USEFULNESS OF OPTICAL COHERENCE TOMOGRAPHY AS AN ADJUNCT DIAGNOSTIC TOOL IN THE DETECTION OF ORAL SQUAMOUS CELL CARCINOMA: An *in vitro* Study

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ABSTRACT

Objective: To correlate OCT imaging with histopathological diagnosis of biopsy specimen; To identify the structural change in oral cancers biopsy specimens by OCT; To evaluate OCT capability of defining the indication for surgical biopsy and measure the accuracy of the OCT findings with the final diagnosis.

Material and method: This prospective study included 52 oral lesions from 44 patients (male = 21, female = 23). Surgical biopsies (excisional = 23, incisional = 29) were obtained under local anesthesia. Two assessors (surgeon and pathologist) examined OCT images of 52 samples in two separate sessions. In first session, they were asked to evaluate the mucosal architectural status including keratin layer (KL), epithelial layer (EP), lamina propria (LP), basement membrane (BM) and the reflection's degree of epithelial layer (EP Re.) and report biopsy need. In the second session, the same two assessors reassessed the OCT images in the same manner of the first session. Firstly, they were asked to give a differential diagnosis for that lesion based only on the brief clinical history and secondly, they were required to score on the five OCT variables and based on that they were asked to report the OCT agreement (agree, disagree) with their clinical differential diagnosis that they had made earlier. The inter-observer agreement (first and second session) and intra-observer agreement were calculated using Kappa scores. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the accuracy of COE, OCT and combined (COE + OCT) were also calculated.

Results: The histopathological results showed there were SCC (14), verrucous carcinoma (1), epithelial dysplasia (7), epithelial hyperkeratosis (1), epithelial hyperplasia (1), and OLP (5). Other lesions included were mucoceles and pyogenic

granuloma (4 each), traumatic eosinophilic ulcer, fibroepithelial polyps and traumatic neuroma (3 each), irritation fibroma, squamous cell papilloma, oral melanotic macule, lymphedema, hemangiolymphangioma and inflamed mucosa (1 each). Sn, Sp, NPV, PPV and Ac for 1st assessor were 86%, 77%, 88%, 73% and 81% (COE), 95%, 80%, 96%, 78% and 87% (OCT), 95%, 77%, 96%, 75% and 85% (combined), while for the 2nd assessor 91%, 70%, 91%, 69% and 79% (COE), 95%, 71%, 96%, 69% and 81% (OCT), 96%, 72%, 95%, 73% and 83% (combined). Kappa score of inter-observer agreement was 0.92 for biopsy need.

Conclusion: Optical coherence tomography (OCT) is a non-invasive technique with a high-resolution capability that is considered a useful tool for obtaining cross-sectional real-time images for different parts of the human body. Basement membrane is considered a key parameter in the detection of oral cancer and for differentiating it from other oral pathological conditions. The EP thickness and its degree of reflection are valuable parameters for recognizing healthy tissues from most pathological conditions, but, they cannot be considered as accurate diagnostic criteria to discriminate between the dysplastic epithelium and benign oral tissues. Overall OCT is a promising optical technique that might be able to define the grades of oral dysplasia in the near future with the continuous increase in its resolution capability.

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Name of Candidate: Dr.Ali Yassen Obade

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Field of Study: ORAL AND MAXILLOFACIAL SURGERY

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CHAPTER ONE
INTRODUCTION

1.1. **Introduction**

Oral cancer is the sixth most common cancer worldwide and more than 90% of oral cancers have been confirmed histopathologically to be a squamous cell carcinoma (SCC) (Choi et al., 2008; Brad W. Neville et al., 2002). Head and neck cancer is relatively rare in the United States, as it is a common disease entity in some parts of the developing world. The greatest incidence of oral cancer has been recorded in most of Melanesian and South Asian countries (Jemal et al., 2011; Saman Warnakulasuriya, 2009). In some Southeast Asian countries such as Bangladesh, India, Pakistan, and Sri Lanka, oral cancer is the most common form of cancer in male populations and accounts up to 23% of the all diagnosed cases of cancer, while for European countries such as United Kingdom, the oral cancer has a lower incidence which accounts for approximately 3% of cancers (Boyle et al., 2005). In Malaysia, the Malaysian-Indian females have a higher rate of oral cancer incidence according to a report taken from the National Cancer Registry (NCR) in Malaysia, 2007 (Zainal Ariffin et al., 2011).

There are many studies that have been done to improve the methods used in the therapeutic field but, the survival rates post intervention has remained the same over the last 50 years (Gómez et al., 2009). This is duo to the delayed diagnosis, for that reason, around half of all oral cancers cases are diagnosed at advanced stages (Saman Warnakulasuriya, 2009). Therefore, oral cancer is a public health problem which carries significant morbidity and mortality (Mccullough et al., 2010). Squamous cell carcinoma (SCC) of the oral cavity accounts for approximately 3% of all cancers worldwide (J. Ferlay et al., 2008).

In the early dysplasia, there are many changes happening in the tissue and cellular levels without breach of the basement membrane. These changes will be represented as oral mucosal lesions (Barnes, 2005; Van Der Waal, 2009). During the clinical oral examination (COE), most precancerous lesions appear visually as white (leukoplkia),

red (erythroplakia) or a combination of red and white (erythroleukoplakia) (M. Lingen et al., 2011; Napier et al., 2008; Van Der Waal, 2009). The early detection of these oral potentially malignant disorders (OPMDs) plays a golden key in reducing the morbidity and mortality (Speight et al., 2006). Meanwhile, histopathological method is the gold standard diagnostic measure for the diagnosis of suspicious oral lesions, but it has a disadvantage of being invasive, painful, expensive and time consuming (Omar Kujan et al., 2007).

In addition, COE alone cannot differentiate between the benign, dysplastic and malignant oral lesions, because many oral malignancies mimic the clinical features of both benign and dysplastic oral lesions (Epstein et al., 2012). Therefore, the clinicians need an adjunct diagnostic tool, which can help in detecting and differentiating between these oral lesions and thus, helping in determining the need for surgical biopsy of the lesion as to help in early diagnosis. They also may play role in the follow-up of these OPMDs. There are some recent adjunct clinical diagnostic tools that have been developed to assist the COE for the early detection of oral SCC (OSCC) and the various OPMDs. These adjunct diagnostic measures include; brush biopsy kits, vital tissue staining (toluidine blue dye, Lugols Iodine), Biomarkers, Oral CDx salivary diagnostics (Chaudhary et al., 2014), DNA ploidy (Torres-Rendon et al., 2009), tissue auto-fluorescence imaging (VELscope Vx) (Awan et al., 2011a), Optical coherence tomography (OCT) (Awan et al., 2011a), tissue reflectance (ViziLite) (Oh et al., 2007), Narrow Band Imaging (NBI) (Watanabe et al., 2008) and various spectroscopy such as Fuorier Transformed Infrared (FT-IR) spectroscopy (Sahu et al., 2005) and micro-Raman spectroscopy (Krishna et al., 2004).

This study aims to evaluate the usefulness of optical coherence tomography (OCT) in the detection of structural changes in squamous cell carcinoma.

1.2. Aim

The aim of this study is to evaluate the usefulness of OCT in the detection of structural changes in squamous cell carcinoma.

1.3 Objectives

1. To correlate OCT imaging with histopathological diagnosis of biopsy specimen
2. To identify the structural change in oral cancers biopsy specimens by OCT.
3. To evaluate OCT capability of defining the indication for surgical biopsy and measure the accuracy of the OCT findings with the final diagnosis.

CHAPTER TWO

LITERATURE REVIEW

2.1. Oral Potentially Malignant Disorders (OPMDs)

The terms pre-cancer, premalignant lesion, potentially malignant, and premalignant condition have been used in many literature data to give a clinical definition for the lesions or the conditions that have the tendency to undergo malignant transformation (Barnes, 2005). However, in 2015 the World Health Organization (WHO) proposed to use the term "oral potentially malignant disorder" instead of the old terms since not all the lesions and conditions defined by this term may become cancer, but still there is a group of morphological changes that may have a tendency for the malignant transformation (S Warnakulasuriya et al., 2007). The recent studies have shown that 5-18% of OPMDs has a tendency for the malignant transformation (Moro et al., 2010; Rana et al., 2012). In this literature review, the focus is on common OPMDs such as leukoplakia, erythroplakia, erythroleukoplakia and oral lichen planus (OLP).

2.1.1. Oral leukoplakia

Oral leukoplakia is potential malignant disorder, which appears clinically as white patch of suspicious risk of malignant transformation excluding other entities that have no risk for developing an oral cancer (S Warnakulasuriya et al., 2007). Recent studies have shown that leukoplakia has tendency that ranges from 3.5-17.9% for neoplastic transformation into SCC (Brouns et al., 2014; Kurokawa et al., 2002; Liu et al., 2012; Liu et al., 2010).

Oral leukoplakia has a prevalence that accounts for 2-3% worldwide, and it is usually more predominant in the male population, but the possibility of its existence in female is also present (Saman Warnakulasuriya, 2009). In addition, the oral leukoplakia is more common in the tobacco smokers by six times more than non-smokers, and it usually affects specific parts of oral cavity such as floor of the mouth, ventrolateral part of the

tongue (Choi et al., 2008; Brad W. Neville et al., 2002).

It is not clear yet about the etiological factor for oral leukoplakia, but there are many studies that have reported smoking, alcohol abuse, long term mechanical injuries, and infection by *Candida albicans* as the most critical risk factors for developing oral leukoplakia (Grajewski et al., 2009; Ribeiro et al., 2010). Since the etiology of oral leukoplakia is still unclear, the therapy for these lesions is not just difficult but also challenging (Suter et al., 2007; Yeh, 2000).

Oral leukoplakia can be classified into to two types: (i) homogenous and (ii) non-homogenous leukoplakia. The homogenous leukoplakia, is defined as a flat and uniformly white through the whole lesion (Thomson, 2012) whereas, the non-homogenous leukoplakia appears as an irregularly flat lesion which can either be white or sometimes mixed white and red lesions (erythroleukoplakia), while in other cases may appear nodular (speckled), or verrucous (Van Der Waal, 2010; Van Der Waal et al., 2002).

Erythroleukoplakia consists of mix of nodular leukoplakia patches on erythroplakic background of oral mucosa. Comparing to the leukoplakia it is less commonly found in the oral cavity (Thomson, 2012). Mostly all the diagnosed cases of erythroleukoplakia show epithelial dysplasia or carcinomas (Brad W Neville, 2009). Therefore, erythroleukoplakia has a high malignant transformation rate which is about 18%-47% (Yu et al., 2009). Recent statistical studies that have been done in Taiwan, showed that the risky habits of tobacco smoking and areca quid chewing considered to be a major factor in the malignant transformation of oral leukoplakia and erythroleukoplakia (A.M.-F. Yen et al., 2007; A.M.F. Yen et al., 2008).

Therefore, the early detection of oral erythroleukoplakia is highly necessary because of its high tendency for the malignant transformation (Chen et al., 2012).

2.1.2. Erythroplakia

It is an oral lesion that appears as bright or fiery red, velvety patch, which may have a smooth, depressed surface, or granular surface that can be differentiated easily from the surrounding normal mucosa and it cannot be accounted for any other recognized lesions.

Comparing to the oral leukoplakia, erythroplakia seems to be less common in the oral cavity and it commonly affects the floor of the mouth, soft palate and ventral surface of tongue (Lapthanasupkul et al., 2007; Thomson, 2012; S Warnakulasuriya et al., 2007). Elderly men around the ages of 65-74 years have the highest rate of developing this highly potential malignant disorder. The cause for erythroplakia is unknown. However, smoking, tobacco and alcohol consumption are the major risk factors in developing oral erythroplakia (Trock, 2000). A study has shown that around 40% of erythroplakia might develop into SCC (Ridge et al., 2008). Thus, the rate of malignant transformation of erythroplakia is relatively high (Thomson, 2012).

2.1.3. Oral lichen planus

Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous abnormality that has unknown etiology (Baccaglini et al., 2013; Lodi et al., 2012; M. Roopashree et al., 2010). It has many types; plaque type, ulcerative, erosive and the reticular type, which is the most predominant one. OLP occurs in different parts of oral cavity, while the buccal mucosa, tongue, and gingiva are most common sites for this disease (Bethke et al., 2005; Edwards et al., 2002; Escudier et al., 2007; Piboonniyom et al., 2005).

Study has shown that the incidence of OLP ranges from 0.5%-3% (Farhi et al., 2010). The common age for its occurrence ranges from 30-60 years, and female population tends to have a higher incidence rate for this disease (Nagao et al., 2005). The rate of OLP malignant transformation into SCC is relatively low which ranges from 1-5.3%

(Shi et al., 2010).

Clinically OLP appears as a white striation, plaques or nodules located at the affected oral mucosa, and this characteristic appearance results from the hyperkeratosis (Thomson, 2012). Histopathologically, OLP can be defined as disorder of the oral mucosa that has a feature of dysfunctional basal epithelium and accumulation of T-cell lymphocytes immediately subjacent to altered oral epithelium. (Joel B Epstein et al., 2003; Payeras et al., 2013; M.R. Roopashree et al., 2010; Scully et al., 2008).

2.2. Oral squamous cell carcinoma (OSCC) and the importance of early detection

Oral cancer occurrence ranks the eighth position in the cancer incidence worldwide, and the third common malignancy within the area of South-Central Asia (Petersen, 2003). Oral squamous cell carcinoma (OSCC) is categorized as the most common cancer around the world that shows malignant neoplastic changes arising from the oral mucosal epithelium (Konkimalla et al., 2007). It accounts 3% of all the malignancies all over the world (J Ferlay et al., 2008). The recent statistical studies showed approximately 500,000 new cases of OSCC are recorded annually (Durmus et al., 2013; Lin et al., 2014).

Approximately 90% to 95% of all malignant lesions that happen within the oral cavity are diagnosed as SCC and occurs most commonly within the tongue, notably at the posterior border and ventral surfaces. Therefore, the tongue accounts for 40% of the all cases of OSCC diagnosed within the oral cavity, while the floor of the mouth occupies the second most intraoral place of the OSCC incidence followed by the gingiva, buccal mucosa, labial mucosa and the hard palate (Brad W. Neville et al., 2002; Silverman, 2001).

In most cases, men aged over 50 years show a high risk of developing OSCC, especially those with the previous history of excessive use of tobacco and alcohol (Friedlander et

al., 1998; Llewellyn et al., 2001). However, many studies have shown that nowadays an increase in the frequency of the OSCC occurs in the younger age group (Chow et al., 2007; Marocchio et al., 2010; Patel et al., 2011).

The 5-year survival rate for the oral cancer patients continues to be less than 50%. Therefore, the early detection and the proper management for these oral cancers will have a great effect in improving the survival rate for these patients (Remmerbach et al., 2009). The prognosis of the oral cancer that is detected in advanced stages (stage III-IV) ranges from 30-50%, while for the early stage (stage I) the survival rate accounts for 80% (Koch et al., 2011b). Hence, the stage of oral cancer is a key factor in these patients. Besides, its pathological stage during the diagnosis is considered an essential element that can decide the morbidity and mortality of those patients (Horowitz et al., 2000).

Clinical oral examination (COE) for the oral cancer patients under the incandescent light is insufficient and difficult because of the nature of the oral lesion that might appear clinically as normal mucosa (Marx et al., 2003). In addition, histopathology is still the gold standard diagnostic tool for suspicious oral lesions (Omar Kujan et al., 2007), but it has some drawbacks such as artefacts that may happen during the processing of tissue samples, which may lead to the change of normal cellular morphology or even to complete destruction of the biopsy sample (Meghana et al., 2007).

The survival chance of these patients can be improved significantly through the early detection of oral cancer and other OPMDs (Alfano et al., 2001). Therefore, the early detection and diagnosis for oral cancer play a key role in improving the survival rate and the quality of life (Garg et al., 2012).

2.3. Most common non-invasive methods used for early detection of OSCC

The oral surgical biopsy and the histopathological analysis remain the most reliable method for the accurate diagnosis of oral cancer and OPMDs, but it is still believed that it has a disadvantage of being a time consuming, invasive and painful method (Nair et al., 2012). Therefore, adjunct clinical diagnostic tools have been recently developed to assist the COE for the early detection of OSCC and OPMDs.

Here we discuss the most recent diagnostic non–invasive methods that have been used in the early detection of oral cancer and OPMDs, which include; Visually Enhanced Lesion Scope (VELscope), Identafi 3000, Brush cytology, Toluidine blue (TB), ViziLite technique, Optical coherence tomography (OCT).

(a) Visually Enhanced Lesion Scope (VELscope)

The Visually Enhanced Lesion scope (VELscope) is a portable hand-held device that is used by dentists to screen for oral tissue abnormalities including OPMDs and oral cancer. It was developed by LED Medical Diagnostics Inc. The VELscope technology uses the blue excitation light wavelength (400-460 nm) and aims it at the oral tissue which results in the stimulation of epithelial cells of the oral mucosa and stroma. This leads to the self/auto-fluorescence of these tissues that helps us in detecting any changes happening in the morphological structure and composition of these tissues. The normal mucosa emits pale green auto-fluorescence, while the cancerous and dysplastic tissues appear dark because of the reduced normal auto-fluorescence (Thomson, 2012).

The recent studies have shown a great value of using this new technology to enhance the efficiency of discovering any new oral lesions that might develop in already diagnosed high risk patients (Kois et al., 2006; Poh et al., 2007). According to the recent studies, the diagnostic accuracy of VELscope in the early detection of OSCC and other dysplastic lesions ranges between 30%-100% (Awan et al., 2011a; Marzouki et al.,

2012; Rana et al., 2012). Sensitivity evaluation of VELscope was at high peak when combining both COE and VELscope rather than doing any of them alone (Camile S. Farah et al., 2012). A recent study has shown that VELscope has sensitivity (84%) and a low specificity (15%) in differentiation between oral dysplasia and other benign lesions (Awan et al., 2011a). Overall the VELscope imaging still shows some unreliability in terms of differentiation between oral benign lesions, dysplasia, and oral cancers (Koch et al., 2011a).

(b) Identafi 3000

Identafi is a technique that combines both reflectance spectroscopy and autofluorescence idea for the detection of OPMDs and oral cancers (Rethman et al., 2010). Comparing to the VELscope, the identafi has the advantage of being able to examine all parts of the oral cavity and its small size which allows easy handling during the oral tissue examination (Roblyer et al., 2009).

Both Identafi and VELscope emit a shiny light to excite the oral tissue being examined to generate the fluorescence. However, the VELscope emits a blue light, while the Identafi emits violet one. Using the Identafi 3000 with a violet light (405 nm) will cause the abnormal oral tissue to look black or dark brown, which is used to differentiate it from the normal oral tissue (Rethman et al., 2010). A recent study evaluating the ability of Identafi 3000 in differentiating between the neoplastic and non-neoplastic tissues shows that this method has 82% sensitivity and 87% specificity and (Schwarz et al., 2009). However, this method has a promising future in the field of oral cancer and OPMDs detection, but it has a disadvantage that has been reported by some studies, stating that the light emitted by this method might undergo some undesirable reflections that will affect the visualization capability and thus affecting its benefits (Ob Kujan, 2013).

(c) Brush cytology

Brush cytology was developed in 1999 to assist in the evaluation of suspicious lesions of the oral cavity by its ability in identifying dysplastic changes that might happen during the pathological process in these oral lesions (Masthan et al., 2012). It is an inexpensive and well-tolerated method which help the clinicians to define the necessity for obtaining a surgical biopsy in some suspicious oral lesions that clinically mimic the benign features (Trullenque-Eriksson et al., 2009; Walling et al., 2003). It uses a type of brush, which is made specifically to get cells from the mucosa of oral lesions (Eisen, 2002; Eisen et al., 2005). The specimen obtained by this method was examined to identify the presence of any structural abnormality in these cells (Sciubba et al., 1999). In terms of the abnormal cells identification, brush cytology test has a sensitivity of 52% and specificity of 29% (Hohlweg-Majert et al., 2009). Overall, this test has a disadvantage of having a great number of false positive results (Bhoopathi et al., 2009).

(d) Toluidine blue (TB)

Toluidine blue (TB) is a metachromatic dye that has the binding ability to the diseased oral tissues such as premalignant, malignant and other inflammatory tissues as well as binding to the DNA of those pathological sites (Allegra et al., 2009). Toluidine blue is (TB) used in many clinical applications and it has been proven the unique ability of this stain to bind selectively to the malignant and premalignant oral lesions (Joel B. Epstein et al., 2003; Guo et al., 2001; Zhang et al., 2005). For that reason, this sensitive stain (TB) has been used as an adjunct method for the early detection of the dysplastic changes that might happen in oral tissue and OSCC (Joel B. Epstein et al., 2003; Martin et al., 1998; Mashberg et al., 1995; Onofre et al., 2001). The sensitivity of TB ranges from 78-100%, while the specificity ranges from 31-100% (Awan et al., 2012; M.W.

Lingen et al., 2008). Overall, TB has a low specificity as it shows some characteristic behaviour of binding with the cells of those inflammatory oral issues

(e) ViziLite technique

This method was first approved by Food and Drug Administration to be used in United States of America in November 2001 (Messadi, 2013). ViziLite technique consists of a kit which includes a capsule that emits a bluish light when activated, and a T-blue acetic acid swab (Ram et al., 2005; Ujaoney et al., 2012). At beginning, the intraoral mucosa will be rinsed thoroughly with 1% acetic acid to clear the glycoprotein barrier and dry the oral mucosa. Then the capsule will be bent to activate the bluish-white light source (490-5510 nm), which will be used to examine the oral tissue. The cells of normal tissue will absorb the light and will look bluish, while the abnormal cells will reflect the light and will have the aceto-whitish appearance with well-marked borders (Huber et al., 2004). A study conducted in 2011 to evaluate the use of VizilLite technique in the early detection of OPMDs and benign keratosis has reported sensitivity of 77.3% and specificity of 27.8% (Awan et al., 2011b). Overall, ViziLite test has a disadvantage of being insufficient to differentiate between benign oral lesions, dysplasia and oral cancer (Camile S Farah et al., 2007; Lestón et al., 2010; Oh et al., 2007).

(f) Optical coherence tomography (OCT)

Optical coherence tomography (OCT) was first developed by Fujimoto et al. in 1991 (Huang et al., 1991). It is a non-invasive as well as nonradioactive diagnostic measurement based on interferometers (Fujimoto, 2003). OCT and ultrasound share the same idea (Tadrous, 2000), in which both have the capability of providing a real time cross-sectional sub-surface tissue images, but the difference is that the OCT uses broadband light, while the ultrasound uses sound waves (Ding et al., 2002; Huang et al.,

1991). Besides, the modern OCT systems overweigh the ultrasound by having around 10 times higher axial resolution (Schmitt, 1999).

The idea of OCT is based on using a low-coherence broadband near-infrared light source, which allows getting an excellent spatial resolution (~20 μm) and real-time images (Fujimoto, 2003; Wojtkowski et al., 2005). The light emitted by the OCT system passes through a fibre coupler or a beam splitter, which divides it into two arms: a reference arm (sending the light to a mirror) and a sample arm (used for scanning an object). The beam that goes though the reference arm is reflected from a mirror at a measured specific distance. An interference pattern is created by merging the light reflected from the mirror and the other one that is reflected from the different layers of the object being scanned. Meanwhile, the depth of the reflection is calculated within the object. This pattern then goes through the OCT image detector and is shown as a representative image pixel. During the OCT scanning process the sample reflects a considerable amount of light that creates a high-resolution image. The OCT sample arm moves spatially along the tissue sample, which produces a B-mode scan. By accounting the intensity and the echo time delay in scattered OCT light, a cross-sectional imaging of microstructures of tissue *in situ* is produced (Fujimoto, 2003; Jerjes et al., 2010; Pitris et al., 2000; Rubinstein et al., 2009; Schmitt, 1999).

This modern technology allows obtaining an optical biopsy that may assist clinician to avoid conventional biopsy procedures that include either incision or excision of the lesion and processing of specimens. Therefore, the optical biopsy is a non-invasive technique that aids in identifying the structural changes in the epithelial lesions (Drexler et al., 2008).

Optical coherence tomography is considered one of the amazing imaging tools in many medical aspects. Its earliest use in the medical field was first reported in the ophthalmology which involves taking the images for the retina to detect the

pathological structural changes happening in the macula, and for measuring the thickness of cornea (Feng et al., 2008). Moreover, it has been used *in vivo* and *in vitro* for the purpose of examining many different kinds of tissues such as gastrointestinal tract (Bouma et al., 2000), breast tissue (Hsiung et al., 2007), skin (Avanaki et al., 2013) and many other kinds of tissues and oral cavity (Ridgway et al., 2006).

In the gastrointestinal field, the OCT has been used to define the structural changes that happen in mucosa and sub-mucosa in many organs such as colon and stomach (Park et al., 2005). While in the field of dermatology, OCT has been used to get cross-sectional real images for the structural layers of skin and to detect many pathological conditions that affect the skin such as psoriasis, melanomas and other skin diseases (Mogensen et al., 2009).

The early detection of the hidden dentinal caries is still considered one of the most highly impact feature of OCT and its uses in dentistry, adding to that, its potential feature of interproximal surfaces examination of molar and premolar teeth and detecting the potency and integrity of the dental fillings (Featherstone et al., 2001; Fried et al., 2002).

Despite the great value of its applications in different medical fields and in dentistry, some significant limitations that might dramatically affect its diagnosis capability have been reported. These limitations include its inefficacy to get enough details in terms of nuclear size, shape, and other cellular structures (Hamdoon et al., 2012a). Moreover, OCT still has a limited penetration depth that is around 2-3 mm which is a limitation in the medical diagnostic field (Abtahian et al., 2012).

2.4. OCT in oral cancer and OPMDs

There are some studies that prove the importance of OCT in the diagnosis of oral disease (Matheny et al., 2004). It was found that OCT could give precise

microstructural information on epithelial layer, integrity of basement membrane and lamina propria. In addition, OCT images have defined clear areas of normal and altered mucosal structures for each pathological condition (Ridgway et al., 2006). Therefore, OCT has a unique ability to identify the layers of oral mucosa and easily differentiate oral cancer from other benign lesions (Jerjes et al., 2010).

In malignancy, there is an increased cellular density which will lead to an equal increase in the refractive index of that specific tissue structure therefore; the refractive index will be increased as it corresponds to the density of the cells. Hence, the refractive index is considered a major factor in the OCT field and the contrast obtained in the OCT images is produced by the reflection of different tissue structures that have different refractive indices (Bista et al., 2011). The great advantage of using OCT technology lies in its ability in detecting the structural changes in keratin layer (KL), epithelial layer (EP), lamina propria (LP) and the basement membrane (BM) (Adhi et al., 2013).

According to a recent study, OCT images of oral cancer of buccal mucosa showed a destruction of the basement membrane integrity as well as some irregularity of epithelial layer thickness with clear invasion down into the lamina propria. Additionally, the statistical analysis of the same study has reported that the OCT sensitivity was 93% and specificity was 97% in identifying the SCC from other oral pathological entities (Wilder-Smith et al., 2009). Another study showed that both carcinoma *in situ* and oral dysplastic tissues lacked regular architectural pattern (Tsai et al., 2009). The dysplastic cells, nucleus size, and the other cellular structures will be organised haphazardly in the diseased oral epithelium (Chi et al., 2009). Therefore, the OCT images of oral epithelium of dysplastic tissue will tend to have a higher degree of signal intensity compared to the normal oral mucosa. In addition, standard deviation (SD) can give us a clear description of the morphological structure of dysplastic and healthy oral epithelium. The SD calculation methods also have been used as a

diagnostic measurement of oral submucous fibrosis. In that case, it is noticed that collagen accumulation in LP results in noticeable reduction in the SD value for that layer. However, the random arrangement of dysplastic cells in the oral epithelium of precancerous lesions might result in an increased SD value (Lee et al., 2009). Therefore, OCT could be a useful adjunctive tool that can be used during the examination and follow-up of different oral lesions, as well as guiding the oral surgical biopsies (Prestin et al., 2012).

The main advantages of using OCT include micron-scale resolution imaging, real-time, direct imaging of tissue morphology and function, imaging depth of up to 3mm, no processing or any special preparation is needed of the sample or subject and highly safe procedure due to its non- ionising radiation feature (Rubinstein et al., 2009).

CHAPTER THREE

MATERIAL AND METHOD

3.1. Sample collection

This prospective study was carried out in patients (subjects) with different oral lesions (benign lesions, OPMDs and oral cancer) who were referred to the Department of Oro-Maxillofacial Surgical and Medical Science, Faculty of Dentistry, University of Malaya within the period 2014 to 2016.

Fifty-two oral lesions from 44 patients (male = 21, female = 23) were included in this study. Surgical biopsies (excisional = 23, incisional = 29) were obtained under local anesthesia. Informed written consent was obtained from each subject. This protocol was approved by the Medical Ethics Committee, Faculty of Dentistry, University of Malaya [DF OS1519/0077 (p)].

3.1.1. Clinical Oral Examination (COE)

The patient's socio-demographic details such as gender and age were recorded. All these subjects underwent a comprehensive clinical examination that included extra-oral and intraoral examination. An oral surgeon carried out the COE under an incandescent light source to assess the site, size, and the clinical characteristics of these oral lesions.

3.1.2. Obtaining the surgical biopsy

Before obtaining a biopsy, marking of biopsy site with a disposable surgical marker was carried out to include normal mucosa together with the lesion as shown in Figure 3.1. Each biopsy specimen therefore had some amount of normal tissue at its edges. Intra-oral photographs of these oral lesions with their markings were documented using a digital Samsung camera (16 megapixel). An incisional or excisional biopsy was performed on each patient under a local anesthesia. Each biopsy specimen was orientated immediately and its edges were sutured and taped to microscopic slide. These slides were photographed to record the orientation and ensure an accurate co-

registration (Figure 3.2, Figure 3.3), then they were placed in containers filled with normal saline and they were transferred to the laboratory within 45 minutes.

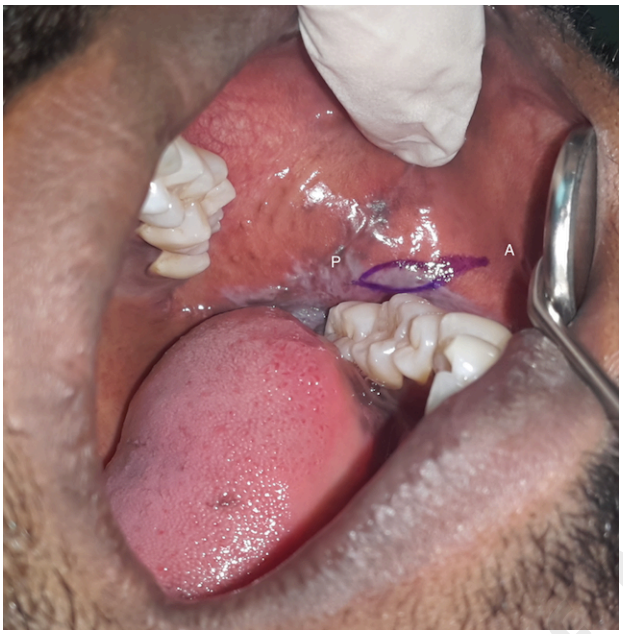

Figure 3.1: An image showing the surgical marking to define the site of biopsy.

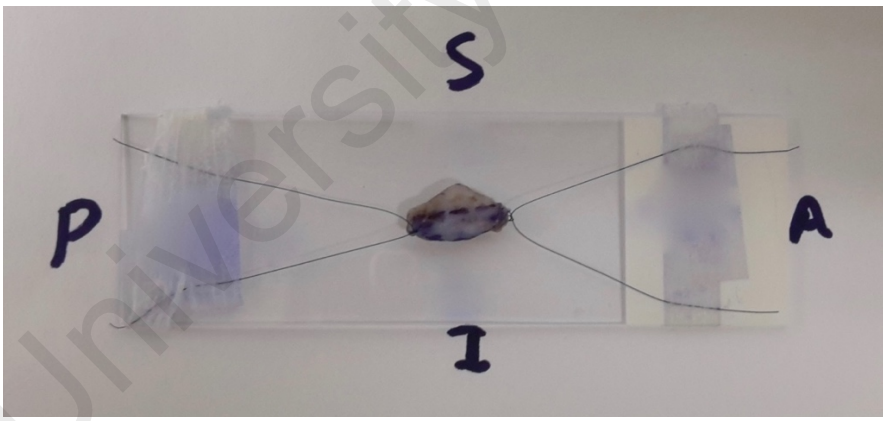

Figure 3.2: An image showing the biopsy specimen laid down on the glass slide and fixed by sutures to ensure accurate orientation. A blue line has been marked on the sample to ensure the histopathlogist ability to identify OCT scanned plan.

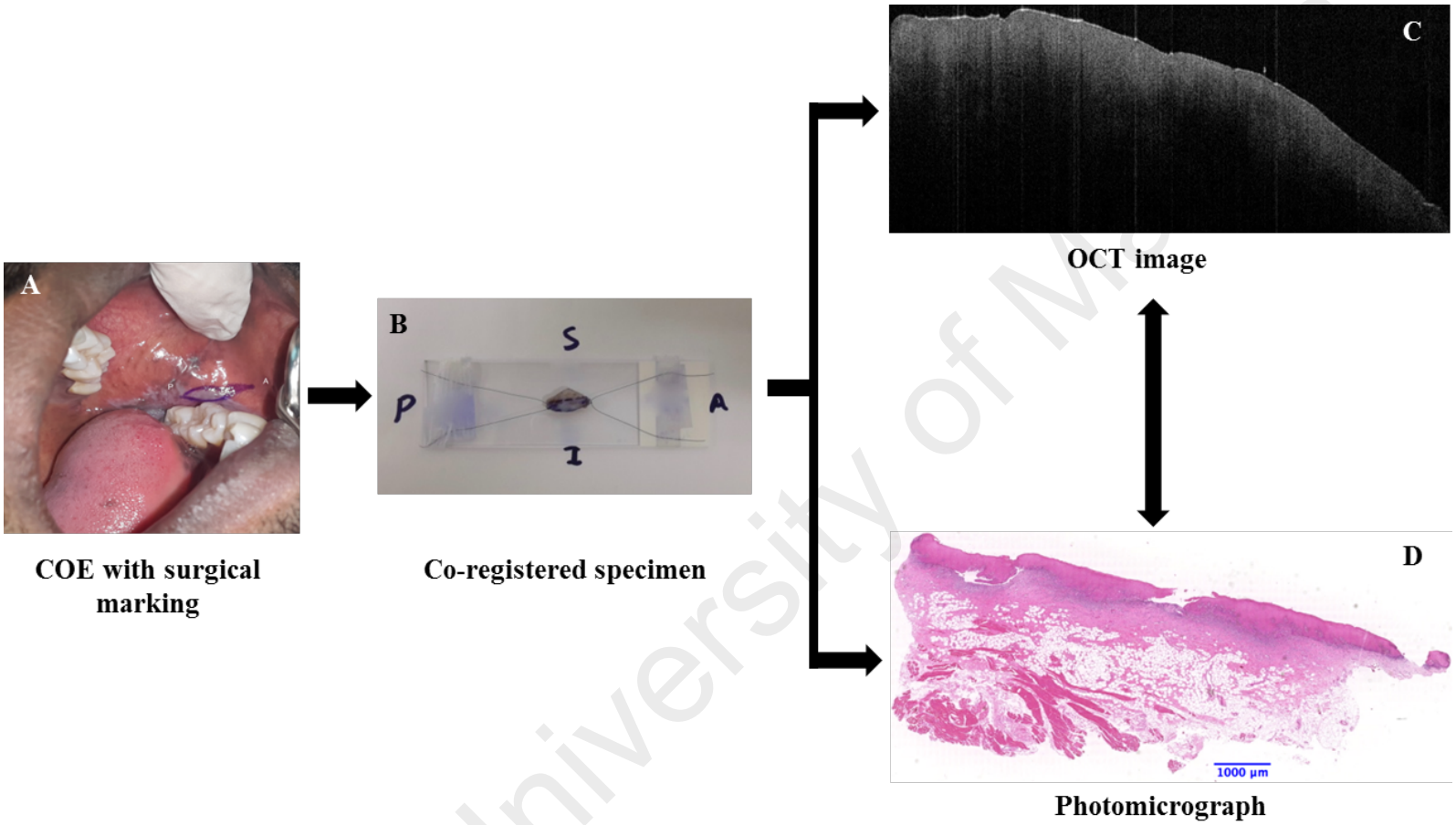

Figure 3.3: Photograph shows (A) lesion sub-site in the oral cavity with the surgical marking. (B) Biopsied tissue sample sutured and taped to the slide with orientation to ensure an accurate co-registration (P- Posterior, S- Superior, I-Inferior, A-Anterior). (C) OCT image of the biopsied tissue. (D) Photomicrograph of the biopsy sample. (Stain: H and E; Original magnification 1.5x).

3.2. OCT scanning

In the laboratory, the specimens were dried and a line was drawn on the specimen using a disposable surgical marker to ensure that the histopathologist grossed the specimens in the same plane as the OCT scanned plane for the accurate comparison between both OCT and histopathological image of that specific specimen as shown in Figure 3.2.

In this study, Swept Source OCT System (Thorlabs OCS1300SS Swept Source OCT System, USA) (Figure 3.4) was used with an imaging wavelength of 1325 nm. The OCT captured B-mode scans of the biopsy specimen, which was 10 mm wide, while the longer biopsy samples needed more than one scan along the long axis of the laser OCT probe. Immediately after scanning with OCT, the specimen was kept in a bottle with 10% buffered formalin for fixation. The samples were sent for histopathological analysis that included specimen embedding in paraffin wax, staining with haematoxylin and eosin, and examination by light microscope for the confirmative histopathological diagnosis.

Figure 3.4: Photograph of (A) THORLABS CS1300SS OCT Imaging System (USA) Components. (B) Thorlabs OCS 1300 SS engine and imaging module. (C) Probe and stand.

3.3. OCT training session and image analysis

Two assessors (oral surgeon and oral pathologist) were trained and calibrated using a collection of OCT and histopathological images of normal and different pathological conditions to enhance their knowledge in the field of OCT images interpretation. Both assessors were required to comment on five variables of the OCT images which included changes in the keratin layer (KL), changes in the epithelial layer (EP), changes in the lamina propria (LP), basement membrane identification (BMI) and the degree of reflection of epithelial layer (EP Re.). These OCT variables help the assessors in the detection of any architectural changes happening in the oral tissue. They also were required to report whether there was a need for a biopsy or not depending on the finding of any abnormality of the five OCT variables of each OCT image.

Comparing between the OCT images of each biopsy specimen, any thickness increase in Kl or EP was marked as (↑), thickness decrease was marked as (↓), while no changes in the thickness were indicated as (No). Our training set shows that KL layer in normal oral mucosa appears as thin layer of hyper reflection (Figure 3.5-A), while in the pathological oral mucosa such as in hyper–keratinization, KL appears thicker, brighter and very hyper-reflective (Figure 3.5-B).

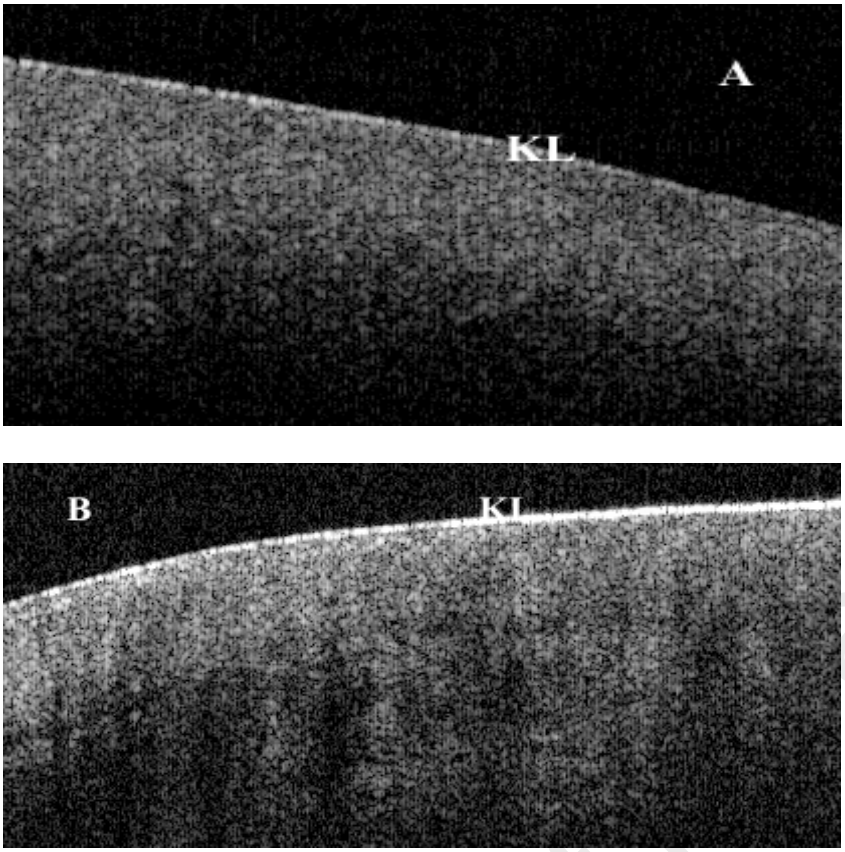

Figure 3.5: (A) OCT image of normal oral mucosa. (B) OCT image showing hyper keratinization.

Our training set also shows that the epithelial layer (EP) often looks translucent or less hyper–reflective than the upper keratin layer and lower lamina propria (Figure 3.6-A) while, the pathological oral tissues the EP tends to be brighter and more hyper-reflective than the lower LP and its thickness would mostly be increased in some pathological conditions (Figure 3.6-B).

The lamina popria (LP) was examined for any changes and marked as (Yes) for inhomogeneous LP and (No) for homogenous LP. Based on the training set, the homogenous LP looked well organized and clearly demarcated from the upper epithelial layer (Figure 3.6-A), while the inhomogeneous LP looked disorganized and difficult to identify from the upper EP, with some area of BM breach as shown in all the SCC condition (Figure 3.6-B).

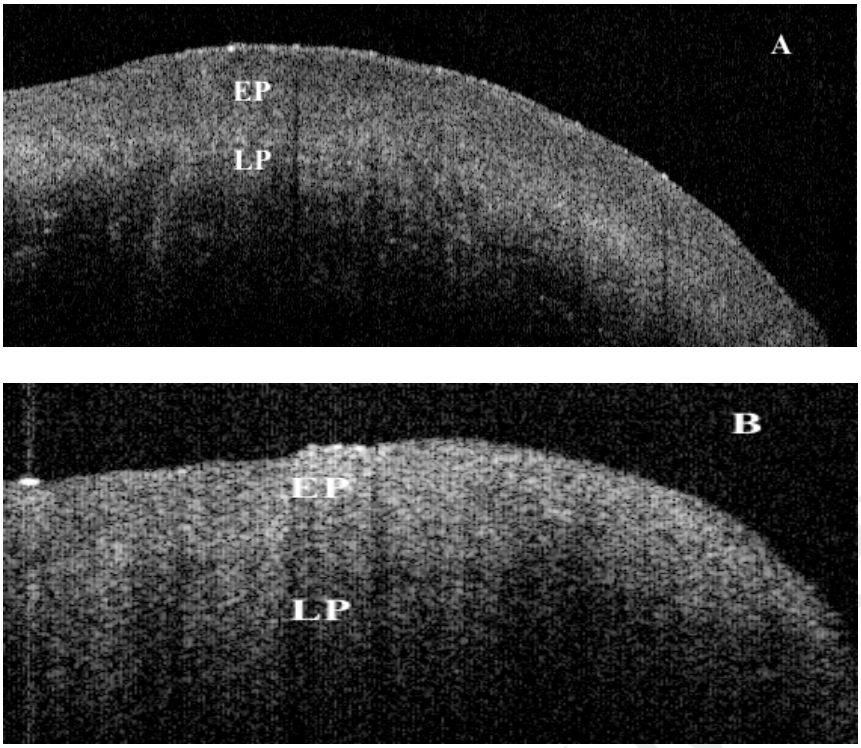

Figure 3.6: (A) OCT image of normal oral mucosa. (B) OCT image of oral dysplasia.

The basement membrane (BM) was marked as completely identified (C), partially identified (P), Breached (B), or not identified (No). Our training set shows that BM can usually be defined as an area between two different contrasts (EP and LP) and it can be identified clearly in most normal and benign oral mucosal tissues (Figure 3.7-A), while in the pathological tissues such as oral cancer, the BM appears interrupted and breached and sometimes hardly to identify clearly (Figure 3.7-B).

The fifth variable; EP Re. was marked as normal reflection (N) or hyper-reflective (↑). The normal reflection meant that EP layer looked less bright than the LP that we concluded from our training set (Figure 3.6-A). Hence, the determination of EP Re status was based on the comparison between EP and LP brightness. The scoring of the OCT variables is shown in Table 3.1.

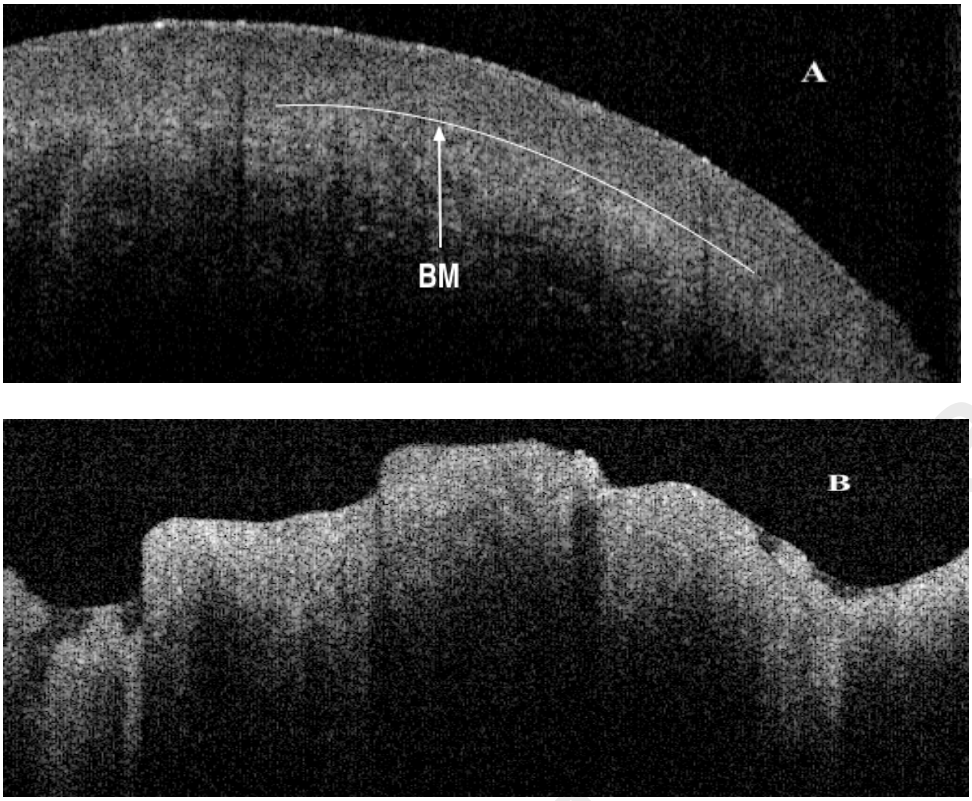

Figure 3.7: (A) OCT image of normal ventral surface of tongue showing intact BM. (B) OCT image of SCC showing breached BM.

Table 3.1. Scoring of the OCT variable.

OCT variables	Scoring			
KL	↑ (Increased thickness)	↓ (Decreased thickness)	No (No changes)	
EP	↑ (Increased thickness)	↓ (Decreased thickness)	No (No changes)	
LP	Yes (Inhomogeneous)	No (Homogenous)		
BMI	C (Completely identified)	P (Partially identified)	B (Breached)	No (Not identified)
EP Re.	N (Normal reflection, translucent)	↑ (Hyper-reflective, brighter than LP)		

K: keratin layer, EP: epithelial layer, BMI: basement membrane identification, LP: lamina propria, EP Re.: epithelial reflection.

The scoring of OCT images of the 52 specimens was carried out in two separate sessions to avoid faulty readings due to eye fatigue of the OCT assessors that could result by continuous looking at many OCT images at the same time, which might affect the OCT scoring accuracy. All the cases have been randomised in both sessions. The purpose of conducting the first session was to evaluate the usefulness of OCT as stand-alone diagnostic tool. Both assessors were blinded to the clinical details, clinical and histopathological diagnosis. They were only provided with the anatomical sites from where the biopsy samples were taken. Both were asked to score the five OCT variables and to report on the biopsy need based on the structural changes that they found during their OCT image assessments. In the first session, any changes in at least three variables

in each case indicated the biopsy need, except for the cases in which the change in the basement membrane (identified partially –P) was neglected and case 9 (Appendix 3) in which the first assessor defined the BM as (not identified-NO) but still did not recommend a biopsy and the histopathological diagnosis was squamous cell papilloma.

The purpose of conducting the second session was to evaluate the usefulness of OCT as an adjunctive diagnostic tool. In this session, the same two assessors were blinded to the clinical and histopathological diagnosis and were given a brief clinical history of the oral lesion being assessed. They were asked to give a differential diagnosis followed by scoring for the five OCT variables. Based on their scoring, they were asked to report on the OCT agreement (agree, disagree) with their clinical differential diagnosis. They were also required to report on the biopsy need based on both the clinical diagnosis that they made at first and the OCT findings.

3.4. Statistical analysis

The inter-observer agreement for the first and the second session and intra-observer agreement were calculated using Kappa scores (poor = 0.00-0.40, good = 0.41-0.70, very good = 0.71-0.80, excellent = 0.81-1). The sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and the accuracy (Ac) of COE, OCT and combined (COE + OCT) were also calculated.

CHAPTER FOUR

RESULTS

4.1. Demographics of subjects included in the research

Fifty-two biopsy samples (excisional = 23, incisional = 29) from 44 patients (male = 21, female = 23) in the age range of 4 years to 87 years were included in this study and the biopsies were taken from different sub-sites of the oral cavity which include; dorsal surface of tongue (n = 4), lateral border of tongue (n = 12), ventral surface of tongue (n = 5), gingiva (n = 9), labial mucosa (n = 9), buccal mucosa (n = 13). The site and type of biopsy performed on subjects included in the study are shown in Table 4.1

The biopsy specimens were kept in normal saline then dried and scanned by OCT within (45 minutes).

Table 4.1: The site and type of biopsy performed on subjects included in the study (n= 52).

Site of biopsy	Total	Incisional 29	Excisional 23
Dorsal surface of tongue	04		
Ventral surface of tongue	05		
Lateral border of tongue	12		
Buccal mucosa	13		
Labial Mucosa	09		
Gingiva	09		
Total	52		

4.2. First session results

The data obtained in the first session was stored in prepared sheets (Excel 2011). The kappa scores of agreement between the two assessors were calculated using (IBM SPSS statistics v.22). The assessment of the five OCT variables (KL, EP, LP, BMI, EP Re.) and the need for biopsy recorded by the first assessor (oral surgeon) and second assessor (oral pathologist) in the first session are shown in appendices 1 and 2 respectively.

4.3. The Second Session Results

During this session, the same two assessors were briefed with clinical history of each oral lesion and were required to give a differential diagnosis for each case and rescore the five OCT parameters (KL, EP, LP, BMI, EP Re.). They were also required to report on the biopsy needs as in the first session. Additionally, they were required to report on the agreement between the OCT imaging and the clinical diagnosis that they have carried out earlier. The summary of OCT images assessment, biopsy needs, clinical diagnosis, and agreement of clinical diagnosis based on OCT imaging by the first assessor (oral surgeon) and second assessor (oral pathologist) in the second session and the histopathological diagnosis are shown in appendices 3 and 4 respectively.

The kappa scores of inter-observer agreement between the two assessors in the first and second session for each of the OCT characteristic that was assessed and for biopsy needs are shown in Table 4.2. In the first session, there was an excellent agreement on KL status and biopsy need as well as a very good agreement on EP, LP, EP Re. and BM, while in the second session, there was an excellent agreement on biopsy need, EP Re. as well as LP and very good agreement on KL, EP and BM. The intra-observer agreements of both assessors for each of the OCT characteristic that was assessed and for biopsy needs are shown in Table 4.2. The kappa scores of intra-observer showed that there was an excellent agreement on Kl, EP, LP, BM, EP Re. and biopsy need for the oral surgeon, while for the oral pathologist there was a good agreement on EP Re. and an excellent agreement on KL, EP, LP, BM and biopsy need. Sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and accuracy (Ac) of COE, OCT findings by both assessors compared to the histopathological diagnosis are shown in Tables 4.3, 4.4, respectively, while those for combined (COE + OCT) for the first and second assessors were shown in Tables 4.5, 4.6 respectively.

Table 4.2: The kappa score for the inter-observer and intra-observer agreement between the two assessors in the 1st and 2nd sessions for each of the OCT characteristics that was scored and the biopsy needs.

	Inter-Observer Agreement		Intra-Observer Agreement	
	First Session	Second Session	1st Assessor	2nd Assessor
KL	0.83	0.78	0.83	0.87
EP	0.75	0.72	0.87	0.92
LP	0.78	0.83	0.91	0.87
BMI	0.79	0.78	0.93	0.90
EP Re.	0.75	0.81	0.88	0.70
Biopsy Need	0.88	0.92	0.85	0.90

KL: Keratin Layer; EP: Epithelial Layer; LP: Lamina Propria; BMI: Basement Membrane Identification; EP Re: Degree of Reflection of Epithelial layer.

Table 4.3: Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of COE findings by 1st and 2nd assessor compared to the histopathological diagnosis.

		Histopathological diagnosis							
		1st Assessor				2nd Assessor			
		Dysplasia & SCC	Non-Dysplasia	Total		Dysplasia & SCC	Non-Dysplasia	Total	
COE	OPMDs & SCC	19 (TP)	7 (FP)	26	73% (PPV)	20 (TP)	9 (FP)	30	69% (PPV)
	Non-OPMDs	3 (FN)	23 (TN)	26	88% (NPV)	2 (FN)	21 (TN)	22	91% (NPV)
	Total	22	30	52		22	30	52	
		86% (Sn)	77% (Sp)		81% (Ac)	91% (Sn)	70% (Sp)		79% (Ac)

Sn: Sensitivity, Sp: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, Ac: Accuracy, TP: True Positive, TN: True Negative, FP: False Positive, FN: False Negative).

Table 4.4: Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of OCT findings by 1st and 2nd assessor compared to the histopathological diagnosis.

		Histopathological diagnosis							
		1st Assessor				2nd Assessor			
		Dysplasia & SCC	Non-Dysplasia	Total		Dysplasia & SCC	Non-Dysplasia	Total	
OCT	Abnormal	21 (TP)	6 (FP)	27	78% (PPV)	20 (TP)	9 (FP)	29	69% (PPV)
	Normal	1 (FN)	24 (TN)	25	96% (NPV)	1 (FN)	22 (TN)	23	96% (NPV)
	Total	22	30	52		21	31	52	
		95% (Sn)	80% (Sp)		87% (Ac)	95% (Sn)	71% (Sp)		81% (Ac)

Sn: Sensitivity, Sp: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, Ac: Accuracy, TP: True Positive, TN: True Negative, FP: False Positive, FN: False Negative.

Table 4.5: Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of combined (COE and OCT) findings by 1st assessor compared to the histopathological diagnosis.

		Histopathological diagnosis			
		1st Assessor			
		Dysplasia & SCC	Non-Dysplasia	Total	
COE +OCT	Non-OPMDs + Abnormal OPMDs & SCC + Abnormal OPMDs & SCC + Normal	21 (TP)	7 (FP)	28	75% (PPV)
	Non-OPMDs + Normal	1 (FN)	23 (TN)	24	96% (NPV)
	Total	22	30	52	
		95% (Sn)	77% (Sp)		85% (Ac)

Sn: Sensitivity, Sp: Specificity, PP: Positive Predictive Value, NPV: Negative Predictive Value, Ac: Accuracy, TP: True Positive, TN: True Negative, FP: False Positive, FN: False Negative), Abnormal group: represents the biopsy samples that assessors noticed architectural changes in their OCT images assessment during the 2nd session and indicate them for biopsy, Normal group: represents the biopsy samples that assessors did not indicate their need for biopsy in the 2nd session, Abnormal group: represents the biopsy samples that assessors indicate their need for biopsy in the 2nd session.

Table 4.6: Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of combined (COE and OCT) findings by 2nd assessor compared to the histopathological diagnosis.

		Histopathological diagnosis			
		2nd Assessor			
		Dysplasia & SCC	**Non-Dysplasia**	**Total**	
COE +OCT	**Non-OPMDs + Abnormal**	22 (TP)	8 (FP)	30	73% (PPV)
	OPMDs & SCC + Abnormal				
	OPMDs & SCC + Normal				
	Non-OPMDs + Normal	1 (FN)	21 (TN)	22	95% (NPV)
	Total	23	29	52	
		96% (Sn)	72% (Sp)		83% (Ac)

Sn: Sensitivity, Sp: Specificity, PP: Positive Predictive Value, NPV: Negative Predictive Value, Ac: Accuracy, TP: True Positive, TN: True Negative, FP: False Positive, FN: False Negative) Abnormal group: represents the biopsy samples that assessors noticed architectural changes in their OCT images assessment during the 2nd session and indicate them for biopsy, Normal group: represents the biopsy samples that assessors did not indicate their need for biopsy in the 2nd session, Abnormal group: represents the biopsy samples that assessors indicate their need for biopsy in the 2nd session.

4.4 Histopathological diagnosis

Histopathological diagnosis showed there were SCC (14), verrucous carcinoma (1), epithelial dysplasia (7), epithelial hyperkeratosis (1), epithelial hyperplasia (1) and oral lichen planus (5). Other lesions included were mucoceles and pyogenic granuloma (4 each), traumatic eosinophilic ulcer, fibroepithelial polyps and traumatic neuroma (3 each), irritation fibroma, squamous cell papilloma, oral melanotic macule, lymphedema, hemangiolymphangioma and inflamed mucosa (1 each).

CHAPTER FIVE

DISCUSSION

Surgical biopsy is the gold standard diagnostic method used for the detection of structural and cytological changes that happen in OPMDs and oral cancers (Wilder‐Smith et al., 2004). However, it is apparently obvious that the surgical biopsy has a disadvantage of being invasive, painful, expensive and time consuming (Nair et al., 2012). Besides, it has some drawbacks because of artifacts that may happen during the processing of tissue samples, which may lead to the change of normal cellular morphology or even complete destruction of the biopsy sample (Meghana et al., 2007).

In addition, clinicians often depend on COE to visually select the most suspicious spot for biopsy. Thus, premalignant spots can often be missed, resulting in late detection of oral malignancy (Wilder‐Smith et al., 2009). Moreover, COE alone cannot differentiate between the benign, dysplastic, and malignant oral lesions, because many oral malignancies mimic the clinical features of both benign and dysplastic oral lesions (Epstein et al., 2012). There is a need therefore, for adjunct diagnostic tools to help the clinician to select the area of biopsy. A special scanning technique that can detect the structural changes in the pathological oral tissues is required. The available scanning methods such as ultra sound (100–500 μm), CT (500–1000 μm) and MRI (500–1000 μm) are still insufficient for an accurate identification of the structural changes that happen during the pathological process at epithelial level (Ridgway et al., 2006). Compared to the other available scanning techniques, OCT shows the highest resolution (Sinescu et al., 2008). It is considered the only optical method that is available with a resolution of up to 10 μm (Ridgway et al., 2006). In addition, the OCT provides cross-sectional image of the healthy and pathological tissues without the necessity of obtaining biopsy or subjecting the patient to an ionizing radiation (Wilder‐Smith et al., 2004).

A study that has shown the interesting capability of OCT in detecting the structural changes that happen in different premalignant, malignant, and benign laryngeal

pathological conditions (Wong et al., 2005). This potential capability will enhance the OCT in identifying any structural changes as well as the integrity of BM, thus will help in identifying the early invasion of oral cancer into the LP. Therefore, the use of an optical high-resolution technique such as OCT might be used clinically as a promising useful adjunctive tool to complement the standard COE.

5.1. Criteria used in the OCT images analysis

Five variables were used in this study to interpret the OCT images by both assessors. These variables include; KL status, EP status, LP status, BMI and EP Re. Each assessor was required to comment on the status of each variable to evaluate the structural changes in the oral mucosal tissue of the biopsy specimen and report the biopsy need.

5.2. OCT images characteristics of normal oral mucosa

This study shows that scanning different parts of normal oral mucosa revealed that the KL usually appears as a thin layer of hyper-reflection and looks brighter than the EP and LP as shown in (Figure 5.1-A), whilst in the pathological oral mucosa such as in hyper–keratinization, KL appears thicker, brighter and very hyper-reflective as shown in (Figure 3.5-B). An OCT study was conducted to assess the structural changes of oral mucosa stated; that the KL layer was hyper-reflective in the mild and moderate oral dysplasia, while it was hypo-reflective in severe dysplasia and carcinoma *in situ* (Hamdoon et al., 2012b). However, our study shows that KL status was variable in different pathological conditions and that its status alone was not very important in identifying the pathological changes, while it was more informative when it was combined with the other variables.

Normally the epithelial layer (EP) often looks translucent or less hyper–reflective than the upper keratin layer and lower lamina propria (Figure 5.1-A), while in the

pathological oral tissues the EP tends to be brighter and more hyper-reflective than the lower LP and its thickness would mostly be increased in some pathological conditions as shown in (Figure 5.2-B).

In normal mucosa, lamina propria (LP) appears as a homogenous layer and is slightly brighter than the upper EP, with well-defined demarcation from the EP (Figure 5.1-A), while in the SCC it looks inhomogeneous and can hardly be identified from EP (Figure 5.3-B and E). The basement membrane (BM) can usually be defined as an area between two different contrasts (EP and LP) and it can be identified clearly in most normal and benign oral mucosal tissues (Figure 5.1-A, 5.4-B and E). Sometimes, BM shows some extensions down to the LP, which represents rete pegs such as in the dorsal surface of the tongue (Figure 5.1-C).

5.3. OCT finding discussion

In this study, kappa scores show that there is a very good agreement on identifying BM status. Therefore, even in the first session in which the assessors were blinded completely to the clinical history, the OCT was still able to identify all the SCC tissue samples that showed breached BM, and discriminate them from all other potentially premalignant, benign and other normal samples where the BM was identified partially or completely without any signs of breach. Therefore, the BM status was a key parameter in detecting the early invasion of oral cancer down into LP (Figure 5.3-B and E).

The kappa scores also show a very good agreement on defining the degree of reflectivity of EP (EP Re.). The epithelial layer (EP) was hyper-reflective in all dysplasia and SCC and some benign cases, while it was translucent in all the normal and some benign tissue biopsies (Figure 5.1-A). Therefore, the degree of EP reflectivity

is a good parameter in differentiating normal tissue from the other pathological conditions.

A study was carried out in laryngeal mucosa which showed a double increase in EP thickness in moderate dysplasia, a triple increase in carcinoma in situ, while invasive carcinoma showed 6 times increase in EP thickness compared to normal laryngeal mucosa (Arens et al., 2007). According to the similarity in epithelial structure, it is greatly possible that the same idea will be true for the oral cavity.

In addition, a study conducted to evaluate the OCT in the assessment of oral mucosa tissue stated that the epithelium thickness was increased dramatically in malignant lesions, while its thickness was variable in oral dysplasia, therefore, drawing some definitive criteria for differentiation between benign and early pre-cancer was difficult (Hamdoon et al., 2013). Our study also shows that EP was increased in thickness and more hyper-reflective than LP in all the OCT images of oral dysplasia and SCC. However, the EP thickness and EP Re. still cannot be definitive diagnostic parameters for the differentiation between oral dysplasia and other benign lesions, as our study shows the EP thickness and its degree of reflection were also increased in some benign conditions.

Therefore, future studies are recommended to generate an informative bank for the measurements of epithelial thickness of oral mucosa of different pathological conditions.

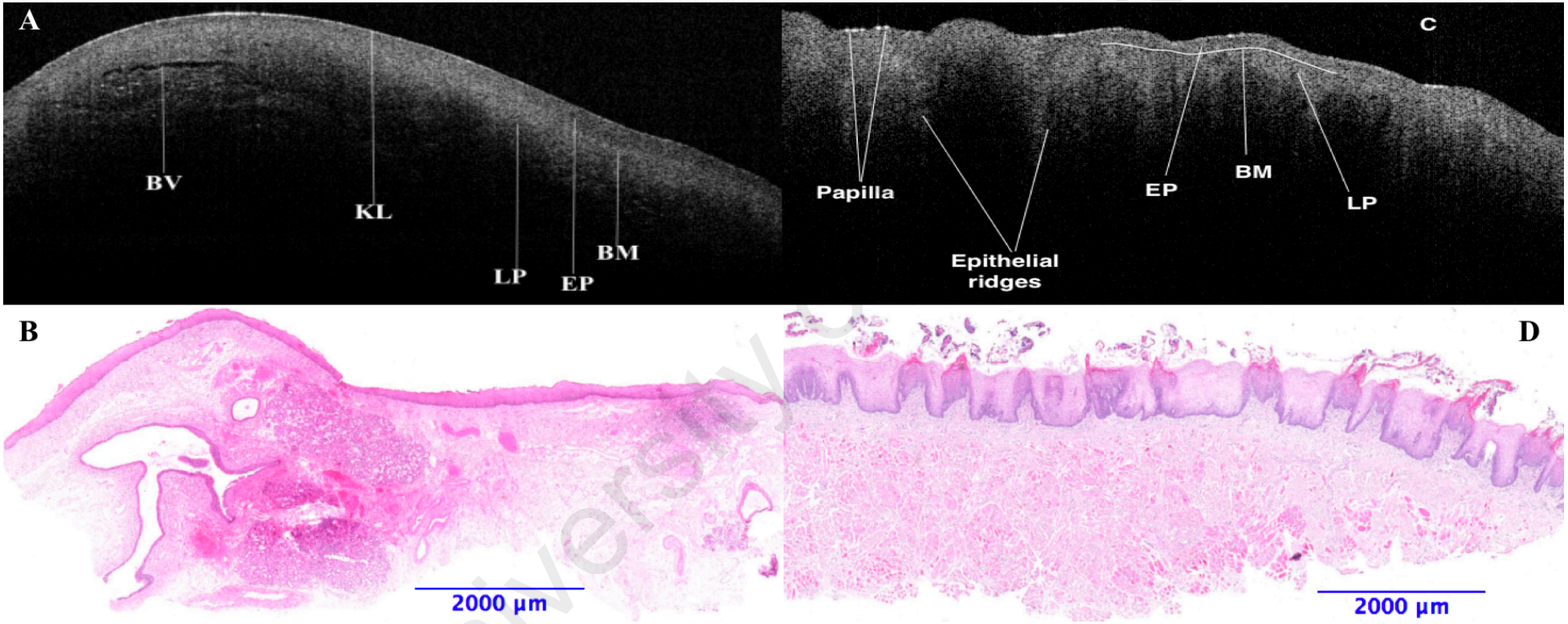

Figure 5.1: (A) OCT image of normal floor of mouth showing keratin layer (KL), epithelial layer (EP) and lamina propria (LP) which are clearly identified. EP looks translucent and less reflective than LP. The basement membrane (BM) is intact, clear and identified fully. (B) Photomicrograph of normal floor of mouth (Stain: H and E; Original magnification 1.0x**)**. (C) OCT image of dorsal surface of the tongues showing papilla and epithelial ridges. (D) Photomicrograph of normal dorsal surface of tongue (Stain: H and E; Original magnification 1.0x**)**.

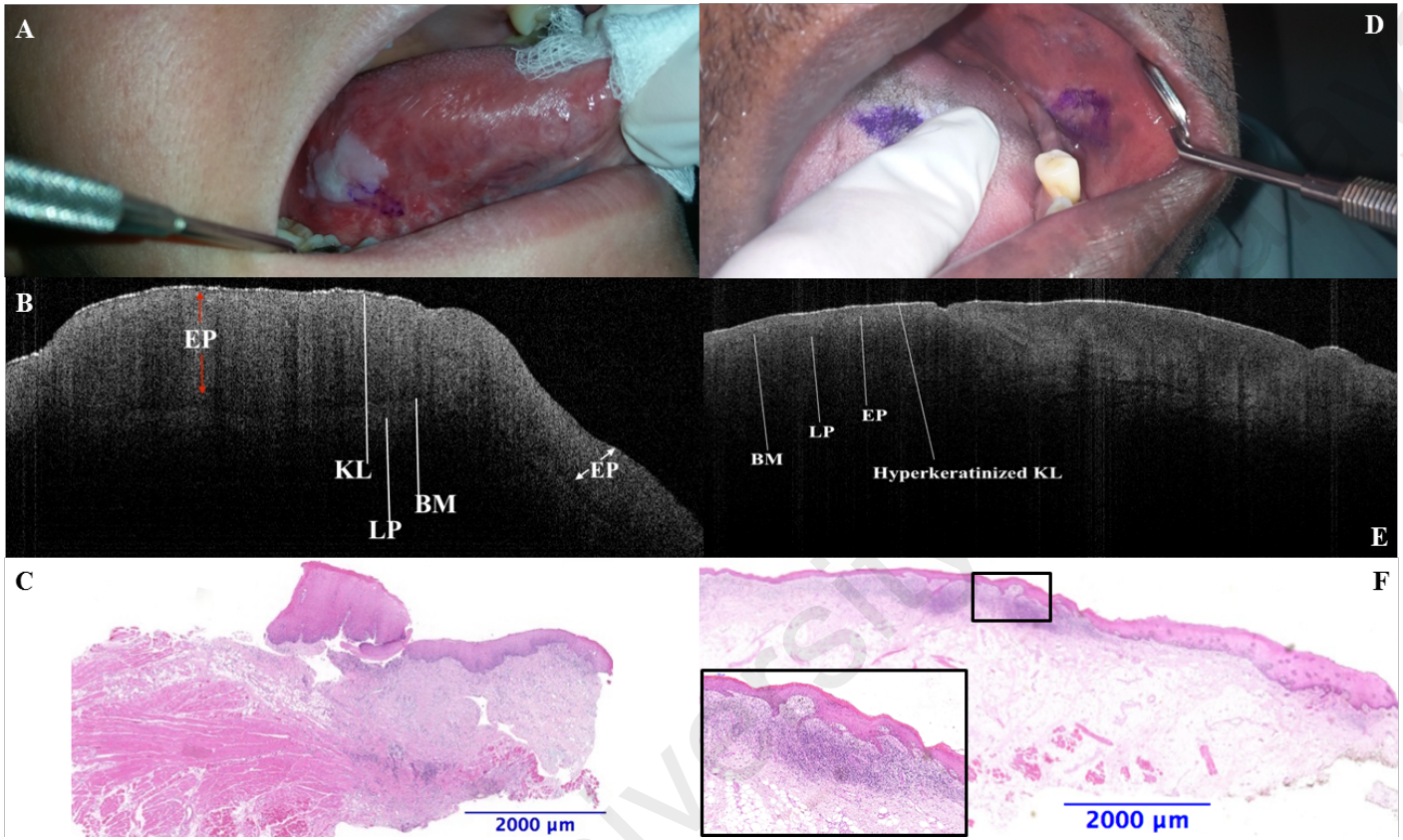

Figure 5.2: (A) Photograph of a homogenous leukoplakia on right lateral border of tongue. (B) OCT image of right lateral border of the tongue. Keratin layer (KL), epithelial layer (EP) and lamina propria (LP) are clearly identified. Basement membrane (BM) looks intact and identified clearly and completely. The epithelial layer (EP) increased in thickness (area defined by red arrows) when it compared to its thickness on the right side of OCT image (area defined by white arrows). (C) Photomicrograph of lateral border of the tongue showing sever epithelial dysplasia (Stain: H and E; Original magnification 1.0x). (D) Photograph of a lesion on the left buccal mucosa that was clinically diagnosed as OLP. (E) OCT image of left buccal mucosa showing keratin layer (KL), epithelial layer (EP) and lamina propria (LP) all are clearly marked. The basement membrane (BM) is intact and identified clearly and completely. (F) Photomicrograph of left buccal mucosa showing OLP (Stain: H and E; Original magnification 0.8x, Inset- Stain: H and E; Original magnification 10x).

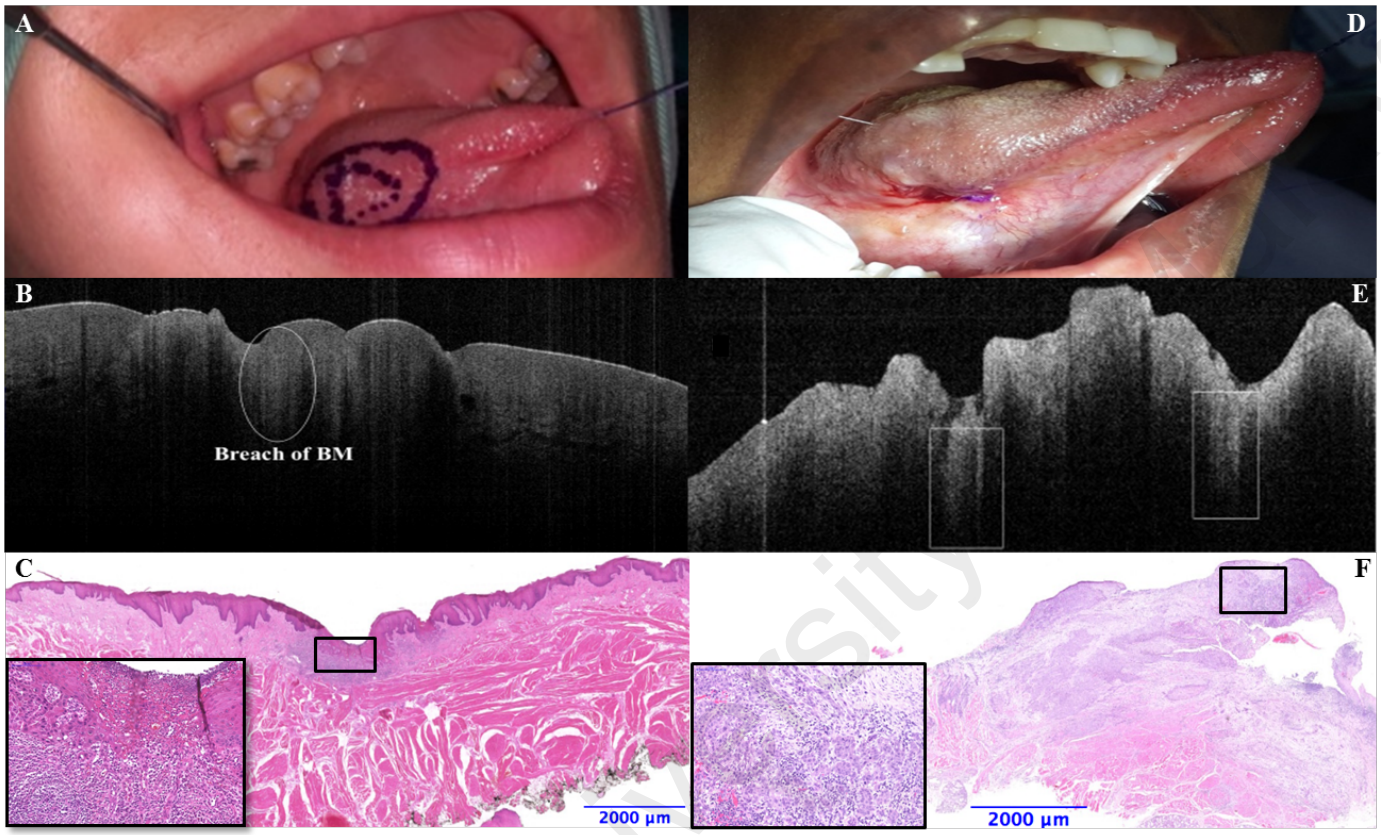

Figure 5.3: (A) Photograph of oral lesion on right lateral border of the tongue (B) OCT image of right lateral border of tongue. The oval area shows the breach in basement membrane (BM) and an uneven surface epithelium. (C) Photomicrograph of right lateral border of the tongue. The area in black rectangle shows the invasion of the epithelial tumor cells into the underlying connective tissue (Stain: H and E; Original magnification 0.8x, Inset- Original magnification 20x). (D) Photograph of non-healing ulcer on right ventral surface of the tongue. (E) OCT image of right ventral surface of the tongue. The areas in the white rectangles represent the focal invasion and breach of basement membrane (BM). The epithelial layer (EP) looks very bright and its thickness is increased dramatically in the area of focal invasion. (F) Photomicrograph of right ventral surface of tongue showing moderately differentiated SCC (Stain: H and E; Original magnification 0.8x, Inset- Original magnification 20x).

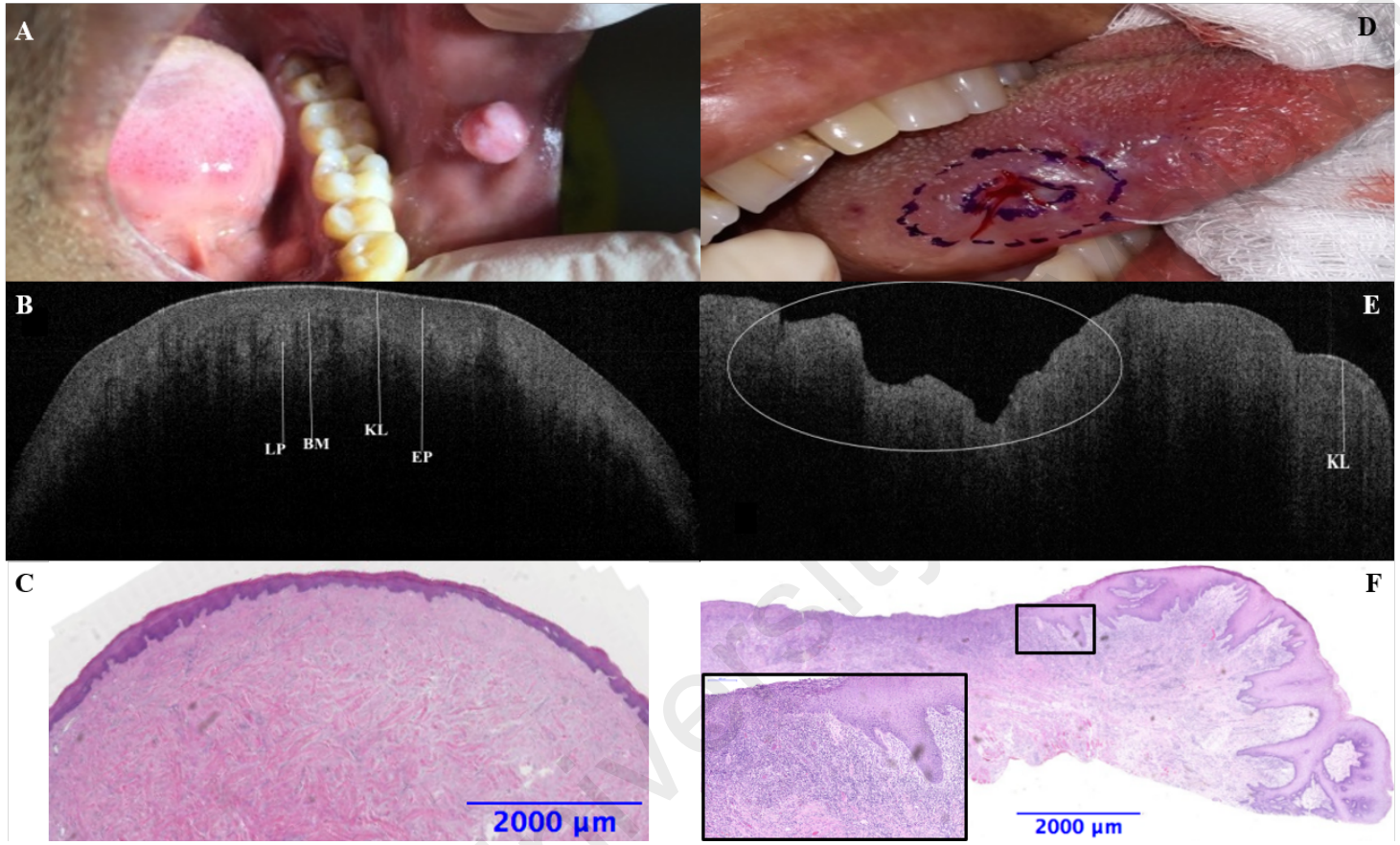

Figure 5.4: (A) Photograph of a fibrous lump on the left buccal mucosa that was clinically diagnosed as a fibro-epithelial polyp. (B) OCT image of left buccal mucosa showing keratin layer (KL), epithelial layer (EP), lamina propria (LP), and basement membrane (BM) are all identified clearly. The epithelial layer (EP) looks translucent and less bright than LP. (C) Photomicrograph of left buccal mucosa showing fibro-epithelial polyp (Stain: H and E; Original magnification 0.8x). (D) Photograph of tongue showing an ulcer on the right lateral border of tongue that was clinically diagnosed as SCC. (E) OCT image of right lateral border of tongue does not show a breach of basement membrane (BM). (F) Photomicrograph of right lateral border of tongue showing eosinophilic traumatic granuloma (Stain: H and E; Original magnification 0.8x, Inset- Original magnification 10x).

5.4. Important research outcome

The main outcome of this study shows that the BM status is a key parameter in the detection of SCC and for differentiating it from other pathological conditions. Besides, this study also shows that Sn, Sp, PPV, NPV, and Ac for both assessors were increased when COE and OCT findings were combined compared to COE alone, except for the Sp for the 1st assessor which remains the same (77%). Therefore, these findings suggest the usefulness of OCT as an adjunct diagnostic tool when combined with standard COE.

5.5. Study limitations.

There were some limitations that were encountered in this study such as (i) the correlation between histopathological and OCT images was not always applicable due to the amount of shrinkage that the biopsy samples were subjected to during its preservation in formalin, (ii) the resolution of OCT images was affected by the *in vitro* nature of the study and as such, the use of OCT technology *in vivo* might improve the quality of OCT image in terms of resolution and clear identification of epithelial and sub-epithelial structures of oral mucosa, (iii) the sample size was small, which represented as a challenging factor to establish diagnostic criteria for differentiating between oral dysplasia and other benign conditions, therefore further studies with larger sample size of dysplastic tissues are required.

CHAPTER SIX

CONCLUSION

Optical coherence tomography (OCT) is a non-invasive technique with a high-resolution capability that is considered a useful tool for obtaining cross-sectional real-time images for different parts of the human body. Basement membrane is considered a key parameter in the detection of oral cancer and for differentiating it from other oral pathological conditions. The EP thickness and its degree of reflection are valuable parameters for recognizing healthy tissues from most pathological conditions, but they cannot be considered as accurate diagnostic criteria to discriminate between the dysplastic epithelium and benign oral tissues. Overall OCT is a promising optical technique that might be able to define the grades of oral dysplasia in the near future with the continuous increase in its resolution capability.

REFERENCES

Abtahian, F., & Jang, I.-K. (2012). Optical coherence tomography: basics, current application and future potential. *Current Opinion in Pharmacology, 12*(5), 583-591.

Adhi, M., & Duker, J. S. (2013). Optical coherence tomography–current and future applications. *Current Opinion in Ophthalmology, 24*(3), 213.

Alfano, M. C., & Horowitz, A. M. (2001). Professional and community efforts to prevent morbidity and mortality from oral cancer. *The Journal of the American Dental Association, 132*, 24S-29S.

Allegra, E., Lombardo, N., Puzzo, L., & Garozzo, A. (2009). The usefulness of toluidine staining as a diagnostic tool for precancerous and cancerous oropharyngeal and oral cavity lesions. *Acta Otorhinolaryngologica Italica, 29*(4), 187.

Arens, C., Glanz, H., Wönckhaus, J., Hersemeyer, K., & Kraft, M. (2007). Histologic assessment of epithelial thickness in early laryngeal cancer or precursor lesions and its impact on endoscopic imaging. *European Archives of Oto-rhino-laryngology, 264*(6), 645-649.

Avanaki, M. R., & Hojjatoleslami, A. (2013). Skin layer detection of optical coherence tomography images. *Optik-International Journal for Light and Electron Optics, 124*(22), 5665-5668.

Awan, K., Morgan, P., & Warnakulasuriya, S. (2011a). Evaluation of an autofluorescence based imaging system (VELscope™) in the detection of oral potentially malignant disorders and benign keratoses. *Oral Oncology, 47*(4), 274-277.

Awan, K., Morgan, P., & Warnakulasuriya, S. (2011b). Utility of chemiluminescence (ViziLite™) in the detection of oral potentially malignant disorders and benign keratoses. *Journal of Oral Pathology & Medicine, 40*(7), 541-544.

Awan, K., Yang, Y., Morgan, P., & Warnakulasuriya, S. (2012). Utility of toluidine blue as a diagnostic adjunct in the detection of potentially malignant disorders of the oral cavity–a clinical and histological assessment. *Oral Diseases, 18*(8), 728-733.

Baccaglini, L., Thongprasom, K., Carrozzo, M., & Bigby, M. (2013). Urban legends series: lichen planus. *Oral Diseases, 19*(2), 128-143.

Barnes, L. (2005). *Pathology and genetics of head and neck tumours* (Vol. 9): IARC.

Bethke, G., & Reichart, P. (2005). [Assessment of severity of oral lichen planus using a new clinical index]. *Mund-, Kiefer-und Gesichtschirurgie: MKG, 9*(3), 152-160.

Bhoopathi, V., Kabani, S., & Mascarenhas, A. K. (2009). Low positive predictive value of the oral brush biopsy in detecting dysplastic oral lesions. *Cancer, 115*(5), 1036-1040.

Bista, R. K., Uttam, S., Wang, P., Staton, K., Choi, S., Bakkenist, C. J., . . . Liu, Y. (2011). Quantification of nanoscale nuclear refractive index changes during the cell cycle. *Journal of Biomedical Optics, 16*(7), 070503-070503-070503.

Bouma, B. E., Tearney, G. J., Compton, C. C., & Nishioka, N. S. (2000). High-resolution imaging of the human esophagus and stomach in vivo using optical coherence tomography. *Gastrointestinal Endoscopy, 51*(4), 467-474.

Boyle, P., & Ferlay, J. (2005). Cancer incidence and mortality in Europe, 2004. *Annals of Oncology, 16*(3), 481-488.

Brouns, E., Baart, J., Karagozoglu, K., Aartman, I., Bloemena, E., & Waal, I. (2014). Malignant transformation of oral leukoplakia in a well-defined cohort of 144 patients. *Oral Diseases, 20*(3), e19-e24.

Chaudhary, R., Shah, A., Shah, D. M., Singh, S., Thakkar, P., & Goyal, S. (2014). Advanced Diagnostic Aids in Detection of Oral Cancer. *Int J Dent Med Res| SEPT-OCT, 1*(3), 139.

Chen, H.-M., Yu, C.-H., Lin, H.-P., Cheng, S.-J., & Chiang, C.-P. (2012). 5-Aminolevulinic acid-mediated photodynamic therapy for oral cancers and precancers. *Journal of Dental Sciences, 7*(4), 307-315.

Chi, A., Neville, B., Damm, D., Allen, C., & Bouquot, J. (2009). Epithelial Pathology In Oral and Maxillofacial Pathology. *Noida: Elsevier*, 433-439.

Choi, S., & Myers, J. N. (2008). Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. *Journal of Dental Research, 87*(1), 14-32.

Chow, C. W., Tabrizi, S. N., Tiedemann, K., & Waters, K. D. (2007). Squamous cell carcinomas in children and young adults: a new wave of a very rare tumor? *Journal of Pediatric Surgery, 42*(12), 2035-2039.

Ding, Z., Ren, H., Zhao, Y., Nelson, J. S., & Chen, Z. (2002). High-resolution optical coherence tomography over a large depth range with an axicon lens. *Optics Letters, 27*(4), 243-245.

Drexler, W., & Fujimoto, J. G. (2008). *Optical coherence tomography: technology and applications*: Springer Science & Business Media.

Durmus, K., ApuhAn, T., & OzEr, E. (2013). Transoral robotic surgery for retromolar trigone tumours. *Acta Otorhinolaryngologica Italica, 33*(6), 425.

Edwards, P. C., & Kelsch, R. (2002). Oral lichen planus: clinical presentation and management. *Journal-Canadian Dental Association, 68*(8), 494-499.

Eisen, D. (2002). Brush biopsy'saves lives'. *Journal of the American Dental Association (1939), 133*(6), 688, 690, 692-688, 690, 692.

Eisen, D., & Frist, S. (2005). The relevance of the high positive predictive value of the oral brush biopsy. *Oral Oncology, 41*(7), 753-755.

Epstein, J. B., Feldman, R., Dolor, R. J., & Porter, S. R. (2003). The utility of tolonium chloride rinse in the diagnosis of recurrent or second primary cancers in patients with prior upper aerodigestive tract cancer. *Head & Neck, 25*(11), 911-921.

Epstein, J. B., Güneri, P., Boyacioglu, H., & Abt, E. (2012). The limitations of the clinical oral examination in detecting dysplastic oral lesions and oral squamous cell carcinoma. *Journal of the American Dental Association (1939), 143*(12), 1332-1342.

Epstein, J. B., Wan, L. S., Gorsky, M., & Zhang, L. (2003). Oral lichen planus: progress in understanding its malignant potential and the implications for clinical management. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 96*(1), 32-37.

Escudier, M., Ahmed, N., Shirlaw, P., Setterfield, J., Tappuni, A., Black, M., & Challacombe, S. (2007). A scoring system for mucosal disease severity with special reference to oral lichen planus. *British Journal of Dermatology, 157*(4), 765-770.

Farah, C. S., & McCullough, M. J. (2007). A pilot case control study on the efficacy of acetic acid wash and chemiluminescent illumination (ViziLite™) in the visualisation of oral mucosal white lesions. *Oral Oncology, 43*(8), 820-824.

Farah, C. S., McIntosh, L., Georgiou, A., & McCullough, M. J. (2012). Efficacy of tissue autofluorescence imaging (VELScope) in the visualization of oral mucosal lesions. *Head & Neck, 34*(6), 856-862.

Farhi, D., & Dupin, N. (2010). Pathophysiology, etiologic factors, and clinical management of oral lichen planus, part I: facts and controversies. *Clinics in Dermatology, 28*(1), 100-108.

Featherstone, J. D. B., & Fried, D. (2001). Fundamental Interactions of Laserswith Dental Hard Tissues. *Medical Laser Application, 16*(3), 181-194.

Feng, Y., & Simpson, T. L. (2008). Corneal, limbal, and conjunctival epithelial thickness from optical coherence tomography. *Optometry & Vision Science, 85*(9), E880-E883.

Ferlay, J., Shin, H., Bray, F., Forman, D., Mathers, C., & Parkin, D. (2008). GLOBOCAN v1. 2, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010.

Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., & Parkin, D. M. (2008). Cancer incidence and mortality worldwide: IARC cancerbase no. 10 (Internet). lyon, france: international agency for research on cancer; 2010. *GLOBOCAN v12*.

Fried, D., Xie, J., Shafi, S., Featherstone, J. D. B., Breunig, T. M., & Le, C. (2002). Imaging caries lesions and lesion progression with polarization sensitive optical coherence tomography. *Journal of Biomedical Optics, 7*(4), 618-627.

Friedlander, P. L., Schantz, S. P., Shaha, A. R., Yu, G., & Shah, J. P. (1998). Squamous cell carcinoma of the tongue in young patients: A matched-pair analysis. *Head & Neck, 20*(5), 363-368.

Fujimoto, J. G. (2003). Optical coherence tomography for ultrahigh resolution in vivo imaging. *Nature Biotechnology, 21*(11), 1361-1367.

Garg, P., & Karjodkar, F. (2012). "Catch Them before it Becomes Too Late"—Oral Cancer Detection. Report of Two Cases and Review of Diagnostic Aids in Cancer Detection. *International Journal of Preventive Medicine, 3*(10), 737.

Gómez, I., Seoane, J., Varela-Centelles, P., Diz, P., & Takkouche, B. (2009). Is diagnostic delay related to advanced-stage oral cancer. A meta-analysis. *European Journal of Oral Sciences, 117*(5), 541-546.

Grajewski, S., & Groneberg, D. (2009). [Leukoplakia and erythroplakia--two orale precursor lesions]. *Laryngo-rhino-Otologie, 88*(10), 666-672; quiz 673-665.

Guo, Z., Yamaguchi, K., Sanchez-Cespedes, M., Westra, W. H., Koch, W. M., & Sidransky, D. (2001). Allelic losses in OraTest-directed biopsies of patients with

prior upper aerodigestive tract malignancy. *Clinical Cancer Research, 7*(7), 1963-1968.

Hamdoon, Z., Jerjes, W., Al-Delayme, R., McKenzie, G., Jay, A., & Hopper, C. (2012a). Structural validation of oral mucosal tissue using optical coherence tomography. *Head & Neck Oncology, 4*(1), 1-9.

Hamdoon, Z., Jerjes, W., Al-Delayme, R., McKenzie, G., Jay, A., & Hopper, C. (2012b). Structural validation of oral mucosal tissue using optical coherence tomography. *Head & Neck Oncology, 4*(1), 1.

Hamdoon, Z., Jerjes, W., Upile, T., McKenzie, G., Jay, A., & Hopper, C. (2013). Optical coherence tomography in the assessment of suspicious oral lesions: An immediate ex vivo study. *Photodiagnosis and Photodynamic Therapy, 10*(1), 17-27.

Hohlweg-Majert, B., Deppe, H., Metzger, M. C., Schumm, S., Hoefler, H., Kesting, M. R., . . . Wolff, K.-D. (2009). Sensitivity and specificity of oral brush biopsy. *Cancer Investigation, 27*(3), 293-297.

Horowitz, A. M., Drury, T. F., Goodman, H. S., & Yellowitz, J. A. (2000). Oral pharyngeal cancer prevention and early detection: dentists' opinions and practices. *The Journal of the American Dental Association, 131*(4), 453-462.

Hsiung, P.-L., Phatak, D. R., Chen, Y., Aguirre, A. D., Fujimoto, J. G., & Connolly, J. L. (2007). Benign and Malignant Lesions in the Human Breast Depicted with Ultrahigh Resolution and Three-dimensional Optical Coherence Tomography 1. *Radiology, 244*(3), 865-874.

Huang, D., Swanson, E. A., Lin, C. P., Schuman, J. S., Stinson, W. G., Chang, W., . . . Puliafito, C. A. (1991). Optical coherence tomography. *Science, 254*(5035), 1178-1181.

Huber, M. A., Bsoul, S. A., & Terezhalmy, G. T. (2004). Acetic acid wash and chemiluminescent illumination as an adjunct to conventional oral soft tissue examination for the detection of dysplasia: a pilot study. *Quintessence International (Berlin, Germany: 1985), 35*(5), 378-384.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: A Cancer Journal for Clinicians, 61*(2), 69-90.

Jerjes, W., Upile, T., Conn, B., Hamdoon, Z., Betz, C. S., McKenzie, G., . . . Sandison, A. (2010). In vitro examination of suspicious oral lesions using optical coherence tomography. *British Journal of Oral and Maxillofacial Surgery, 48*(1), 18-25.

Koch, F. P., Kaemmerer, P. W., Biesterfeld, S., Kunkel, M., & Wagner, W. (2011a). Effectiveness of autofluorescence to identify suspicious oral lesions—a prospective, blinded clinical trial. *Clinical oral Investigations, 15*(6), 975-982.

Koch, F. P., Kunkel, M., Biesterfeld, S., & Wagner, W. (2011b). Diagnostic efficiency of differentiating small cancerous and precancerous lesions using mucosal brush smears of the oral cavity—a prospective and blinded study. *Clinical Oral Investigations, 15*(5), 763-769.

Kois, J. C., & Truelove, E. (2006). Detecting oral cancer: a new technique and case reports. *Dentistry today, 25*(10), 94, 96-97.

Konkimalla, V. B., Suhas, V. L., Chandra, N. R., Gebhart, E., & Efferth, T. (2007). Diagnosis and herapy of oral squamous cell carcinoma.

Krishna, C. M., Sockalingum, G., Kurien, J., Rao, L., Venteo, L., Pluot, M., . . . Kartha, V. (2004). Micro-Raman spectroscopy for optical pathology of oral squamous cell carcinoma. *Applied Spectroscopy, 58*(9), 1128-1135.

Kujan, O. (2013). What dentists should know about oral cancer screening. *European Journal of General Dentistry, 2*(3), 229.

Kujan, O., Khattab, A., Oliver, R. J., Roberts, S. A., Thakker, N., & Sloan, P. (2007). Why oral histopathology suffers inter-observer variability on grading oral epithelial dysplasia: an attempt to understand the sources of variation. *Oral Oncology, 43*(3), 224-231.

Kurokawa, H., Yamashita, Y., Takeda, S., Tomoyose, T., Funaki, K., Takano, H., . . . Takahashi, T. (2002). Relationship between epithelial dysplasia and the development of squamous cell carcinoma in oral leukoplakia. *Asian Journal of Oral and Maxillofacial Surgery, 14*(4), 197-201.

Lapthanasupkul, P., Poomsawat, S., & Punyasingh, J. (2007). A clinicopathologic study of oral leukoplakia and erythroplakia in a Thai population. *Quintessence International (Berlin, Germany: 1985), 38*(8), e448-455.

Lee, C.-K., Chen, H.-M., Wang, Y.-M., Yang, C., Chiang, C.-P., Tsai, M.-T., & Lee, H.-C. (2009). Diagnosis of oral submucous fibrosis with optical coherence tomography. *Journal of biomedical optics, 14*(5), 054008-054008-054007.

Lestón, J. S., & Dios, P. D. (2010). Diagnostic clinical aids in oral cancer. *Oral Oncology, 46*(6), 418-422.

Lin, C.-H., Kang, C.-J., Tsao, C.-K., Wallace, C. G., Lee, L.-Y., Lin, C.-Y., . . . Liao, C.-T. (2014). Priority of fibular reconstruction in patients with oral cavity cancer undergoing segmental mandibulectomy. *PloS One, 9*(4).

Lingen, M., Pinto, A., Mendes, R., Franchini, R., Czerninski, R., Tilakaratne, W., . . . Woo, S. B. (2011). Genetics/epigenetics of oral premalignancy: current status and future research*. *Oral Diseases, 17*(s1), 7-22.

Lingen, M. W., Kalmar, J. R., Karrison, T., & Speight, P. M. (2008). Critical evaluation of diagnostic aids for the detection of oral cancer. *Oral oncology, 44*(1), 10-22.

Liu, W., Shi, L.-J., Wu, L., Feng, J.-Q., Yang, X., Li, J., . . . Zhang, C.-P. (2012). Oral cancer development in patients with leukoplakia–clinicopathological factors affecting outcome. *PloS one, 7*(4), e34773.

Liu, W., Wang, Y.-F., Zhou, H.-W., Shi, P., Zhou, Z.-T., & Tang, G.-Y. (2010). Malignant transformation of oral leukoplakia: a retrospective cohort study of 218 Chinese patients. *BMC cancer, 10*(1), 685.

Llewellyn, C. D., Johnson, N. W., & Warnakulasuriya, K. (2001). Risk factors for squamous cell carcinoma of the oral cavity in young people—a comprehensive literature review. *Oral Oncology, 37*(5), 401-418.

Lodi, G., Carrozzo, M., Furness, S., & Thongprasom, K. (2012). Interventions for treating oral lichen planus: a systematic review. *British Journal of Dermatology, 166*(5), 938-947.

Marocchio, L. S., Lima, J., Sperandio, F. F., Corrêa, L., & Sousa, S. O. M. d. (2010). Oral squamous cell carcinoma: an analysis of 1,564 cases showing advances in early detection. *Journal of Oral Science, 52*(2), 267-273.

Martin, I. C., Kerawala, C. J., & Reed, M. (1998). The application of toluidine blue as a diagnostic adjunct in the detection of epithelial dysplasia. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 85*(4), 444-446.

Marx, R. E., & Stern, D. (2003). Oral and maxillofacial pathology. *Chicago: Quintessence*.

Marzouki, H. Z., Tuong, V. V. T., Ywakim, R., Chauvin, P., Hanley, J., & Kost, K. M. (2012). Use of fluorescent light in detecting malignant and premalignant lesions in the oral cavity: a prospective, single-blind study. *Journal of Otolaryngology-Head & Neck surgery. Le Journal d'oto-rhino-laryngologie et de chirurgie cervico-faciale, 41*(3), 164-168.

Mashberg, A., & Samit, A. (1995). Early diagnosis of asymptomatic oral and oropharyngeal squamous cancers. *CA-A Cancer Journal for Clinicians, 45*(6), 328-351.

Masthan, K. M. K., Babu, N. A., Dash, K. C., & Elumalai, M. (2012). Advanced diagnostic aids in oral cancer. *Asian Pacific Journal of Cancer Prevention, 13*(8), 3573-3576.

Matheny, E. S., Mina-Araghi, R., Brenner, M., Hanna, N. M., Jung, W., Chen, Z., & Wilder-Smith, P. (2004). Optical coherence tomography of malignancy in hamster cheek pouches. *Journal of Biomedical Optics, 9*(5), 978-981.

McCullough, M. J., Prasad, G., & Farah, C. S. (2010). Oral mucosal malignancy and potentially malignant lesions: an update on the epidemiology, risk factors, diagnosis and management. *Australian Dental Journal, 55*(s1), 61-65.

Meghana, S., & Ahmedmujib, B. (2007). Surgical artefacts in oral biopsy specimens: Punch biopsy compared to conventional scalpel biopsy. *Journal of Oral and Maxillofacial Pathology, 11*(1), 11.

Messadi, D. V. (2013). Diagnostic aids for detection of oral precancerous conditions. *International Journal of Oral Science, 5*(2), 59-65.

Mogensen, M., Thrane, L., Jørgensen, T. M., Andersen, P. E., & Jemec, G. B. E. (2009). *Optical coherence tomography for imaging of skin and skin diseases. Paper presented at the Seminars in Cutaneous Medicine and Surgery.*

Moro, A., Di Nardo, F., Boniello, R., Marianetti, T. M., Cervelli, D., Gasparini, G., & Pelo, S. (2010). Autofluorescence and early detection of mucosal lesions in patients at risk for oral cancer. *Journal of Craniofacial Surgery, 21*(6), 1899-1903.

Nagao, T., Ikeda, N., Fukano, H., Hashimoto, S., Shimozato, K., & Warnakulasuriya, S. (2005). Incidence rates for oral leukoplakia and lichen planus in a Japanese population. *Journal of Oral Pathology & Medicine, 34*(9), 532-539.

Nair, D. R., Pruthy, R., Pawar, U., & Chaturvedi, P. (2012). Oral cancer: Premalignant conditions and screening-an update. *Journal of Cancer Research and Therapeutics, 8*(6), 57.

Napier, S. S., & Speight, P. M. (2008). Natural history of potentially malignant oral lesions and conditions: an overview of the literature. *Journal of Oral Pathology & Medicine, 37*(1), 1-10.

Neville, B. W. (2009). *Oral and maxillofacial pathology*: Elsevier Brasil.

Neville, B. W., & Day, T. A. (2002). Oral cancer and precancerous lesions. *CA: A Cancer Journal for Clinicians, 52*(4), 195-215.

Oh, E. S., & Laskin, D. M. (2007). Efficacy of the ViziLite system in the identification of oral lesions. *Journal of Ooral and Mmaxillofacial Surgery, 65*(3), 424-426.

Onofre, M. A., Sposto, M. R., Navarro, C. M., & Paulo, S. (2001). Reliability of toluidine blue application in the detection of oral epithelial dysplasia and in situ and invasive squamous cell carcinomas. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 91*(5), 535-540.

Park, B., Pierce, M. C., Cense, B., Yun, S.-H., Mujat, M., Tearney, G., . . . de Boer, J. (2005). Real-time fiber-based multi-functional spectral-domain optical coherence tomography at 1.3 μm. *Optics Express, 13*(11), 3931-3944.

Patel, S. C., Carpenter, W. R., Tyree, S., Couch, M. E., Weissler, M., Hackman, T., . . . Chera, B. S. (2011). Increasing incidence of oral tongue squamous cell carcinoma in young white women, age 18 to 44 years. *Journal of Clinical Oncology, 29*(11), 1488-1494.

Payeras, M. R., Cherubini, K., Figueiredo, M. A., & Salum, F. G. (2013). Oral lichen planus: focus on etiopathogenesis. *Archives of Oral Biology, 58*(9), 1057-1069.

Petersen, P. E. (2003). The World Oral Health Report 2003: continuous improvement of oral health in the 21st century–the approach of the WHO Global Oral Health Programme. *Community Dentistry and Oral Epidemiology, 31*(s1), 3-24.

Piboonniyom, S.-o., Treister, N., Pitiphat, W., & Woo, S.-B. (2005). Scoring system for monitoring oral lichenoid lesions: a preliminary study. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 99*(6), 696-703.

Pitris, C., Jesser, C., Boppart, S. A., Stamper, D., Brezinski, M. E., & Fujimoto, J. G. (2000). Feasibility of optical coherence tomography for high-resolution imaging of human gastrointestinal tract malignancies. *Journal of Gastroenterology, 35*(2), 87-92.

Poh, C. F., Ng, S. P., Williams, P. M., Zhang, L., Laronde, D. M., Lane, P., . . . Rosin, M. P. (2007). Direct fluorescence visualization of clinically occult high-risk oral premalignant disease using a simple hand-held device. *Head & Neck, 29*(1), 71-76.

Prestin, S., Rothschild, S. I., Betz, C. S., & Kraft, M. (2012). Measurement of epithelial thickness within the oral cavity using optical coherence tomography. *Head & Neck, 34*(12), 1777-1781.

Ram, S., & Siar, C. H. (2005). Chemiluminescence as a diagnostic aid in the detection of oral cancer and potentially malignant epithelial lesions. *International Journal of Oral and Maxillofacial Surgery, 34*(5), 521-527.

Rana, M., Zapf, A., Kuehle, M., Gellrich, N.-C., & Eckardt, A. M. (2012). Clinical evaluation of an autofluorescence diagnostic device for oral cancer detection: a prospective randomized diagnostic study. *European Journal of Cancer Prevention, 21*(5), 460-466.

Remmerbach, T. W., Meyer-Ebrecht, D., Aach, T., Würflinger, T., Bell, A. A., Schneider, T. E., . . . Böcking, A. (2009). Toward a multimodal cell analysis of brush biopsies for the early detection of oral cancer. *Cancer Cytopathology, 117*(3), 228-235.

Rethman, M. P., Carpenter, W., Cohen, E. E. W., Epstein, J., Evans, C. A., Flaitz, C. M., . . . Koch, W. M. (2010). Evidence-based clinical recommendations regarding screening for oral squamous cell carcinomas. *The Journal of The American Dental Association, 141*(5), 509-520.

Ribeiro, A. S., Salles, P. R., da Silva, T. A., & Mesquita, R. A. (2010). A review of the nonsurgical treatment of oral leukoplakia. *International Journal of Dentistry, 2010*.

Ridge, J. A., Glisson, B. S., Lango, M. N., Feigenberg, S., & Horwitz, E. M. (2008). Head and neck tumors. *Cancer management: A Multidisciplinary Approach, 11*.

Ridgway, J. M., Armstrong, W. B., Guo, S., Mahmood, U., Su, J., Jackson, R. P., . . . Chen, Z. (2006). In vivo optical coherence tomography of the human oral cavity and oropharynx. *Archives of Otolaryngology–Head & Neck Surgery, 132*(10), 1074-1081.

Roblyer, D., Kurachi, C., Stepanek, V., Williams, M. D., El-Naggar, A. K., Lee, J. J., . . . Richards-Kortum, R. (2009). Objective detection and delineation of oral neoplasia using autofluorescence imaging. *Cancer Prevention Research, 2*(5), 423-431.

Roopashree, M., Gondhalekar, R. V., Shashikanth, M., George, J., Thippeswamy, S., & Shukla, A. (2010). Pathogenesis of oral lichen planus–a review. *Journal of Oral Pathology & Medicine, 39*(10), 729-734.

Roopashree, M. R., Gondhalekar, R. V., Shashikanth, M. C., George, J., Thippeswamy, S. H., & Shukla, A. (2010). Pathogenesis of oral lichen planus–a review. *Journal of Oral Pathology & Medicine, 39*(10), 729-734.

Rubinstein, M., Schalch, P., Di Silvio, M., Betancourt, M. A., & Wong, B. J. (2009). Optical coherence tomography applications in otolaryngology. *Acta Otorrinolaringologica (English Edition), 60*(5), 357-363.

Sahu, R., & Mordechai, S. (2005). Fourier transform infrared spectroscopy in cancer detection.

Schmitt, J. M. (1999). Optical coherence tomography (OCT): a review. *Selected Topics in Quantum Electronics, IEEE Journal, 5*(4), 1205-1215.

Schwarz, R. A., Gao, W., Redden Weber, C., Kurachi, C., Lee, J. J., El-Naggar, A. K., . . . Gillenwater, A. M. (2009). Noninvasive evaluation of oral lesions using depth-sensitive optical spectroscopy. *Cancer, 115*(8), 1669-1679.

Sciubba, J. J., & Group, U. S. C. O. S. (1999). Improving detection of precancerous and cancerous oral lesions: computer-assisted analysis of the oral brush biopsy. *The Journal of The American Dental Association, 130*(10), 1445-1457.

Scully, C., & Carrozzo, M. (2008). Oral mucosal disease: Lichen planus. *British Journal of Oral and Maxillofacial Surgery, 46*(1), 15-21.

Shi, P., Liu, W., Zhou, Z.-T., He, Q.-B., & Jiang, W.-W. (2010). Podoplanin and ABCG2: malignant transformation risk markers for oral lichen planus. *Cancer Epidemiology Biomarkers & Prevention, 19*(3), 844-849.

Silverman, S. (2001). Demographics and occurrence of oral and pharyngeal cancers: the outcomes, the trends, the challenge. *The Journal of the American Dental Association, 132*, 7S-11S.

Sinescu, C., Negrutiu, M., Todea, C., Hughes, M., Tudorache, F., & Podoleanu, A. G. (2008). *Fixed partial dentures investigated by optical coherent tomography. Paper Presented at The Biomedical Optics* (BiOS) 2008.

Speight, P. M., Palmer, S., Moles, D., Downer, M., Smith, D., Henriksson, M., & Augustovski, F. (2006). The cost-effectiveness of screening for oral cancer in primary care. *Health Technology Assessment.*

Suter, V. G., Morger, R., Altermatt, H. J., Spieler, P., & Bornstein, M. M. (2007). [Oral erythroplakia and erythroleukoplakia: red and red-white dysplastic lesions of the oral mucosa--part 2: cytodiagnosis, pathogenesis, therapy, and prognostic aspects]. *Schweizer Monatsschrift fur Zahnmedizin Revue Mensuelle Suisse D'odonto-stomatologie Rivista Mensile Svizzera di Odontologia Stomatologia, 118*(6), 510-518.

Tadrous, P. J. (2000). Methods for imaging the structure and function of living tissues and cells: 1. Optical coherence tomography. *The Journal of Pathology, 191*(2), 115-119.

Thomson, P. (2012). *Oral Precancer: diagnosis and management of potentially malignant disorders*: John Wiley & Sons.

Torres-Rendon, A., Stewart, R., Craig, G., Wells, M., & Speight, P. (2009). DNA ploidy analysis by image cytometry helps to identify oral epithelial dysplasias with a high risk of malignant progression. *Oral Oncology, 45*(6), 468-473.

Trock, B. (2000). Out of the mouths of babes: oral premalignant lesions and use of alternative tobacco products. *Cancer Epidemiology Biomarkers & Prevention, 9*(7), 637-638.

Trullenque-Eriksson, A., Muñoz-Corcuera, M., Campo-Trapero, J., Cano-Sánchez, J., & Bascones-Martínez, A. (2009). Analysis of new diagnostic methods in

suspicious lesions of the oral mucosa. *Med Oral Patol Oral Cir Bucal, 14*(5), E210-216.

Tsai, M.-T., Lee, C.-K., Lee, H.-C., Chen, H.-M., Chiang, C.-P., Wang, Y.-M., & Yang, C.-C. (2009). Differentiating oral lesions in different carcinogenesis stages with optical coherence tomography. *Journal of Biomedical Optics, 14*(4), 044028-044028-044027.

Ujaoney, S., Motwani, M. B., Degwekar, S., Wadhwan, V., Zade, P., Chaudhary, M., . . . Mamtani, M. (2012). Evaluation of chemiluminescence, toluidine blue and histopathology for detection of high risk oral precancerous lesions: A cross-sectional study. *BMC Clinical Pathology, 12*(1), 6.

Van der Waal, I. (2009). Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. *Oral Oncology, 45*(4), 317-323.

van der Waal, I. (2010). Potentially malignant disorders of the oral and oropharyngeal mucosa; present concepts of management. *Oral oncology, 46*(6), 423-425.

van der Waal, I., & Axell, T. (2002). Oral leukoplakia: a proposal for uniform reporting. *Oral Oncology, 38*(6), 521-526.

Walling, D. M., Flaitz, C. M., Adler-Storthz, K., & Nichols, C. M. (2003). A non-invasive technique for studying oral epithelial Epstein–Barr virus infection and disease. *Oral Oncology, 39*(5), 436-444.

Warnakulasuriya, S. (2009). Global epidemiology of oral and oropharyngeal cancer. *Oral Oncology, 45*(4), 309-316.

Warnakulasuriya, S., Johnson, N., & Van der Waal, I. (2007). Nomenclature and classification of potentially malignant disorders of the oral mucosa. *Journal of Oral Pathology & Medicine, 36*(10), 575-580.

Watanabe, A., Taniguchi, M., Tsujie, H., Hosokawa, M., Fujita, M., & Sasaki, S. (2008). The value of narrow band imaging endoscope for early head and neck cancers. *Otolaryngology-Head and Neck Surgery, 138*(4), 446-451.

Wilder-Smith, C. H., Wilder-Smith, P., Kawakami-Wong, H., Voronets, J., Osann, K., & Lussi, A. (2009). Quantification of dental erosions in patients with GERD using optical coherence tomography before and after double-blind, randomized treatment with esomeprazole or placebo. *The American Journal of Gastroenterology, 104*(11), 2788-2795.

Wilder-Smith, P., Jung, W. G., Brenner, M., Osann, K., Beydoun, H., Messadi, D., & Chen, Z. (2004). In vivo optical coherence tomography for the diagnosis of oral malignancy. *Lasers in Surgery and Medicine, 35*(4), 269-275.

Wilder-Smith, P., Lee, K., Guo, S., Zhang, J., Osann, K., Chen, Z., & Messadi, D. (2009). In vivo diagnosis of oral dysplasia and malignancy using optical coherence tomography: preliminary studies in 50 patients. *Lasers in Surgery and Medicine, 41*(5), 353-357.

Wojtkowski, M., Srinivasan, V., Fujimoto, J. G., Ko, T., Schuman, J. S., Kowalczyk, A., & Duker, J. S. (2005). Three-dimensional retinal imaging with high-speed ultrahigh-resolution optical coherence tomography. *Ophthalmology, 112*(10), 1734-1746.

Wong, B. J., Jackson, R. P., Guo, S., Ridgway, J. M., Mahmood, U., Su, J., . . . Armstrong, W. B. (2005). In vivo optical coherence tomography of the human larynx: normative and benign pathology in 82 patients. *The Laryngoscope, 115*(11), 1904-1911.

Yeh, C. J. (2000). Simple cryosurgical treatment for oral lesions. *International Journal of Oral & Maxillofacial Surgery, 29*(3), 212-216.

Yen, A. M.-F., Chen, S.-C., & Chen, T. H.-H. (2007). Dose-response relationships of oral habits associated with the risk of oral pre-malignant lesions among men who chew betel quid. *Oral Oncology, 43*(7), 634-638.

Yen, A. M. F., Chen, S. C., Chang, S. H., & Chen, T. H. H. (2008). The effect of betel quid and cigarette on multistate progression of oral pre-malignancy. *Journal of Oral Pathology & Medicine, 37*(7), 417-422.

Yu, C. H., Lin, H. P., Chen, H. M., Yang, H., Wang, Y. P., & Chiang, C. P. (2009). Comparison of clinical outcomes of oral erythroleukoplakia treated with photodynamic therapy using either light-emitting diode or laser light. *Lasers in Surgery and Medicine, 41*(9), 628-633.

Zainal Ariffin, O., & Nor Saleha, I. (2011). National cancer registry report: Malaysia cancer statistics-data and figure 2007. *Putrajaya, Ministry of Health Malaysia*.

Zhang, L., Williams, M., Poh, C. F., Laronde, D., Epstein, J. B., Durham, S., . . . Le, N. D. (2005). Toluidine blue staining identifies high-risk primary oral premalignant lesions with poor outcome. *Cancer Research, 65*(17), 8017-8021.