characterized by non-keratinizing, basaloid cells which resemble tonsillar epithelium rather than oral epithelium.

Although, HPV DNA has been detected in premalignant oral lesions, OSCC has a fundamentally different etiology compared to HPV-SCC where viral oncoproteins E6 and E7 play a pivotal role. HPV E6 marks p53 for ubiquitination; E7 suppresses Rb function, leading to overexpression of p16 as a compensatory mechanism. Unlike HPV-SCC, p16 overexpression has not been consistently found in OSCC (Chi et al, 2015). Most importantly, patients with HPV driven tumors respond far better to treatment and possess significantly higher overall survival. In a study of 810 patients with head and neck squamous cell carcinoma, Huang and colleagues found that the 5–year overall survival rate was ranged from 30%-70% for all stages while the range was 74-88% for HPV-SCC (88%, 78%, 71%, and 74% for stage I, II, III, and IV respectively) (Huang et al, 2015). The fundamental differences between OSCC and HPV-SCC are highlighted by the recent

changes to the WHO blue book and American Joint Committee on Cancer (AJCC) cancer staging system. For these reasons, HPV-SCCs have been excluded in this study.

1.3 PROGNOSIS IN ORAL SQUAMOUS CELL CARCINOMA

1.3.1 Pathologic staging (TNM)

Tumor stage is the most critical determinant of prognosis. OSCC staging is determined by a combination of clinical examination, histopathology evaluation, and radiologic findings. The system universally used by the global health community is the tumor (T), node (N), metastases (M) system American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC). The TNM system designates the following: T- primary tumor size in centimeters, N- number and location of lymph nodes involved, M- presence of metastasis to distant sites. For OSCC, the T (T1, T2, T3, T4) is determined by the both greatest dimension of the tumor and the depth of invasion; the N (N1, N2, N3) refers to the number and laterality of lymph nodes involved; M (M0, M1) refers to the presence of distant metastatic deposits (AJCC, 2017).

Treating clinicians typically refer to OSCC in stages (I, II, III, IV) rather TNM system is primarily used by pathologists. Stage I correlates with T1N0 cancer where the primary tumor is less than 2 centimeters in its greatest dimension and has not spread to regional lymph nodes. Stage II is defined as carcinoma 2-4 centimeters in size with no evidence of spread to lymph nodes. Stage III cancers may refer to locally advanced disease with a tumor size of greater than 4 centimeters, or a tumor of any size with one ipsilateral lymph node involved (N1). Stage IV oral cancer is the most advanced with the primary tumor invading vital structures and tunneling through adjacent

anatomy, or the cancer has involved more than one lymph node or a node on the contralateral side of the neck. Of course, if distant metastasis is present, the OSCC is automatically Stage IV. For the most part, Stage IV cancers cannot undergo complete surgical section due to the extent of the primary tumor.

Early stage OSCC has a reported locoregional recurrence rate ranging 15-40%. The 5-year relative survival for localized OSCC is 83.7%, and tumors with regional and distant spread have rates of 65% and 39.1%, respectively (Camisasca et al, 2011, Ganly et al, 2012; Wang et al, 2013). Large-scale studies and multivariate analyses have consistently demonstrated that nodal spread is an independent factor for overall survival. One 17-year retrospective study of 227 patients revealed that the difference in survival outcomes between pN0 and pN1 disease was significantly greater than the difference between pN2 to pN3 disease (Don et al, 1995; Qian et al, 2018).

With localized disease (pN0), the concern for most patients with proper treatment is loco-regional recurrence rather than the patient succumbing to the primary tumor itself. In many instances, the recurrent tumor presents at a later stage and bears a poorer prognosis in comparison to an initial malignancy.

1.3.2 Histopathologic parameters of prognostic relevance

Whereas the staging criteria relies on macro features of tumor spread, the histologic parameters of disease aggressiveness are dictated by microscopic characteristics of the tumor such as histologic grade, depth of invasion, and perineural and angiolymphatic involvement. The World Health Organization (WHO) segments OSCC into 3 different histologic differentiation grades: well, moderate, and poor (Pindborg et al, 1997). Broadly speaking, the pathologist decides on the histologic grade by determining how much the tumor resembles the tissue of origin. For OSCC,

the degree of keratin production, cellular pleomorphism, aberrant mitotic figures, and overall cohesiveness of the cells are all taken into consideration. Interestingly, researchers have not reached a consensus on the importance of histologic grade to overall prognosis. Although some studies have found a significant correlation between well-differentiated tumors and a higher 5-year overall survival, others did not find a convincing association between histologic grade and prognosis or response to treatment (Kademani et al, 2005; Scully & Bagan, 2008; 47 Jerjes et al, 2010). One of the reasons stated by studies for this lack of correlation may be due to inaccuracies in grading for a number of cases wherein the small biopsies were not representative of the entire tumor (Pindborg et al, 1997; Al-Rajhi et al, 2000).

Depth of invasion (DOI) was recently incorporated into the T-staging criteria AJCC 8th edition for all OSCC based on results from several large-scale studies which showed that DOI conferred strong prognostic significance for overall survival (Yuen et al, 2000; Almangush et al, 2014; Masood et al, 2018). These new guidelines advise that measurements in millimeters should be taken from the adjacent benign basement membrane to the deepest island of tumor cells. According to the 8th edition, T1 tumors have a DOI value of 5 mm or less; T2 cancers have a DOI between 5 and 10 mm; a DOI of greater than 10mm is T3. One such collaborative effort from 11 international tertiary care centers identified DOI as an independent predictor of disease-specific ($P < .001$) and its inclusion in the T-category stratified patients into more clear-cut prognostic stages (ICOR, 2014).

Perineural and angiolymphatic refers to tumor cells sitting adjacent to neural structures or growing inside a vessel, respectively. Because carcinomas frequently spread via neural, lymphatic, or vascular channels, the association between perineural and/or angiolymphatic invasion and overall prognosis has been well studied. Many studies suggest that the presence of these histologic

parameters significantly increase the likelihood of nodal involvement, locoregional recurrence, and overall survival (Brandywein-Gensler et al, 2005; Jerjes et al, 2010). Approximately one-quarter of all OSCC have either perineural or angiolymphatic invasion; however, this number decreases for T1 and T2 OSCCs to occur in 5-20% of cases (Almangush et al, 2014). For angiolymphatic involvement, one study revealed that 48.8% (21/43) patients experienced locoregional recurrence within 5 years (Jerjes et al, 2010). The same study found recurrence in only 20.9% (9/43) of cases with perineural invasion. Correlations of prognosis with perineural invasion appear to be more equivocal because some studies only report an association with prognosis if the tumor involves a large nerve bundle versus smaller nerves (Weijers et al, 2004; Brandwein-Gensler et al, 2005).

1.3.3 Treatment for OSCC

Treatment varies greatly depending on the cancer stage. Stage I and II tumors, which makeup approximately 31% of cases (SEER, 2017), are prototypically treated with curative intent via surgery alone, or surgery with adjunct radiation therapy (RT) in larger tumors where clear margins cannot be ascertained. For patients with late stage disease (III, IV), the cancer has spread to regional lymph nodes, or complete resection of the tumor involves extensive post-treatment complications and poor quality of life. These individuals undergo multiple modalities of treatment, which typically consists of a combination of RT, chemotherapy (CT), and surgery (Marur et al., 2016).

In the United States, two-thirds of all OSCC cases are treated with surgery alone; one-third with surgery and RT, and about 10% with surgery, RT, and CT (Schwam & Judson, 2016). In patients with early stage OSCC, roughly half of the cases underwent an elective neck dissection

(END). In early stage OSCC treated with surgery alone, margins without carcinoma cannot be achieved in about 7-8% of cases. The presence of positive margins has been consistently associated with poorer outcomes in large scale studies involving the National Cancer Database with a reported hazards ratio of 1.27-1.39 (Luryri et al, 2015; Schwam & Judson, 2016).

The question of whether to perform a dissection is a more controversial subject in OSCC treatment. An elective neck dissection (END) is performed to detect subclinical nodal metastasis which evade clinical and radiographic exam. Patients typically present with no clinical and radiographic evidence of nodal involvement. One study (n=47) which recommended prophylactic END reported the prevalence of subclinical nodal metastasis to be 38% (Hadaddin et al, 1999). However, other studies did not find a significant correlation between END and lower rate of 5-year disease-free survival (Schwam & Judson, 2016; D'Cruz et al, 2009). Some suggested that an END only benefits survival in cases of identified perineural invasion because this phenomenon suggests a more aggressive phenotype that increases likelihood of occult metastases (Tai et al, 2012; Kim et al, 2018). Nonetheless, the majority of tertiary care centers in the United States consider neck dissection elective rather than the standard of care for T1, clinically negative neck patients.

1.3.4 Post-treatment surveillance

Post-treatment surveillance for OSCC is structured for early detection of loco-regional recurrence to increase survival outcomes. Previous research demonstrates that most recurrences occur within the first 3 years of the initial presentation of malignancy. Follow-up regimens are structured to be more frequent in the first 3 years of treatment (every 3 months), decrease in years 3-5 (every 6 months), and gradually taper off after year 5 (every 12 months). The rationale for this timing is

due to multiple studies that suggest that the majority of recurrences occur within the first 3 years of the initial presentation of malignancy, and decrease there afterwards (de Visscher & Manni, 1994; Brennan et al, 2018). One large-scale study involving 603 patients found a rate of recurrence of 25% at 5-years of follow-up and a nominal increase of 3% in detection after 5-years (Haas et al, 2002).

2.0 OBJECTIVES

Dental professionals hold a unique position in oral cancer screening because of their ability to diagnose early abnormal pathological changes within the oral cavity and to follow up with patients at frequent intervals. The primary aims of our study are as follows: (1) To describe the characteristics of patients with early stage (T1N0) oral cavity squamous cell carcinoma. (2) To compare the clinicopathologic features between the locoregional recurrence and the disease-free group. (3) To determine whether select clinicopathological factors (age, sex, site, histologic differentiation, depth of invasion, history of dysplasia, treatment modality) increases the risk of locoregional recurrence. Our findings may provide valuable insights to help manage post-treatment surveillance and allow for better stratification of patients into high and low risk groups for recurrence.

3.0 MATERIALS AND METHODS

3.1 DATA COLLECTION

After approval (PRO17100554) from the University of Pittsburgh's Institutional Review Board, we utilized the University Dental Health Services (UDHS, faculty practice service of University of Pittsburgh School of Dental Medicine) specimen database stored through CoPathPlus pathology reporting software. Cases of OSCC diagnosed by oral and maxillofacial pathologists over a period of 13 years (2000-2012) in the adult patient population (>18 years old) were retrieved. We included patients of both sexes and all ethnicities. With respect to oral site, we excluded cases from the lip because most of these tumors have a cutaneous rather than mucosal origin and develop from excessive exposure to UV light. After extensive review of the pathology reports for these patients, only cases with T1N0 tumors, as determined by AJCC 5^{th}-7^{th} editions, without high-risk features such as perineural or angiolymphatic invasion, were selected. Additional exclusion criteria consisted of patients who received less than 5 years follow-up, cases with positive resection margins, or were treated with CT and/or RT in addition to surgery as determined by a review of the UPMC electronic medical record. We chose 5 years as the cutoff for follow-up because the vast majority (80-90%) of locoregional recurrences take place within 5 years of curative treatment for the index tumor (de Visscher & Manni, 1994; Brennan et al, 2018). Table 1 (below) provides our inclusion/exclusion criteria.

Table 1: Inclusion and Exclusion Criteria for Study

Inclusion Criteria	Exclusion Criteria
T1N0/Stage I OSCC	Positive resection margins
Conventional squamous cell carcinoma histology	Lip mucosa
Negative for high-risk HPV	Less than 5 years follow-up information
Treatment with surgery alone	Treatment with CT and/or RT

The medical records and available H&E slides pertaining to each patient were reviewed, and the following clinical and histologic data were collected: sex, age, site, histologic grade, depth of invasion, history of oral dysplasia, treatment modality (surgery +/- elective neck dissection). For patients who experienced loco-regional recurrence, the number of recurrences and the time interval between the index tumor and first episode of recurrence were collected.

All data were collected and stored on password-encrypted, secure computers with the University Of Pittsburgh Dental Medicine Department Of Diagnostic Sciences. No patient identifiers were collected, and there were no contacts with the subjects other than accessing their present medical records for the study. The records were de-identified and identification key was kept in a secure location for the principal researchers to access. The data files with patient information were only accessed by the principal investigator (Y.L.) listed on the IRB.

3.1.1 Clinical parameters

Clinical parameters such as the age, sex, oral site, history of dysplasia, the onset of loco-regional recurrence, and treatment modality were collected through review of the patient's medical record and pathology reports.

3.1.2 Histopathologic parameters

Histopathologic parameters such as histologic grade and depth of invasion were primarily obtained through review of the H&E slides. However, for cases in which the H&E slide is missing from the record, the values on the pathology report were used or, if this was not also not available, the values were be marked as “N/A” (not available).

3.1.3 Endpoint criteria for disease-free survival

Because of the differences in starting points for our patients, (2000-2012), some subjects were followed for 12 years while others were followed for 5 years. For the purposes of our study, disease-free survival (DFS) is defined as being cured of OSCC after surgical treatment for at least 5-years. Our study endpoint for patients is either disease-free survival for the duration of the time period (2000-2012), or locoregional recurrence (LR), defined as the return of cancer cells of the same histologic type at a site same site, adjacent site, or regional lymph nodes after a minimum 3-month disease free period.

3.2 STATISTICAL ANALYSES

Chi-squared and Fisher's exact tests were used to determine univariate associations between our categorical clinicopathologic parameters and locoregional recurrence. A two-sample student's T-test was used to compare continuous variables. Statistically significant variables were then used to generate a logistic regression model to calculate an odds ratio and generate a receiver operating characteristic (ROC) curve.

4.0 RESULTS

4.1 CHARACTERISTICS OF THE STUDY POPULATION

Table 2: Clinicopathologic features of Stage I OSCC patients

Feature		# of Patients	% of Total (n=65)
Age	≥60	37	56.9%
	<60	28	43.1%
Sex	M	30	46.2%
	F	35	53.8%
Oral Site	Tongue	32	49.2%
	Mandible	9	13.8%
	Floor of mouth	8	12.3%
	Buccal mucosa	7	10.8%
	Maxilla	5	7.7%
	Palate	4	6.2%
Histologic grade	Well	28	43.1%
	Moderate	37	56.9%
	Poor	0	0.0%
Depth of Invasion	≥1.7 mm	31	47.7%
	<1.7 mm	34	52.3%
History of dysplasia	Yes	22	33.8%
	No	43	66.2%
Treatment modality	Excision	37	56.9%
	Excision + END	28	43.1%
Locoregional recurrence	Yes	22	33.8%
	No	43	69.2%

Table 3: Clinicopathologic features of OSCC Patients (LR vs DFS group)

Feature		DFS group	% of DFS group	LR group	% of LR group
Age	≥60	23	53.5%	14	63.6%
	<60	20	46.5%	8	36.4%
Sex	M	23	53.5%	7	31.8%
	F	20	46.5%	15	68.2%
Oral Site	Tongue	26	60.5%	6	27.3%
	Mandible	4	9.3%	5	22.7%
	Floor of mouth	5	11.6%	3	13.6%
	Buccal mucosa	3	7.0%	4	18.2%
	Maxilla	3	7.0%	2	9.1%
	Palate	2	4.7%	2	9.1%
Histologic grade	Well	19	44.2%	9	40.9%
	Moderate	24	55.8%	*13*	*59.1%*
Depth of Invasion	≥1.7 mm	18	43.9%	8	42.1%
	<1.7 mm	23	56.1%	11	57.9%
History of dysplasia	Yes	7	16.3%	15	68.2%
	No	36	83.7%	7	31.8%
Treatment modality	Excision	24	55.8%	13	59.1%
	Excision + END	19	44.2%	9	40.9%

A total of 279 cases of OSCC were retrieved from UDHS over a period of 13 years (2000-2012). 243 cases of OSCC remained after excluding the lip as a site of development for reasons explained in Section 3.1. After review of the medical record and applying our exclusion criteria, a total of 65 cases of early stage (T1N0) OSCC remained for our study.

Table 2 and Table 3 summarize the clinicopathologic features evaluated for our patients after a minimum follow-up time of 60 months. Table 2 provides an overview of the characteristic of the entire cohort of patients (N=65). 22/65 (33.8%) patients experienced locoregional recurrence with a median time to the second tumor of 35 months; 43/65 (69.2%) of patients remained disease free for 5 or more years. Table 3 separates the disease-free and loco-regional recurrence groups.

The average depth of invasion for our cases was 1.7 mm; this value was calculated from 60 cases since 5 cases did not have H&E slides available for review. This average value (mm) was used as a point of comparison.

4.2 KEY FINDINGS

Figure 3: Test for association: sex vs locoregional recurrence

	SEX		
RECURRENCE	F	M	Total
No	20	23	43
%	46.51	53.49	100.00
Yes	15	7	22
%	68.18	31.82	100.00
Total	35	30	65
%	53.85	46.15	100.00

Pearson chi2(1) = 2.7501 Pr = 0.097

Although males comprised a larger portion (53.5%) of the disease-free cohort while females made up a larger portion of the LR cohort (68.2%), this difference not statistically significant (alpha=0.05).

Figure 4: Age in LR vs DFS group

```
------------------------------------------------------------------------------
   Group | # of cases  Mean age    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
     DFS |      43    60.81395    1.709609    11.21066    57.36382    64.26408
      LR |      22    62.86364     2.35515    11.04663    57.96583    67.76144
---------+--------------------------------------------------------------------
Combined |      65    61.50769    1.378252    11.11182    58.75432    64.26107
---------+--------------------------------------------------------------------

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.2430         Pr(|T| > |t|) = 0.4859          Pr(T > t) = 0.7570
```

The mean age at the time of the index tumor of the disease-free group was 60.8 years while the LR group was 62.8 years old. This difference was not found to be statistically significant (alpha=0.05).

Figure 5: Test for association: Histologic grade vs locoregional recurrence

```
           |   HISTOLOGIC GRADE
RECURRENCE |  Moderate       Well |     Total
-----------+----------------------+----------
        No |        24         19 |        43
         % |     55.81      44.19 |    100.00
-----------+----------------------+----------
       Yes |        13          9 |        22
         % |     59.09      40.91 |    100.00
-----------+----------------------+----------
     Total |        37         28 |        65
         % |     56.92      43.08 |    100.00

          Pearson chi2(1) =   0.0637   Pr = 0.801
```

All 65 cases were either moderately or well-differentiated; no poorly-differentiated OSCC were present. A larger portion of moderately-differentiated tumors was found in both the disease-free and LR cohorts, 55.8% and 59.09%, respectively. A statistically significant association was not found between histologic grade and LR.

Figure 6: Depth of invasion (mm) in LR vs DFS group

Group	# cases	Mean(mm)	Std. Err.	Std. Dev.	[95% Conf.	Interval]
DFS	41	1.731707	.2067884	1.324092	1.313772	2.149642
LR	19	1.647368	.312261	1.361114	.9913324	2.303404
combined	60	1.705	.1710457	1.324914	1.362738	2.047262
diff		.0843389	.3706918		-.6576814	.8263592

Ha: diff < 0 | Ha: diff != 0 | Ha: diff > 0

Pr(T < t) = 0.5896 | Pr(|T| > |t|) = 0.8208 | Pr(T > t) = 0.4104

The overall average depth of invasion was 1.7 mm for our T1N0 OSCC cases. We excluded cases for which the H& E slide could not be located. The mean depth of invasion for the disease-free cohort was 1.73 mm and 1.64 mm for the LR group. This difference was not found to be statistically significant (alpha =0.05).

Figure 7: Test for association: treatment modality vs locoregional recurrence

	TREATMENT		
RECURRENCE	Excision	+END	Total
No	24	19	43
%	55.81	44.19	100.00
Yes	13	9	22
%	59.09	40.91	100.00
Total	37	28	65
%	56.92	43.08	100.00

Pearson chi2(1) = 0.0637 Pr = 0.801

Out of our 65 cases, 56.9% of patients underwent surgery *without* END while 43.1% of patient underwent surgery *with* END. 13/37 (35.1%) of patients with excisional surgery alone experienced loco-regional recurrence while 9/28 (32.1%) of patients with surgery + END experienced loco-regional recurrence. No significant associations were found between loco-regional recurrence and treatment modality.

Figure 8: Association of oral site of OSCC development and LR. FOM= floor of mouth; Gingiva = mandible and maxilla.

	RECURRENCE		
Site	No	Yes	Total
buccal mucosa	3	4	7
%	42.86	57.14	100.00
fom	5	3	8
%	62.50	37.50	100.00
gingiva	7	7	14
%	50.00	50.00	100.00
palate	2	2	4
%	50.00	50.00	100.00
tongue	26	6	32
%	81.25	18.75	100.00
Total	43	22	65
%	66.15	33.85	100.00

Fisher's exact = 0.092

Figure 9: Association of high-risk vs low/moderate risk sites and LR by. Group 1 =Buccal mucosa, gingiva, palate. Group 2 = Tongue, floor of mouth.

Site	Recur No	Yes	Total
Group 1	12	13	25
%	48.00	52.00	100.00
Group 2	31	9	40
%	77.50	22.50	100.00
Total	43	22	65
	66.15	33.85	100.00

Pearson chi2(1) = 5.9795 Pr = 0.014

We found no significant association between locoregional recurrence and oral site when each site (tongue, floor of mouth, buccal mucosa, gingiva, palate) was evaluated separately. The sites were clustered to increase the size of each group and to examine recurrence in the context of risk of development of OSCC. Traditionally high-risk sites (tongue, floor of mouth) were grouped together, and low/moderate risk sites (gingiva, palate, buccal mucosa) were arranged in another group. With this clustering, we found a statistically significant difference between the oral site and locoregional recurrence with areas at lower risk for developing OSCC at higher risk for experiencing locoregional recurrence.

Figure 10: Chi-Squared test of association for history of dysplasia vs LR

	HX OF DYSPLASIA	
RECURRENCE	No	Yes	Total
No	36	7	43
	83.72	16.28	100.00
Yes	7	15	22
%	31.82	68.18	100.00
Total	43	22	65
%	66.15	33.85	100.00

Pearson chi2(1) = 17.5103 Pr = 0.000

History of dysplasia was recorded by looking at the patient's medical record for biopsy proven dysplasia or epithelial atypia in the region where the cancer developed. The vast majority (83.7 %) of the disease-free cohort while only a minority (31.8%) of LR cohort did not have previous epithelial dysplasia. This association of history of dysplasia to loco-regional recurrence was found to be statistically significant ($p < 0.001$).

4.3 LOGISTIC REGRESSION AND RECEIVER OPERATATING CHARACTERISTIC (ROC) ANALYSIS

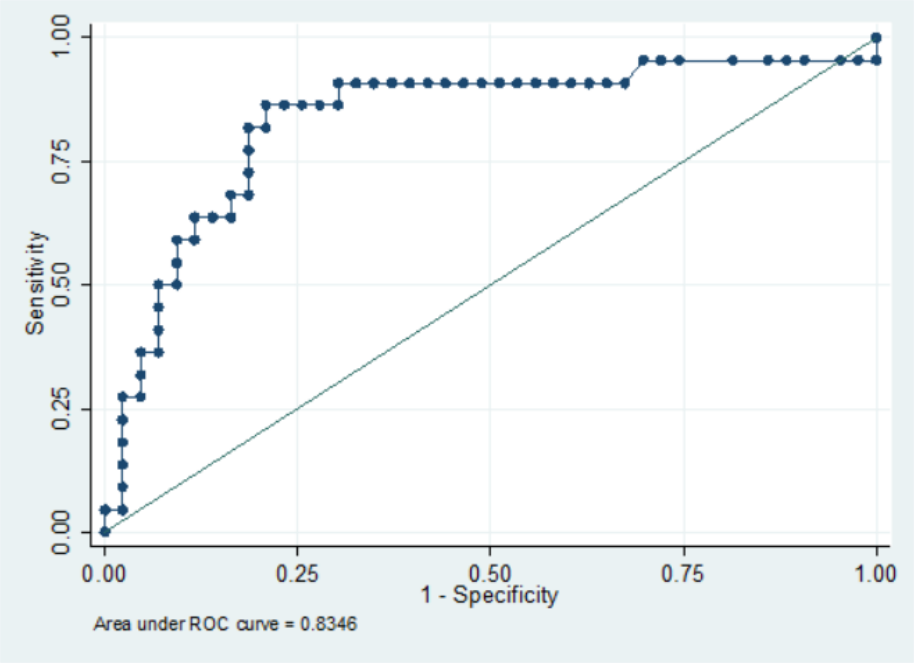
Figure 11: Logistic regression model controlling for age and sex. Using history of dysplasia and oral site as independent variables, we calculated the odds ratio for LR.

Logistic regression — Number of obs = 65
LR chi2(4) = 23.14
Prob > chi2 = 0.0001
Log likelihood = -30.028211 — Pseudo R2 = 0.2782

recur	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
age	1.002756	.0289718	0.10	0.924	.9475499	1.061178
sex	.7902981	.5359273	-0.35	0.729	.2092006	2.985512
hx of dysplasia	12.00301	8.180695	3.65	0.000	3.156099	45.64883
Group_2 sites	.235641	.163538	-2.08	0.037	.0604652	.9183247
_cons	.3956383	.7618237	-0.48	0.630	.0090837	17.23196

Based on our logistic regression model, the odds of developing a loco-regional recurrence in a patient with a history of dysplasia is 12 times greater compared to those without a history of dysplasia. The odds of recurrence are 76% lower for patients with OSCC on Group 2 sites (tongue, floor of mouth) compared to other sites.

Figure 12: ROC curve analysis using oral site and history of dysplasia as predictors of LR. The area under the curve or AUC = 0.83



The area under the curve for the ROC is 0.834 which indicates that using oral site and history of dysplasia, our model can accurately discriminate between LR and disease-free survival in 83.4% of cases for our study population.

5.0 DISCUSSION

The three objectives of the present study were (1) to examine the characteristics of early stage (T1N0) OSCC, (2) to compare select clinicopathologic factors (age, sex, histologic grade, history of dysplasia, tumor site, DOI, treatment modality) between the disease-free and locoregional recurrence groups, and (3) to evaluate which, if any, of the selected clinicopathologic parameters increases the risk of locoregional recurrence. By limiting our study to early stage cancers, we were able to control for pathologic stage and nodal disease, which is the most significant predictor of disease-free survival and overall survival. The rate of locoregional recurrence in our entire cohort was 29.2% at the 5-year mark and 33.8% at the 10-year mark. These values fall within the reported range of recurrence rates (~15-40%) in the literature. The wide range in values can be attributed to differences in methodology and follow-up times among different studies. The latest National Cancer Institute statistics indicate that the median age at diagnosis is 63 with the largest percentage of new cases in the age group 55-64. The average age of our patients was 61.5 years (range: 43-90), which falls into the age ranges reported by most studies.

In contrast to other researchers who report significantly more male subjects, females made up a majority (53.8%) of our patient population. This occurrence may be related to the fact that although males comprise of a significant majority in OSCC, females are overrepresented in early stage OSCC at a F:M ratio of 1.8: 1 (SEER, 2017).

With regard to oral site of development, the national cancer database reports that tongue, lip, and floor of mouth are the most common sites for OSCC in the United States (SEER, 2017). We excluded the lip for reasons mentioned in Section 3.1, but similar to other reports, noted that

the most prevalent site for development of Stage I OSCC is tongue followed by the mandible and floor of mouth; the hard palate was the least common site.

A slight majority (57%) of tumors were classified as moderately-differentiated compared to well-differentiated. Although a step-wise relationship between histologic grade and an overall worse prognosis has not been established, researchers have reached a general consensus that poorly-differentiated tumors spread faster compared to well and moderately differentiated OSCC, and these cancers present more often with late-stage disease (Roland et al, 1992). Of note, none of the patients in our study had poorly-differentiated tumors. This result can be explained with the existing data on histologic presentation of OSCC. Thomas et al. found that only 13.3% of Stage I and II OSCCs represent poorly-differentiated tumors, and these cancers carry a 3x greater risk of death compared to well or moderately differentiated tumors (Thomas et al, 2014).

The average depth of invasion (DOI) for our cases was 1.7 mm (range: 0.2-4.0). The difference in DOI between the disease-free and LR group was not significant. Our results confirm the existing dogma that a DOI less than 5mm does not add discerning prognostic information. A systemic review of the effect of DOI on disease-free survival conducted by Pentenero and colleagues suggests that the effect of DOI only becomes significant when the value exceeds 3mm (Pentenero et al, 2005). With the 8th edition of the AJCC, DOI greater than 5 mm signifies the differences between a T1 and T2 tumor. Thus, since the DOI in our study ranged from 0.2-4.0 mm, we did not expect to find significant differences in disease-free survival.

Currently, the medical community has not reached a consensus regarding the inclusion of elective neck dissection (END) in early stage oral cavity carcinomas. Certain studies report improved survival for patients who underwent END while other found no difference in T1N0 tumors, if properly staged (Woolgar et al, 1999; Cannis et al, 2012). We observed that the treatment

of choice for 56.9% of patients was surgery alone while 43.1% decided on an ipsilateral limited neck dissection in conjunction with the surgical resection. The motivation for more extensive treatment is based on reports of occult cervical lymph node metastasis which frequently evade detection clinically or by imaging. As previously mentioned, the spread of carcinoma to the lymphatics upstages the cancer and worsens prognosis. In comparing these two treatment modalities, we did not find an association with disease-free survival, and the data indicates that END did not improve overall survival or disease-free survival for our patients with T1 tumors and clinically N0 necks. The absence of tumors which had greater than 5 mm DOI may have affected our finding since this pathologic parameter is a strong predictor of cervical metastasis.

Although we did not find an association between age, sex, histologic grade, treatment modality, DOI with locoregional recurrence, oral site and history of dysplasia did emerge as significant predictors of disease-free survival. Similar to previous reports, our data suggest that OSCC from distinct oral sites experience different rates of loco-regional recurrence, with buccal mucosa having the highest rate (4/7) and tongue the lowest (6/26). Despite these differences, no statically significant association was found when each oral site was analyzed separately; our small sample size may not have enough power to distinguish the differences for the effect of oral subsite. Interestingly, the grouping of site of development into low and high-risk sites yielded a statistically significant association. Exposure of the oral mucosa to carcinogens is strongly linked to OSCC, and researchers believe that OSCC development in certain anatomic regions such as floor of mouth and tongue are more prevalent because these areas have the heaviest exposure to alcohol and tobacco in the Western world. In addition, with thin layers of keratin within the stratum corneum, the ventral and lateral surfaces of the tongue along with the floor of mouth are designated as “high-risk” sites for OSCC development. However, using a logistic regression model, we found that

traditionally high-risk sites for oral cancer development such as the tongue and floor of mouth were 76% less likely to experience locoregional recurrence compared to low-risk sites such as the gingiva, palate, or buccal mucosa.

This finding that low-risk sites of OSCC development have a higher risk of locoregional recurrence appears counterintuitive at first glance. Nonetheless, there are a few explanations from the literature which can resolve the oddity of our result. Firstly, several studies have described a higher rate of occult cervical lymph node metastasis in buccal mucosa, palate, and gingiva compared to tongue and floor of mouth (Diaz et al, 2003; Lin et al, 2006). In addition, a number of studies show that OSCC of the tongue has a higher 5-year disease-free and overall survival compared to other sites (Montes et al, 2008; Wang et al, 2016; Thomas et al, 2014). One unique aspect of the tongue which may be protective in the spread of cancer is that the intrinsic muscles, which makeup a bulk of tongue tissue and exist to alter the shape of the tongue, do not communicate with vital head and neck fascial spaces or bone unlike the buccal mucosa or gingival/palatal tissue (Kitamura et al, 2018)

Lastly, our results support a very robust association between history of dysplasia and locoregional recurrence ($p<.001$). Our logistic regression model showed that the odds of developing locoregional recurrence in patients with a history of epithelial dysplasia are 12 times higher with a 95% C. I. [3, 46] compared to those without. This finding was surprising; to the best of our knowledge, previous studies have not evaluated disease-free survival in the context of history of dysplasia for OSCC. Obviously, further research beyond the scope of this project is required to delve into the possible cellular mechanisms behind this phenomenon and answer the question of why certain individuals are more prone to progression to malignancy.

Currently, we know that a host of molecular and genomic alterations can exist in clinically "normal" appearing tissue before high-grade dysplasia or carcinoma develops. However, to date, researchers have not identified a clinically validated set of biomarkers which can predict the relentless progression to carcinoma that occurs in some patients (Lingen et al., 2011). One area of interest in evaluating the root cause of locoregional recurrence is looking at field cancerization. This notion first proposed by Slaughter in 1953 refers to the observation that non-cancerous epithelial cells adjacent to the tumor have pre-malignant phenotypes and genomic alterations (Slaughter et al, 1953). These cancer-primed cells can remain at the site of tumor or migrate to an adjacent site, and recurrent tumors or second primaries can develop from these abnormal cells. Therefore, the effect of field cancerization may explain the strong association between history of dysplasia and locoregional recurrence (Angadi et al, 2012). Certain patients may be intrinsically more prone to recurrence because they possess a larger spatial field of cancer primed cells, some of which may have also migrated into adjacent oral tissues. Furthermore, since the effects of field cancerization is not clinically or even histologically apparent, these abnormal cells likely get left behind during surgery when the primary tumor is removed.

5.1 LIMITATIONS OF STUDY

Our moderate sample size was the primary weakness. By limiting the study to a very specific OSCC patient population, we were able to examine 65 cases, the majority (32/65) of which were early stage tongue cancers. In addition, social habits (smoking, alcohol) strongly linked to the development of OSCC were not consistently reported in the medical record. Thus, we had to exclude these relevant parameters from our study.

Another study limitation is the difference in follow-up interval for patients. Due to the study design, all patients were followed for a minimum of 5 years post curative surgical treatment. We chose 5 years as the minimum period of follow-up because previous studies demonstrated the vast majority (80-90%) of recurrences occur within this time frame. However, since patient data were collected up to 2012 to accrue a viable sample size, some patients were followed for 5 years while others for 10 years or longer. In addition, we did not contact patients for this study, so medical records became the sole indication of whether patients experienced locoregional recurrence or remained disease-free.

6.0 CONCLUSION

In our present study, we found history of dysplasia and “low-risk” oral site to be significant independent predictors of locoregional recurrence (LR) for early-stage OSCC. Based on our findings, we believe LR can be stratified into high-risk group and a low-risk group in T1N0 cancers. High-risk individuals have tumors of gingiva, buccal mucosa, or palate with a previous history of oral dysplasia. Cases without a history of oral dysplasia and which occur in the tongue or floor of mouth are at a lower risk for LR. These findings should be confirmed with a larger number of cases. These findings taken in the proper clinical context can provide insights on how to improve upon the current post-treatment follow-up protocols for OSCC.

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