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Title: The description of diagnosed cases of Oral Epithelial Dysplasia at the Tygerberg Oral Health Centre

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Key words

Oral epithelial dysplasia

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Degree of dysplasia

Oral Squamous cell carcinoma

Oral Potentially Malignant Disorders

Abstract

Oral epithelial dysplasia (OED) is a growth anomaly which occurs as a result of atypical, abnormal proliferation and a change in the architecture and cytological features of cells of epithelial origin, which ultimately results in the formation of a lesion with disturbed differentiation and maturation.

The purpose of this study was to describe the OED cases diagnosed at Tygerberg Oral health centre in a 7-year period between 2012 and 2019. The patients' medical records from Tygerberg Oral Health Centre and National Health Laboratory Service (NHLS) were reviewed. All diagnosed cases of OED were identified and the data retrieved for further assessment and comparison. The individual medical records and follow up data were assessed.

Seventy cases of OED were diagnosed in the period assessed. Of those 70 cases, the median age was 58 and the interquartile range was from 48 – 62. Thirty-six of the diagnosed patients were female and thirty-four were males. The majority of lesions diagnosed with OED were found on the tongue, floor of the mouth (FOM) and buccal mucosa. Majority of the lesions were found in non-smokers and non-alcohol consumers. These two categories both presented with mild cases of OED.

From the results, it was derived that OED has no intra-oral location predilection. Moreover, OED is not directly associated with smoking.

Declaration

I Nocwaka Charlotte Nkomo hereby declare that this report is my own work. It is being submitted for a Masters in Science degree at the University of the Western Cape, it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Dr. NC Nkomo

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I acknowledge the role played by my supervisor Dr Jeftha and co-supervisor Dr Opperman. Thank you for your guidance, patience and assistance. A huge appreciation goes to Dr Kimmie for all the data analysis.

Dedication

To my family for always inspiring me, for pushing me to learn and be more. To my grandmother for the unending prayers and encouragement. To my friends for always holding me accountable.

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LIST OF ABBREVIATIONS

OED: Oral epithelial dysplasia

OPMDs: Oral potentially malignant disorders

OSCC: Oral Squamous Cell Carcinoma

HPV: Human Papilloma virus

EBV: Epstein Barr Virus

WHO: World Health Organization

NHLS: National Health Laboratory Service

OR: Odds ratio

Chapter 1: Introduction

This retrospective study aims to describe Oral epithelial dysplasia (OED) cases over a 7-year period and analyse the link between the clinical appearance of the cases to demographics and risk factors. "Oral epithelial dysplasia is a growth anomaly which occurs as a result of atypical, abnormal proliferation and a change in the architecture and cytological features of cells of epithelial origin, which ultimately results in the formation of a lesion with disturbed differentiation and maturation" (Ranganathan K. and Kavitha L., 2019). Rigorous follow up of patients diagnosed with oral epithelial dysplasia is essential to assess for malignant transformation, early detection and intervention. Early diagnosis and intervention in turn improves the overall life expectancy and quality of life due to less invasive treatment regimes Ranganathan K. and Kavitha L., 2019). Currently most patients present quite late and those diagnosed with OED do not follow rigorous follow up protocols. This follow up is often difficult in resource poor settings and amongst patients who hail from resource constraint backgrounds.

OED is characterized by a sequence of cellular and molecular events that may resolve or end in neoplasia. Clinically, OED is found in certain lesions, these lesions are called Oral Potentially Malignant Disorders (OPMDs). The causes/risk factors of OPMDs and OED are similar to that of OSCC. There are numerous risk factors that have been implicated in the formation of OED, the common ones being tobacco use (smoking and/or chewing) and alcohol consumption (WHO, 2017). Other suggested factors include infections (Candida and HPV infections), hematic deficiencies and genetic diseases such as dyskeratosis congenita (Porter S. *et al*., 2018).

The estimates of the malignant transformation rates of OPMDs vary in literature from site to site and from population to population (Pereira J.S. *et al*., 2011). The progression time is unpredictable and it ranges from 6 months to 8 years, however it may take up to 30 years to progress carcinoma, therefore the reported transformation rates are also dependant on the duration of the study (Feller L. and Lemmer J., 2011).

OED is not associated with any specific clinical appearance; however, it has been classically associated with leukoplakia and erythroplakia (Jaber M.A., 2010). Leukoplakia has been reported to have a malignancy transformation rate of 1 – 3%, for all type of leukoplakias. Erythroplakia has a low prevalence rate (0.01% - 0.2%), it however has a high malignant transformation of approximately 51 – 66% (Patait M. *et al*., 2016). Proliferative verrucous leukoplakia has been linked to a 100% transformation rate (Liu W. *et al*., 2011). In South Africa, 60 000 new cases of OSCC are reported yearly. The areas of highest incidence of OSCC are Eastern Europe, South and Southeast Asia, Caribbean and in Pacific, region parts of Western Europe and parts of Latin America. India accounts for the majority of these cases, with 100 000 cases reported yearly (Abram M.H., 2013).

50% of all cancers develop from precursor lesions. The 5-year survival rate of oral cancers in most countries is below 50%. This high failure rate is due to the delay in the diagnosis and the emergence of secondary tumours (Sathiasekar A. *et al*., 2017). Therefore, the understanding of the progressive, multistep process of genetic changes in tumour formation, invasion and metastasis and the awareness in the epidemiology of OED and OSCC, together with highlighting the importance for clinicians to identify, diagnose and follow on OPMDs may help provide effective and appropriate treatment plans to reduce mortality and improve the quality of life (Ranganathan K. and Kavitha L., 2019).

This study identifies and describes the OED diagnosed cases over a 7-year period to highlight the important role that clinicians play in the identification, follow up on these cases and their long-term management. Moreover, it will assess the risk factors in relation to patients diagnosed with OED to investigate the role played by those risk factors in the aetiology and malignant transformation. It has been also said that most patients present late for assessment (Sathiasekar A. et al., 2017) and as a result of late diagnosis, most OPMDs would have transformed to malignancy; this study will bring light to the incidence of the diagnosed cases and the ages of the patients at the time of diagnosis.

Chapter 2: Literature review

The term dysplasia refers to abnormal epithelial activity that can is seen on a histopathological level. (Jain A, *et al.*, 2016). The term was first introduced in 1958 by Reagon, in relation to cells extracted from the uterine cervix (Rastogi V. *et al.*, 2013). "It is a histopathology feature which shows abnormal cell activity in normal epithelium" (Jain A. *et a*l., 2016). WHO Classification of Head and Neck Tumours (4th ed.) defines dysplasia as a spectrum of architectural and cytological epithelial changes caused by an accumulation of genetic changes that can be associated with an increased likelihood of progression to squamous cell carcinoma (WHO, 2017). This results in the formation of a lesion with disturbed differentiation and maturation (Tilakaratne W.M. *et al*., 2019). Carmo M.A.V. *et al*. described dysplasia as "a development disorder in a stratified squamous epithelium, architectural disturbances affecting the normal maturity and stratification may occur" (Kujan O. *et al*., 2006). Epithelial dysplasia, dyskeratosis and atypia are often used interchangeable (Sharma N. *et al.,* 2010).

A definite diagnosis can only be reached after microscopic assessment has been done, a biopsy is therefore necessary and important (Chandran R., 2012). It can affect the full epithelium thickness or part; the cells show variable degrees of cellular atypia (Tilakaratne W.M. et al., 2019).

OED is a histopathological diagnosis for disorders that present in different forms clinically (Tilakaratne W.M. et al., 2019). Oral Epithelial Dysplastic lesions vary in characteristics and in their clinical appearances. This variability may be in lesion colour, size and texture. The common lesions presenting with OED include but are not limited to oral submucous fibrosis, oral leukoplakia, erythroplakia, smokeless tobacco keratosis, proliferative verrucous leukoplakia, lichen planus, discoid lupus erythematosus, oral submucous fibrosis, actinic chelitis and certain genetic disorders for example Xeroderma pigmentosus and Dyskeratosis congenita (Tilakaratne W.M. et al., 2019). These lesions have been identified as Oral Potentially Malignant Disorders (OPMDs). The worldwide prevalence rate of these lesions ranges between 1 – 5% (Tilakaratne W.M. et al., 2019).

2.1 Leukoplakia and Erythroplakia

Leukoplakia and Erythroplakia are the most common OPMDs (Farah C. S. *et al*., 2014). OED has been classically associated with leukoplakia and erythroplakia (Jaber M.A., 2010).

Leukoplakia has been defined by WHO as a white plaque of questionable risk having excluded other known diseases or disorders that carry no risk (Farah C. S. *et al*., 2014). There are two clinical variants which are the homogenous and non-homogenous types (Feller L. and Lemmer J., 2012). Homogenous lesions are usually flat lesions with smooth or relatively smooth surfaces (Chandran R., 2012). However, these lesions may have surface irregularities and can be further classified according to the surface into pumice like, wrinkled, corrugated and flat. The non-homogenous type can be further classified into:

i. Speckled leukoplakia - red and white but predominantly white
ii. Erythroleukoplakia - red and white, with less white
iii. Nodular leukoplakia - small polypoid outgrowths, rounded red or white excrescences
iv. Verrucous leukoplakia - wrinkled or corrugated surface appearance
v. Proliferative verrucous leukoplakia - multiple, simultaneous leukoplakias (Chandran R., 2012).

Below is a clinical picture of the above-mentioned lesions.

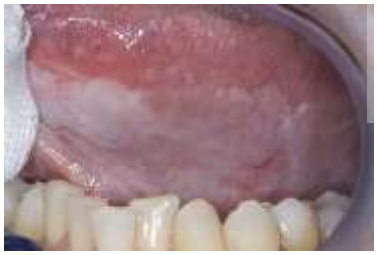


Figure 1: Leukoplakia

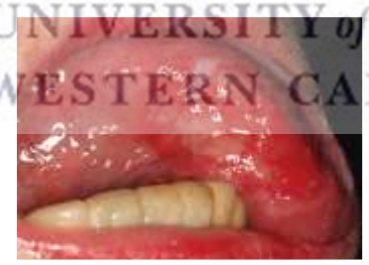


Figure 2: Erythroplakia

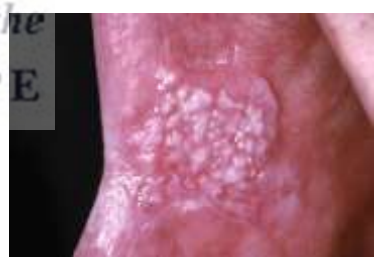


Figure 3: Nodular leukoplakia

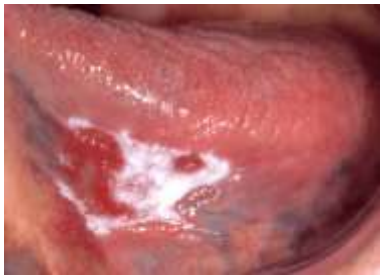


Figure 4: Erythroleukoplakia

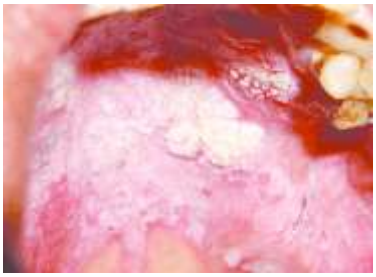


Figure 5: Verrucous leukoplakia

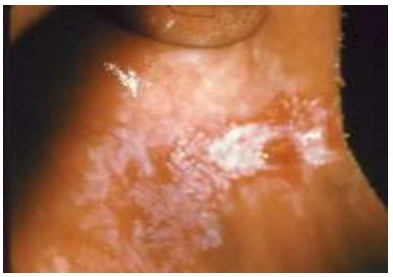


Figure 6: Speckled leukoplakia

From Parlatescu I. *et al.*, 2014, Van der Waal I., 2015 and Woo S., 2019.

These lesions can present as singular and multiple. Multiple lesions may appear clinically on one anatomic site or on various sites (Chandran R., 2012). The size of the lesions also varies

from a few millimetres to centimetres (Feller L. and Lemmer J., 2012). The most common affected site is the buccal mucosa representing 25% of oral epithelial dysplasias, followed by the mandibular gingiva, tongue and floor of mouth at 20%, 10% and 10% respectively. The remainder accounts for the rest of the oral cavity sites (Feller L. and Lemmer J., 2012, Napier S.S. *et al*., 2008). Homogenous lesions have a low risk of malignant transformation whilst the non-homogenous types have a high risk of malignant transformation (Geetha K. M. *et al*., 2015).

Erythroplakia is used to describe a red plaque or macular lesion in the mouth for which a specific clinical diagnosis cannot be established. It is the rarest of all OPMDs, however it has the highest malignancy transformation. Erythroplakia sometimes presents with speckled leukoplakia. It usually presents as carcinoma in situ or invasive carcinoma at time of biopsy (Patait M. *et al*., 2016).

2.2 Etiopathogenesis

The current knowledge on the etiological risk factors is limited (Jaber M.A., 2010). The cause of Oral Epithelial Dysplasia should therefore be linked with each clinical entity (Tilakaratne W.M. *et al*., 2019). Tobacco and areca nut use, independently or synergistically, are associated with 70-90% of oral epithelial dysplasias, whilst the aetiology of the remaining lesions is idiopathic (Feller L. and Lemmer J., 2012). Tobacco's etiological role cannot be established but it remains as a definite risk factor. The relation of tobacco and other known risk factors and malignant transformation remains unclear and controversial (Ho P.S. *et al*., 2009). They however play a role in OSCC development which may occur de novo or on a pre-existing lesion with OED (Warnakulasuriya S. *et al*., 2008, Bouquot J.E. *et al*., 2006, Chandran R. *et al*., 2013).

In a study done by Jaber (2010) which investigated the clinical, demographic, histological and prognostic aspects of OED in non-smoking and non-drinking patients; the presence of OED in these patients suggested that other risk factors other than alcohol and tobacco do exist (Jaber M.A., 2010). The factors associated with the pathogenesis of idiopathic leukoplakic lesions are unknown (Feller L. and Lemmer J., 2012).

The risk factors associated with leukoplakia are alcohol use, chronic irritation and all forms of tobacco (chewable tobacco has the highest risk). Other contributing factors include candida infections, galvanic current from dissimilar metal restorations, syphilis, UV rays and micronutrient deficiencies for example vitamin B complex and iron (Karthiga Kannan S. *et al.,* 2019). However, previous studies have shown that the same etiological factors may produce lesions without OED (Nagao T. *et al*., 2005).

2.3 Risk factors for Oral Epithelial Dysplasia

The role of tobacco use and alcohol consumption as risk factors in the development of OSCCs has been well documented in literature, however limited studies have been done on their roles as risk factors in the development of OED (Jaber M.A., 2010). A study by Morse *et.al* (1996) showed that OED risk was associated with smoking and alcohol consumption. The risk was dependant on the frequency of usage/consumption, with the risk increasing when used synergistically (Sharan R.N. *et al*., 2012).

Individuals who consume 100g of alcohol and smoke 20 cigarettes daily are at an increased risk of developing OED. This risk was found to decline following smoking cessation. Smokers of 10 years or more demonstrated no excess risk compared to non-smokers. Alcohol consumption alone is an insignificant predictor of OED (Jaber M.A., 2010).

2.3.1 Tobacco

a. Smoking tobacco

There are two basic forms of tobacco which are smoking tobacco and smokeless tobacco. Smoking is a linked risk factor in the development of OPMDs, whether used alone or in combination with alcohol, it has a prevalence rate of 1-5%. OED incidence rates vary in tobacco users in relation to non-users, this is a result of the difference in the method and types of tobacco consumption. For example, a study done on Bombay police and Indian industrial workers who smoked and/or chewed tobacco showed that they developed OED at an annual incidence of 5.2/1000 – 30.2/1000 (Napier S.S. *et al.,* 2008).

b. Betel quid/areca nut chewing

Also called smokeless tobacco, there are different modes of use which include snuffing or chewing; this is responsible for the development of OPMDs. Betel quid chewing is a habitual practice common in some parts of the world, which include Taiwan, South Asia and the Pacific islands, these areas have 10% of the world population (Lee C-H. *et al.*, 2003).

A mixture of substances is wrapped in a betel leaf. The 3 common forms are areca nut quid and tobacco products, areca nut quid without tobacco products and tobacco products in dry and moist forms. Other uncommon substances that may be present include spices, various essences and lime; this varies from place to place. Some betel quid forms contain tobacco, for this reason betel quid chewing lesions have been categorized under smokeless tobacco (Sharan R.N. *et al*., 2012). Betel quid/areca nut chewing is a known etiological factor in the development of leukoplakia, however there is 43 - 68% variation in the proportion of betel chewers in individuals with leukoplakia. There are therefore several other etiological factors involved in its pathogenesis (Sharan R.N. *et al*., 2012).

Betel chewing patients are at a greater risk of developing OPMDs and this risk increases with an increase in duration and frequency of use (Lee C-H. *et al.*, 2003, Schwarz F. *et al.*, 2005). Tannins and alkaloids in areca nut have been identified as the causative agent for the development of dense fibrosis and oral submucous fibrosis which in turn leads to dysplastic changes (Sharan R.N. *et al*., 2012). Moreover, it has also been shown to be able to produce DNA breaks and adducts which result in pre neoplastic tissue alteration (Lee C-H. *et al.*, 2003).

An analysis done on tobacco smoking has shown that the increased risk of OED as a result of smoking is attributed to heavy smoking relative to non-smoking (Tilakaratne W.M. *et al*., 2018). In smoking oral leukoplakia has no gender predilection, presents at a later stage, mostly affects the floor of the mouth and is usually of the homogenous type (Chandran R. *et al.*, 2013, Tilakaratne W.M. *et al.*, 2018., Pereira J.S. *et al.*, 2011). However, in erythroplakic cases, both smoking and alcohol are proven risk/etiological factors. Half of these cases present with OED, while the remainder show invasive carcinoma at the time of diagnosis (Napier S.S. and Speight P.M., 2008).

2.3.2 Alcohol

The relationship between OPMDs/ OED and alcohol is not well established. The role of alcohol and the mechanism behind alcohol as a risk factor in OED and the pathogenesis thereof is not well understood. In literature, different studies reveal inconsistent results of a negative as well as positive relationships (Nagao T. *et al*., 2003, Petti S. and Scully C., 2006, Tilakaratne W.M. *et al*., 2018). In the positive studies, the risk depends on the alcohol intake. The greater the intake, the greater the risk. OED development is not influenced by the type of alcohol. The use of heavy alcohol and smoking together are considered as risk factors for OED; their role in the development of OSCC is well established (Tilakaratne W.M. *et al*., 2018).

A few suggestions have been given to explain the role of alcohol as a risk/etiological factor. Acetaldehyde, an alcohol proximate metabolite is a known carcinogen. Alcohol may inhibit the detoxification of carcinogens and interfere with DNA repair. Moreover, ethanol its metabolite has the ability to enhance genotixicity and/or activate other carcinogenic agents. Alcohol use is also known to reduce immune function, it also enhances the penetrations of carcinogens through the oral cavity tissues. Alcohol also interferes with nutritional intakes and their bioavailability including that of antioxidants (Lee C-H. *et al*., 2003).

2.3.3 Microorganisms

a. Human papilloma virus (HPV)

Recent research has found a relationship between high-risk Human Papilloma virus (HPV) and a subgroup of high-risk OED lesions, more so on lesions found on the floor of the mouth (Feller L. and Lemmer J., 2012). The estimated prevalence of high-risk HPV in oral and oropharyngeal dysplasia has been found to be 24.5% and of those cases, 25.3% were found in oral lesions alone. The detection of HPV was two times more prevalent in men than in women (Dietrich T. *et al*., 2004).

There is however a variation in the prevalence of HPV in OPMDs, this may be due to factors such as the sampling and detection method, ethnicity and geographic location. As a result, the

role of HPV in the development of high-risk OED is not clear and more research is required (Feller L. and Lemmer J., 2012).

50% of proliferative verrucous lesions present with dysplasia at time of diagnosis. Although the cause of these lesions is unknown, a relationship with high risk HPV infection has been established (Bhatia N. *et al.*, 2013).

b. Candida

Chronic hyperplastic candidiasis has been shown to undergo malignant transformation, 15% of the non-dysplastic types progress to a dysplastic lesion. However, other studies have suggested that the presence of Candida may not prove a causal relationship as candidal colonization may have taken place in a pre-existing OED (Bakri M.H. *et al.*, 2010).

Candida produces carcinogens, including nitrosamines, which have an ability to create irregularities in the DNA replication process. Although it has been found that a *Candida ablicans* infection alone is insufficient to cause OED or OSCC, it has been shown to metabolize pro carcinogens and can cause chronic inflammation by modifying the micro environment (Bakri M.H. *et al.*, 2010).

c. Epstein-barr Virus (EBV)

Studies have shown that EBV DNA has been found in OED and OSCC, this has not been found in normal epithelium. The EBV oncogenic potential has been established with reference to nasopharyngeal carcinoma. However, its involvement in the development of dysplasia and in the process of oral carcinogenesis is not clear (Tilakaratne W.M. *et al*., 2019).

d. Ultraviolet (UV) light

Actinic cheilitis is one of the OPMDs. It usually affects the lip and is caused by chronic/prolonged exposure to the sun and it mostly affects fair skinned (Caucasians) men with a prevalence between 0.45% - 2.4%. UV light induced DNA mutations are believed to initiate and promote dysplastic changes of the epidermis/labial mucosa in actinic cheilitis. In 40% -

100% of cases, dysplastic changes have been identified in actinic cheilitis (De Santana Sarmento D.J. *et al.*, 2014).

e. Immunological -mediated disorders

Oral lichen planus and Discoid lupus erythematosus are chronic immunologically mediated diseases with malignant transformation rates of 0.4% - 3.7% and 6.8% respectively. There is no clear data on the rate of OED in these lesions (Fitzpatrick S.G. *et al*., 2014).

f. Miscellaneous

Certain diseases with genetic abnormalities develop malignancies. These include xeroderma pigmentosum, epidermolysis bullosa congenita (only certain forms) and dyskeratosis. It is difficult to determine if they have a precancerous, or potentially malignant stage. In dyskeratosis congenita, the degree of dysplastic change increases with age (Mortazavi H. *et al.*, 2014).

Oral Epithelial dysplastic lesions can regress, remain stable or undergo malignant progression or transformation. Dysplasia is theoretically reversible. Since it is a change in tissue level and atypia as cellular level change, when the causative/underlying stimulus is removed, the alterations revert to normal (Rastogi V. *et al*., 2013). If the causative factors persist, dysplastic cells escape homeostatic and hormonal control to assume autonomy of tumour cell. The irreversible changes are characterized by cell division in an accelerated form which in turn facilitates the accumulation of genetic damage. This further drives towards the transformation path, leading to cell death/neoplasticity (Rastogi V. *et al.*, 2013). Their clinical appearance changes with time and the lesions may change from homogenous to non-homogenous. The progression is unpredictable and may take a few months to a few years (Feller and Lemmer, 2012). It is extremely difficult to predict the risk of malignant progression in any individual patient (Speight P. M. *et al.*, 2018).

Statistically, OPMDs have an increased risk of progressing to malignancy, this however varies according to a different lesion and patient related factors (Speight P. M. *et al.*, 2018). The presence of epithelial dysplasia is an indicator of malignant potential of OPMDs. It is useful in the prediction of the malignant transformation of oral lesions (Ranganathan K. and Kavitha L., 2019). The presence of dysplasia and its grade has been used over the years as a malignancy potential marker. The higher the dysplastic grade, the higher the progression risk (Van Zyl *et al.*, 2012, Ranganathan K. and Kavitha L., 2019).

2.4 Diagnosis of OED

Clinically OPMDs that are suspected of having OED can be assessed. This can be done in various ways, these include the conventional oral examination, detection by toluidine blue staining, by acetic acid, chethiluminesant illumination and optical coherence tomography (Goyal P. *et al.*, 2012).

Histopathology is the gold standard in the diagnosis and is also used in the prediction of the lesion's malignant transformation risk; however, no consensus has been reached in its usage of as tool in predicting malignant transformation (Dost F. *et al.*, 2014). It is used as the gold standard in managing OED lesions. The histopathological findings and reporting are dependent on the quality and correct sampling of the lesions, the role played by clinicians is therefore important (Speight P. M. *et al.*, 2018).

Histological examination and biopsies allowing for the ruling out other diagnoses and allows for the evaluation of any tissue changes (Speight P. M. *et al.*, 2018, Warnakulasuriya S. *et al.*, 2008, Reibel J. *et al.*, 2017). In this diagnosis, other etiological factors and risk factors are excluded; however, alcohol, chronic inflammation, Human Papilloma virus and chronic frictional trauma may contribute to the development of dysplastic lesions (Feller and Lemmer, 2012). The location of the lesion in the oral cavity is mostly related to the etiological factor (Speight P.M. *et al.*,2018). Histopathology is an essential tool in diagnosing dysplasia. It is therefore important to obtain a good biopsy. To prevent the possibility of underdiagnosis and to improve accuracy the biopsy should be obtained from the proper site, be of adequate size

and depth and should be of proper orientation and with minimal distortion of the tissues (Tilakaratne W.N. et al., 2019).

The choice between incisive or excisive biopsy is subjective, however an incision is preferred for large lesions (7cm), multiple lesions, high risk sites and for erythroplakic lesions. An incision biopsy however poses a risk of missing the actual dysplastic stage and the presence of carcinoma (Tilakaratne W.N. *et al*., 2019). Incisional biopsies have a few shortcomings and these can be countered by obtaining multiple biopsies at different sites of the lesion to better represent the entire lesion and to also decrease the rate or possibility of underdiagnosis (Lee J.J. *et al.*, 2007). This however poses a risk of tumour dissemination to blood vessels in cases of invasive carcinoma (Maeda K. *et al.*, 2010).

OED characteristics are cytological atypia and architectural disturbances (Speight P.M. *et al.*,2018). OED is diagnosed and graded in relation to the extent of changes in terms of cytological abnormalities and architectural changes; these are considered together as the criteria used to diagnose dysplasia (Gale N. *et al*., 2005). There are a few different grading and staging systems that deviate from the conventional mild, moderate, severe categorizing. From the several grading systems that exist, no international consensus has been reached on which system to use (Speight P.M. *et al*.,2018).

However, in literature, the usefulness of OED grading has been contested due to the lack of established agreement on the risk of malignant transformation based on histopathology findings (Dost F., *et al.*, 2014). Most studies on the malignant transformation of OED are based on the reinterpretation of specimens re-examined by pathologists at the time of the study and not the original interpreter of the histology findings (Dost F. *et al.*, 2014). Therefore, the evaluation of only the histopathology of lesions in the assessment of malignant transformation risk is a limited approach, various clinical and biological factors should also be considered and employed. Moreover, there is a great challenge in the accuracy of diagnosing and classifying OED lesions, which poses a problem in any OED study (Jaber M.A., 2010)

2.5 Dysplastic grading

The presence of dysplasia is a very important indicator of management and treatment approach (Feller and Lemmer, 2012). However, it is not a reliable marker as some lesions with no dysplasia can progress to OSCC whilst the dysplastic ones can also regress or stabilize. In studies there is a variation in the malignant transformation numbers of OED, it ranges between 1% to 20%, however the reliability of these results is questionable due to the context in which the studies are done, the criteria of case selection, diagnostic criteria, geographic area, the follow up time and the lack of distinguish between non-treated and treated Oral Epithelial Dysplasia (Chandran R., 2012).

Table 1: Cytologic and architectural diagnostic criteria for oral epithelial dysplasia

Architectural changes	*Cytologic changes*
Irregular epithelial stratification	Abnormal variation in nuclear size (anisonucleosis)
Loss of polarity of basal cells	Abnormal variation in nuclear shape (nuclear pleomorphism)
Drop-shaped rete ridges	Abnormal variation in cell size (anisocytosis)
Increased number of mitotic figures	Abnormal variation in cell shape (cellular pleomorphism)
Abnormally superficial mitotic figures	Increased nuclear/cytoplasmic ratio
	Premature keratinization in single cells (dyskeratosis)
	Atypical mitotic figures
Keratin pearls within rete ridges	Increased number and size of nucleoli
Loss of epithelial cell cohesion	Hyperchromasia (hyperstaining)

‘From Reibel *et al.*

OED was traditionally graded according the severity, the number of thirds of the affected epithelium being the judging factor in the defining grade. There are difficulties in the assessment and the standardization of the different degrees of epithelial dysplasia (WHO, 2017). This has led to the development of numerous grading systems to help improve inter/intra observer variability and also improve reproducibility. OED grading is an enormous challenge amongst pathologist as no consensus has been reached over a single classification. These

grading systems are however subjective and there is little or no inter/intra observe agreement. This makes it difficult to predict malignant transformation potential and rates. This has therefore posed a difficulty in the classification and diagnosis of OED lesions (Speight P.M. *et al.*,2018).

The commonly used grading systems are Smith and Pindborg, the 1978 and 2005 WHO classification, Ljubljana and the New binary system classifications (Sadiq *et al.*, 2015). The 2017 WHO classification is the gold standard for the histological diagnosis of OPMDs, it however has its shortcomings (Ranganathan K. and Kavitha L., 2019).

In the 2017 WHO classification, a combination of cytological and architectural features is used to provide a more objective diagnostic approach (Speight P.M. *et al.*,2018). Moreover, this classification does not report the clinical behaviour of the lesions and also does not provide clinical therapeutic protocols (Kujan O. *et al.*, 2006). It has great variability and low reproducibility (Jain A. *et al.*, 2016)

2.5.1 WHO classification

In the WHO system lesions were classified into 5 five groups described below:

1. Hyperplasia: describes a lesion showing an increase in cell number in the spinous layer and/or in the basal/parabasal cell layers. There are regular stratification and no cellular atypia.

2. Mild dysplasia: architectural disturbance only in the lower third of the epithelium with cytological atypia.

3. Moderate dysplasia: architectural changes approaching into the middle third of the epithelium is the main criteria, but the degree of cytological atypia may require advancing it to "severe".

4. Severe dysplasia: architectural changes affecting greater than two thirds of the epithelium, with cytological atypia.

5. Carcinoma in situ: indicates that malignant transformation has started but invasion has not.

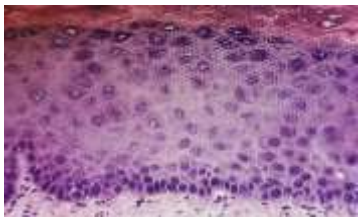


Figure 7: Hyperkeratosis with normal cytology and architecture

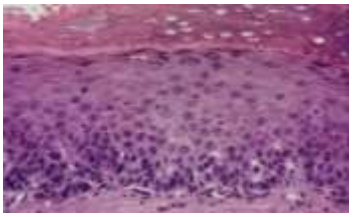


Figure 8: Mild dysplasia: basal cells lack of polarization, variation in nuclear size and shape, increased mitotic figures, hyperchromasia

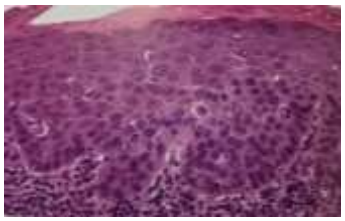


Figure 9: Moderate dysplasia: drop shaped rete ridges, mild abnormal variation in nuclear size & hyperchromatism, increase nuclear/cytoplasmic ratio, mitotic figures in the basal/parabasal area

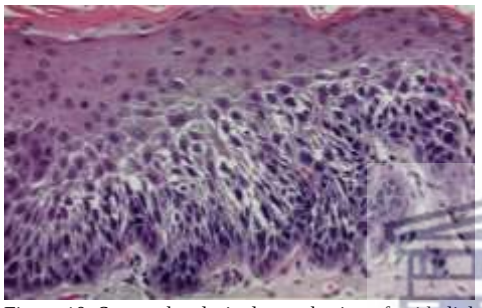


Figure 10: Severe dysplasia: loss cohesion of epithelial cells, loss of polarity of basal cells, marked abnormal variation in nuclear size and shape, abnormal variation in cell shape. Changes extend to the upper third of the epithelium epithelial thickness.

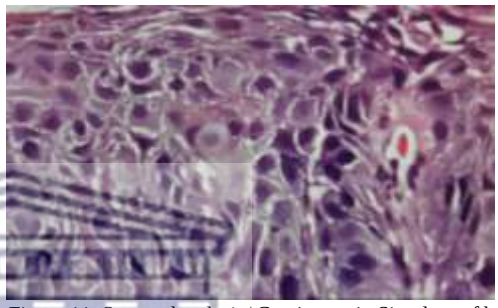


Figure 11: Severe dysplasia/ Carcinoma in Situ: loss of basal cell polarity, epithelial differentiation and cellular cohesion, increased mitotic figures and abnormal variation in nuclear and cellular features of the full epithelial thickness

From WHO, 2017

Full or almost full thickness architectural changes in cellular layers with pronounced cellular atypia. Atypical mitotic figures and abnormal superficial mitoses are common.

2.5.2 Ljubljana grading system

Zerdoner D (2003) evaluated the applicability of the Ljubljana grading system, a classification suggested for grading of epithelial hyperplastic lesions of the larynx, to hyperplastic epithelial lesions originating in the oral cavity.

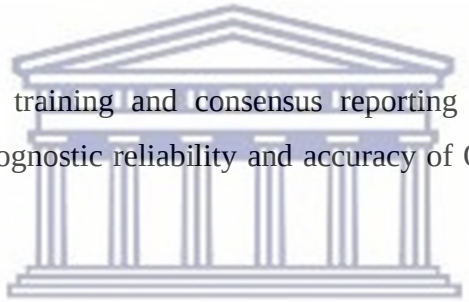
- Simple hyperplasia - is characterized by increased thickness of the spinosum (prickle) cells layer without cellular atypia.
- Abnormal hyperplasia - shows hyperplasia of basal and parabasal cell layers which constitutes up to one half of full epithelial thickness.
- Atypical hyperplasia - characterized by recognizable changes toward malignancy but
- epithelial stratification is unchanged.

2.5.3 Binary grading system

A binary grading system was developed in an attempt to improving grading reliability. Although supported by WHO, further clinical studies and validation evidence is still needed for this system. In the binary system, lesions are divided into high risk or low risk based on the number of presenting histology identified features.

High risk lesions have 4 architectural and 5 cytological features listed in the above table, whilst low risk lesions show fewer changes. Research done by Kujan et al showed that the binary system demonstrated greater inter/intra observer agreement and reliability in comparison to the WHO grading system developed in 2005 (Speight P.M. *et al.*,2018, Kujan O. *et al.*, 2007, Kujan O. *et al*., 2006 Gale N. *et al*., 2005). Moreover, the binary system has superior reproducibility in comparison to the 3-scale grading system that is widely used and also has a better prognostic value. In literature, it is therefore predicted that it will become the standard grading system (Pereira J.S. *et al.*, 2011).

It has been suggested that training and consensus reporting done by a minimum of 2 pathologists can improve prognostic reliability and accuracy of OED grading (Pereira J.S. *et al*., 2011).



2.6 Risk assessment of malignant transformation

a. Clinical prognostic factors

There is a lot of inconsistency in the literature concerning the risk factors associated with OED malignant transformation (Warnakulasuriya S. et al., 2008). Over the years, the dysplastic grade has become highly accepted by many as the independent predictor of malignant transformation.

Various other risk factors have been analyzed and their association with malignant transformation. These were listed by Van der Waal in 2009, they include:

- idiopathic leukoplakia
- long standing leukoplakia

- presence of OED
- presence of Candida ablicans
- location of the lesions, with the FOM and tongue being high risk sites
- Size of lesions, lesions > 200m2
- Non homogenous type
- Gender, females at higher risk (Van der Waal I., 2009)

The above has raised so much debate and controversy and there is therefore still a wide variation in the degree of agreement.

However, age, gender, diet, site of lesion, smoking and alcohol consumption were later shown not to be risk indicators. Holstrup et al however showed that the only significant variables were the size of the lesion (>200mm2) and non-homogenous leukoplakia (Holmstrup P. *et al.*, 2006).

Numerous studies however support the notion that the FOM and lateral aspect of the tongue have the highest incidence of leukoplakia and have an increased risk of malignant transformation (Warnakulasuriya S. *et al.*, 2018, Ho M.W. *et al*., 2012, Dost F. *et al*., 2014).

Compliance plays a huge role in risk assessment. The cessation of habits that pose a high risk such betel quid, tobacco use, alcohol and areca nut use is important in delaying and preventing malignant transformation. Regular long-term monitoring of patients is therefore necessitated and important (Ho M.W. *et al*., 2012).

OED may present in any of the clinical forms of OPMD, the clinical outcome of the different forms is difficult to predict. Similar lesions in two different individuals may have different outcomes regardless of treatment. There is therefore a lot debate on which lesion to treat and when or at what stage to do the treatment (Brennan M. *et al*., 2007).

b. Pathological prognostic factors

A thorough understanding of the pathophysiology of disease progression from dysplasia to OSCC is needed in determining whether this progression will occur in OED lesions (Feller and Lemmer, 2012). To better predict the disease progression, it has been suggested to use of a combination of molecular markers and the clinical and histopathological grading (Ranganathan K. and Kavitha L., 2019).

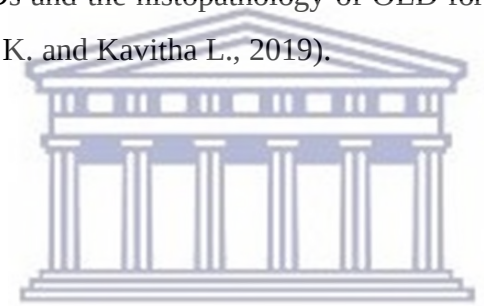
Moderate to severe dysplastic oral epithelium have a double risk of transforming into carcinoma compared to those that show mild dysplasia and hyperplasia. There is no clear knowledge on how many OSSC lesions develop from precursor lesions and how many develop from normal mucosa. Studies have shown that between 16-62% of oral cancers develop from a non-cancerous lesion, whereas a survey in India showed that 80% of cancers were preceded by pre-cancerous conditions and lesions (Feller and Lemmer, 2012).

The high-risk malignant transformation of dysplastic lesions can be associated with the type of pre malignant lesion, the surface texture/appearance and number of lesions, presence of certain local and systemic risk factors and anatomic location of lesion. The ventro lateral surface of the tongue, the floor of the mouth and soft palate have been identified as high-risk anatomic locations (Chandran R., 2012). Large lesions have been identified as having a higher risk of transformation compared to smaller ones. A study done by Holmstrup *et al*, also agrees with this. In their study they established that the only factor that showed statistical correlation with malignant transformation was the size of the lesion. (Jaber M.A. *et al.,* 2003). Lesions larger than 200mm^2 are more likely to progress compared to those less than this (Jaber M.A. *et al.,* 2003).

A dysplastic lesion study done of 630 patients in the UK found that over 95% of the lesions were Leukoplakias. Of those lesions, 42% of these lesions present on the floor of the mouth and on the ventral and lateral surfaces of the tongue. The above-mentioned lesions presented with severe dysplasia. Approximately 21% of the lesions were found on the buccal mucosa and these mostly presented with mild dysplasia (Speight P. M. *et al.,* 2018). They also explained that the site of the lesion was insignificant as a prognostic factor. The size and type of the lesion have a high prognostic significance (Speight P. M. *et al.,* 2018).

OED lesions with a proliferative verrucous appearance that appear in several sites in the oral cavity have higher risk of malignant transformation. Approximately 50% of erythroplakic lesions will have progressed to Oral Squamous Cell Carcinoma at diagnosis (Feller and Lemmer, 2012).

The progression of Oral epithelial dysplasia to carcinoma is highly unpredictably and this may take months to years. The malignancy potential of these lesions cannot be identified clinically. Studies have shown a higher risk in the development of second epithelial dysplastic lesions in non-smokers as compared to smokers, however there has been no link in the development of first lesions (Feller and Lemmer, 2012). It is important to understand the relation between the clinical diagnosis of OPMDs and the histopathology of OED for early diagnosis and clinical management (Ranganathan K. and Kavitha L., 2019).



2.7 Molecular markers

There are specific molecular markers that are indicative of the disease progression risk and these need to be identified. Genetic content alterations and mutations of the oral epithelium are a fundamental part of premalignancy (Ranganathan K. and Kavitha L., 2019). It has been shown that during malignant development, there is an accumulation of genetic and epigenetic alterations (Warnakulasuriya S. *et al*., 2008). Genetic change is a complex process, there is an interaction of the host's genetic factors and the environment carcinogens which leads to the activation of proto-oncogenes and the inactivation of genomic stability and tumour suppressor genes (Rastogi V. *et al*., 2013). Epigenetic changes on the other hand refer to heritable gene expression changes that do not alter the DNA sequence (Rastogi V. *et al*., 2013). Various signalling pathways and genes are involved in the development of OSCC. Molecular markers that correlate OED with malignant transformation include loss of heretozygosity, DNA aneuploidy of specific chromosomal loci, amplification of Cyclin D1, Cytokeratins CK1,8,18, high – risk Human Papillomavirus p16 (Feller L. and Lemmer J., 2011, Ranganathan K. and Kavitha L., 2019).

2.8 DNA aneuploidy

Aneuploidy can be defined as an imbalance in the segregation of chromosomes leading to the lack of uniformity in daughter cell distribution and detachment of parts of the chromosomes (Scully C. *et al*., 2003). Aneuploidy is the most predictable indicator of malignant transformation compared to the presence of dysplasia and staging (Chandran R., 2012). Using high resolution flow cytometric analysis, it was found that approximately 80% OSCC are aneuploid and 20% diploid (Jaber M.A., 2010). A lesion with mild dysplasia with aneuploidy has a high chance of transformation (Van Zyl *et al.*, 2012).

2.9 Field of precancerisation

The discovery of the field of precancerisation and developments on this phenomenon brought an understanding to genetic multistep carcinogenesis (Sathiasekar A. *et al.*, 2017).

Field of precancerisation can be defined as an "area of clinically normal looking epithelium, that appears normal or shows signs of dysplasia when assessed microscopically but in which keratinocytes have undergone cytogenetic alterations" (Chandran R., 2012, Sathiasekar A. *et al*., 2017, Foo J. *et al.*, 2014).

It has been found that clinically appearing mucosa can harbour genetic mutations from a field near the precancerous lesion, malignant transformation can occur from adjacent normal appearing mucosa. These fields extend from 4mm to 7cm (Sathiasekar A. *et al*., 2017).

The entire oral cavity can harbour dysplastic mucosa by the process of field cancerisation. The field of cancerisation effect therefore poses a risk of cancer recurrence and progression even after tumour excision (Foo J. *et al*., 2014). OPMDs may be a clinical manifestation of precancerised epithelial fields (Chandran R., 2012).

This understanding of the development will improve management strategies. The conventional method of surgical excision of lesions should be reconsidered. A few options have been suggested to better manage OPMDs and lesions with OED. These include the importance of thorough examination of the entire oral cavity instead of only the lesional area. Moreover, emphasis has been placed on the long term follow up and monitoring of the patients as it takes approximately 67 – 96 months for the malignant transformation to occur (Mohan and

Jagannathan, 2014, Sathiasekar A. *et al*., 2017). Early detection of malignant changes improves the prognosis. In these follow up visits counselling and reinforcement of habit cessation should be done. Continuous exposure to carcinogens (alcohol, tobacco, HPV to name a few) induces more mutations to the existing precancer field (Sathiasekar A. *et al.*, 2017).

2.10 Epidemiology

The global OED prevalence ranges between 0.5% - 3.46% (Feller and Lemmer, 2012). 10% of OED lesions are idiopathic whilst the other 90% are associated with tobacco and/or areca nut use (Feller and Lemmer, 2012). OED has a more male predilection in comparison to females and there is an increase of prevalence with age but remains uncommon before middle age (Neville B, *et al.*). OED lesions tend to affect individuals above the age of 30 years. (Chandran R., 2012).

The available literature on the relationship between race and OED lesions is sparse and very few studies report the incidence of OED. The reported world prevalence of OED varies from 1 – 5. However, this varies in the different countries with India having a higher prevalence than Western countries. In USA, the OED prevalence in white males is 2.9%. In rural India the OED incident rate is 240 per 100 000 people/year in males and 3 per 100 00 people/year in females. Japan on the other hand has a rate of 409 per 100 000 people/year in males and 70 per 100 000 people/year for females (Feller and Lemmer, 2012).

In South Africa, the clinicopathological features of OED is well categorized for the white population more than the black population. There is also little knowledge about the epidemiology and the demographics concerning OED. In OED prevalence, 86% cases were found in white people, 9% black and 5% Asian, although the population constitutes of mainly black people. This has been attributed to the political history of South Africa that put restrictions of Health care access (Feller and Lemmer, 2012).

In South Africa, black people are diagnosed with OED at a younger age compared to other races, with the idiopathic OEDs having a greater proportion and mostly presenting with non-

homogenous lesions. However, more white people present with dysplastic lesions compared to all the other races, the floor of the mouth and buccal mucosa being the most affected sites (Feller and Lemmer, 2012).

2.11 Management

OED cases can be managed using different approaches after assessing each case and individual thoroughly. A conservative approach can be used or active treatment modalities can be employed. A conservative approach involves close monitoring/surveillance whilst reducing and eliminating risk factors. A serial of biopsies are also taken. This conservative approach is mostly taken in diffuse lesions exhibiting mild dysplasia and in immunocompromised patients. Whereas surgery is the preferred treatment option for smaller lesions presenting with moderate to severe dysplasia and for patients fit for surgery (Nankivell and Mehanna, 2011).

However, in most cases it is not easy to make a definitive/ clear decision. Most patients present late and the treatment approach is further complicated by comorbidities that exist or may arise due associated smoking. poor diet and alcohol habits (Nankivell and Mehanna, 2011).

A recent meta – analysis demonstrates that the yearly malignant transformation rate of OED lesions was 12% (Mehanna H. M. *et al*., 2009). Although long term follow up studies have shown that dysplastic lesions may either remain, regress of transform into malignancy; a study by Pindborg *et al* found out that carcinoma can develop from non - dysplastic lesions suggesting that the aggressive approach used in the treatment of dysplastic lesions may not be necessitated (Gupta P. C. *et al*., 1980, et al., Mehta F. S., 1980).

Other studies have shown that surgical treatment has no advantage over conservative treatment (Arduino P.G. *et al*., 2009). A study done by Holmstrup et al reported slightly higher rate of malignant transformation in oral leukoplakia treated by surgery in relation to those lesions that were left untreated (Holmstrup P. *et al.*, 2006). In light of the above, it may be therefore justified to adopt a conservative observing policy (Tilakaratne W.M. *et al.*, 2019).

2.12 Treatment

There are various treatment options for Oral Epithelial Dysplastic lesions, these include monitoring and observation, surgical excision, cyrotherapy and laser therapy. Any known risk factors should be avoided and ceased, these include smoking, alcohol and HPV associated high risk sexual activities. The risk of OED reduced following smoking cessation (Jaber M.A., 2010).

High risk lesions require intervention whilst low risk lesions do not. Dysplastic lesions that present on the ventral and lateral surfaces of the tongue, the oropharynx, floor of the mouth and soft palate should be ideally treated by complete excision. However, the risk of malignant transformation is not completely eliminated by employing any of the existing treatment modalities (Chandran R., 2012). Moreover, treatment does not eliminate the risk of reoccurrence of the dysplastic lesions (Feller and Lemmer, 2012).

In a Cochrane review in 11 trials, non - surgical regiments for leukoplakia were looked at. The non – surgical modalities were found to be ineffective in preventing the malignant transformation of OPMDs (Lodi G. *et al*., 2006). Although medical treatment may cause resolution in some lesions, relapse and adverse effects were common. The choice of treatment and lack of therefore remains at the clinician's discretion. All risk factors associated which each case should be taken into account (Lodi G. *et al*., 2006).

a. Surgical treatment

Although there is controversy in literature concerning which treatment modality to follow, surgical treatment remains the most popular treatment method for moderate to severe dysplastic lesions. Excision of OPMDs can be done in different ways, these include laser cryosurgery, cold steel and laser.

Cold scapel is the traditional method. It is a cheap method, readily available, and it obtain good margins and depths for histopathology, it however may not be an appealing choice for large diffuse lesions (Chen H. M. *et al*, 2015).

Laser is becoming more common due to the superior wound healing it offers. Furthermore, it produces less dysfunction and achieve great haemostasis. Carbon dioxide laser is most commonly used type, especially for excision. This together with evaporation is more appropriate for larger lesions (Chen H. M. *et al*, 2015).

Cyrosurgery is widely used in the management OPMD's and has been found to play an even more important role in the management of Leukoplakia. A study by Chen showed regression of lesions after multiple cryotherapy sessions (Chen H. M. *et al*, 2015).

b. Non-surgical treatment

Topical and systemic chemo-preventative agents exist and these are aimed and used in the prevention of progression and malignant transformation. These agents/modalities include bleomycin, ketorolac, acitretin, lycopene, Vitamin A, alpha-tocophenol a beta carotene, 13-cis-retinoic acid, mixed tea, and attenuated live viruses. These agents may be used however they have not been established as effective in malignant transformation, more studies have to be done.

OPMDs with dysplasia

Regardless of chemoprevention and/or surgical treatment recurrences occur. It is difficult and almost impossible to predict the outcome of OED. All patients presenting with OPMDs should therefore follow rigorous follow ups. Close appointments should be decided by the presence of risk factors. Moreover, the period and frequency of surveillance may be decided by the degree of dysplasia, method of treatment (and non-treatment thereof) and the evaluation of possible risk for malignant transformation.

A significant amount of scientific literature has accumulated on OED relating to aspects of its diagnosis and management. However, the evidence base is weak because of the significant variability of published research. Poorly described study methods, variability in different OED grading systems. Inter and intra examiner variability causing issues of reliability. Inadequate sample size, inconsistent durations of follow up are the methodological issues contributing to

the failure to provide dependable information. Randomized clinical trials on the malignant transformation potential of OED and its outcomes are limited.

2.13 OSCC

Oral cancers cause more deaths that all orofacial disorders and diseases combined (Abram M.H. *et al*., 2012). OSCC has a wide range age and sex coverage, with the peak incidence in the 6th and 7th decades (Abram M.H. *et al.,* 2012). It however has a more male occurrence and this may be due to their higher indulgence in risk factors (Abram M.H. *et al*., 2012). In studies on younger patients (less than 45 years), a female predilection has been found Abram (M.H. *et al*., 2012). In the literature, there is has been debates of the disease process in younger patients compared to older patients. They have compared the causal factors and disease course in younger and older patients to establish if OSCC in younger patients is a distinct entity on its own, no consensus had been reached (Abram M.H., 2013).

The age and gender OSCC distribution patterns in South Africa correlate with global trends; OSCC mostly affects the elderly and affects more males (Speight P.M, *et al*. 2018). In the world statistics, cancers of the oropharynx and oral cavity account for approximately 220 000 new cases in men and 90 000 in women (Speight P.M, *et al*. 2018).

2.14 OSCC in a South African context

Head and neck tumours are amongst the most aggressive tumours and OSCC represents the vast majority (Dost F. *et al.,* 2014). Of all oral cancers, OSCC accounts for 90% of them, oral cancer is the term therefore used in reference to OSCCs**.** OSCC is the most common cancer in the world (Ranganathan K. and Kavitha L., 2019). It is mostly common in developing countries. OSCC has been identified as the 6th leading cause in cancer deaths (Liu W. *et al.*, 2011). This is attributed to the high tendency metastases due to the close proximity of local and regional local lymph nodes and the uninhibited infiltration (Dost F. *et al.,* 2014). Consequently, the survival rates over a 5-year period are reportedly as low as 9% for some parts of the oral cavity (Dost F. *et al*., 2014).

OSCC is an important disease burden in South Africa. Cancers of the mouth form 1.04% of all cancers in the country as reported by the National Cancer Registry of South Africa in 2011 (Khamissa *et al*., 2014).

The knowledge on the epidemiological, histopathological and clinical features of OSCC of the various ethnic groups in South Africa is limited and the data skew (Khamissa *et al*, 2014). According to Ayo-Yusuf et al, South African women, especially Indians have a high OSCC incidence and this is linked to betel nut chewing; this is similar to a smaller black subgroup that uses oral snuff (Mohangi T., 2016).

Sub-Saharan Africa is experiencing a rapid increase in the burden of cancer related mortality and morbidity (Faggons P. J. *et al*., 2015). In South Africa OSCC is the 5th most common cancer in males and the 10th most common in females. The rural populations have a lower OSCC incidence that the urban counterparts (Botha C. E. *et al*., 2018)

There also has been an increase incidence of OSCC in younger patients. The mortality rate associated with OSCC has remained mostly unchanged for decades, with an average 5-year survival rate of 50%. Early detection and monitoring of the patient is therefore essential to avoid the costly and invasive treatments.

Chapter 3: Methodology

3.1 Aim

The aim of this study is to describe cases of oral epithelial dysplasia diagnosed at the Tygerberg Oral Health Centre between 2012 and 2019 and to determine the demographics, clinical picture of these cases.

3.2 Objectives

- To determine the number of diagnosed cases of oral epithelial dysplasia over the 7-year period.
- To describe the demographics of patients diagnosed with oral epithelial dysplasia.
- To assess the degree of dysplasia in relation to the location of the biopsied lesion.
- To assess the degree of dysplasia in relation to contributing risk factors such as reported smoking habit and alcohol consumption.

3.3 Hypothesis

Oral epithelial dysplasia has no predilection in terms of intra-oral location and is not directly associated with smoking.

3.4 Study design

This is a cross sectional retrospective records-based descriptive analytical review.

3.5 Sampling/data collection

Medical records from Tygerberg Oral Health Centre and NHLS were reviewed for diagnoses of OED. All diagnosed cases of OED were identified from NHLS for sampling and choosing in accordance with the inclusion and exclusion criteria. Thereafter, the patient's files were

retrieved. The individual medical records and follow up data were assessed. The following information was drawn out for further analysis and assessment:

- The diagnostic pathology results and grading
- Existence of any predisposing factors and patient habits
- Anatomic sites of OED

3.6 Inclusion and exclusion criteria

- Any patients with incomplete data will be excluded.
- Any patient that has been previously diagnosed with head and neck cancer will also be excluded.

3.7 Data extraction

1. Patient demographics
    - Sex
    - Age
2. Habits
    - Smoking habits
    - Drinking habits
3. Clinical information
    - Date of diagnosis
    - Location of lesion
    - Diagnosis
    - Pathological description and grade

3.8 Analysis

Summary statistics was performed using frequencies and percentages. Associations between the variables was performed using a suitable test of correlation. All tests will be deemed statistically significant at $p < 0.05$. All statistical tests were conducted using StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC.

3.9 Ethical approval

Approval to conduct this study was obtained from the Biomedical Research Ethics Committee of the University of the Western Cape. Patient anonymity was assured as each patient record was given a code. The original list of patient records was kept in a password protected computer, accessible only to the researcher. Permission to access records was also sought from the Dean of the Faculty of Dentistry.

Chapter 4: Results

4.1 Frequency

Overall, 99 cases of OED were diagnosed in the 2012 to 2019 period. However, 29 cases were excluded due to incomplete data. Only 70.7% of the overall cases was therefore reported on. As shown in table 2 and graph 1, in the 2012 to 2019 period, 70 cases were diagnosed with OED. The lowest number of cases were diagnosed in 2012 and 2019 and the highest number in 2018. Majority of the OED cases were diagnosed between year 2015 and 2018.

Table 2: Diagnosed cases per year

DIAGNOSED YEAR	FREQUENCY	PERCENTAGE
2012	5	7.14
2013	7	10
2014	6	8.57
2015	13	18.57
2016	10	14.29
2017	9	12.86
2018	15	21.43
2019	5	7.14
Total	**70**	**100**

As seen in the graph below, there is a gradual increase in the number of cases from 2012 till 2015, where there is a sudden decrease. 2019 exhibits the highest decrease in the diagnosed OED cases.

Figure 12: OED diagnosis per year

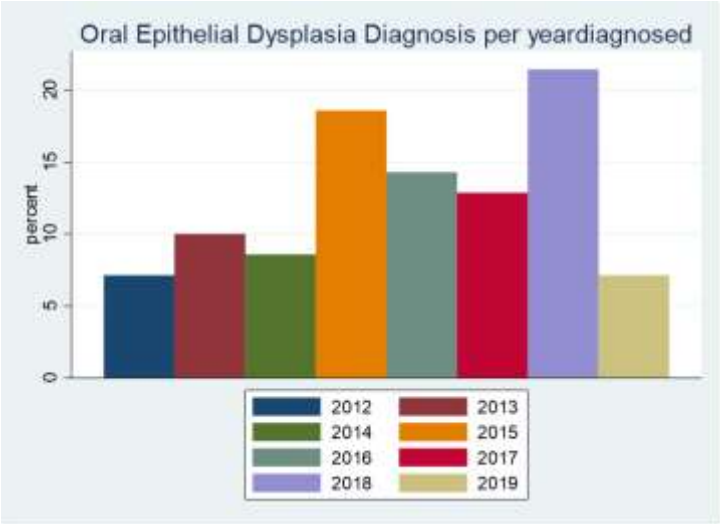
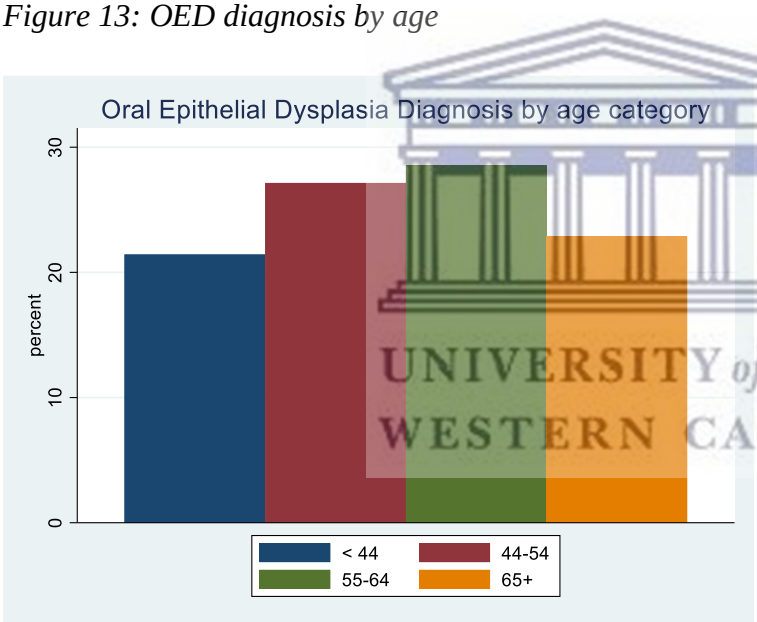


Figure 13: OED diagnosis by age



The number of diagnosed cases increase gradually with the increase in age group category, it however decreases in the 65+ age group. Most OED cases fell in the 55 – 64 categories, whilst the <44 age category had the least number of cases.

Key
Frequency Row percentage Column percentage

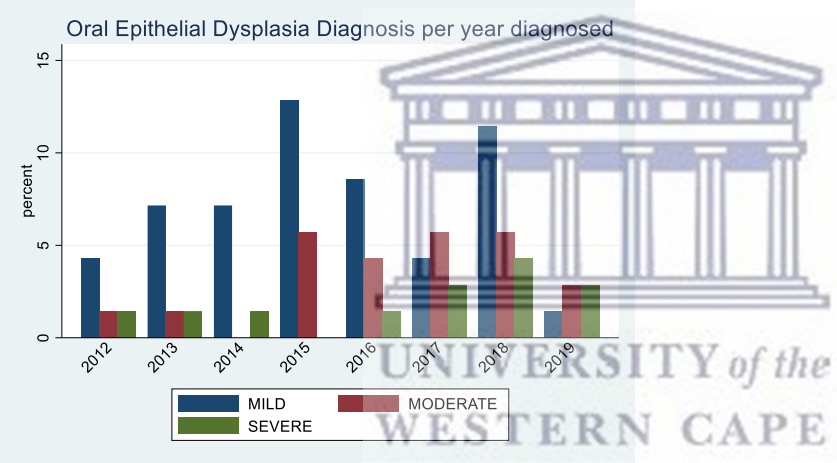
Table 3: Degree of OED per diagnosed year

	DEGREE			
Diagnosed year	**MILD**	**MODERATE**	**SEVERE**	**Total**
2012	3 60 7.5	1 20 5.26	1 20 9.09	5 100 7.14
2013	5 71.43 12.50	1 14.29 5.26	1 14.29 9.09	7 100 10
2014	5 83.33 12.50	0 0 0	1 16.67 9.09	6 100 8.57
2015	9 69.23 22.50	4 30.77 21.05	0 0 0	13 100 18.57
2016	6 60 15	3 30 15.79	1 10 9.09	10 100 14.29
2017	3 33.33 7.5	4 44.44 21.05	2 22.22 18.18	9 100 12.86
2018	8 33.33 7.50	4 44.44 21.05	3 20 27.27	15 100 21.43
2019	1 20 2.50	2 40 10.53	2 40 18.18	5 100 7.14
Total	40 57.14 100	19 27.14 100	11 15.71 100	70 100 100

Pearson chi2(14) = 11.6186 Pr = 0.637
Fisher's exact = 0.530

The OED cases were further split by degree of severity and year diagnosed. Of the 5 cases diagnosed in 2012, 3 of them were mild, 1 moderate and 1 severe (60% mild, 20% moderate and the other 20% severe). Of all the mild cases 7,5% of the cases were diagnosed in 2012. The majority of mild cases (at 22.5%) were diagnosed in 2015 and the minority of mild cases (at 2.5%) were diagnosed in 2019. Of all the moderate cases, the majority of the cases (at 21.05%) were diagnosed in 2014, 2017 and 2018. There were no moderate cases diagnosed in 2014. Of all severe cases, the majority were diagnosed in 2018 (at 27.27%), there were no severe cases diagnosed in 2015.

Figure 14: Oral Epithelial dysplasia diagnosis per year diagnosed



Overall, the highest amount of cases were mild cases diagnosed in 2015, followed by the mild cases diagnosed in 2019. When the cases are separated by year, in 2014 more that 80% of the cases were mild, no moderate cases and just under 20% of the cases were severe.
A Chi square and fisher tests were done and there was statistical significance. In 2019 there were high moderate and severe cases compared to mild, in comparison to all the other years (with 2017 being an exception) that had majority mild cases.

4.2 Demographics

4.2.1 Age

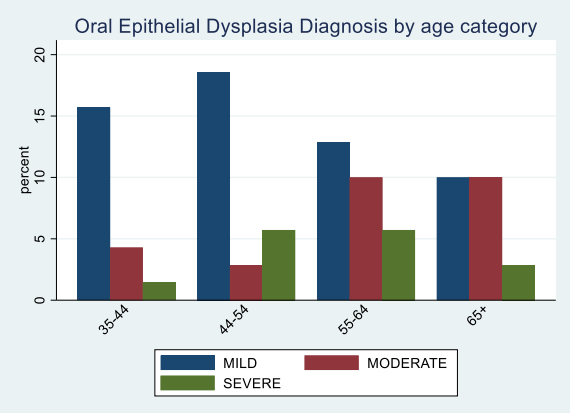
Table 4: OED cases and age

AGE	FREQUENCY	PERCENTAGE	CUMMULATIVE
< 44	15	21.43	21.43
45 - 54	19	27.14	48.57
55 - 64	20	28.57	77.14
>=65	16	22.86	100
TOTAL	**70**	**100**	

The median age of the participants was 58 with an inter-quartile range from 48 to 62. The youngest participant was 11 and the eldest was 82. The age is not evenly distributed. It is therefore being distributed as a median and inter - quartile range.

Percentiles		Smallest		
1%	11	11		
5%	17	13		
10%	32.5	16	Obs	70
25%	48	17	Sum of Wgt.	70
50%	55		Mean	53.07143
		Largest	Std. Dev.	15.2328
75%	62	76		
90%	68.5	78	Variance	232.0383
95%	76	81	Skewness	-.8497275
99%	82	82	Kurtosis	3.730972

Figure 15: Oral Epithelial Dysplasia Diagnosis by age category



In the moderate cases, there is an increase in the 55 – 64 age group and it more or less the same in the 65+ category. For mild and severe cases there seems to be a decrease in the cases after the 44 – 54 age categories. There is no statistically significant difference between age and degree of severity.

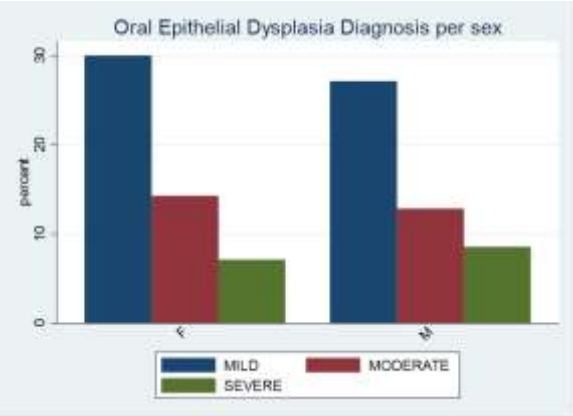
4.2.2 Sex

Table 5: Diagnosed Oral Epithelial Dysplasia cases per sex

GENDER	FREQUENCY	PERCENTAGE	CUMMULATIVE
FEMALE	36	51.43	51.43
MALE	34	48.57	100
TOTAL	**70**	**100**	

There were 36 females diagnosed with OED and 34 males, there is therefore no big difference in the sex cases.

Figure 16: Oral Epithelial Dysplasia diagnosis per sex



As shown in the graph above both males and females had the highest number of mild cases followed by moderate cases and the severe cases were the lowest in each sex group. Females in comparison to males, have more mild and moderate OED cases and less severe cases.

Key
frequency row percentage column percentage

Table 6: Degree of Oral Epithelial Dysplasia and sex

	DEGREE			
SEX	**MILD**	**MODERATE**	**SEVERE**	**Total**
F	21 58.33 52.50	10 27.78 52.63	5 13.89 45.45	36 100 51.43
M	19 55.88 47.50	9 26.47 47.37	6 17.65 54.55	34 100 48.57
Total	40 57.14 100	19 27.14 100	11 15.71 100	70 100 100

Pearson chi2(2) = 0.1866 Pr = 0.911
Fisher's exact = 0.946

In females, 58.33% of the cases were mild, 27.78% moderate and 13.89% severe cases. In males on the other hand, 55.88% of the cases were mild, 2647% cases moderate and 17.65% severe. The males had 54.55% severe OED cases compared to the 45.45% of females. The Pearson chi test was done and there was no statistically significant difference between sex and the degree of severity.

4.3 Location

In categorizing the biopsied areas, all tongue lesions were combined. Certain pathological reports did not specify sites, these sites were listed as oral mucosa.

Figure 17: Location of diagnosed lesion

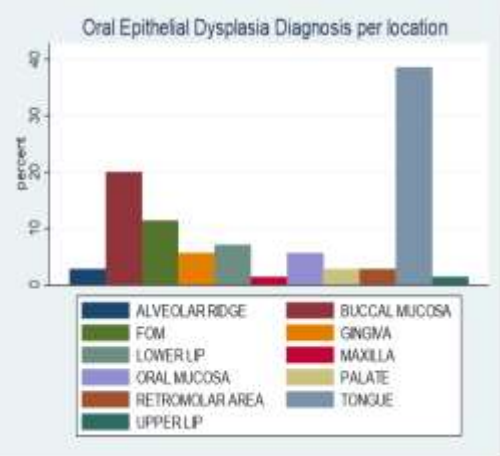


Table 7: Location of diagnosed lesions

LOCATION	FREQUENCY	PERCENTAGE
Tongue	27	38.57
Buccal mucosa	14	20
FOM	8	11.43
Lower lip	5	7.14
Gingiva	4	5.71
Oral mucosa	4	5.71
Palate	2	2.86
Retromolar area	2	2.86
Alveolar ridge	2	2.86
Upper lip	1	1.43
Maxilla	1	1.43
TOTAL	**70**	**100**

The tongue has the highest prevalence at 38.57% followed by the buccal mucosa at 20% then the FOM at 11.43%, the upper lip and maxilla had the least number of OED cases at 1.43% respectively.

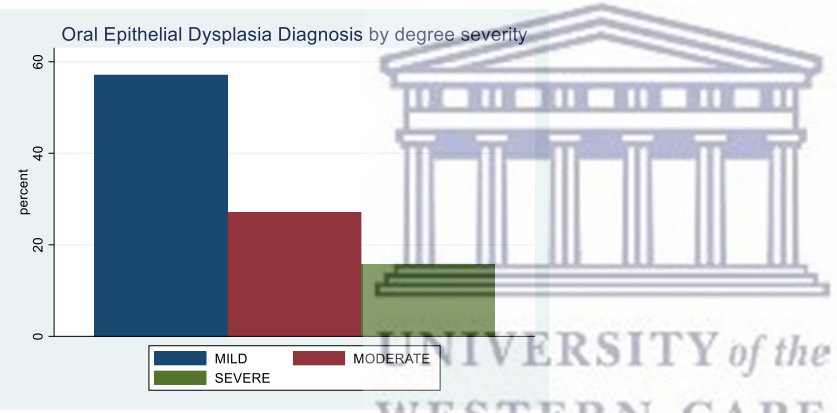
4.4 Degree of dysplasia

Table 8: OED diagnosis by degree severity

DEGREE	FREQUENCY	PERCENTAGE	CUMMULATIVE
Mild	40	57.14	57.14
Moderate	19	27.14	84.29
Severe	11	15.71	100
Total	**70**	**100**	

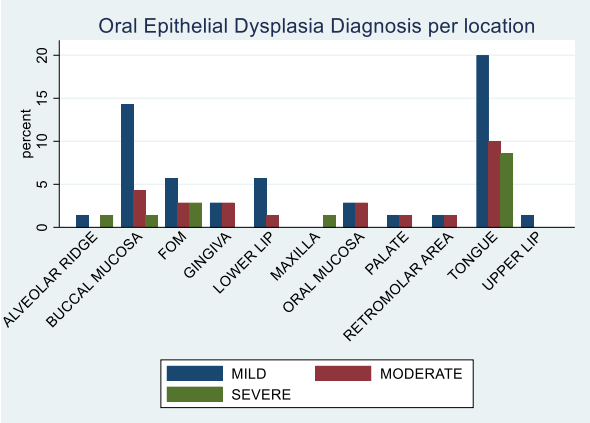
As shown in Table 6 and graph 6, majority of the cases were mild at 57.14%, followed by moderate at 27.14% and then severe comprising of only 15.71% of the cases.

Figure 18: OED diagnosis by degree severity



4.4.1 Degree of Dysplasia and location of biopsied area

Figure 19: OED diagnosis and severity per location



The tongue presented with the highest number of cases. Moreover, it also has the highest number of cases in each severity category. The upper lip only had mild cases. No severe

cases were found on the gingiva, lower lip, oral mucosa, palate, upper lip and retromolar pad. The alveolar ridge, maxilla and upper lip had no moderate cases. The maxilla had only severe cases.

Table 9: OED severity diagnosis per location

	DEGREE			
LOCATION	**MILD**	**MODERATE**	**SEVERE**	**TOTAL**
Alveolar ridge	1 50 2.50	0 0 0	1 50 9.09	2 100 2.86
Buccal mucosa	10 71.43 25	3 21.43 15.79	1 7.14 9.09	14 100 20
FOM	4 50 10	2 25 10.53	2 25 18.18	8 100 11.43
Gingiva	2 50 5	2 50 10.53	0 0 0	4 100 5.71
Lower lip	4 80 10	1 20 5.26	0 0 0	5 100 7.14
Maxilla	0 0 0	0 0 0	1 100 9.09	1 100 1.43
Oral mucosa	2 50 5	2 50 10.53	0 0 0	4 100 5.71
Palate	1 50 2.50	1 50 5.26	0 0 0	2 100 2.86
Retromolar area	1 50 2.50	1 50 5.26	0 0 0	2 100 2.86
Tongue	14 51.85 35	7 25.93 36.84	6 22.22 54.55	27 100 38.57
Upper lip	1 100 2.5	0 0 0	0 0 0	1 100 1.43
Total	40 57.14 100	19 27.14 100	11 15.71 100	70 100 100

Pearson chi2 (20) = 16.5315 Pr = 0.683 Fisher's exact = 0.838

Key
Frequency Row percentage Column percentage

Out of all the lesions on the tongue, 51, 85% are mild, 25,93% are moderate and the remainder 22.22% are severe. 35% of all mild cases were found on the tongue, whilst 25% of all cases were found on the buccal mucosa. Of all lesions found on the buccal mucosa, 71.43% are mild, 21.43% were moderate and 7.14% mild. Of all lesions found on the FOM, 50% are mild, 25% were moderate and 25% mild. An Exacts test was done and there is no statistically significant difference between degree of dysplasia and location of lesion.)

4.5 Risk factors

4.5.1 Alcohol

As shown in Table 8 and Graph 8, 45 of the patients did not consume alcohol whilst 25 of the patients consumed alcohol. Therefore, there were more non-alcohol consumers diagnosed with OED than alcohol consumers.

Table 10: OED diagnosis and alcohol usage

ALCOHOL USER	FREQUENCY	PERCENTILE	CUMMULATIVE
N	45	64.29	64.29
Y	25	35.71	100
Total	**70**	**100**	

Figure 20: OED diagnosis per alcohol usage

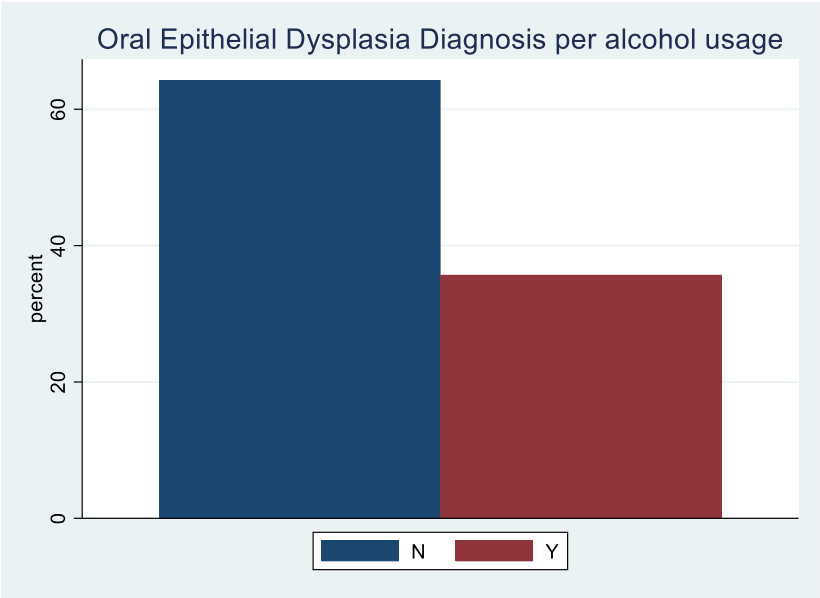
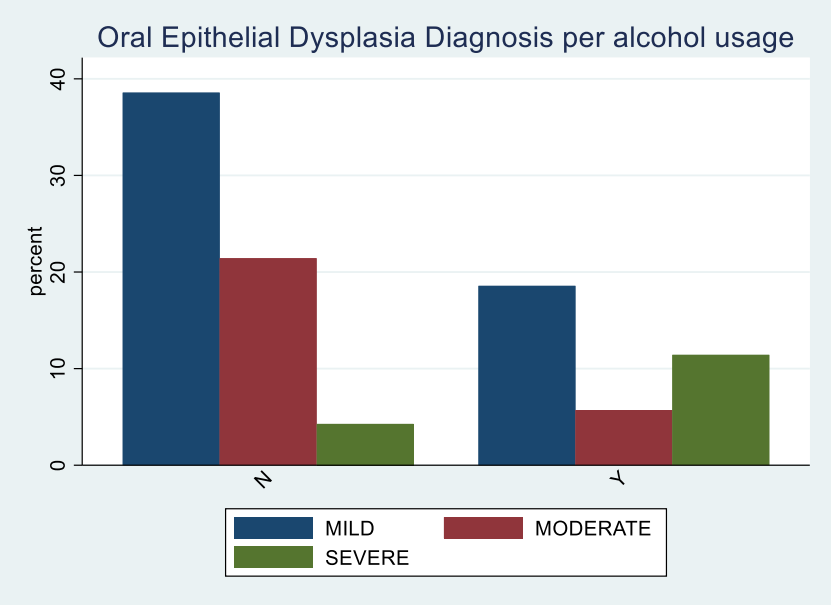


Figure 21: OED diagnosis severity per alcohol usage



Non-alcohol consumers had majority mild dysplasia cases, followed by moderate cases. In alcohol consumers on the other hand had more severe than moderate cases. Alcohol consumers had more cases of severe dysplasia.

Table 11: OED diagnosis severity per alcohol usage

Key
Frequency Row percentage Column percentage

Enumerating sample-space combinations:
stage 3: enumerations = 1
stage 2: enumerations = 8
stage 1: enumerations = 0

	DEGREE			
ALCOHOL USER	**MILD**	**MODERATE**	**SEVERE**	**Total**
N	27 60 67.50	15 33.33 78.95	3 6.67 27.27	45 100 64.29
Y	13 52 32.50	4 16 21.05	8 32 72.73	25 100 35.71
Total	40 57.14 100	19 27.14 100	11 15.71 100	70 100 100

In non-alcohol users, 60% of the cases were mild, 33.33% moderate and 6.67% severe. Of all mild cases, 67.50% were found in non-alcohol users, whilst 78.95% of all moderate cases were found in non-alcohol users. 27.27% of all severe cases were found in non-alcohol users. In alcohol users on the other hand, 52% of the cases were mild, 16% moderate and 32% severe. Of all mild cases, 32.50% were found in alcohol users, whilst 21.05% of all moderate cases were found in alcohol users, 72.73% of all severe cases were found alcohol users.

4.5.2 Smoking

As seen in table 10 and graph 10, 54.29% of cases were found in non-smokers whilst smokers had 45.71% of the cases.

Table 12: OED diagnosis per smoking status

SMOKER	FREQUENCY	PERCENTAGE	CUMMULATIVE
Non – smoker	38	54.29	54.29
Smoker	32	45.71	100
Total	**70**	**100**	

Figure 22: OED diagnosis per smoking status

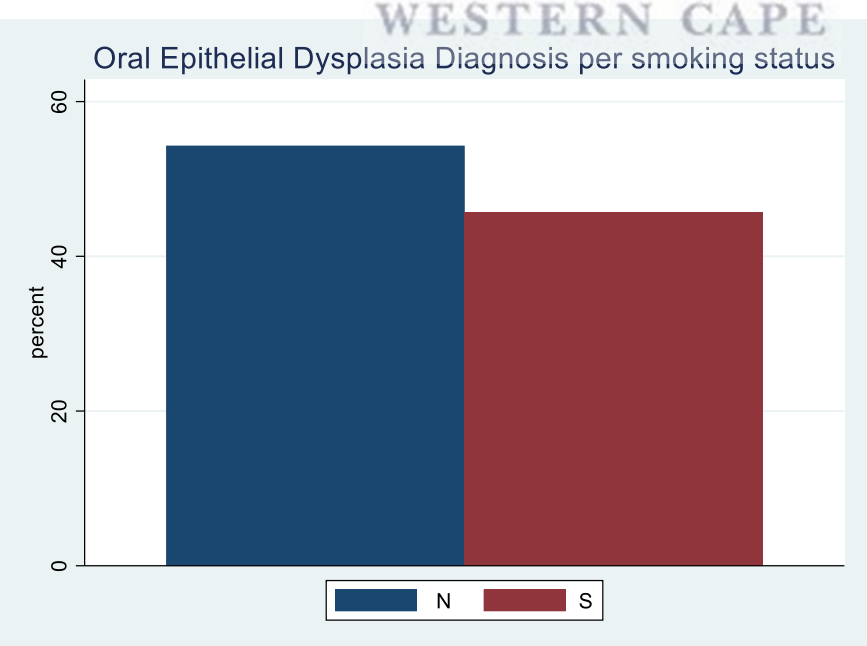
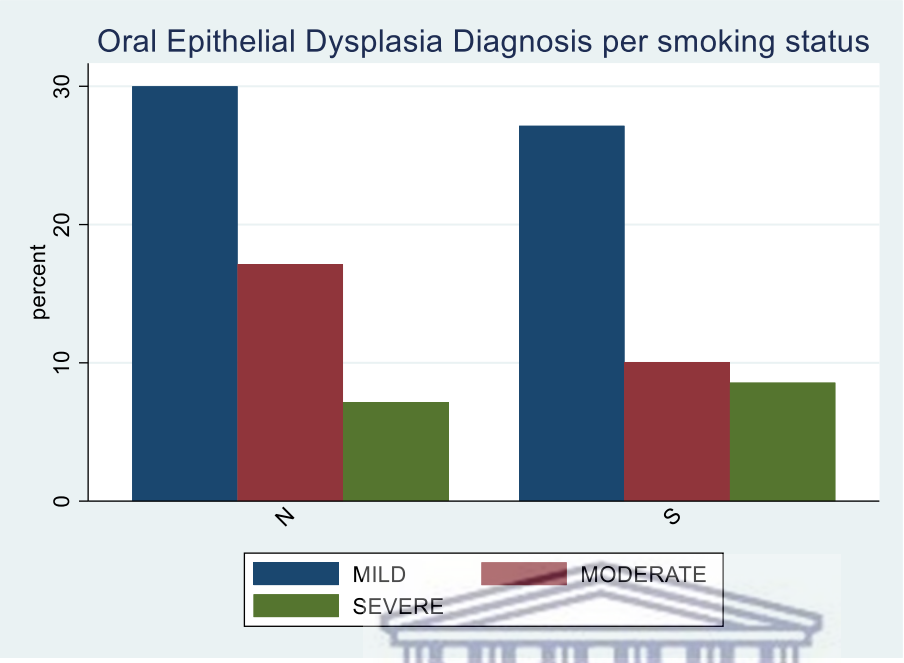


Figure 23: OED diagnosis severity per smoking status



In the non-smokers, majority of the cases were mild, followed moderate then severe. This pattern is the same for the smokers as well. The smokers had fewer moderate cases compared to non-smokers but they have more severe cases compared to the non-smokers.

Key
frequency row percentage column percentage

Enumerating sample-space combinations:
stage 3: enumerations = 1
stage 2: enumerations = 3
stage 1: enumerations = 0

Table 13: OED diagnosis severity per smoking status

	DEGREE			
SMOKER	**MILD**	**MODERATE**	**SEVERE**	**Total**
N	21	12	5	38
	55.26	21.58	13.16	100
	52.50	63.16	45.45	54.29
S	19	7	6	32
	59.38	21.88	18.75	100
	47.50	36.84	54.55	45.71
Total	40	19	11	70
	57.14	27.14	15.71	100
	100	100	100	100

Pearson chi2(2) = 0.9998 Pr = 0.607

Fisher's exact = 0.597

Non-smokers had higher collective percentages of mild and moderate cases compared to smokers, whilst smokers had higher percentage of severe cases.

4.5.3 Alcohol vs smoking

Table 14: OED diagnosis severity per smoking status and alcohol use

	ALCOHOL USER		
SMOKER	**N**	**Y**	**Total**
N	29	9	38
	76.32	23.68	100
	64.44	36	54.29
S	16	16	32
	50	50	100
	35.56	64	45.71
Total	45	25	70
	64.29	35.71	100
	100	100	100

Key
frequency row percentage column percentage

Pearson chi2(1) = 5.2398 Pr = 0.022 9

76.32% of all cases were found in non-smokers who also did not consume alcohol. 64.44% of all cases were found in non-alcohol consumers. 50% of all cases were found in non-smokers who also did not consume alcohol. 50% of all cases were found in smokers who also consumed alcohol. 64% of those smokers, also consumed alcohol. There is a statistically significant difference between smokers and alcohol consumers.

4.5.3 Age as a risk factor

Figure 24: Oral epithelial dysplasia diagnosis by age category

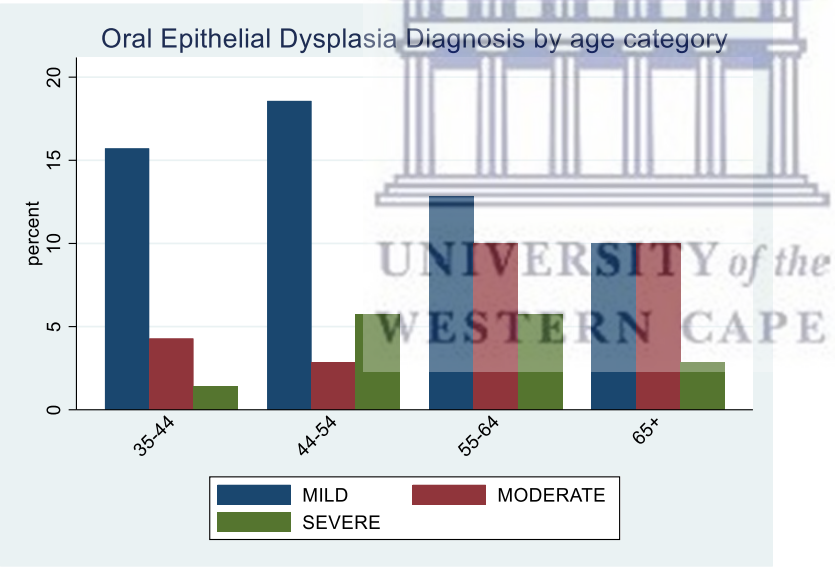


Table 15: Oral epithelial dysplasia diagnosis by age category

Key
frequency row percentage column percentage

Enumerating sample-space combinations:
stage 4: enumerations = 1
stage 3: enumerations = 44
stage 2: enumerations = 767

stage 1: enumerations = 0

	DEGREE			
Age	**MILD**	**MODERATE**	**SEVERE**	**Total**
< 44	11 73.33 27.50	3 20 15.79	1 6.67 9.09	15 100 21.43
44 - 54	13 68.42 32.50	2 10.53 10.53	4 21.05 36.36	19 100 27.14
55 - 64	9 45 22.50	7 35 36.84	4 20 36.36	20 100 28.57
65+	7 43.75 17.50	7 43.75 36.84	2 12.50 18.18	16 100 22.86
Total	40 57.14 100	19 27.14 100	11 15.71 100	70 100 100

Ordered logistic regression — Number of obs = 70
LR chi2(6) = 5.61
Prob > chi2 = 0.4681
Log likelihood = -64.712516 — Pseudo R2 = 0.0416

Table 16: Oral epithelial dysplasia diagnosis by age risk factors and age categories

Degree	**Odds ratio**	**Std. err**	**Z**	**p> z**	**95% conf. interval**	
Smoker S	0.756	0.418	-0.506	0.613	0.255	2.236
Sex M	0.966	0.516	-0.064	0.949	0.339	2.751
Alcohol Y	2.026	1.112	1.287	0.198	0.691	5.940
Age 44-54	1.309	1.022	0.344	0.731	0.283	6.051
55-64	3.079	2.271	1.525	0.127	0.726	13.069
65+	2.366	1.825	1.116	0.264	0.522	10.729
/cut 1 /cut 2	1.001 2.478	0.617 0.680			-0.208 1.146	2.210 3.811

Note: Estimates are transformed only in the first equation.

For subjects who drink alcohol relative to subjects who do not drink alcohol, the (odds ratio) OR for moderate and severe dysplasia to mild dysplasia would be expected to increase by a factor of 2.026. This odds ratio can be as low as 0.691 and as high as 5.94, and included the null value of 1, p = 0.198, which implies that the OR is not statistically significant.

For subjects who are in the non-smoker group relative to subjects who are below 44, the OR for moderate and severe dysplasia to mild dysplasia would be expected to increase by a factor of 3.079.

For subjects who are in the 55 – 64-year age group relative to subjects who are below 44, the OR for moderate and severe dysplasia to mild dysplasia would be expected to increase by a factor of 3.079.

Females had a greater dysplasia compared to males of 0.04. Non-smokers had a greater increase in factor of 24.4%. Non-smokers had more severe cases of OED compared to mild and moderate cases. However, the results are not statistically significant.

Chapter 5: Discussion

In the literature the difficulty of accurately classifying and diagnosing OED cases has been highlighted, this becomes an inherent limitation in any OED study. The inter observer irregularities and variabilities have been largely attributed to this as the pathologist's assessment of the specimen which is largely based on their training and previous experiences (Jaber M., 2010). The validity and uniformity are therefore affected.

5.1 Frequency

In this study, we are describing the diagnosed cases of OED in the Tygerberg oral health centre in the 7-year span. A total of 70 cases of OED were diagnosed. There was a gradual increase in the number of cases diagnosed. Out of those 70 cases, 57.14% were mild, 27.14% were moderate and the remainder (15.71%) fell under the severe category. In the literature it has been reported that mild dysplasia is the more prevalent grade than severe and moderate dysplasia (Reibel J. *et al*., 2003, Dost F. *et al.* ,2014).

5.2 Age

The median age was 58. The age was not evenly distributed and the interquartile range was 48 -62. The oldest patient was 82 and the youngest was 11 years of age. The mean age has been reported to be between the age of 50 – 69. 23.43% of the patients fell in the < 44 age group, literature on the other hand, 5% of OED diagnoses has been made on patients below the age of 30 (Pereira J. S. *et al.*, 2011)

There is an increase in the number of OED cases with age increase. The 55 – 64 age group had the highest number of cases. In the literature two authors have reported that the peak incidence of all grades of OED is in the 3rd - 5th decade (Speight P.M, *et al.* 2018, Pereira J. S. *et al*., 2011). Mincer on the other hand reported a higher peak incidence of 6th – 7th decade (Mincer H.H. *et al.,* 1972)

There was also an increase in the number of severe cases with age, more severe cases were found in older patients. Subjects in the 55 – 64 age group in comparison to those below 44 had

an increased OR of 3.079 for developing dysplasia. Studies have shown age to be a risk factor, the older the patient, the higher risk of developing OED (Pereira J. S. *et al.*, 2011).

5.3 Sex

OPMDs are less common in females (Speight P.M, *et al*. 2018). There is almost an even distribution of dysplasia between the two sexess, with males diagnosed with 48.57% of cases and females 51.43% respectively. This female predominant contradict with what the literature reports as it has been reported that OED has a male predilection (Jaber M. A., 2010). Studies done by Pereira in the other hand reported a female predominance (Pereira J. S. *et al.*, 2011). Females have greater dysplasia compared to males by factor 0.04. Although OED has been mostly reported to have a male predilection, there has been a decrease in the male: female ratio (Jaber M. A., 2010).

The females had less severe (45.45%) cases compared to males (54.55%). A Pearson chi test was done ($p = 0.1866$) and there is no statistical significance between sex and the degree of severity. In literature, there is variation in the sexe distribution of OED. For example, in Western countries and Europe, OED had a female predilection whilst in India there is a more male predilection. This is attributed to the cultural and habits ((Ranganathan K. and Kavitha L., 2019).

5.4 Location

The tongue had the greatest number of cases followed the buccal mucosa and then the floor of the mouth. The maxilla and upper lip were the least affected. This pattern corresponds with a systematic review conducted by Ariyawardana and Warnakulasuriya which reported that on a global basis, the most common site overall was the buccal mucosa at 18.4% of lesions, followed by the tongue which accounted for 16.14% of cases.

The tongue and floor on the mouth combined also formed part of the common sites with 14.85% cases (Speight P.M, et al. 2018) However, they recognised that the data may camouflage certain geographic variations as they may apply to populations with the most common habits of tobacco and alcohol use (Speight P.M, et al. 2018). Another study identified the buccal mucosa as the site mostly affected by OPMDs, followed by the gingiva then the

tongue (Feller L. and Lemmer J., 2012). Previous studies have reported high rates of oral mucosal lesions at this site, which is likely to be associated with placement of local tobacco and betel products; however, this was not a predisposing factor for OED development (Dost F. *et al.* ,2014).

The tongue had majority of the severe cases, with 54.55% of all severe cases being diagnosed on the tongue. followed by the tongue then the FOM. Spreight and Pereira *et al.* also identified the tongue and the FOM as the sites that are mostly affected by OED and these cases fell under the severe category (Spreight P.M. *et al*., 2018, Pereira J. S.*et al*., 2011).

Majority of the mild cases were found on the tongue (35%), followed by the buccal mucosa (25%) and the FOM and the lower lip (10% respectively). This slightly differs from what is reported in the literature that reports that mild cases are more likely to be found on the lower lip (Pereira J. S. *et al*., 2011). The distribution of OL within the oral cavity differs in different parts of the world. The intraoral locations varied depending upon the chewing and smoking habits involved (Karthiga Kannan S. *et al*., 2019).

An Exacts test was done (0.838) and there is no statistically significant difference between degree of dysplasia and location of lesion, this corresponds with what Pereira *et al*. reported (Pereira J. S.*et al.*, 2011).

5.5 Risk factors

5.5.1 Alcohol

There were no statistically significant associations with smoking in males or with alcohol. More diagnoses were done non-alcohol consumers (64.29%) than alcohol consumers (34.71%). Alcohol consumers had majority severe cases and these formed 72.73% of all diagnosed severe cases. The non-alcohol consumers on the other hand has majority moderate cases and these formed 78.95% of all diagnosed moderate cases. In alcohol consumers, the OR for OED development increased by factor 2.026, with a value of 0.198; this was however statistically insignificant.

This corresponds with a cross sectional study that reported an association of alcohol consumption with OED with an OR of 2.4, even with controlled associated risk factors (Morse

D. E. *et al*., 1996). In another prospective cohort study, alcohol was reported as an independent risk factor for OPMDs and OED. Another Indian cross-sectional study found alcohol consumption to be a significant risk factor to OED, even in non-tobacco users and more in females (Hashibe M. *et al*., 2000, Maserejian N.N. *et al.,* 2006).

However, there have been other studies that have denied the association between OED and alcohol consumption. This inconsistency in the results with both negative and positive relationship has led to the conclusion that, the relationship between alcohol and OED therefore is not well established. The risk associated then depends on the level of alcohol consumption, the type of alcohol is irrelevant (Diettrich T. *et al.*, 2004, Li L. *et al*., 2011).

5.5.2 Smoking

The majority of OED cases (54.29%) diagnosed in non-smokers. This correlates with what is reported in the in the literature. Jaber found that there is no excess risk for OED development for smokers versus non-smokers (Jaber M.A., 2010).

However, Leukoplakia is the most common OPMD, there is evidence-based link between smoking and leukoplakia had not been well established. However, non prospective observational studies have highlighted the association between smoking and oral leukoplakia (Arduino P.G. *et al*., 2013). Individuals with tobacco related lesions have an increased risk of OED (Dombi C., *et al*, 2001).

Both the smokers and non-smokers had majority mild OED cases at 59.38% and 55.26% respectively. A pearson chi test was done and the p value was 0.998, there was however no statistical significance. The non-smokers had more mild cases (55.26%) compared to moderate and severe cases. The smokers on the other hand had more severe cases (54.55%) compared to non-smokers. There is a variation of OPMDs incidence in literature. The increased risk of OED from smoking is attributed to heavy smoking of 20 cigarettes/day with an OR of 4.38 relative to non-smokers (Schepman K. P. *et al.,* 2001).

5.5.3 Alcohol and smoking

Alcohol and smoking are known risk factors that have a synergistic effect when combined. The risk of OED development declines with smoking cessation. In non-smokers, the consumption of alcohol is not a significant predictor of OED (Jaber M.A., 2010).

41% were identified as non-smokers and non-alcohol consumers This was much higher than the 4.4% reported by Farshadpour *et al.* (Farshadpour F. *et al*., 2007). Wei *et al.* on the other hand reported a much higher figure at 31% (Wey P.D. *et al.,* 1987).

More mild OED cases were found on subjects who did not smoke nor consume alcohol than in smokers and alcohol drinkers. This corresponds with the literature that reports that non-alcohol and tobacco users had majority mild cases. (Kaugars G.E. *et al.*, 1989, McGuirt W.F. *et al.*, 1983). More moderate OED cases were observed in smokers and alcohol drinkers in comparison to subjects who did not smoker or consume alcohol (Jaber M.A., 2010).

The presence of OED in non-smokers and non-alcohol consumers suggests that there are other risk factors other than smoking and drinking (Jaber M.A., 2010).

In the non-smokers, consumption of alcohol was not a significant predictor of OED. However, there was a synergistic effect of alcohol when combined with some aspects of tobacco smoking. In our study, the role of tobacco as a risk factors was not established, however Jaber's results identified the role of tobacco in the aetiology of OED. The role of alcohol, however, is principally only important in conjunction with tobacco use (Jaber M. A., *et al*., 1998)

Chapter 6: Limitation

The sample size is the first limiting factor. A few of the patients were excluded due to incomplete information. Several studies have shown great inter and intra examiner variability in the assessment of the absence/ presence and in the grading of OED (Jaber M. A., 2010). This lack of reproducibility poses a difficulty in the diagnosing and classification of OED and becomes a problem in any OED study. It has been suggested to use the same pathologist in a n attempt to overcome these challenges, this could however not be done in this study as the TBOHC uses different pathologist, this variable could therefore not be controlled.

Moreover, the type and frequency and usage practice of smoking and alcohol consumption was not recorded for most patients. This information is important is assessing the amount of risk posed by these habits.

In categorizing the biopsied areas, all tongue lesions were combined. Certain pathological reports did not specify sites, these sites were listed as oral mucosa. Proper reporting on specific sites would have been helpful.

Most of the results were statistically insignificant, although some of the findings correlated with that of previous studies/literature.

Chapter 7: Conclusion

The sites that had the highest lesions diagnosed with OED were the buccal mucosa and the FOM, although this was found to be statistically insignificant. Studies however agreed with these findings and this has been associated with the patients' habits. OED therefore has no intra-oral location predilection.

Majority of OED diagnosed lesions were found in non-smokers. Jaber also found that there is no excess risk for OED development for smokers versus non-smokers. There is no statistical significance between smoking and OED development. There OED is not directly associated with smoking.

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