Clinical and Histopathological Correlation Between Dysplastic Changes in Oral Potentially Malignant Lesions and Presence of Candida albicans

By
Mahgub Abdulkareem Mahmoud

Supervisor: Professor Ali Mohamed Elmurtadi
Co-supervisor: Professor Mohamed SH Ingafou

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Faculty of Dentistry

Department of oral medicine, oral Pathology, oral Radiology and Diagnosis

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This dissertation was successfully defended and approved. Date: 10 / 04 /2021

Examination Committee Signatures

Supervisor: Prof Ali M Emurtadi

Signature……

Prof.Azam A Sultan (Internal Examiner)

Signature ……..

Prof. Sanousi M Taher (External Examiner)

Signature ……

Dean of Dental Faculty **Director of Graduate studies and training**

…… ……

Dedication

I dedicate my thesis

To the soul of my father,

Abdulkareem El-Sakallia

Mahgub Abdulkareem Mahmoud

Declaration

This is to certify that the work presented in this thesis represents original research carried out by Mahgub Abdulkareem Mahmoud Submitted for partial fulfillment of the requirements for the degree of Master of Science in Oral Pathology according to the regulations of the University of Benghazi.

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List of abbreviations

Term	Meaning
Act	Actinic cheilitis
CanLeu	Candidal leukoplakia
EryP	Erythroplakia
GMS	Grocott's methenamine silver
H&E	Haemtoxylin & eosin
HPVs	Human papilloma viruses
LeukP	Leukoplakia
OED	Oral Epithelial Dysplasia
OEP	Oral Erythroplakia
OLD	Oral lichenoid dysplasia
OLEP	Oral leukoplakia
OLP	Oral Lichen planus
OSCC	Oral Squamous Cell Carcinoma
OSMF	Oral Submucous Fibrosis
PAS	Periodic Acid- Schiff
PMOL	Potentially Malignant Oral Lesion
PVL	Proliferative Verrucous leukoplakia
VH	Verrucous hyperplasia
VL	Verrucous leukoplakia
WHO	World Health Organization

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Abstract

Background: The concept of a two-step process of cancer development in the oral mucosa, starting with an initial presence of a precursor (premalignant, precancerous) lesion which is subsequently develops into frank cancer, is a well-established concept,red lesions such as erythroplakia, leukoerythroplakia, verrucous lesions and ulcerative lesions may represent higher risk, whereas homogenous leukoplakia carries a lower risk dysplasia or malignancy at diagnosis. *Candida albicans* is a highly versatile commensal organism that is well adapted to its human host transition from one of commensals to pathogen, the role of *Candida albicans* as a possible etiological factor in leukoplakia and its possible role in malignant transformation is still unclear.

Aims of this study is to look at the epidemiological features of PMOLs and their clinical presentation and to determine the prevalence of fungal hyphae in 30 tissue sections.

Materials and methods: Data of the clinically suspected and histopath-ologically confirmed 90 (4.7%) cases from total of 1894 biopsied lesions in the period from 1998 to 2010 were obtained from the department archives. Furthermore, thirty biopsies from those were stained with (H & E) first then with PAS stain to look for candidal hyphae.

Results: From these 90 cases, 62 (68.8%) of them were OLP, 20 (22.2%) OLEP, 3 (3.3%) of VL and 2 (2.2%) cases of OSMF. Whereas OEP, actinic cheilitis. Candidial leukoplakia were detected in only one case. Dysplastic changes with varying degrees were noticed in almost all the biopsies except one. Mostly mild 20 cases, moderate 6 cases and severe in 3 biopsies. Candida hyphae were detected in only 2 cases the yeast (blastospore) represented 6.6% of these case in candidal leukoplakia and verrucous leukoplakia cases.

Conclusion: Fungal hyphae in the potentially malignant lesions and conditions is not useful indicator in predicting malignant transformation. The presence of epithelial dysplasia is more important in predicting malignant transformations than the clinical characteristics of the lesion.

INTRODUCTION

Chapter: 1 INTRODUCTION

1.1. Overview

Oral cancer constitutes about 3-20% of all malignancies and poses significant worldwide health problem, especially in developing countries, representing a leading cause of death. According to World Health Organization (WHO), carcinoma of oral cavity in males in developing countries, accounts for the 6th commonest cancer after lung, prostrate, colorectal, stomach and bladder cancer, while in females, it is the 10th commonest site of cancer after breast, colorectal, lung, stomach, uterus, cervix, ovary, bladder, and liver. About 95% of the carcinomas of the oral cavity are of squamous cell type. The 5-year survival rate remained at approximately 50% for several decades (Vokes et al., 1993). The prognosis is far more better for the lesions, which detected and intervened in early stages (Epsteinet al., 2002).

A compelling evidence, including clinical, experimental, and morphological data, support the concept that squamous cell carcinoma of the upper aerodigestive tract (OSCC) are preceded by or coexist with potentially non-invasive lesions of the mucosa. These lesions encompass a histological continuum between the normal mucosa at one end and high-grade dysplasia/carcinoma in situ, at the other end, establishing a model of neoplastic progression. This continuum of pre-invasive neoplasia is encountered in many other epithelia, including the lower respiratory tract and the cervix of uteri (Tepperman et al., 1981).

The identification of pre-neoplastic lesions of the upper aerodigestive tract through clinical, morphological and molecular means helps in the early detection and treatment of head and neck squamous cell carcinoma. Moreover, understanding and documenting the morphological and molecular abnormalities associated with this progression may shed light on the biology of these tumors (Axèll, 1994).

1.1. History of cancer

The idea of precancer has been a slowly changing and often confusing concept, beginning with the 1805 suggestion by European panel of physicians that there are benign diseases which will always develop into invasive malignancy if followed long enough. (Epstein et al., 2002).

Historically, the definition of an oral premalignant lesion dates back to 1973, when it was defined by the World Health Organization (WHO) as "*a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart*" The precancerous condition in its turn is "a generalized state associated with a significantly increased risk of cancer". Examples of precancerous conditions are sideropenic dysphagia, submucous fibrosis and possibly, lichen planus(WHO, 1973).

1.2. The concept of premalignancy

Oral precancer, in particular, have a rich and fascinating literature extending as far back as the 1870s, when James Paget (one of England's most renowned surgeons), proposed that "leukokeratosis" or "smoker's patch" of the hard palate (nicotine palatinus), or the tongue, in inveterate pipe smokers carried an increased risk of eventual cancer transformation. He mentioned that he saw his first cancer transformation in this disease in 1851. Ironically, we no longer consider nicotine palatinus to be a pre-cancer.Another white keratotic lesion, leukoplakia, has demonstrated a far greater risk of malignant transformation, a risk which has been discussed since before 1876, when the Hungarian dermatologist, Schwimmer, first coined the term (Bosatra et al., 1997).

The international consensus on terminology and definitions set in 1997 has subdivided oral precancer into precancerous lesions and pre-cancerous conditions. The concept of a two-step process of cancer development in the oral mucosa, where the initial presence of a precursor (premalignant, precancerous) lesion subsequently developing into cancer, is well-established. (Axéll et al., 1996)

1.3. Premalignant Lesions/Conditions of oral epithelial tissue

Although arising de novo in many instances, a significant proportion of OSCC develop from the so-called premalignant lesions and conditions. A wide range of conditions have been implicated in the development of oral cancer, including leukoplakia, Erythroplakia, palatal lesion of reverse cigar smoking, oral lichen planus like lesions, oral submucous fibrosis, discoid lupus erythematosus, and some hereditary disorders such as dyskeratosis congenita and epidermolysis bullosa (Anonymous 1974).

The concept of a two-step process of cancer development in the oral mucosa, starting with an initial presence of a precursor (premalignant, precancerous) lesion which is subsequently develops into frank cancer, is a well-established concept in literature. Thus, the identification of high-risk oral premalignant lesions and intervention at

premalignant stages could constitute one of the keys to reducing mortality, morbidity and cost of treatment associated with OSCC (Epstein, Zhang et al., 2002).

1.4. Histopathological changes associated with premalignancy

Cancer being a genetic disorder involves multiple alterations of the genome progressively accumulated during a protracted period, the overall effects of which surpass the inherent reparative ability of the cell. In the course of its progression, visible physical changes are taking place at the cellular level (atypia) and at the resultant tissue level (dysplasia). These alterations include genetic changes, epigenetic changes, surface alterations and alterations in intercellular interactions. The sum of these physical and morphological alterations is of diagnostic and prognostic relevance and are designated as 'precancerous' changes. In many situations, the relationship between these changes and the progression towards neoplasia is not understood. Nevertheless, it seems probable that these changes are ultimately involved in driving cells further along the path to neoplastic transformation (Speight and Torres-Rendon; 2011).

1.5. Terminology in the moniker *"potentially malignant lesion"*

A potentially malignant oral lesion (PMOL) has been defined as a *''morphologically altered tissue in which cancer is more likely to develop than in its apparently normal counterpart'',* whereas a precancerous condition is defined as *" generalized state associated with a significantly increased risk of cancer"*. The latter definition signifies that the cancer can arise in any part of the oral cavity and not necessarily in a pre-existing lesion(Warnakulasuriya et al., 2008).

The term ''potentially malignant" was preferred to ''premalignant" or "precancerous". The WHO has periodically organized an international workshop to redefine the term "precancer" and the various oral precancerous lesions. In the workshop organized in London in 2005, the term "precancer" was eliminated and replaced by the presumably more illuminating term "potentially malignant lesion" for oral lesions as the use of the terms such as "oral precancer lesion" and "oral premalignant lesion"" in itself poses problems, since this terminology signifies an inevitable development of cancer from such diseases (Speight, 2007). This is due to an important paradigm about premalignancy, the presence and grade of epithelial dysplasia plays a significant role for future malignant development (Banoczy, 1984; Warnakulasuriya et al.,

2008). Thus, the use of term "potentially malignant" signifies more precisely, what is actually meant. (Johnson et al., 1993).

Oral epithelial dysplasia (OED) is an early cellular change in squamous epithelium usually associated with an increased risk of transformation of the benign oral mucosal lesions into malignancy. *Candida albicans* is an opportunistic infection can be detected in many oral lesions, but its role in the process of malignant transformation is under investigation(Warnakulasuriya et al., 2008).

1.6. Importance of studying potentially malignant lesions

The main purpose of identifying oral premalignant lesions is to prevent malignant transformation in these presumed potentially malignant lesions by initiating adequate intervention, which is widely based on histopathological features of a biopsy of the lesions.For a long time, epithelial dysplasia makes the paradigm whole mark of the potential malignant changes in the lesion. The presence of epithelial dysplasia usually imposes more aggressive approach than in case of no dysplasia (Bouquot at al., 1994).

The diagnosis of precancerous lesion is primarily based on morphology and its grading on histology (dysplasia). Even though this estimation is subjective and therefore carries a low prognostic value of an impending malignancy, it is still widely practiced to assess the risk of malignant potential of such lesions. Because of this inherent discrepancy, such lesions may well be designated as potentially malignant.

A premalignant phase in the development of oral cancer is predicted by the classic model of experimental epithelial carcinogenesis. Virtually all oral squamous cell carcinomas arise from a premalignant precursor, but it is difficult to specifically define the term 'premalignant'. Oral pathologists use the term epithelial dysplasia to indicate microscopic features in a biopsy specimen that are associated with a risk of malignant change and then assign a grade of severity. There is good correlation between higher grades of dysplasia and increasing risk of cancer but less so with the lower grades(Bouquot at al., 1994).

The studies evaluating the prevalence rate of PMOLs associated with increased risk of malignant transformation had helped scientists in conducting awareness programs at community centers and they further helped to promote prevention activities in order to reduce the morbidity and mortality rates especial attention to elevated prevalence in our situation as lichen planus in addition given an idea about PMOLs in our field and further investigated by microbiological, cytological and histopathological

studies supposed to perform with better facility and equipment(Tilakaratne et al., 2019)..

The present study tries to explore any clinical or histopathological evidence of the association between epithelial dysplasia and the presence of *Candida albicans* in several potentially malignant lesions such as leukoplakia, lichen planus, erythroplakia, actinic cheilitis and submucous fibrosis.

REVIEW OF LITERATURE

Chapter: 2 REVIEW OF LITERATURE

2.1. Malignant transformation

James Paget was the first to describe malignant transformation of an oral lesion into carcinoma. In 1877, Schwimmer reported the similar findings. Later, in 2005, WHO coined team "potentially malignant disorders" to describe such lesions (Pallaet al., 2015). Such transformation has a prevalence of 1-5% o in such sites. According to the many researchers, most of PMOLs are detected in the age groups from 30 to 50 years (Saraswathi, 2006, Pindborg, 1977) and that the buccal mucosa, tongue, gingiva and the floor of the mouth are the most sites of occurrence of the PMOLS. According to many studies the buccal mucosa is involved in (47- 77.4%), and the tongue in about (17.4% to 28.8%) (Bhurgri et al in 2005, by Shyam et al in 2014, Suvarna et al., 2018).

2.1.1. Factors associated with malignant transformation of the lesion

Studies on prevalence and rate of malignant transformation gave different results because of differences in the length of observation period, the type of study population, and the therapeutic approach (Pindborg, Reibel et al., 1985,Axell, 1987, Hari Vinay et al., 2014).

It is apparent that in addition to tobacco use, intake of specific nutrients and their deficiency may have a role in the development and progression of oral precancerous lesions. Ascorbic acid (vitamin C) appeared to be protective against leukoplakia with the halving of associated risk of malignant transformation.

2.1.2. Epithelial dysplasia and malignant transformation

Epithelial dysplasia is defined as "a precancerous lesion of stratified squamous epithelium characterized by cellular atypia and loss of normal maturation and stratification short of carcinoma in situ". The term 'epithelial dysplasia' is confined to histopathological changes associated with an increased risk of malignant development. The frequency of epithelial dysplasia in leukoplakia varies between < 1 and > 30% (Mehta et al., 1969).

The individual cellular changes are referred to as atypia, (Pindborg et al., 1997), oral epithelial dysplasia is not associated with any specific clinical appearance, thus

white, red, or mixed white and red changes are those most frequently revealing epithelial dysplasia.

Furthermore, erythroplakia has a high grade of dysplasia or even early invasion carcinoma and it can be as high as 80%. The presence of epithelial dysplasia is generally accepted as one of the most important predictors of malignant development in premalignant lesions (Bouquot et al., 2006).

Oral lesions with epithelial dysplasia more often develop into carcinoma than those without dysplasia (Pindborg et al., 1977), furthermore, features of epithelial dysplasia are commonly seen in epithelium adjacent to oral carcinomas (Katz et al., 1985). However, epithelial dysplasia will not necessarily develop into cancer, and some may even regress (Mincer et al., 1972). Oral epithelial dysplasia is traditionally graded as one of the three categories mild, moderate and sever dysplasia with the use of the architectural changes and cellular changes in the evaluation, (Speight and Torres-Rendon; 2011).

Hyperorthokeratosis, epithelial dysplasia and inflammatory cells in the connective tissue were observed in 87%, 23% and 55% of the biopsies collected from Srikakulam district Melanin deposits were noted in the lamina propria of most of them. The epithelium was atrophic in 60% of biopsies from red areas. Epithelial dysplasia was observed in 52% of red areas, 25% of excrescences, 20% of ulcerations, 10% of patches and in 19% of non-pigmented areas(Daftary et al., 2007).

In a six-year follow-up study, palatal changes remained stationary in 75% of individuals; regressed in 14% and were variable in 11%, i.e. they regressed, recurred and regressed again (Gupta, Mehta et al., 1980). The regression rates were higher when the habit was discontinued or reduced substantially. Malignant transformation was observed for 0.3% of the palatal lesions. In a 10-year intervention study in the same area, the malignant potential of various components of palatal changes was evaluated: red areas and patches were found to exhibit a high potential for malignant transformation.

In two studies from India (Gupta et al., 1980, Silverman et al., 1976), rather low annual malignant transformation rates of oral leukoplakia have been reported, 0.3% and 0.06%, respectively. In reports from western countries, usually based on hospital material, somewhat higher figures have been mentioned (Axell 1987).

2.1.3. Clinical significance of OED

OED is a microscopic diagnosis of immense clinical importance. The initial reports of oral potentially malignant disorders with oral epithelial dysplasia transforming to oral cancer helped in understanding the nature of oral malignancies. Since then, clinical studies on oral potentially malignant disorders have combined microscopic findings of oral epithelial dysplasia to assess the malignant transformation potential of different grades of epithelial dysplasia (Tilakaratne et al., 2019).

Banoczy and co associates studied 500 leukoplakia patients in order to define the characteristics of epithelial dysplasia and to correlate the findings with the clinical data. They appointed that epithelial dysplasia was found in 120 cases (24%) and was graded as mild, moderate, or severe. The occurrence of dysplasia was highest in the group of erosive lesions. The majority of lesions of severe dysplasia were found on the tongue and lips. Follow-up studies on sixty-eight leukoplakia patients with histologic dysplasia revealed carcinoma in nine cases (13.2%) during the mean observation period of 6.3 years. Leukoplakias of the tongue showed the highest incidence of malignant change (Banoczy and Csiba 1976).

The prevalence of transmission from OED to malignancy was studied in different parts of the world. (Bosatra et al., 1997) had retrospectively followed-up for 30 months the evolution of 97 cases of epithelial dysplasia in the head and neck region and mean Dysplastic mucosal areas were observed in the oral cavity in 11 cases, in the pharynx (oro- and hypopharynx) in 39 cases and in the larynx (supraglottic and glottic regions) in 47 cases. Fifty out of the 97 patients developed a squamous carcinoma in the same area, demonstrating a significant direct correlation between age and neoplastic evolution. A direct correlation was also observed between severity of dysplasia and carcinomatous evolution. Further direct correlations were observed between degree of dysplasia, carcinomatous evolution and amount of exposure to cigarette smoke and alcohol.

2.1.4. Grading of potential malignant lesions and of epithelial dysplasia

At a workshop coordinated by the WHO Collaborating Centre for Oral Cancer and precancer in the United Kingdom issues related to potentially malignant disorders of the oral cavity were discussed by an expert group. The consensus views of the working group are presented in a series of papers and agreed for three classification schemes (oral epithelial dysplasia scoring system, squamous intraepithelial neoplasia and

Ljubljana classification for epithelial dysplasia grading) for routine use. Although most oral pathologists possibly recognize and accept the criteria for grading epithelial dysplasia, firstly based on architectural features and then of cytology, there is great variability in their interpretation of the presence, degree and significance of the individual criteria(Warnakulasuriya et al., 2008).

Several studies have shown great interexaminer and intraexaminer variability in the assessment of the presence or absence and the grade of oral epithelial dysplasia. The Working Group considered the two-class classification (no/questionable/ mild - low risk; moderate or severe - implying high risk) and was of the view that reducing the number of choices from 3 to 2 may increase the likelihood of agreement between pathologists. The utility of this need to be tested in future studies. The variables that are likely to affect oral epithelial dysplasia scoring were discussed and are outlined here; these need to be researched in longitudinal studies to explore the biological significance of a low-risk or high-risk dysplasia (Warnakulasuriya, Tilakaratne et al., 2018).

Epithelial dysplasia is usually assessed subjectively and considerable inter-observer variability exists in its interpretation. Principally, what is lacking in this exercise is objectivity and lack of reproducibility. A consensus decision is, by and large, adopted in the management of individual lesions and based on the presence of dysplastic features, epithelial dysplasia is usually divided into three categories: mild, moderate and severe., there is Overall substantial intra- and interobserver consistency and almost perfect conformity in the grading of OED. But an appropriate statistical method is necessary to determine the degree of observer agreement. (Brothwell et al., 2003).

2.1.5. The malignant potentiality of different PMOLs

The indicted charges of an inherent premalignant disposition of OLP is largely vindicated by the supporters of the concept of lichenoid dysplasia as a distinct entity and many cases of lichenoid or lichen planus may represent in fact lichenoid dysplasia (Eversole, 1992), but Oral lichenoid dysplasia (OLD) as proposed by Krutchkoff and Eisenberg, has not found universal acceptance by the pathology community (Sanketh,et al., 2017), although some investigators believe that The malignant transformation rate is higher for oral lichenoid lesions (4.4%) than OLP (1.2%). If the first biopsy (showed intraepithelial Neoplasia (Casparis et al., 2015).

2.1.6. Risk factors for malignant transformation

The use of tobacco is considered as the main risk factor for malignant transformation among high risk individuals are those who have had previous cancer of the upper aerodigestive tract, where recurrent rate within 2 years in these persons is about 10-22% (Tepperman and Fitzpatrick 1981).

2.1.7. Clinical examination for oral premalignant lesions and SCC

Clinical examination for oral premalignant lesions and SCC should include thorough head, neck and intraoral examination with examination of the lymph nodes and visual examination and palpation of the oral mucosal surfaces.

The location, size, border, color and surface characteristics of any lesion should be recovered for future comparisons. Although the clinical appearance alone is not diagnostic and may be confusing due to similarity to various inflammatory and traumatic lesions (Axel et al., 1996, (Bouquot 1994; Bouquot and Whitaker 1994), Silverman et al., 1984).

Red lesions such as erythroplakia, leukoerythroplakia, verrucous lesions and ulcerative lesions may represent higher risk, whereas homogenous leukoplakia carries a lower risk of dysplasia or malignancy at diagnosis (Bouquot and Ephoros, 1995).

2.1.8. Histopathological examination

Histopathological examination of the PMOLs makes a substantial bulk of the total number of the biopsied oral lesions according to several studies (Warnakulasuriya et al., 1984 (4.2%), (Rao et al., 1998) (3.98%), (Lim et al., 2003) (4.2%), (Hari et al., 2014) (4.2%), and (Suvarna et al., 2018) (4.26).

Fragments of the plaque material may be smeared on a microscopic slide, and the organisms may be cultured in a variety of media, including blood agar, cornmeal agar and Sabouraud's broth. Histologic sections of a biopsy from a lesion of oral candidiasis show the presence of the yeast cells and hyphae or mycelia in the superficial and deeper layers of involved epithelium. These are more easily visualized if the sections are stained with PAS or methenamine silver, since the organisms are positive in both instances. Chlamydospores are seldom seen on oral smears or histologic sections.

According to several authors, Candida infection not only causes epithelial hyperplasia but may also induce epithelial atypia, leading to malignant change (Renstrup, 1970). Epithelial atypia was reported in 71% cases of speckled leukoplakia, with 40% of all candidal leucoplakias demonstrating epithelial atypia. In animal models, Candida

infection can induce epithelial dysplasia when inoculated into non-dysplastic hyperplastic lesions (Zhang et al., 1994).

Candidia, (in the presence or absence of PMOL), is naturally existing organism as a commensally harmless microbe in more than 40% of people and it only prevail if there is favorable conditions of unbalanced oral environment which decrease the host immunity. The increased fungal virulence thus induced by local adaptation for invasion by alteration of epithelial layers by the lesions through roughness its surface.

2.1.9. Staging of PMOLs

The value of histology as an indicator of cancer risk is time tested not only in the oral cavity or other head and neck regions, but also in other sites, including the uterine cervix, lung, breast, skin, and esophagus. The evaluation of molecular markers is extensively used as gradual extension of the classical histological and clinical approaches. An increase in abnormality in either histology or genetic markers was associated with an increase in cancer risk. Some investigators advocated the estimation of DNA content in dysplastic lesions with progression risk of oral precancer.

For the sake of simplicity, this hypothetical model only includes pathological and molecular markers (clinical features such as appearance, site, and size of the lesion, are not included but should be part of an eventual screening system). In this model, the majority of lesions would be placed into stage 1, with the lowest probability of progressing to cancer. Such lesions would contain both low-risk pathology (P1) and genetic patterns (G1). The emergence of intermediate-risk patterns in either histology (P2) or genetic profile (G2) would place a lesion into stage 2, while Stage 3 would contain lesions with a high-risk genetic or histology pattern. It should be noted that the greatest impact of such a staging system would be on lesions in stage 3. Such lesions would now include cases with a relatively benign phenotype (without or with minimal dysplasia) but a high-risk genotype (e.g. P1-G3).

2.2. Oral leukoplakia

Oral leukoplakia is *"predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion"* (Axèll, 1996). It is the most common potentially malignant lesion of the oral mucosa (Bouquot and Gorlin 1986). However, the usage of this term should be limited exclusively to the clinical context by the exclusion of other lesions, which present as oral white plaques.

It is not known how many oral squamous cell carcinomas arise from precursor lesions and however many develop from apparently normal oral mucosa. Studies have shown that between 16 and 62% of oral carcinomas are associated with leukoplakic lesions when diagnosed (Bouquot et al., 1988), and an Indian house-to-house survey showed that about 80% of oral cancers were preceded by oral pre-cancerous lesions or conditions (Gupta et al., 1989). Others consider the vast majority of oral cancers to arise from otherwise clinically normal mucosa (Cowan et al., 2001).

There is a general agreement that a pre-cancerous lesion is defined as "a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart", whereas a pre-cancerous condition is defined as "a generalized state associated with a significantly increased risk of cancer".

2.2.1. Etiology

Leukoplakia occurs more frequently in smokers of tobacco than in nonsmokers. There is a dose-response relationship between tobacco usage and the prevalence of oral leukoplakia. Reducing or cessation of tobacco use may result in the regression or disappearance of oral leukoplakia (Guptaet al., 1995). On the other hand, disappearance of oral leukoplakia has occasionally been reported in patients who continued to smoke. Tobacco was most often chewed as an ingredient in betel quid (smokeless tobacco or *paan*) in India. The *paan*-chewers' lesion consists of a thick, brownish-black encrustation on the buccal mucosa at the site of the placement of betel quid. It is often seen in heavily addicted betel quid chewers. It could be scraped off with a piece of gauze (leukoplakia); it regresses spontaneously when the habit is discontinued. Due to these reasons the *paan-chewer's* lesion does not deserve the designation of leukoplakia. This is a specific entity and rarely progresses to leukoplakia. Whether the use of alcohol by itself is an independent etiological factor in the development of oral leukoplakia, is still questionable. Its effect, at best, may be synergistic to other well-known etiological factors (physical irritants).

The possible contributory role of viral agents (human papilloma virus strains (HPV) numbers 16 and 18) in the pathogenesis of oral leukoplakia, particularly with regard to exophytic verrucous leukoplakia is advocated by many investigators. The clinical significance of human papilloma virus-associated epithelial dysplasia, so-called 'koilocytic dysplasia' remains under investigation.

In a study from India, serum levels of vitamin A, B12, C, beta-carotene and folic acid were significantly decreased in patients with oral leukoplakia compared to controls, whereas, serum vitamin E was not. Relatively little is yet known with regard to possible genetic factors in the development of oral leukoplakia.

2.2.2. Histopathological aspects of leukoplakia

It should be emphasized that leukoplakia is a clinical term, and its use carries no implications with regard to the histological findings. However, it is recommended that a histological report should always include a statement on the presence or absence of epithelial dysplasia, and if present, the assessment of its severity.

The hallmarks of the histopathological aspects of leukoplakia are epithelial hyperplasia and surface hyperkeratosis. Epithelial dysplasia, if present, may range from mild to severe. In some instances, carcinoma in situ and even squamous cell carcinoma are encountered histologically. The term 'lichenoid dysplasia' (OLD) is sometimes used when the dysplastic epithelium may show features that, to some extent, resemble those of lichen planus. Compared with other oral lichenoid conditions, OLD lesions are at a particularly high risk of malignant transformation and should be managed based on the presence of dysplasia and not the lichenoid inflammatory infiltrate. OLP demonstrates a relatively low rate of malignant transformation. Diagnostic histopathology is important for discriminating OLP from OLD.

The term '*chevron'* type of keratinization is used when it is associated with use of tobacco. Microabscesses may be observed in the superficial layers of the epithelium in the presence of *Candida albicans* and inflammatory cell infiltration is commonly seen. Some of the exophytic, verrucous or papillomatous lesions, despite the absence of epithelial dysplasia, may in time progress to squamous cell carcinoma and that long-term follow-up should be considered. The final diagnosis of a white lesion of the oral mucosa can often only be made through a close dialogue between the clinician and the pathologist. Even then, cases may remain unsettled.

2.2.3. Epidemiology

The prevalence of oral leukoplakia according to many epidemiological data from different countries over the last 30 years varies from 1% to 13% with a mean value of 3%. (Dambi et al., 2001). The prevalence of leukoplakia in Ernakulam district (Kerala, India) was 17 per 1,000; it was highest (61 per 1,000) among people with mixed habits.

The annual age adjusted incidence rate was 2.1 per 1,000 among men and 1.3 per 1,000 among women; the highest incidence (6.0 per 1,000) was among men who both chewed and smoked. In an adult Swedish population a 3.6% prevalence rate was recorded (Axell 1976). Various studies have estimated that the prevalence of leukoplakia to be between 0.2 to 3.6%, with vast regional variations. It was in India (0.2-4.9%), in (Sweden (3.6%), in Germany (1.6%) and in Holland (1.4%). Generally, the rate of dysplastic or malignant alterations in oral leukoplakia has been reported to be between 15.6% and 39.2% (Mishra et al., 2005; Schepman et al., 1996).

Almost all leukoplakia in India occur in tobacco users. A definite dose-response relationship between leukoplakia and various forms of tobacco use in this area has been demonstrated. The dose-response relationship was stronger for the smoking habit than for the chewing habit and remained significant after taking account of age, gender and type of tobacco habit. The onset of leukoplakia usually takes place after the age of 30 years; resulting in a peak incidence above the age of 50 years. The gender distribution in most studies variesranging from a strong male predominance in different parts in India, to almost 1:1 in the western world (Gupta et al.,1989).

2.2.4. Clinical aspects

Leukoplakia typically occurs in 2-4% of adults and represents more than 80% of all precancerous lesions of the head and neck mucosa. It is more prevalent in males and becomes more common with advanced age. Leukoplakia lesions commonly occur on the vermilion border of lips, the buccal mucosa, and the gingiva (Bouquet and Whitaker 1994).

"Preleukoplakia" is defined as a low-grade or very mild reaction of the oral mucosa, appearing as a gray or grayish-white, but never completely white area with a slightly lobular pattern and with indistinct borders blending into the adjacent normal mucosa. Patients with oral leukoplakia carry a fivefold higher risk of developing oral cancer than controls. Tobacco habits seem to play a major role in malignant transformation rates of oral leukoplakia. The clinical process of leukoplakia is described below (Shirani et al., 2014):

1- Thin leukoplakia appears in a white-gray or gray plaque and may have fissures and wrinkle appearance.

2- Thick leukoplakia is a white plaque with obvious thickness, leathery palpation and numerous deepen fissures.

3- Nodular/glandular leukoplakia is more sever with more surface irregularities.

4- Verrucous/verruciform leukoplakia has sharp or blunt projections.

5- Proliferative verrucous leukoplakia is a high-risk type with multiple keratotic plaque and roughened surface projections.

2.2.5. Diagnosis

Leukoplakia represents an area localized in distribution, hyperkeratotic in nature and white in appearance due to wetting of the keratotic patch while in contact with saliva. It should be stressed that the diagnosis of leukoplakia denotes mainly, that the mucosa is irritated by either mechanical, chemical or galvanic means, and it is trying to adapt to the noxious stimuli by undergoing hyper keratinization of its surface.

A provisional diagnosis of leukoplakia is made when a lesion at clinical examination cannot be clearly diagnosed as any other disease of the oral mucosa with a white appearance. Frictional trauma should be excluded. A definitive diagnosis is made when it is histopathologically examined. The term preleukoplakia is sometimes used when the whiteness is not very distinct and should not be confused with leukoedema. When a biopsy is taken, the term leukoplakia should be replaced by the diagnosis obtained histologically. In other words, leukoplakia denotes a negative diagnosis based on exclusion criteria (Schepman et al 1996).

2.2.6. Clinical classification

On the clinical presentation leukoplakia lesions can either be homogenous or non-homogenous. The homogeneous type is usually asymptomatic, whereas the non-homogeneous (mixed white and red) leukoplakia is often associated with mild complaints of localized pain or discomfort (Mishra, 2005).

In the presence of redness or palpable induration, malignancy may already be present ‘non-homogeneous leukoplakia’ is applicable both to the aspect of color, (i.e. mixture of white and red changes) *"erythroleukoplakia"* and to the aspect of texture, (i.e. exophytic, papillary or verrucous). Regarding the latter lesions, no reproducible clinical criteria can be provided to distinguish (proliferative) verrucous leukoplakia from the clinical aspect of verrucous hyperplasia or verrucous carcinoma (Barns et al., 2005).

2.2.7. Classification and staging system for oral leukoplakia

A proposal for a modified classification and staging system for oral leukoplakia (OLEP) has been presented by (Van der Waal 2018) in which the size of the leukoplakia and the presence or absence of epithelial dysplasia are taken into account. Altogether four clinical stages are recognized:

L1 — Size of leukoplakia <2 cm

L2 — Size of leukoplakia 2–4 cm

L3 — Size of leukoplakia >4 cm

Lx — Size not specified P —

Pathology

P0 — No epithelial dysplasia

P1 — Distinct epithelial dysplasia

Px — Dysplasia not specified in the pathology report.

2.2.8. oral leukoplakia Staging System

Stage I — L1 P0

Stage II — L2 P0

Stage III — L3 P0 or L1 L2 P1

Stage IV — L3 P1

The proposed system should facilitate uniform reporting of treatment or management results of OLEPs in which a biopsy has become available. The system can easily be adjusted by replacing the histopathological criteria of epithelial dysplasia by a clinical subdivision in homogeneous and non-homogeneous leukoplakia for cases in which no biopsy is available. It also could serve as a means for epidemiological studies. It has yet to be shown whether such a staging system may also be helpful in providing guidelines for the management of oral leukoplakia.

2.2.9. Natural History

Oral leukoplakia is a lesion of considerable clinical variability and its appearance may change overtime, with some lesions increasing and some become more severe looking and others become smaller or perhaps disappear altogether (Speight et al., 2011).

2.2.10. Idiopathic leukoplakia

Non-tobacco associated leukoplakia (leukoplakia in non-smokers). It is also referred to as "idiopathic leukoplakia." which is the commonest type of leukoplakia (Pindborg et al., 1997).

2.2.11. Tobacco- induced leukoplakia

Include smoker's palate (leukokeratosis nicotine palatinus), palatal keratosis in reverse smokers and snuff dippers. These lesions are clearly related to tobacco use and, therefore, are usually listed as 'lesions. These lesions are being regarded as 'definable lesions' and are traditionally not described as leukoplakia. Nevertheless, some of these lesions may transform into cancer or it may regress in case of cessation of smoking.

2.2.12. Tobacco-associated leukoplakia

It is also denoted as (leukoplakia in smokers), over a period of time the 'tobacco-induced white lesions' on the continual use of tobacco in patients who smoke cigarettes, cigars or pipes these lesions become persistent and given the term 'tobacco-associated leukoplakia' (Schepman et al., 1996).

2.2.13. Candidal leukoplakia

A hyperplastic epithelial lesion in which the presence of *Candida albicans* is demonstrated. It is also called candida-associated leukoplakia (hyperplastic candidiasis). Clinically, the lesions are symptomless and regress after appropriate antifungal therapy and correction of underlying nutritional or other deficiencies. If the lesions are untreated, a minor proportion may demonstrate dysplasia and develop into carcinomas. In the absence of clinical response to antifungal treatment, it seems preferable to consider such lesion as leukoplakia.

2.2.14. Proliferative Verrucous Leukoplakia and its Related Lesions

Proliferative verrucous leukoplakia (PVL) and verrucous hyperplasia (VH) are two related oral mucosal lesions. The terms; however, are not clinically or pathologically interchangeable. The term PVL is preferably a clinical one, but VH is preferably histological term.

PVL was first described by Hansen et al., in 1985, as a particularly aggressive form of oral idiopathic leukoplakia that has a considerable morbidity and a strong potential for malignant transformation. Diagnosis is often made late in the protracted course of PVL with the disease in an advanced stage when it is especially refractory to

treatment. The histologic spectrum of PVL includes VH, (a histologically defined lesion with varying degrees of dysplasia) to three forms of squamous cell carcinoma (i.e. verrucous, conventional and papillary squamous cell carcinoma). In fact, the absence of epithelial dysplasia in initial stages of histopathologic of PVL, prevents such a white lesion from being recognized as potentially malignant and being suited for an aggressive treatment (Sudbø et al., 2001).

2.3. Erythroplakia

Erythroplakia (*Erythroplasia of Queyrat*) is chronic bright red velvety mucosal plaques which cannot be characterized clinically or pathologically to any other specific diagnosis. Erythroplakia most frequently occurs in males aged 50–70. It is rare in comparison with leukoplakia which is a relatively common condition, with prevalence rate of in the oral cavity of about 1 per 2500 adults. In contrast to leukoplakia, erythroplakia is almost always associated with premalignant changes histologically and is, therefore, the most important precancerous lesion. Many studies have demonstrated that 80–90% of erythroplakia are histopathologically either severe epithelial dysplasia, *carcinoma in situ*, or even invasive carcinoma (Bouquot J et al.,1995).

Erythroplakia has no apparent sex predilection; most cases reported have occurred in the sixth and seventh decades. The etiology of erythroplakia is unknown, although it seems likely that smoking and alcohol abuse are important etiological factors. The Common sites of involvement are the floor of the mouth, tongue, retromolar pad, and soft palate. (Neville et al., 2002).

Just as there are many oral lesions that present clinically there are a number of conditions that appear as red areas such as some dermatoses, inflammatory conditions due to local infection, or a more general subacute or chronic stomatitis associated with the presence of dentures, tuberculosis, fungus infection and other conditions. Some red plaques prove to be early squamous cell carcinomas. The red patches that cannot be classified in any of these classes fall into the group of erythroplakia (a negative diagnosis refer leukoplakia).

The term '*erythroplasia'* was originally used by *Queyrat* to describe a precancerous red lesion that develops on the penis. Oral erythroplakia is clinically and histopathologically similar to the genital process. Whereas red lesions of the oral mucosa have been noted for many years, the use of the term "Erythroplakia" in this context was rare. Erythroplakia lesions are easily overlooked and the true prevalence of the condi-

tion is not known. This blatant underreporting probably reflects the fact that leucoplakias are more likely to be biopsied and emphasizes the lack of appreciation of the significance of erythroplakia clinically. Several clinical variants of erythroplakia have been described by different investigators, but there is no general agreement on classification. Shear, in 1972, described "homogeneous erythroplakia, interspersed with patches of leukoplakia, and granular or speckled erythroplakia".

2.4. Erythroleukoplakia

It is a white-and-red non-homogeneous leukoplakia (erythroleukoplakia) that may be either irregularly flat, nodular (exophytic or verrucous leukoplakia). These types of leukoplakia are often associated with mild complaints of localized pain or discomfort. Proliferative verrucous leukoplakia is an aggressive type of leukoplakia that almost invariably develops into malignancy. This type is characterized by widespread and multifocal appearance, often in patients without known risk factors. In general, non-homogeneous leukoplakia has a higher malignant transformation risk, but oral carcinoma can develop from any leukoplakia (Bouquot,J.E et al., 1994).

The suggestion has also been made to change the term *speckled leukoplakia* to *speckled erythroplakia*, to emphasize the frequency with which this particular lesion is associated with cellular atypia. Many of these lesions are irregular in outline, and some contain islands of normal mucosa within areas of erythroplakia, a phenomenon that has been attributed to coalescence of a number of precancerous foci. The high rate of premalignant and malignant changes noticed in erythroplakia is true for all clinical varieties of this lesion and not solely a feature of speckled erythroplakia (Mishra Met al.,2005).

Histopathologically, epithelium shows a lack of keratin production and is often atrophic, but it may be hyperplastic. This lack of keratinization, especially when combined with epithelial thinness, allows the underlying microvasculature to show through, thereby causing the red color. The underlying connective tissue often demonstrates chronic inflammation.

2.5. Palatal Changes Associated with Reverse Smoking

Reverse *chutta* (crude form of cigar) smoking practiced especially among females of Srikakulam district of Andhra Pradesh, recorded a prevalence of 8.8% of leukoplakia, 4.6% preleukoplakia and 17.9% leukokeratosis nicotine palate. The annual

age-adjusted incidence rates of palatal changes are 24.9 per 1,000 among men and 39.6 per 1,000 among women and the peak incidence was in the 55–64-year age group (Srikakulam data). Palatal changes comprise several components:

1- Keratosis—diffuse whitening of the entire palatal mucosa

2- Excrescences—1–3 mm elevated nodules, often with central red spots

3- Patches—well defined, elevated white plaques

4- Red areas—well defined reddening of the palatal mucosa

5- Ulcerated areas—crater-like areas covered by fibrin

6- Non pigmented areas—areas of palatal mucosa that are devoid of pigmentation.

2.6. Oral Lichen Planus-and lichenoid lesions

Oral Lichen Planus is a relatively common mucocutaneous disorder with varied clinical presentation ranging from white keratotic lesions to red atrophic affections. White variety Lesions are rarely symptomatic, although some degree of soreness and roughness may be experienced while the erosive lesion associated usually with soreness and discomfort (Ingafou et al., 2006, Elmurtadi and Ingafou, 2017).

2.6.1. Epidemiology

The prevalence of this OLP prevalence worldwide 1-2% with no apparent racial predilection and slight female predilection. Lichenoid lesions may be associated with the use of certain drugs such as oral hypoglycemic, anti-hypertensive or NSAIDs or it may be seen as a reactive lesion of buccal mucosa to amalgam restorations (Porter et al., 1997; Brown et al., 1993).

2.6.2. Clinical Aspects

The lesion always occurs on the buccal mucosa and mandibular groove, areas which are in intimate contact with the betel quid. Sometimes seen on the palate and lateral aspects of the tongue.

2.6.3. Histological Features

The lesion shows hyperparakeratinized, or atrophic epithelium, intimate intermingling of lymphocyte cells with an indistinct liquefactive degeneration of the basal cell layer and a band-like inflammatory cell infiltrate containing lymphocytes and plasma cells at lamina propria. There is jagged or saw-toothed rete pegs.

2.6.4. Natural History

Oral lichen planus lesions can persist for a long time and complete remission is rare. Some reported cases of resolution recorded after the removal of amalgam restorations. The question of malignant transformation of such lesions has not been resolved, but certainly some cases of malignancy has arose from long standing oral lichen planus lesions but is most certainly small (Eversole, 1992; Eisenberg and Krutchoff, 1992).

2.6.5. Association with candida

(Jainkittivonget al., 2007) found candida in 76.7% OLP patients and 40% of the controls. They indicated that topical steroids induce Candida growth and the associated risk factors are age, medication use, and the wearing of dentures.

2.6.6. Association with dysplasia

(Odukoyaet al., 1985), studied 100 cases of oral lichen planus were reviewed with 100 nonspecific oral mucosal inflammatory lesions as a control group for the presence of dysplasia. Mild dysplasia was found in 57% of cases versus 32% of cases in control, moderate dysplasia in 9% versus 10% in control, and severe dysplasia in 2% of cases versus no cases in control. It has been suggested that, while mild or moderate dysplasia may not indicate precancerous potential, severe dysplasia in lichen planus may signify the development of a precancerous lesion.

2.7. Oral Submucous Fibrosis

Oral submucous fibrosis (OSF) is a chronic, progressive, scarring disease, that predominantly affects people of South-East Asian origin. This condition was described first by Schwartz (1952) while examining five Indian women from Kenya. The WHO definition for an oral precancerous condition “a generalized pathological state of the oral mucosa associated with a significantly increased risk of cancer accords” well with the characteristics of OSF. According to Pindborg 1977, if only the fibrous band was the criterion for diagnoses, the prevalence rate would have been about 1.6% with general female preponderance.

The onset is insidious, over two to five years. This includes a burning sensation in the mouth when consuming spicy food, appearance of blisters especially on the palate, ulcerations or recurrent generalized inflammation of the oral mucosa, excessive salivation, defective gustatory sensation and dryness of the mouth. As the disease progresses, the oral mucosa becomes blanched and slightly opaque, and white fibrous bands appear. The buccal mucosa and lips may be affected at an early stage, although it was

thought that the palate and the faucial pillars are the areas involved first. The oral mucosa is involved symmetrically (with possible exception) and the fibrous bands in the buccal mucosa run in a vertical direction

There is compelling evidence to implicate the habitual chewing of areca nut with the development of OSF. It occurs predominantly in the Indian subcontinent where the habit is more prevalent. The frequency of this habit in population affected by OSF ranged from 34–100% (Bhonsle et al., 1987).

On the basis of the histopathological appearance of stained (H&E) sections, OSF can be grouped into four clearly definable stages: very early, early, moderately advanced and advanced. These stages are based not only on the amount and nature of the subepithelial collagen, but also on the following criteria taken together.

2.8. Actinic cheilitis

Actinic cheilitis is lip changes induced by chronic exposure to ultraviolet radiation and shows solar elastosis, a feature that has been associated with mast cell infiltrates (Santana, Nagata et al., 2020). Fair-skinned individuals whose skin does not tan well and who sustain prolonged occupational or recreational exposure to direct sunlight are at greater risk of developing squamous cell carcinoma of the lower lip. The prevalence is highest in Australia, where a light-skinned population is common and outdoor sports are very popular activities (Drake et al., 1995). Overall, actinic keratosis is estimated to be present in 40-60% of the Australian population older than 40 years (Frost and Green, 1994). The prevalence of actinic cheilitis is higher in men than in women, due to the greater likelihood of men have an outdoor occupation and thus have greater cumulative UV exposure (Alasche, 2000).

The epithelium of the lip undergoes a series of preneoplastic changes that become progressively worse as the dose of actinic radiation accumulates and the patient ages. The sharp ridge or line of demarcation at the vermilion and cutaneous junction on the lower lip is replaced by a puffy, rounded margin, and the skin develops multiple vertical creases. The exposed mucosal surface becomes mottled, consisting of red (atrophy) and white (hyperorthokeratosis) patches, and displays prominent superficial blood vessels (telangiectasia). This accumulation of changes is termed actinic cheilitis (also termed solar cheilosis, solar keratosis, or solar elastosis). But No significant differences in the thicknesses of lamina propria and zone of solar elastosis were observed according to the grade of epithelial dysplasia (Gonzaga,et al., 2020).

As time and exposure progress, recurring chronic ulcers frequently develop on the lip, usually lateral to the midline. Eventually the ulcers stop healing, at which point a biopsy usually reveals that a superficial well-differentiated squamous cell carcinoma has developed. Treatment of the altered tissue before the development of malignancy usually consists of superficial surgical removal of the damaged tissue (lip stripping, lip shave). When biopsy reveals the presence of invasion, surgical wedge resection is usually adequate treatment unless metastasis has occurred.

2.9. Syphilitic glossitis

In syphilitic glossitis, the surface of the tongue gets broken up by fissures due to atrophy and fibrosis of tongue musculature; and hyperkeratosis frequently follows. Syphilitic glossitis is found almost exclusively in males. The predilection for syphilitic glossitis to undergo carcinomatous transformation has been recognized for many years. The incidence of such malignant transformation has been as high as 30% in various reported series. However, in the series of Meyer and Shklar, the development of epidermoid carcinoma in luetic glossitis occurred in only 19% of the cases. In a separate study, the same authors reported that only 7.5% of the patients in a series of 210 cases of carcinoma of the tongue had a history of syphilis. The prominent apparent decrease in the relationship between syphilis and lingual carcinoma was suggested to be related to the early and intensive treatment of the disease with antibiotics (Shafer,et al.,2012)

2.10. Plummer-Vinson Syndrome

Plummer-Vinson syndrome (Paterson-Brown-Kelly syndrome (sideropenic dysphagia) is one manifestations of iron-deficiency anemia and was first described by Plummer in 1914 and by Vinson in 1922 (Soames et al.,1998).

Iron deficiency is the most prevalent single deficiency state on a worldwide particularly in women and children, in some parts of the world, this may reach 50%. Men are only rarely affected however; this deficiency is more common than has been realized. Changes include formation of an esophageal web, spooning of the nails (koilonychia), normoblastic arrest in the bone marrow and microcytosis, anisocytosis, and hypochromia of the erythrocytes in the peripheral blood. Sore tongue, similar to that found in nicotinic acid and riboflavin deficiencies, has been described in the iron deficiency anemia. The iron deficiency leading to this anemia usually arises through chronic blood loss as in patients with a history of profuse menstruation or inadequate dietary

intake or faulty iron absorption and in cases of increased requirements for iron, as during infancy, childhood and adolescence and during pregnancy.

Few studies have been reported on the histopathologic changes occurring in the tissues of human beings or experimental animals with iron deficiency anemia. Iron deficiency is an exceedingly prevalent form of anemia, particularly in females. The syndrome is associated with high risk of development of OSCC in esophagus and larynx.

2.11. Oral Epithelial Dysplasia

Oral Epithelial Dysplasia (OED) is a loss in uniformity of the individual cells, as well as a loss in their architectural orientation. Dysplasia is encountered principally in the epithelia. It comprises Dysplastic cells exhibit considerable pleomorphism (variation in size and shape) and often possess deeply stained (hyperchromatic) nuclei, which are abnormally large for the size of the cell. The nuclear: cytoplasmic ratio increases from 1:4 to 1:1, at the expense of the cytoplasmic volume. Mitotic figures are more abundant than usual, although almost invariably they conform to normal pattern. Frequently, the mitoses appear in abnormal locations within the epithelium and may appear at all levels rather than in its usual basal location.

OED is a spectrum of architectural and cytological epithelial changes caused by accumulation of genetic changes The usual proliferative organization of the epithelium is lost and is replaced by a disorderly arranged scramble of cells with varying degrees of differentiation arrest, and is associated with an increased risk of progression to squamous cell carcinoma (Tilakaratne et al., 2019).

2.11.1. Etiology

Dysplasia “Dysplasia is characteristically associated with protracted chronic irritation or inflammation”, of which oral cavity is a common site.

Due to its peculiar anatomic location and its constant presence of endogenous and exogenous microorganisms, a sustained state of chronic subclinical infection is maintained at the oral cavity. Overlying physical trauma consistently inflicted on the oral mucosa, compounds the existing situation.

A role of Human papillomaviruses (HPVs) which were implicated in the etiology of many benign and malignant mucosal lesions in both human beings and animals was suggested in the etiology epithelial dysplasia as well (Abdelsayed 1991).

"*Dysplasia is a reversible*, and therefore presumably a controlled, cellular alteration. When the underlying inciting stimulus is removed, the dysplastic alterations revert to normal." However, there is an important and significant cellular change discerned at the morphological level, which cautions its likelihood of subsequent neoplastic transformation. The rate at which epithelial dysplasia will progress from its mildest to its most severe forms will vary considerably among individuals and may range from months to years. In some cases, removal appears to slow the rate of progression to a more severe form. It appears doubtful that the moderate and severe forms of epithelial dysplasia can regress by simply removing the causative factor. When the dysplastic cells breach the basement membrane and enter the adjacent connective tissue, it is considered to be a superficially invasive or microinvasive squamous cell carcinoma (Sapp et al., 2004).

The line of demarcation when the cell surpasses the point of no return is rather faint and the underlying mechanism poorly defined. In short, dysplasia giving rise to neoplasia is akin to cellular changes in response to a noxious stimulus. As it is evident it traverses the stage of cellular adaptation, reversible damage, and finally, irreversible cell death. When a susceptive cell is exposed to a carcinogen it probably tries to adapt to it, depending on the extent and duration of stimuli. An increase in cell proliferation, diminishing the cytosolic volume and the associated organellar load, could be an attempt in this direction.

2.11.2. Pathogenesis

In the context of oral epithelium, an accelerated growth phase depicted by broadening the progenitor compartment (hyperplasia) is the earlier sequela of exposure to an irritant. When the irritant persists, the epithelium shows features of cellular atrophy, again a well characterized feature of adaptation (atrophy). At a later stage when the stages of adaptation and reversible cell damage surpass, the cells progressively slip into a stage of irreversible cell damage; manifests either as cell death or neoplastic transformation. It appears that some mysterious line is crossed, whereby dysplastic cells escape the normal homeostatic controls and assume the autonomy of a tumor cell. The accelerated pace of cell division noted at the earlier stages of transformation as part of an adaptive response (to replace the damaged cell pool) is, in a way, facilitative of the accumulation of further genetic damage, thereby driving the cells further along the path of transformation.

There is considerable overlap between the stages of cellular adaptation, reversible damage and atypia. Precise morphologic and genetic criteria delineating this line of demarcation between atypia (reversible damage) and neoplasia is unfortunately unknown and is the scope of the study of tumor biology replication readiness with roughly equal cell cycle times and their migration towards the surface layers is simply by physical process of forward push induced during waves of cell division. Experimental work to elucidate these underpin concepts and radical revisions are made (Leblond et al., 1964). Between 1969 and 1975, a number of workers showed that these concepts were too simple, which suggested that a complex pattern of migration from the basal layer to the surface had to occur to maintain this highly ordered and non-random cell stacking. This, in turn, focused attention on the pattern of cell proliferation in the basal layer and led to the concept of the existence of a number of proliferative subpopulations, of which the stem cell population seemed to be of particular importance for tissue homeostasis and disease. This concept envisages that not all cells in the basal layer can divide, that a number are instead already differentiating, that cells capable of division comprise a number of subpopulations and finally, that cell migration is not caused by cell proliferation pressure but is an independent biological process.

The loss of epithelial antigens in the dysplastic oral epithelium has long been suggested as a predicting sign for the malignant potential the oral lesions (Bovopoulou, Sklavounou et al., 1985), as well as the presence of an abnormal DNA content in oral epithelial dysplasia lesions with an increased risk of progression to carcinoma (Sudbo J.et at ., 2001).

The histologic connotation of dysplasia to premalignancy is marked by aberrant and uncoordinated cellular proliferation depicted basically at cellular level (atypia), reflections of which could be discerned at tissue levels too (dysplasia). Frequently it is the forerunner of cancer, the underlying mechanism in cellular transformation is not clear. (Speight et al., 2011).

2.11.3. Diagnosis of OED

The features of OED have been well-described and other architectural features, in particular verrucous and papillary architecture, bulky epithelial proliferation and epithelial atrophy. Proliferative leukoplakia, verrucous or otherwise, often show only hyperkeratosis in early lesions, with development of OED occurring over time, and squamous cell carcinoma developing in the majority of cases over time (Woo 2019).

Traumatic/frictional keratoses are often mistaken clinically for leukoplakia and it is important for the pathologist to recognize and report them as such (Woo 2019).

1% toluidine blue (tolonium chloride) solution applied topically with a swab or as an oral rinse can be helpful in differentiation between different types of lesions with malignant change and other early squamous cell carcinomas from benign inflammatory lesions of the oral mucosa. This technique gives excellent results in detecting epithelial dysplasia with false-negative (underdiagnosis) and false positive (overdiagnosis) rates of well below 10%.

The concept of hyperkeratosis without features of OED and that is not reactive, is likely a precursor to the dysplastic phenotype. Many cases of leukoplakia exhibiting OED are associated with a band of lymphocytes at the interface and these should not be mistaken for oral lichen planus. (Woo 2019).

CD24 immunostaining intensity scoring may serve as a helpful technique to assist with the histological recognition of dysplasia in oral biopsies, but not for distinguishing between grades of dysplasia.(Abdulmajeed, Dalley et al., 2013). Strong expression of maspin in the middle third of the epithelium may be considered a diagnostic sign of mild-to-moderate dysplasia and an indication of carcinoma in the upper third. The correlations between maspin and controlling factors (e.g. p63 and p53) may be events with key roles in the development of tongue carcinoma (Veredet al., 2009).

2.11.4. Grading of epithelial dysplasia.

Epithelial dysplasia is graded as mild, moderate, severe, and carcinoma in situ. Distinctions between mild, moderate, and severe are made on the basis of a histological examination. Knowledge of the degree of dysplasia assists with diagnostic decision-making and helps to predict whether the lesion will progress to cancer or will resolve on its own after removal of the irritant.

It is recommended that the histological report of a leukoplakia should include a statement on the absence or presence of epithelial dysplasia and an assessment of its severity. The practical value of the grading of epithelial dysplasia is questionable. Although leukoplakia with moderate or severe epithelial dysplasia shows a greater disposition for malignant transformation than in the absence of dysplastic features, carcinomatous transformation may also take place in non-dysplastic leukoplakia(Warnakulasuriya 2001).

2.11.5. Histopathological stages in epithelial potential malignant lesions

1- *Squamous hyperplasia*: This may be in the spinous layer (acanthosis) and/or in the basal/parabasal cell layers (basal cell hyperplasia); the architecture shows regular stratification without cellular atypia.

2 -*Mild dysplasia*: The architectural disturbance is limited to the lower third of the epithelium accompanied by cytological atypia.

3 -*Moderate dysplasia*: The architectural disturbance extends into the middle third of the epithelium; consideration of the degree of cytological atypia may require upgrading.

4 -*Severe dysplasia:* The architectural disturbance involves more than two thirds of the epithelium; architectural disturbance into the middle third of the epithelium with sufficient cytologic atypia is upgraded from moderate to severe dysplasia.

5 *Carcinoma in situ*: Full thickness or almost full thickness architectural disturbance in the viable cell layers accompanied by pronounced cytological atypia. It is the highest grade of dysplasia and consists of abnormal cells that have not invaded adjacent tissue.

2.11.6. Clinical significance

OED had long been implicated in many cases of malignancy in different parts of the body, namely uterine cervix, lungs and oropharynx (Alvarez 1968), (Asteet al., 1986).

Treatment of severe OED lesions had significantly reduced cancer progression, and phenotypic changes at the site of the disease had significant predictive value for cancer progression (Zhanget al., 2016).

Oral epithelial dysplasia does not follow a predictable sequential progression from mild to moderate to severe. It is not uncommon for a mild dysplasia to rapidly progress to an invasive carcinoma; however, not all epithelial dysplasia will develop into carcinoma. (Shulman, and Gonzales, 2008).

2.11.7. Histopathological identification and diagnosis

Flores-Hidalgo, 2019 studied the infiltrating intraepithelial T lymphocytes and suggested the localization of CD8+ cells can be potentially useful as an adjunctive diagnostic procedure to distinguish oral epithelial dysplasia from other inflammatory entities, such as lichen planus.(Flores-Hidalgoet al., 2019).

2.11.8. Malignant transformation of oral lesions with OED

A direct correlation was also observed between severity of dysplasia and carcinomatous evolution. Further direct correlations were observed between degree of dysplasia, carcinomatous evolution and amount of exposure to cigarette smoke and alcohol (Bosatra et al., 1997).

A wide range of molecular changes associated with progression of dysplasia to squamous cell carcinoma were found. These include loss of heterozygosity, dysregulation of apoptosis, aberrant DNA expression, and altered expression of numerous tissue markers. Based on the literature search, no single molecular pathway has been identified as the primary factor in progression of dysplasia to squamous cell carcinoma (Brennan, et al., 2007).

2.11.9. Worldwide studies on dysplasia and oral cancer

(Gupta et al., 1998) carried out a population-based case control study in Gujarat, India found 318 cases with leukoplakia among 5,018 male tobacco users. (Daftary et al., 2007) found that palatal involvement was noted in 422 (85%) of the 497 leukoplakia cases and in 168 (57%) of the 296 preleukoplakias, and of course in all of the cases of leukokeratosis nicotina palatinus. Palatal changes associated with reverse smoking thus exhibited a spectrum of clinical changes, and it was not satisfactory to group them under leukoplakia, preleukoplakia or leukokeratosis nicotina palatinus. Accordingly, a newer classification for palatal changes encompassing the entire spectrum of clinical components was proposed by (Mehta et al., 1977).

Singh et al evaluated the correlation between presence of Candida organisms and epithelial dysplasia in various oral mucosal lesions associated with areca nut and tobacco use. In 50 individuals aged 19-70 years and found that out of 50 biopsy specimens stained for presence of Candida using PAS stain, samples of only 2 participants demonstrated presence of Candida in hyphal form, whereas the biopsy specimens stained for demonstrating dysplastic changes using H&E stain displayed various levels of cellular atypia in samples of 16 participants. Out of these 12 were mild, 3 were moderate & 1 displayed severe dysplastic changes and concluded that a statistically non-significant correlation between the presence of Candida and epithelial dysplasia in oral mucosal lesions (Singh et al., 2014).

In a study of 223 biopsies a statistically significant positive association between fungal infection and moderate and severe epithelial dysplasia was observed (Barrett et al., 1998). An analysis of subsequent biopsies showed that epithelial dysplasia associ-

ated with fungal infection significantly worsened over time in comparison with non-infected epithelial dysplasia. Another study by McCullough (using the oral rinse technique) also described a significant correlation between epithelial dysplasia and the overall degree of oral yeast carriage in 223 patients. Intriguingly, in 44.6% of patients who had a histopathological diagnosis of either epithelial dysplasia or oral squamous cell carcinoma, the frequency of oral yeast carriage was significantly greater ($p < 0.001$) than that in those without such histopatholgically demonstrable lesions.

Santana and co-associates have evaluated the epithelial dysplasia in 52 cases of actinic cheilitis and the solar elastosis and found that the mast cell density increased with epithelial dysplasia worsening and this was not associated with elastosis area or collagen loss. (Santana et al., 2020).

2.12. Oral fungal infection

Fungi are eukaryotic microorganisms that are more closely related to humans than bacteria at cellular level. They belong to the group Eumycota, and are chemoheterotrophs with a chitinous cell wall. More than 100,000 species have been described. Most species grow as multicellular filaments called hyphae-forming mycelium such as molds; some species also grow as single cells like yeasts. Some groups of fungi are pathogenic to humans and require control measures. Human fungal pathogens belong to four main groups, namely *zygomcetes, ascomycetes, deuteromycetes, and basidiomycetes. Candida sp*, *Fusarium sp,* is normally avirulent in healthy people but could be disseminated to deep tissue and cause fatal disease in unhealthy people (Chakrabarti 2005; Reedy et al., 2007).

Candida exists in three forms namely, pseudohyphae, yeast, and chlamydospore forms. (Hayens and Westerneng 1996). It reproduces by asexual budding and forms pseudo hyphae. These species grow rapidly at 25–37°C. In general, *candida* species differ from one another but can be identified by the formation of pseudo hyphae or by biochemical test.

It has been shown repeatedly that this microorganism is a relatively common inhabitant of the oral cavity, gastrointestinal tract, and vagina of clinically normal persons. When the favorable condition develops, the organism transforms into pathogenic form (i.e. that is yeast form transformed into hyphae). Thus, it appears that the mere presence of the fungus is not sufficient to produce the disease.

This disease is said to be the most opportunistic infection in the world. Its occurrence has increased remarkably since the prevalent use of antibiotics, which destroy the normally inhibitory bacterial flora and immunosuppressive drugs, particularly corticosteroids.

Oral candidiasis, commonly referred to as "thrush," is an opportunistic fungal infection that commonly affects the oral mucosa. The main causative agent, *Candida albicans,* is a highly versatile commensal organism that is well adapted to its human host; however, changes in the host microenvironment can promote the transition from one of commensalism to pathogen. This transition is heavily reliant on an impressive repertoire of virulence factors, most notably cell surface adhesins, proteolytic enzymes, morphologic switching, and the development of drug resistance.

2.12.1. Virulence and Pathogenicity of candida

Pathogenesis is the ability of a microorganism to infect the host and produce disease resulting from interaction of pathogen with host via expression of certain factors on both sides. Determinants of pathogenicity are called virulence factors. Several determinants including genes or gene products such as enzyme molecules known as virulence factors are involved in this relationship, producing superficial to invasive infections in humans.

Virulence refers specifically to a property of the pathogen and, according to modern definitions, virulence is the ability of a pathogen to multiply and cause harm to its host (Casadevall 2007). Like any other microbial pathogen, fungal infection also involves some basic steps such as:

1- Entry and adherence to the host tissue:Fungi rarely cause disease in immunocompetent hosts, though often exposed to infectious spores. Disease results when fungi accidentally penetrate host barriers or when immunologic defects or other debilitating conditions exist that favor fungal entry and growth (Hogan et al., 1996). There must be actual penetration of the tissues, although such invasion is usually superficial and occurs only under certain circumstances. Infection of a host starts with the adherence of fungi at epithelial surface layers and further dissemination to different host sites. Invasion of various tissues and resistance to attack by the host immune system is necessary for a pathogen to establish infection. Adherence of candida cells to host tissues is a complex multifactorial phenomenon utilizing several types of adhesins expressed on morphogenetically changing cell surfaces. However, the striking feature of Candida

cells is the formation of biofilms in host tissue, resulting in enhanced adherence. (Ramage et al., 2006).

2- Invasion of the host tissue:When fungi enter the mammalian host, their life-style changes from saprophytic to parasitic and start to induce transcriptional and translational changes that promote survival under the newest environmental conditions. Fungi often develop morphogenetic virulence mechanisms, e.g., formation of yeasts, hyphae, and spherules that facilitate their multiplication within the host at higher temperature. Yeast cells of many Candida species form filamentous pseudo hyphae and hyphae in tissues. As Carbon siderophores, high affinity iron chelators, to be efficiently bind host iron into fungal cytoplasm (Haas et al., 2008). *Candida albicans*, the response to hypoxia is dependent on coordination of specific transcriptional regulators; for example, transcription factor ACE2 represses oxidative metabolic processes and promotes filamentation (Mulhern et al., 2006).and metal ions are lacking in host tissues; fungi encode certain mechanisms by employing.

3- Multiplication, colonization and dissemination in the tissues:As saprophytes, fungi survive in an environment with a moderate ambient temperature and pH, essential nutrients such as carbon and metal ions, and atmospheric concentrations of carbon dioxide and oxygen. A fungus utilizes various mechanisms to deceive or destroy the immune cells and spread to various organs. Dissemination depends on interactions of factors from host and fungi facilitated by severe endocrinopathies and immune disorders.

4- Evasion of the host immune system and damage to the tissues: Candidal infections are a serious problem in individuals with weakened immune defense. The overall, severity of disease depends on factors such as inoculum size of the attacking pathogen, magnitude of tissue damages, ability of fungi to multiply in the tissue, and the immune status of the host cells. For a fungus to survive in its niche it has to adapt to constantly changing parameters.

2.12.2. Host Factors

Immune cells are the major antagonists to the survival of fungal pathogens inside the host. However, primary resistance to fungal invasion and colonization is contributed by cutaneous and mucosal physical barriers. The non-specific host defenses include (1) the antifungal activity of saliva and sweat, (2) the competition for space and nutri-

ents by the normal microbiota, and (3) the mechanical barrier of the skin and mucous membranes which prevent entry of fungi. (4) Inflammatory systems by the action of neutrophils, mononuclear phagocytes, and other granulocytes beside the cell-mediated immunity regulated by T-lymphocytes (Wanner et al., 1996).

In addition, healthy individuals employ a second line of defense formed by neutrophilic granulocytes. They mainly attack hyphae, which are too large for ingestion. These in turn are killed by oxidative and non-oxidative mechanisms, including different defensins.

2.12.3. Fungal Factors

Production of extracellular enzymes such as *keratinases, collagenases, gelatinases, phospholipases, lipases,* and *acid proteinases* by *Candida species,* is considered to be the fungal-associated factor that helps fungi in nutrient uptake, tissue invasion, adherence, and dissemination inside the host. The pathogenicity of fungal pathogens such as adherence, dimorphism, phenotypic switching, secretion of hydrolytic enzymes, biofilm formation, and ability to adapt at host body temperature are some of the well-known virulence factors among pathogenic fungi and are discussed in relation to *Candida albicans.*

Virulence factors help the pathogen to grow at elevated temperatures, facilitate adherence, penetration and dissemination, or assist in resistance against innate immune defenses, e.g., phagocytosis and complement, evasion from adaptive immune defenses, or nutritional and metabolic factors, necrotic factors, or morphology variation.

As a commensal. Candida resides in yeast form and multiplies by budding into blastospores, but during weakened immunity of the host it transforms into the hyphal form as the start of pathogenesis (Cladera one and Fonzi, 2001).

Filamentous forms are more adhesive due to increased expression of adhesins on the surface, and also secretion of a higher amount of hydrolytic the enzyme enhances invasiveness results in deep tissue penetration and the establishment of infections. biofilms-forming capacity has greatly increased the potency of Candida to convert from the commensal stage into a virulent pathogen.

2.12.4. Clinical features of Oral Candidiasis

Candidiasis encompasses secondary or opportunistic infections ranging from acute, sub-acute, and chronic to life-threatening mycoses. The four clinical patterns seen are:

Pseudomembranous Candidiasis.This clinically appears as white to yellowish white plaques which can be easily scraped off, exposing red areas. The lesions are usually extensive, involving more than one site in the oral cavity. It may also extend to involve the oropharynx and esophagus.

Erythematous Candidiasis.This is clinically seen as red lesions, which are commonly located on the dorsum of the tongue, palate, and buccal mucosa. Tongue lesions are also referred to as central papillary atrophy.

Hyperplastic Candidiasis lesions are characterized by white plaques which cannot be removed by scraping. Diagnosis can be confirmed by biopsy, which demonstrates the fungal hyphae in the keratinized layers of the epithelium. This lesion can be differentiated from other oral white lesions as these respond to topical or systemic antifungal therapy mixed infection of *Candida albicans* and Staphylococcus or candida alone.

*Angular Cheilitis*Erythema and/or fissuring and/or scaling of the angles of the mouth clinically characterize this lesion. Microbiologically, the lesion can be due to mixed infection of *Candida albicans* and *Staphylococcus aureus* or Staphylococcus or Candida alone.

*Denture Stomatitis.*The pathological reactions of the denture-bearing palatal mucosa appear under several titles and terms such as denture-induced stomatitis, denture sore mouth, denture stomatitis, inflammatory papillary hyperplasia, and chronic atrophic candidiasis. The term denture stomatitis is used with the prefix Candida-associated if the yeast Candida is involved. In the randomized populations, the prevalence of denture stomatitis is about 50% among complete denture wearers.

2.12.5. Diagnosis

Candidiasis is diagnosed by its clinical appearance, PAS staining for candida hyphae, of biopsied tissue or smears from lesion, or culturing the organism on Sabouraud's agar.

2.12.6. The relationship between candida and leukoplakia

The role of *Candida albicans* as a possible etiological factor in leukoplakia and epithelial dysplasia and its possible role in malignant transformation is still unclear.

(Cawson, and Lehner, 1968) were the first to suggest a possible etiological relationship between leukoplakia and candidal infection on finding mycelial elements of *Candida albicans* infiltrating the cornified layer of the epithelium in some cases of human oral leukoplakia. But other authors have regarded *Candida albicans* as a secondary invader rather than the cause of the leukoplakia in those cases in which it is found.

About 10% of oral leucoplakias satisfy the clinical and histological criteria for chronic hyperplastic candidiasis (candidal leukoplakia). Epithelial dysplasia was reported to occur four to five times more frequently in Candida leukoplakia than in leukoplakia in general. However, this change is more common in the speckled variant than in homogeneous leukoplakia and carcinomatous change is more a characteristic of the speckled lesion than that of candidal superinfection. Various kinds of evidence have been presented to justify an etiologic role for Candida in neoplastic transformation, which includes, among others, the catalytic transformation in vitro of the carcinogenic nitrosamine, N-nitrosobenzyl-methylamine, by strains of *Candida albicans* demonstrated to be selectively associated with leukoplakia. Candida and epithelial dysplasia have long been associated with oro-mucosal lesions (Singh et al., 2014).

2.12.7. The role of candida in oral cancer

The role of fungal infections has been studied in this respect and holds much promise as such an indicator. (Cawson, 1966). Studies on the association of Candida and leukoplakia date back to 1970s. There is a longstanding discussion whether Candida infection is a cause of leukoplakia or if it is a superimposed infection in a preexisting lesion.

Among the fungi, *Candida albicans* is the most common microorganism to pose a possible risk factor for the malignant transformation in premalignant lesions and conditions. Few studies have also indicated that presence of candidal infection may increase the risk of a premalignant lesion and conditions turning malignant (Neville and Day, 2002).

Leukoplakia on clinical ground that were histologically chronic hyperplastic candidiasis showed a higher rate of malignant transformation on follow-up. Animal studies have shown that Candida can cause epithelial hyperplasia and cellular atypia.

It has been shown that, upon treatment, non-homogeneous candida infected leukoplakia converted into homogeneous lesion, and some lesions even regressed. Leukoplakia with candidal infection has a higher rate of malignant transformation than

uninfected leukoplakia. The ability of *Candida albicans* to colonize, penetrate, and damage host tissues depend on the imbalance between *Candida albicans* virulence factors and host defenses, often due to specific defects in the immune system. Certain strains of *Candida albicans* and other yeasts play a causal role in the development of oral cancer by means of endogenous nitrosamine production.

The association of Candida with various precancer and cancer lesions has been reported as a causative agent. Candida can then produce carcinogenic compounds, like nitrosamines, N-nitrosobenzylmethylamine. Strains with high nitrosation potential were isolated from lesions with more advanced precancerous changes.

The yeast cells in such cases extends from the mucosal surface to the deeper epithelial cell layers representing transport and deposition of precursors like nitrosamines to deeper layers. This showed that certain strains of *Candida albicans* play a key role in the development of dysplasia. The association of Candida with premalignant states has been studied extensively and many authors have shown an increase in Candida colonization in these lesions as compared to controls. Further, this persistent infection along with other cofactors may also induce epithelial atypia and dysplasia leading to malignant change. The majority, if not all potentially malignant conditions are systemic disorders that result in atrophy of the oral mucosa resulting in increased penetrance of carcinogens will ultimately progress to invasive carcinoma and as such warrant early aggressive treatment from those that will regress and can safely be left alone (Neville and Day, 2002). Nevertheless, literature shows very few studies linking the association of dysplasia and related lesions with Candida. The association of *Candida albicans* with potentially malignant and malignant cases has been investigated by various authors under microbiological (Ariyawardana, 2007) cytological (Rashmi et al., 2009) and histopathological studies (Jarvensivu, 2006).

Hence, this study was designed to evaluate the prevalence of oral potentially malignant lesions and conditions among Libyan people through proximate twelve years and assessment the relations ship between fungal hyphae in most common potentially malignant lesions and conditions.

2.12.8. Histochemistry in study of candidiasis

Most fungi in sections may be demonstrated at least in part by Gram's method. Gridley's method (1953) is the best for their selective demonstration, florescent method of Culling and Vassar, gives bright yellow fungi on a dark back ground. All fungi are

periodic acid- Schiff (PAS) positive these techniques may be used for general demonstration. The reactivity of the PAS technique is based on the structure of the monosaccharide units.

In a histopathological study of 44 cases, Wang studied oral candidiasis and found out that the micro abscess in superficial epithelium is the histologic symbol of oral candidiasis. (Wang, 1991). So, for those cases with micro abscess, PAS stain should be done in order to make definite diagnosis.

PAS stains consist of 1% periodic acid and Schiff's reagent (Basic fuchsin, Potassium, metabisulphate, Hydrochloric acid, Deactivated charcoal and Distilled water). The reaction is based on the fact that certain tissue elements are oxidized by the periodic acid, one of the reaction products being an aldehyde. Such aldehydes are then demonstrated with Schiff reagent to produce colored product.

Basic fuchcin in the mixture reacts with the newly formed aldehyde groups in the tissue producing a bright magenta color. Hematoxylin is then typically used as a counterstain to visualize other tissue elements. PAS stain histochemistry of carbohydrates in the tissue thus used in demonstration of the following:

1) Polysaccharides: The technique is commonly used to identify polysaccharides. The main polysaccharide identified via histology staining in human and animal tissue sections is glycogen, this is present in numerous tissues,
2) Neutral mucus substances: It is also commonly used to stain and identify neutral mucus substances. These can include glycoproteins, glycolipids, and neutral mucins, which are produced by epithelial cells in different organs.
3) Tissue basement membranes: These PAS-positive thin layers of reticular connective tissue anchor and support epithelium and endothelium to underlying connective tissue.
4) Fungal organisms: The cell walls of some fungal organisms contain high levels of carbohydrate, and also stain PAS-positive. However, this only works on living fungi while Grocott's methenamine silver (GMS) will stain both living and dead fungal organisms can be used to help in diagnosis of:
    a) -glycogen storages diseases: These are conditions in which excessive quantities of glycogen are stored in the liver, muscles, or kidney tumors: Glycogen granules can also be present in some tumors.
    b) -fungal infection: PAS can be used to visualize some fungal organisms in tissue sections.

c) -Basement membranes: Since PAS can be used to highlight the basement membranes of tissues, it can be used to identify disorders in which there is weakness or improper functioning of basement membranes.

2.12.9. Types of PAS stain technique

1) Hotchkiss' buffered alcoholic PAS technique (1948).
2) periodic acid- methenamine sliver technique (Gomori, 1946; Grocott, 1955) result in PAS positive black brown and background is light yellow green.
3) Gridley method (1955) result in PAS stain hyphae deep blue, candida give rose to purple.

Aims of the study

Chapter: 3 AIMS OF THE STUDY

1-To study the demographic features of potentially malignant cases such as age, sex and their clinical presentation.

2- To examine the correlation between the presence of *Candida albicans* and the epithelial dysplasia in a number of potentially malignant lesions.

MATERIAL AND METHODS

Chapter: 4 MATERIAL AND METHODS

The present study was undertaken through retrieving archived records from of the Oral Pathology in faculty of Dentistry University of Benghazi representing all the cases in oral pathology department during 12 years.

4.1. General Clinical characteristics of PMOLs

The study employing clinically suspected and histopathologically diagnosed ninety (90) cases of potentially malignant lesions and conditions comprising sixty-two (62) cases of lichen planus, (20) leukoplakia, (3) verrucous leukoplakia, (2) oral submucous fibrosis, (1) erythroplakia, (1) actinic cheilitis and (1) case of candidal leukoplakia.

4.2. Setting

This study was carried out using the facilities provided by the histopathology laboratory in the faculty of Dentistry University of Benghazi.

4.3. Materials

1. The clinical and laboratory records of 1894 cases diagnosed in the department and retrieved from its achieves in the period between 1998 and 2010..
2. Biopsies retrieved from the paraffin-embedded tissue blocks in the department stores and prepared by the techniques described below.
3. Light microscope type (Leica).
4. Digital camera
5. Computer software.

4.4. Criteria for inclusion in the study

There were no definitive criteria taken in selection of the cases such as exclusion diabetic patients, immune compromising patients and patient receiving any corticosteroid therapy. It should be noted that all cases included cases of oral lichen planus are only those which were severe, amenable to therapy,unusual site or previously found to have some degree of epithelial dysplasia.

Positive control used in this study was a section of the case of known candida leukoplakia, whereas negative control was a normal oral mucosa.

4.5. Histopathological examination

The second step of the study is a histopathological examination of 30 biopsies diagnosed clinically and confirmed histopathologically as potentially malignant oral lesion obtained from the archives of the Oral Pathology department involved. The cases included represented lichen planus (12), leukoplakia (10), verrucous leukoplakia (3), oral submucous fibrosis (2), erythroplakia (1), actinic cheilitis (1), and candida leukoplakia (1).

Paraffin-embedded tissue blocks of previously diagnosed cases of thirty (30) potentially malignant lesions and conditions. Relevant information of age, sex and histopathological grading of dysplasia was obtained from the records of the patients.

4.6. Tissue preparation

Tissue preparation was started with cutting serial sections of paraffin-embedded 4 µm thick histological slides from tissue blocks by rotary microtome machine. The cut sections were stained with H & E to confirm the diagnosis and to check the availability of the tissue, so to decide whether to include them. The sections were inspected and examined by light microscope.

After that, thirty slides were cut at the beginning and stained with hematoxylin and Eosin to explore the behavior of epithelium in regard to presence of epithelial hyperplasia, hyperkeratosis, superficially located microabscess or chronic inflammatory infiltrate in the lamina propria of mucosa and to detected the configuration and degree of epithelial dysplasia.

All section taken included normal mucosa beside the areas affected by the lesion for evaluation of the alteration and the abrupt changes or transition from normal to abnormal tissues such as atrophy or ulceration. Other features such as the location, shape and extension of the rete ridges in relation to lamina propria.

4.6.1. Procedure for staining by Mayer's hematoxylin:

(1) Deparaffinization by using xylene for 5 minutes.

(2) Treated with absolute alcohol 2 changes in one minute.

(3) Wash water.

(4) Stained in hematoxylin in 10 minutes.

(5) Washed in running water.

(6) Stained in 1% aqueous eosin Y for 3 minutes.

(7) Wash in running water one minute.

(8) Dehydrated in 3 changes absolute alcohol in one minute.

(9) Cleared in 2 changes xylene.

(10) Mounting using resinous mounting media e.g. Canada balsam or synthetic resin .

4.6.2. PAS stain Procedure

1-Immersed in distilled water.

2-Oxidize for 5 minutes in 1% aqueous periodic acid.

3-Washed in running water for 5 minutes and rinse in distilled water.

4-Treated with Schiff reagent for 15 minutes.

5- Washed for 10 minutes in running water.

6-counter-stain with Mayer's hematoxylin for 5 minutes.

7-Dehydrate in alcohol and mounted in Canada balsam.

These aldehydes then react with the Schiff reagent to give a purple-magenta color (bright red) while the nuclei is blue. Both these two types of satins hematoxylin, eosin (HE) and periodic acid Schiff (PAS) were visualized in under light microscope for pervious purpose.

4.7. Statistical analysis

The obtained data were tabulated in a spreadsheet and analyzed. Descriptive analysis of the data was done to obtain information about the frequencies, mean, median, standard deviation, range, maximum and minimum and the percentage.

RESULTS

Chapter: 5 RESULTS

5.1. Clinical characteristics of the PMOLs group

The total number of biopsies for various oral conditions done in the period between 1998 and 2010 was 1894. Among them, there were 90 (4.7%) for suspected lesions of being premalignant. The general characteristics of these patients are detailed below.

5.1.1. Gender of the patients with PMOLs

The total number of patients with suspected PMOLs was 90 patients from both sexes. 42(46.6%) of them were males and 48 (53.4%) were females as shown in(Figure: 1).

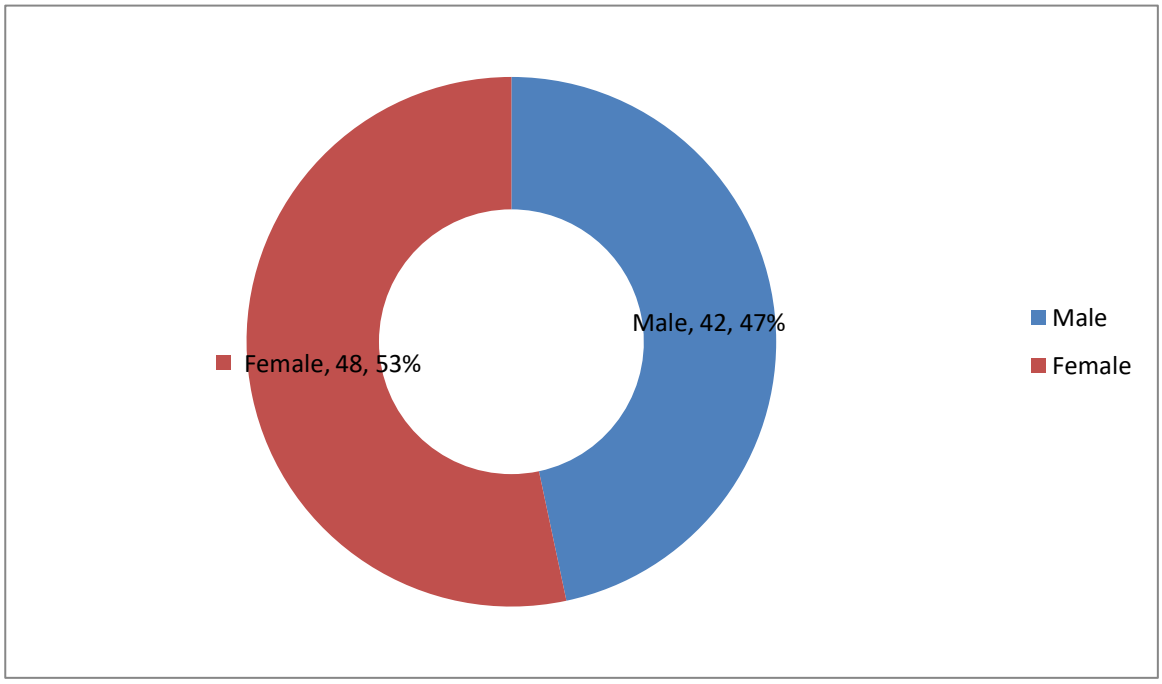

Figure 1: Gender of the patients with PMOLs

5.1.2. The type of PMOLs

The total number of the clinically suspected and histopathologically confirmed cases of potentially malignant oral lesions (PMOLs) was 90 cases, 62 (68.8%) of them were OLP, 20 (22.2%) cases of oral leukoplakia, 3 (3.3%) of VL and 2 (2.2%) cases of OSMF. Whereas erythroplakia, actinic cheilitis and candidial leukoplakia were detected in only 1 lesion for each of them as shown in (Table 1) and (Figure 1).

Table 1: Type of PMOLs

PMOLs*	**No.**	**Percentage**	**Percent from total number of biopsies**
OLP	62	68.8%	3.50%
LeukP	20	22.2%	1.00%
VL	3	3.3%	0.15%
OSMF	2	2.2%	0.10%
EryP	1	1.1%	0.05%
Act	1	1.1%	0.05%
CanLeu	1	1.1%	0.05%
Total	90	100%	4.70%

*OLP=Oral Lichen planus, LeukP =Oral Leucoplakia, VL=Verrucous leucoplakia, OSMF=Oral submucous fibrosis, EryP=Erythroplakia, Act=Actinic cheilitis, CanLeu=Candidal leukoplakia.

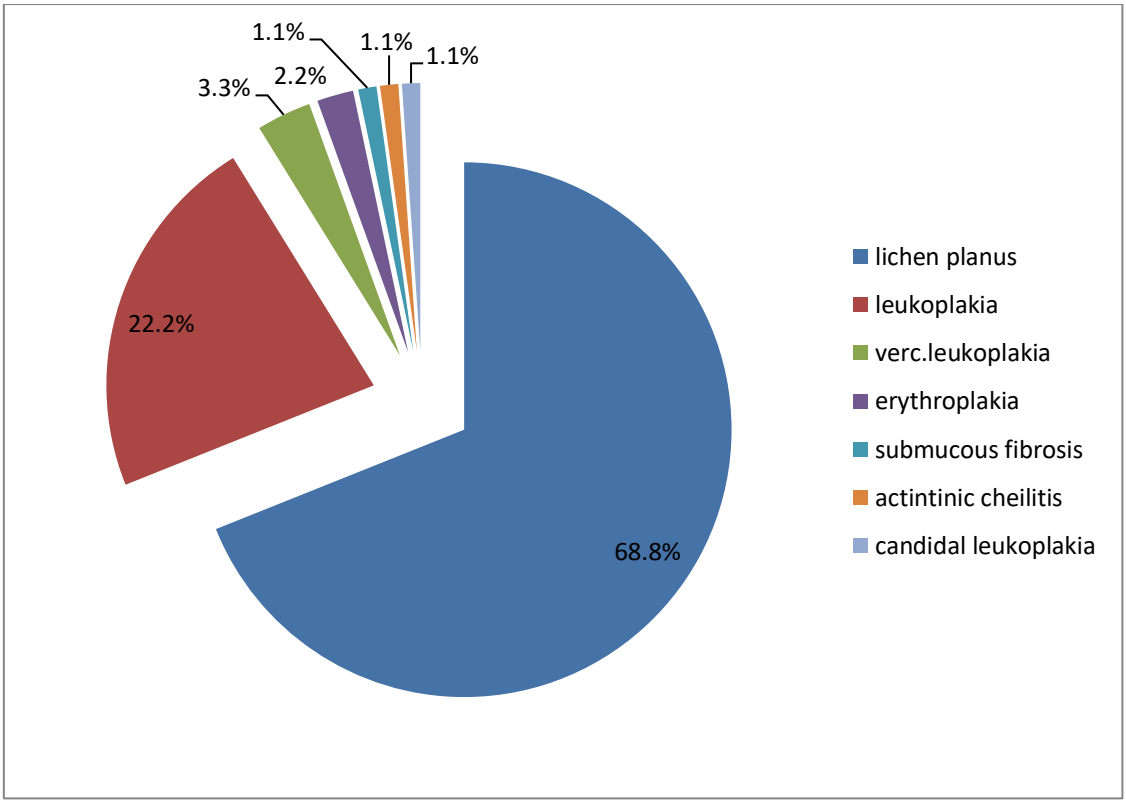

Figure 2: The prevalence of different types of POM lesions

5.1.3. The site of potential malignant lesions

The buccal mucosa alone was involved in 59 patients, labial mucosa alone was involved in 10 cases, tongue alone was involved in (9) 10% patients and the palate alone was involved in 3 cases. Multiple sites involvement was recorded in fewer cases. Both buccal mucosa and tongue were involved together in (5) 5.5% cases, while there was an involvement of buccal mucosa, lips and gingiva in (4) 4%, cases as shown in (Table 2).

Table 2: Site of PMOLs.

Site	**No of cases**	**Percentage**
Buccal mucosa	59	65%
Labial mucosa	10	11%
Tongue	9	10%
Buccal mucosa and tongue	5	5.5%
Buccal mucosa, lips and gingiva	4	4%
Palate	3	3%
Total	90	100%

5.1.4. Age of the patients with PMOLs

Age of the patients was ranged from 15 to 75 years, and the median was 45 years, which indicates that the middle age patients (35-55 years) are more liable to have such lesions. As shown in (Table 3) and (Table 4).

Table 3: Age of the patients with PMOLs

Interval	Frequency	Percentage
15-25 years	8	8%
25-35 years	14	15%
35-45 years	23	25%
45-55 years	22	24%
55-65 years	16	17%
>65 years	7	7%
Total	90	100%

Table 4: Descriptive statistics of ages of the PMOLs.

PMOL	No.	Age		Mean	Median	Standard Deviation
		Minimum	Maxmum			
OLP	62	15	68	45.9	49	15.7
LeukP	20	15	80	43.3	40	16.9
VL	3	26	65	51.6	64	22.2
OSMF	2	11	55	33	33	31.1
EryP	1					
Act	1					
CanLeu	1					

OLP=Oral Lichen planus, LeukP =Oral Leucoplakia, VL=Verrucous leucoplakia, OSMF=Oral submucous fibrosis, EryP=Erythroplakia, Act=Actinic cheilitis, CanLeu=Candidal leukoplakia.

5.1.5. Distribution of PMOLs according to gender

There were 48 cases of females and 42 males. There were more cases of females (38) OLP in comparison to males (24), whereas the cases with leukoplakia more in males (12) in comparison with females (8). there were relatively small number of patients in other varieties for comparison as shown in (Table 5).

Table 5: Distribution of PMOLs according to gender

Type of PMOLs	Female	Male	Total
OLP	38	24	62
LeukP	8	12	20
VL	1	2	3
OSMF	1	1	2
EryP	0	1	1
Act	0	1	1
CanLeu	0	1	1
Total	48	42	90

OLP=Oral Lichen planus, LeukP =Oral Leucoplakia, VL=Verrucous leucoplakia, OSMF=Oral submucous fibrosis, EryP=Erythroplakia, Act=Actinic cheilitis, CanLeu=Candidal leukoplakia.

5.1.6. Sites of different PMOLs

Buccal mucosa was the most site affected by different PMOLs followed by lip then the tongue as it is clear form (Table 6).

Table 6: Sites of different PMOLs

Type of PMOLs	Buccal mucosa	lip	Palate	Tongue	Buccal mucosa &lip	Buccal mucosa & tongue
OLP	45	5	1	3	4	4
LeukP	10	2	2	6	0	0
VL	2	1	0	0	0	0
OSMF	1	1	0	0	0	0
EryP	0	0	0	0	0	1
Act	0	1	0	0	0	0
CanLeu	1	0	0	0	0	0
Total	59	10	3	9	4	5

OLP=Oral Lichen planus, LeukP =Oral Leucoplakia, VL=Verrucous leucoplakia, OSMF=Oral submucous fibrosis, EryP=Erythroplakia, Act=Actinic cheilitis, CanLeu=Candidal leukoplakia.

5.1.7. Age groups of different PMOLs

Most of the lesions were detected at first time at the ages between 35 to 55 years as shown in (Table 7).

Table 7: Age groups of different PMOLs

Type of PMOLs	**Age range (years)**						**Total**
	< 25	25-35	35-45	45-55	55-65	>65	
OLP	4	9	15	18	13	3	62
LeukP	2	3	8	3	1	3	20
VL	0	1	0	0	2	0	3
OSMF	1	0	0	0	0	1	2
EryP	0	0	0	0	1	0	1
Act	0	1	0	0	0	0	1
CanLeu	0	0	0	0	1	0	1
Total	7	14	23	21	18	7	90

OLP=Oral Lichen planus, LeukP =Oral Leucoplakia, VL=Verrucous leucoplakia, OSMF=Oral submucous fibrosis, EryP=Erythroplakia, Act=Actinic cheilitis, CanLeu=Candidal leukoplakia.

5.2. Oral Lichen planus

Only three clinical types of lichen planus were biopsied in this group of patients; namely plaque-like type (35 patient), erosive type (23 patients) and bullous type (4 patients). More than half (51%) of the biopsies were taken from patients aged (35-55) years as shown in (Table 8).

Table 8: Age groups of OLP lesions

Interval (years)	**Frequency**	**Percentage**
15-25	4	6%
25-35	9	14%
35-45	15	24%
45-55	18	29%
55-65	13	20%
65-75	3	3%

5.2.1. Site of lesions of oral lichen planus

Sixty-two biopsies were taken from 38 females and 24 males with OLP lesions involved different sites in the mouth. 45 (72.5%) of them involved buccal mucosa alone, and 3 cases involved the tongue, 5 cases involved the lips and only 1 case involved the palate while multiple site involvement was reported in the remaining 8 cases as shown in (Table 9).

Table 9: Site of lesions of oral lichen planus lesions

Site	**Frequency**	**Percentage**
Buccal mucosa	45	72.5%
Tongue	3	4.8%
Lips	5	8.0%
Buccal and tongue	4	6.4%
Buccal and lip	4	6.4%
Palate	1	1.6%
Total	62	100%

5.2.2. Clinical type of OLP biopsied as suspected PMOL

The most frequent type of OLP lesions biopsied as being suspected PMOLs was plaque like (35) lesions (35), erosive (23) and bullous (4) as illustrated in (Figure 2).

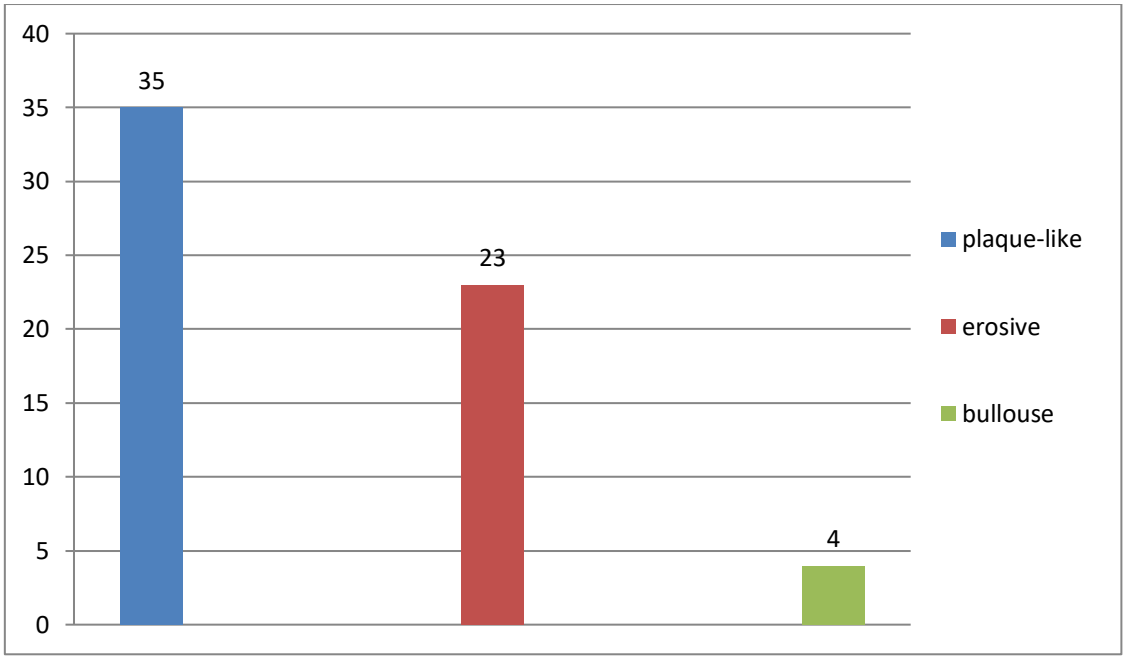

Figure 3: Clinical types of oral lichen planus encountered in this study.

5.3. General features of cases with oral Leukoplakia

There were 20 patients with oral leukoplakia, (8 females and 12 males), their ages ranged from 15-75 years, 55% of the patients in the age group between 35 years and 55 years as shown in (Table 10). Most of the oral leukoplakia lesions were taken from buccal mucosa, tongue and lip as shown in Figure 4.

Table 10: Age groups of leukoplakia patients

Interval	**Frequency**	**Percentage (%)**
15-25	2	10%
25-35	3	15%
35-45	8	40%
45-55	3	15%
55-65	1	5%
65-75	3	15%

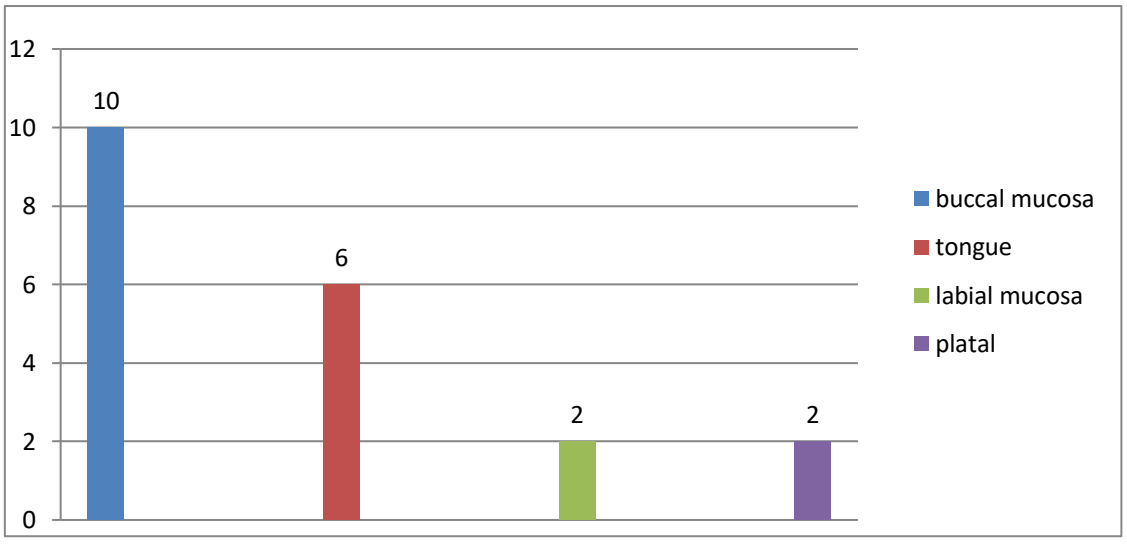

Figure 4: The sites of oral leukoplakia lesions

5.4. Biopsies studied for dysplasia and candida

The second phase of this study is to examine histopathologically a total of 30 case of previously confirmed potentially malignant lesions to look for epithelial dysplasia and the prevalence of existence of candida hyphae in those lesions.

This study included 12 cases of lichen planus, 10 leukoplakia, 3 verrucous leukoplakia, 2 submucous fibrosis, 1 Erythroplakia, 1 Actinic cheilitis and 1 Candidal leukoplakia. Their ages ranged from 25 to 65 years, as shown in (Table 11).

Table 11: Age groups of cases with PMOLs studied for dysplasia and candida

Lesions	**Age range (years)**						**Total**
	<25	25-35	35-45	45-55	55-65	>65	
OLP	1	0	7	4	0	0	12
LeukP	1	2	3	2	1	1	10
VL	0	1	0	0	2	0	3
OSMF	1	0	0	0	1	0	2
EryP	0	0	0	0	0	1	1
Act	0	1	0	0	0	0	1
CanLeu	0	0	0	0	1	0	1
Total	3	4	10	6	5	2	30

OLP=Oral Lichen planus, LeukP =Oral Leucoplakia, VL=Verrucous leucoplakia, OSMF=Oral submucous fibrosis, EryP=Erythroplakia, Act=Actinic cheilitis, CanLeu=Candidal leukoplakia.

5.4.1. Distribution of PMOLs studied for dysplasia and candida

Most biopsies were taken from buccal mucosa; however, the rest of the cases were taken from tongue, lips and palate respectively as shown in (Table 12).

Table 12: Distribution of PMOLs studied for dysplasia and candida

Type of PMOLs	**Buccal mucosa**	**Lip**	**palate**	**Tongue**	**Total**
OLP	8 (26%)	0	1 (3%)	2 (6%)	11 (36.6%)
LeukP	5 (16%)	2 (6%)	1 (3%)	2(6.6%)	10 (33%)
VL	2 (6%)	1 (3%)	0	2 (6%)	3(10%)
OSMF	1 (3%)	1 (3%)	0	0	2 (6.6%)
EryP	0	0	0	1 (3%)	1 (3.3%)
Act	0	1 (3%)	0	0	1 (3.3%)
CanLeu	1 (3%)	0	0	0	1 (3.3%)
Total	17 (56%)	5(16%)	2 (6%)	6(20%)	29(100%

OLP=Oral Lichen planus, LeukP =Oral Leucoplakia, VL=Verrucous leucoplakia, OSMF=Oral submucous fibrosis, EryP=Erythroplakia, Act=Actinic cheilitis, CanLeu=Candidal leukoplakia.

5.4.2. Prevalence of epithelial dysplasia in PMOLs studied for dysplasia and candida

Dysplastic changes with varying degrees were noticed in almost all the biopsies except one. Mild epithelial dysplasia detected in 20 cases, moderate dysplasia in 6 cases and severe epithelial dysplasia in 3 biopsies as shown in (Table 13).

Table 13: Grades of epithelial dysplasia in various types of PMOLs

Lesion	No of cases	Degree of epithelial dysplasia			
		No dysplasia	Mild dysplasia	Moderate dysplasia	Severe dysplasia
OLP	12	1	10	0	1
LeukP	10	0	6	3	1
VL	3	0	1	1	1
OSMF	2	0	2	0	0
EryP	2	0	0	1	0
Act	1	0	1	0	0
CanLeu	1	0	0	1	0
Total	30(100%)	1 (3.3%)	20 (66.6%)	6 (20%)	3 (10%)

OLP=Oral Lichen planus, LeukP =Oral Leucoplakia, VL=Verrucous leucoplakia, OSMF=Oral submucous fibrosis, EryP=Erythroplakia, Act=Actinic cheilitis, CanLeu=Candidal leukoplakia.

5.4.3. Prevalence of fungal hyphae in PMOLs studied for dysplasia and candida

Although almost all the cases had some degree of dysplastic changes except one case, candida hyphae were detected in only 2 cases of them and the yeast (blastospore) represented 6.66% of these cases.

Oral lichen planus revealed various kinds of dysplasia; but no candida was detected in any case of them, whereas in leukoplakia, although dysplastic changes were mild and moderate in some of them, there were no candidal hphae in any one of them.The case of candidal leukoplakia and verrucous leukoplakia had positive candidal hyphae (Table 14).

Table 14: Prevalence of Fungal Hyphae in the Lesion with Dysplastic Change.

Type of PMOLs	**No. of cases**	**Positive hyphae or blastospores**	**Negative hyphae or blastospores**
OLP	11	0	11
LeukP	10	0	10
VL	3	1	2
OSMF	2	0	2
EryP	1	0	1
Act	1	0	1
CanLeu	1	1	0
Total	29	2	27

OLP=Oral Lichen planus, LeukP =Oral Leucoplakia, VL=Verrucous leucoplakia, OSMF=Oral submucous fibrosis, EryP=Erythroplakia, Act=Actinic cheilitis, CanLeu=Candidal leukoplakia.

5.5. Histopathological examination

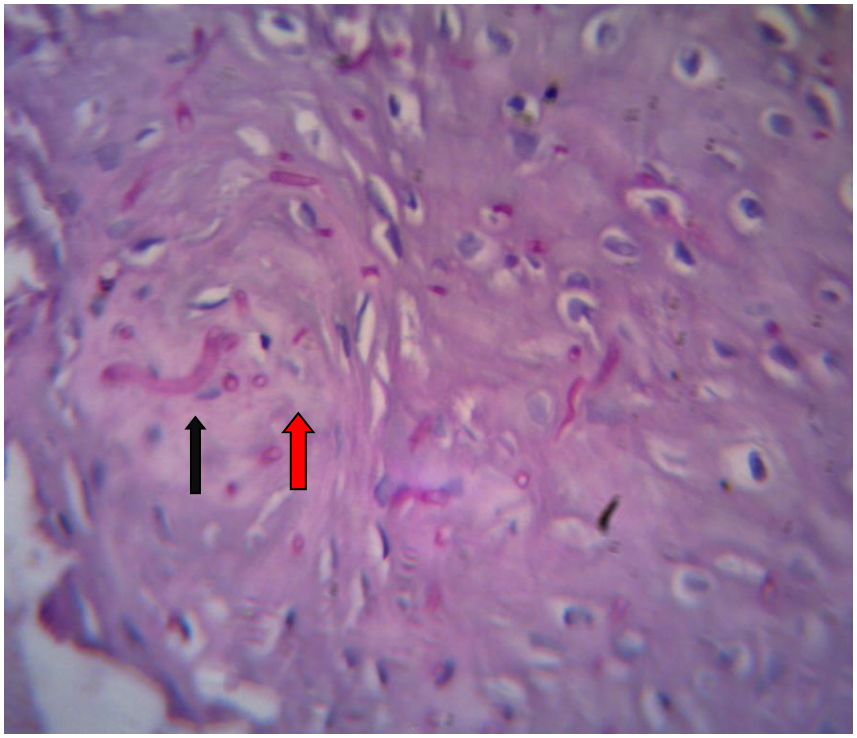

Figure 5: A photomicrograph of candidial leukoplakia showing candida hyphae

This mass of hyphae called “mycelium”; the red arrow was yeast (blastospore) forms of *Candida albicans*. also, in the slide visualized cellular atypia in modality of nuclear hyperchromatism, nuclear pleomorphism with reduction of intercellular adhesion and alteration in nuclear/ cytoplasmic ratio either by area or volume (PAS stain).

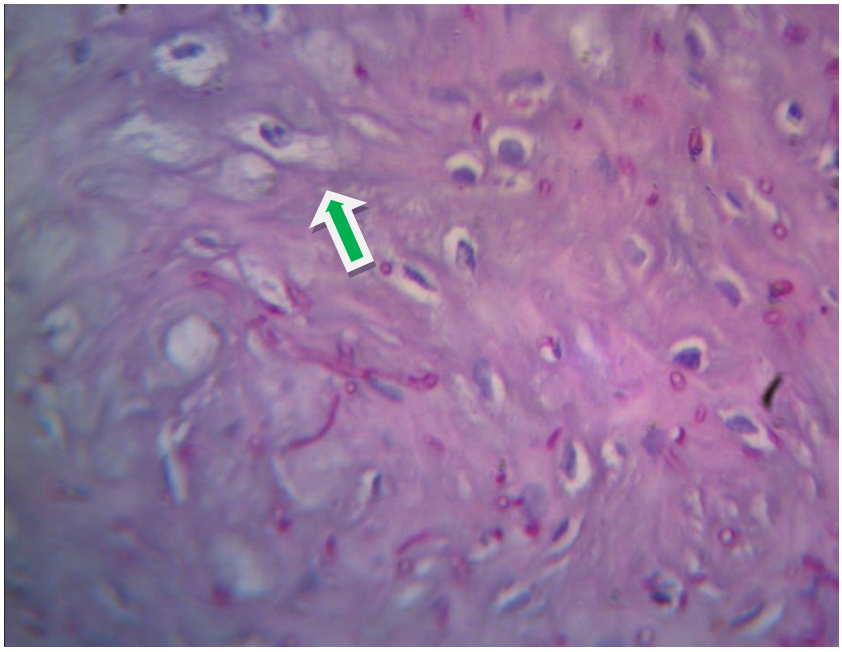

Figure 6: A photomicrograph of candida leukoplakia

This photomicrograph of candida leukoplakia is showing invading fungal hyphae and blastospore in superficial layer in candidial leukoplakia lesion. Many cells in superficial layers of epithelium are separated by edema (green arrow). (PAS stain).

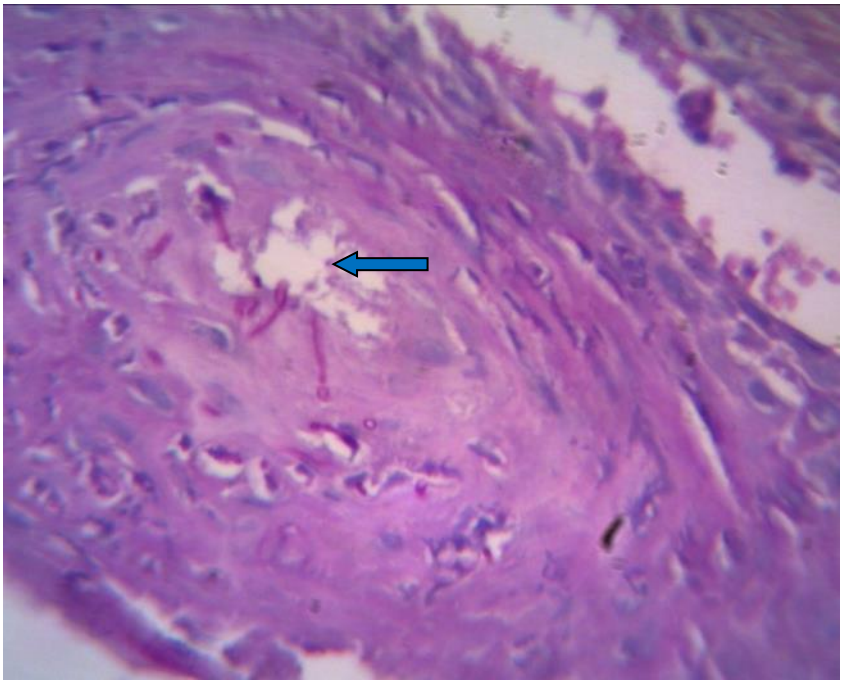

Figure 7: A photomicrograph of candidial leukoplakia

A photomicrograph of candidial leukoplakia showing a superficial colonization of fungal hyphae and microabscess a in superficial layer. Collection of neutrophil leucocytes with candidial hyphae and the edema formed microabscess resulting atrophic area in superficial layers or even missing which responsible for the speckled erythematous appearances seen clinically (PAS stain).

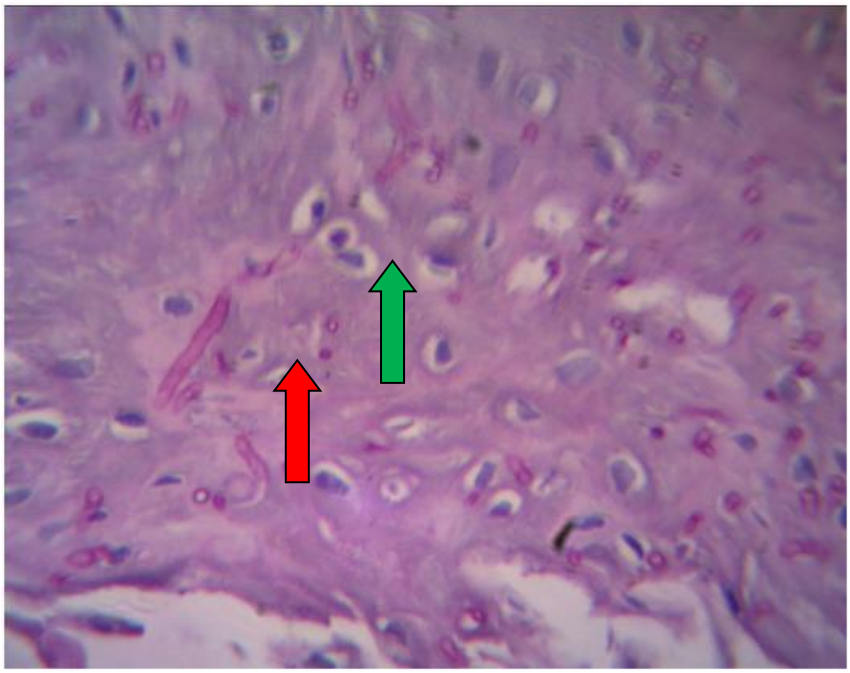

Figure 8: A photomicrograph of intracellular extenuation of *Candida albicans*

This photomicrograph is showing candidal hyphae and blastopore (yeast) invasion the superficial epithelium at right angles to the surface and not penetrated deeper in to prickle cell layers (red arrow). The candida invasion always stops short of penetrating beyond the junction between the parakeratotic layer and the stratum spinosum (prickle cell).

Cellular atypia which is characteristic of epithelial dysplasia demonstrate apparently abnormal form of mitoses, nuclear hyperchromatism, nuclear, cellular pleomorphism and loss of intercellular adhesion or cohesion (green arrow). (PAS stain).

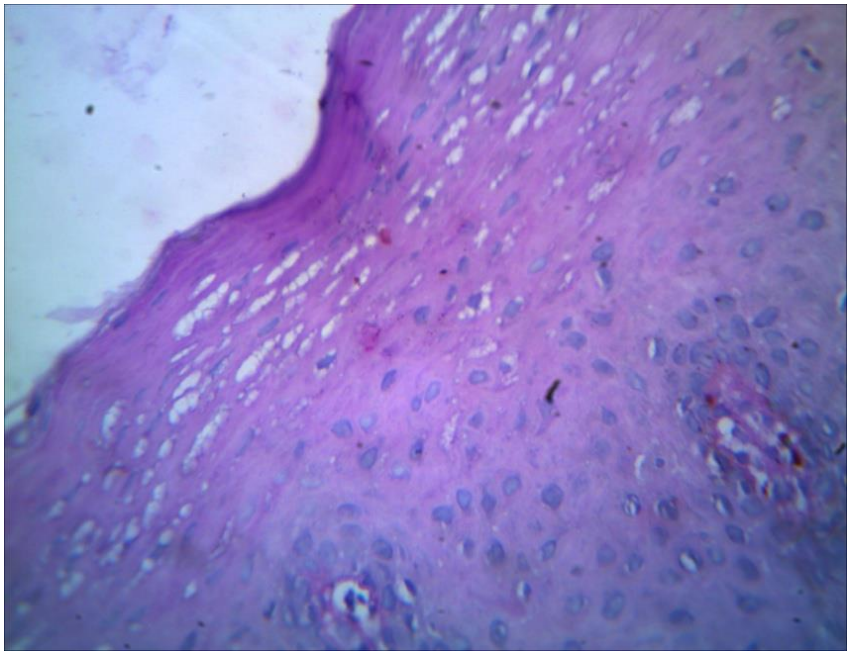

Figure 9: A photomicrograph of candidial leukoplakia

The photomicrograph is showing parakeratosis, prominent acanthosis and epithelial hyperplasia is characteristic of candidial leukoplakia, bubble like form given epithelial rete peg its shape (PAS stain).

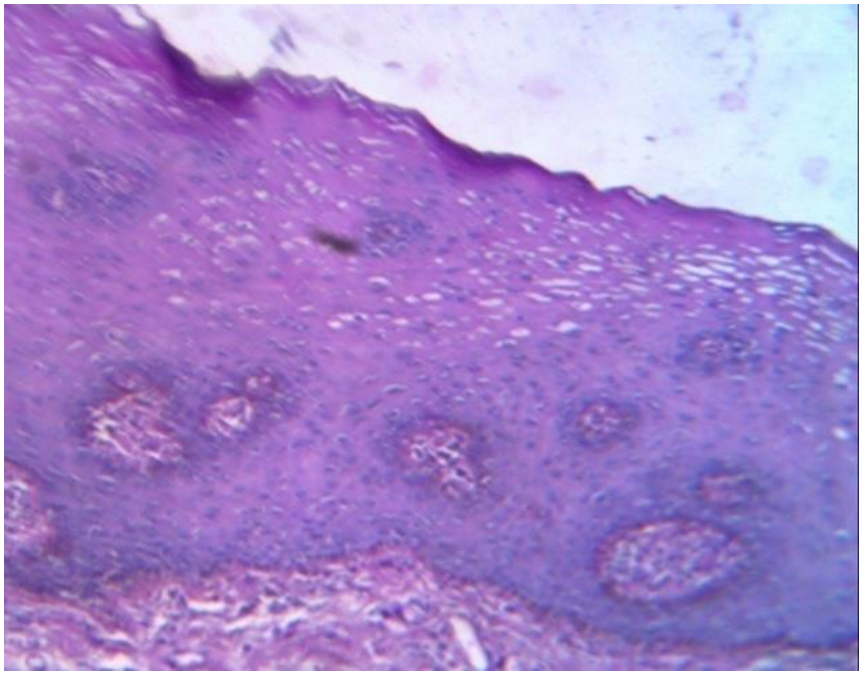

Figure 10: A photomicrograph of candidial leukoplakia

Low magnification in this photomicrograph is showing acanthosis of epithelial layers interface lamina propria, variations in thickness of the epithelium a parakeratosis present. The parakeratotic layer is of variable thickness, about 12 or more cells deep, generally corresponding to the depth of invasion of the hyphae (PAS stain).

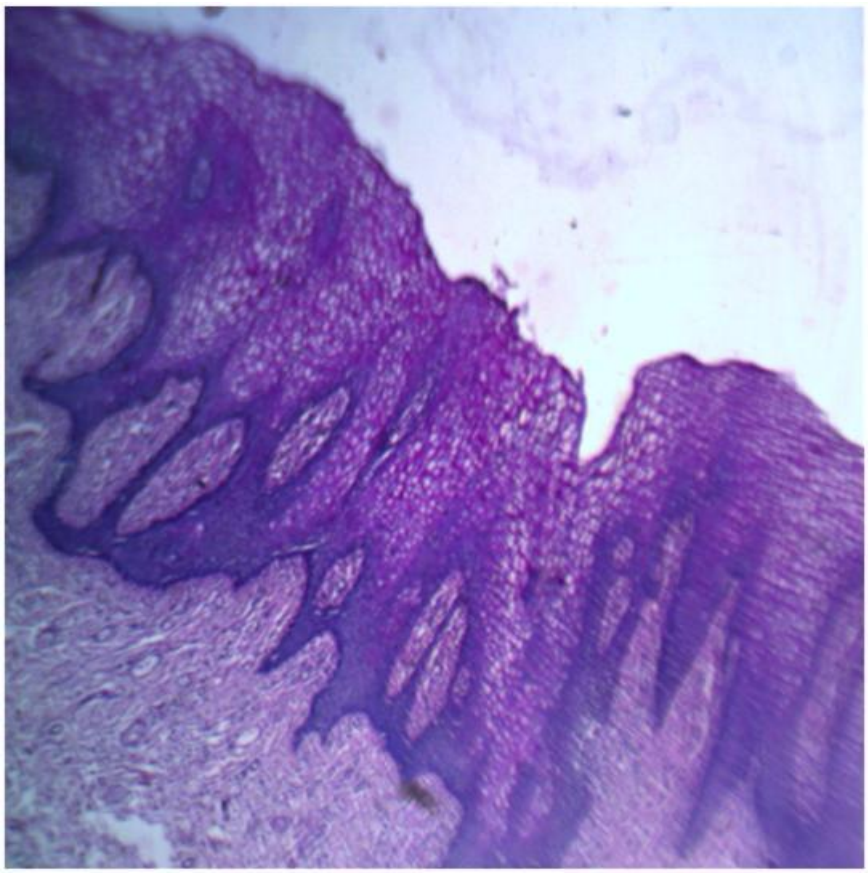

Figure 11: A photomicrograph of verrucous leukoplakia

This photomicrograph is showing exfoliated epithelial rete ridge and hyperplasia, acanthosis of epithelial layer is the most characteristics feature parakeratosis and irregular elongation of epithelial ridges seen in involvement regains (PAS stain).

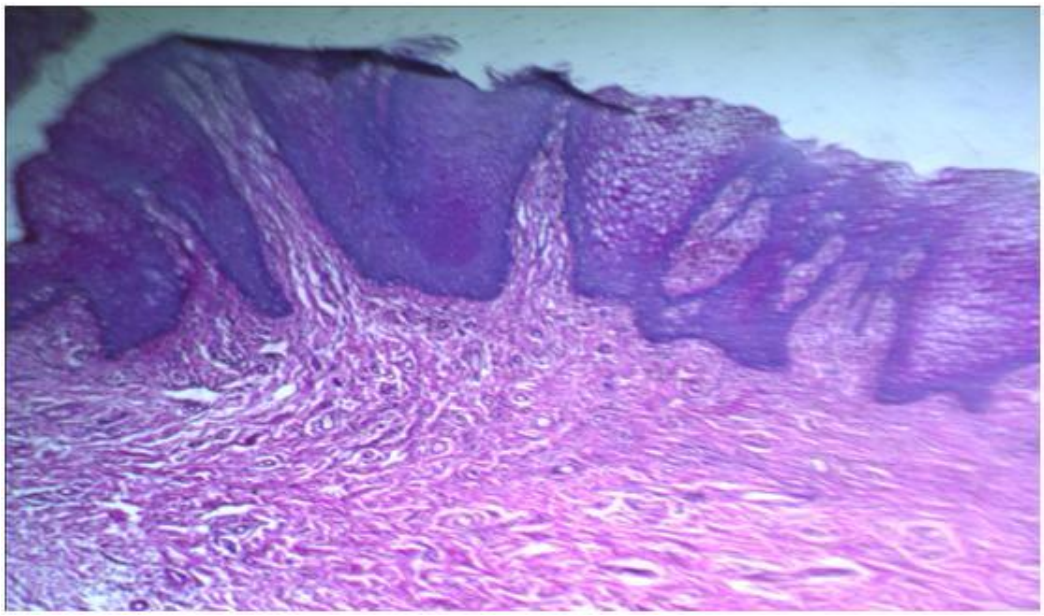

Figure 12: A photomicrograph of verrucous leukoplakia

This photomicrograph is showing papillary hyperplasia with thickrete ridge and (PAS stain).

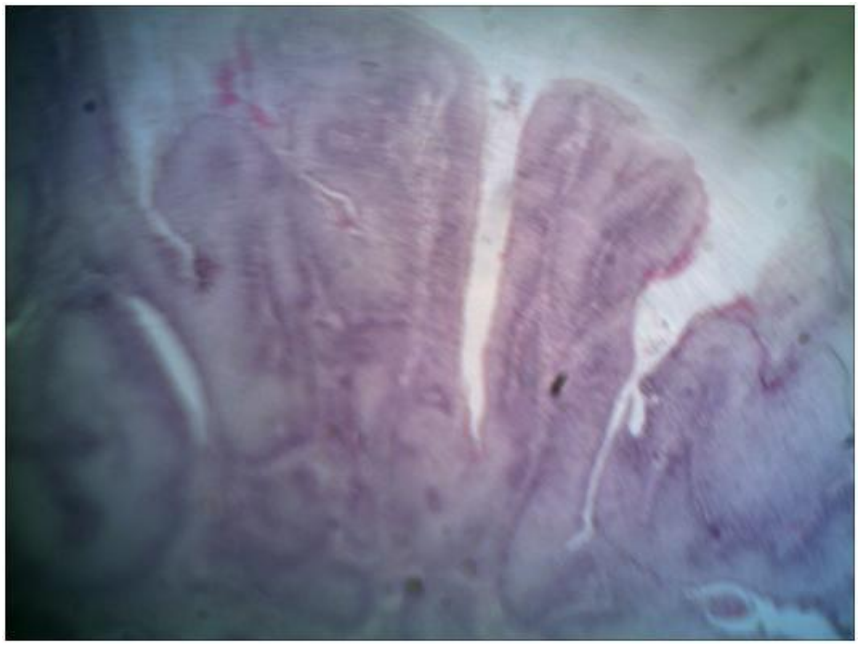

Figure 13: A photomicrograph of proliferative verrucous leukopakia

This photomicrograph is showing proliferated superficial layers to form elongated exophytic papillary pattern of growth with folded irregular appearance with large pushing bulbous rete ridge and clefts and separation between each papillary proliferated(PAS stain).

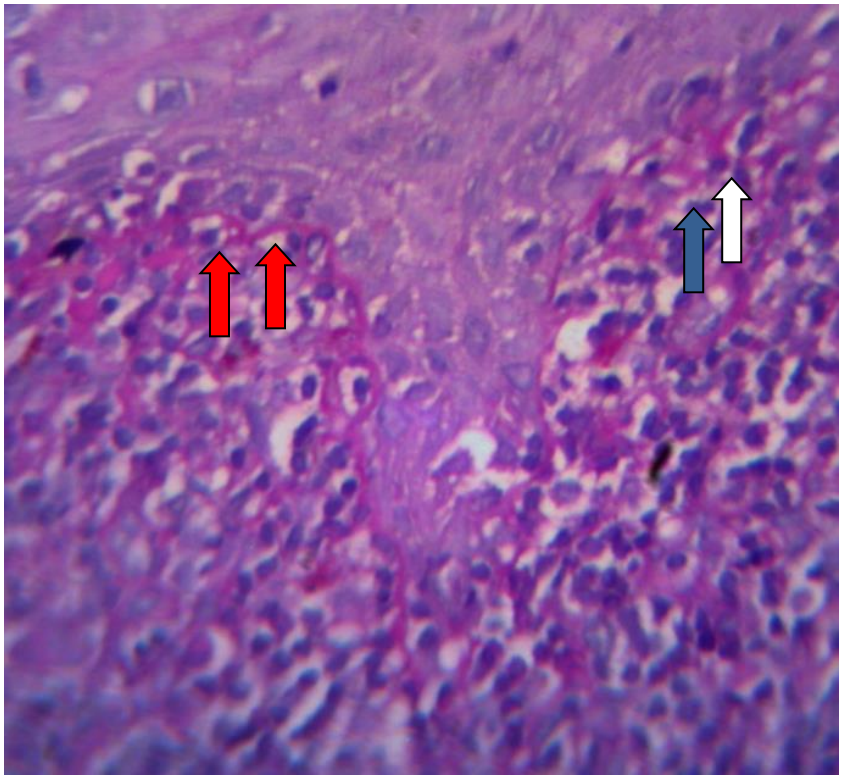

Figure 14: A photomicrograph of lichen planus

A photomicrograph is showing saw tooth appearance with well demarcated basement membrane (red arrow), mononuclear inflammatory cell infiltration in sub epithelium layers (blue arrow), edema due degeneration of basal cells (white arrow). (PAS stain).

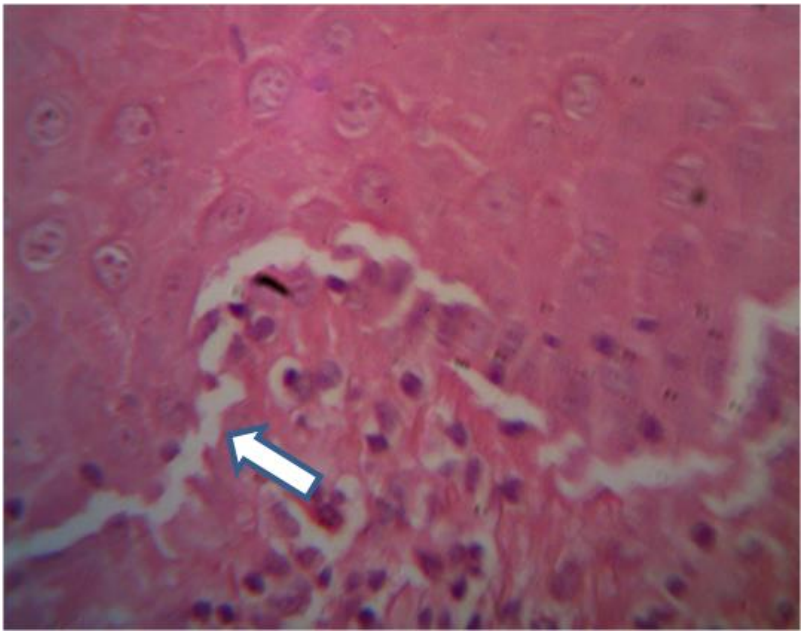

Figure 15: A photomicrograph of lichen planus

A photomicrograph is showing lymphocytes in a band underlying basement membrane associated with edema and formed small cleft (white arrow) inflammatory cells infiltrated in subepithelial area with characteristic of degeneration of the basal region of epithelium. (H & E stain).

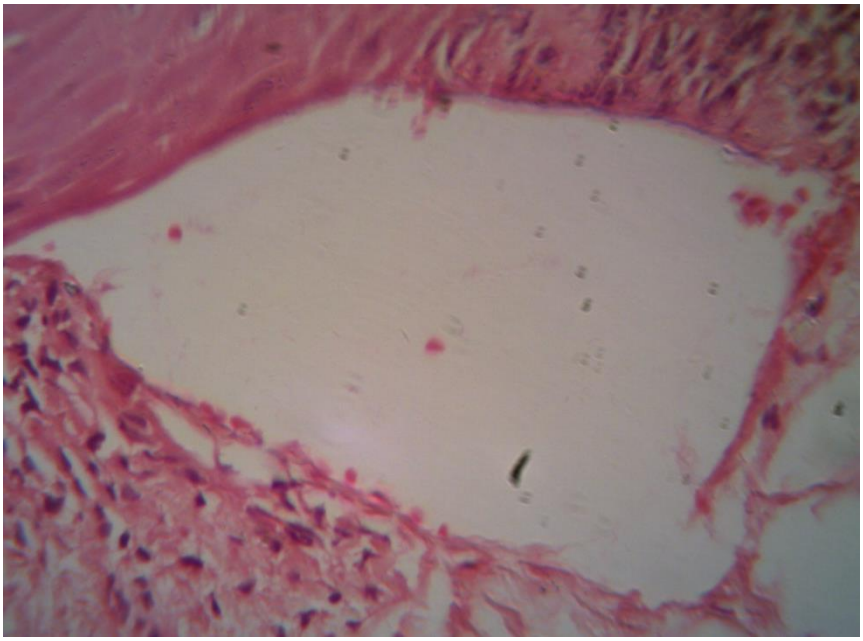

Figure 16: A photomicrograph of bullous Lichen planus

A photomicrograph is showing sub epithelium bullae formed after lake of cohesion between epithelium and lamina propria as result of basal cell degeneration and edema. Lifting of epithelial cells result in formation of subepithelial bullae (H & E stain).

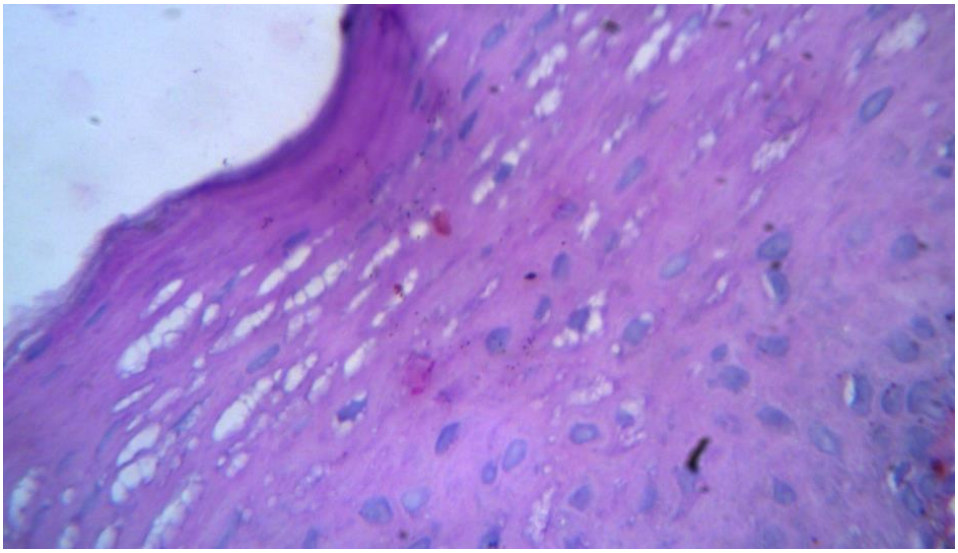

Figure 17: A photomicrograph of moderate dysplasia in leukoplakia

This photomicrograph is showing hyperkeratosis and hyperplasia, moderate dysplasia, hyperchromatism, prominent and enlarged nucleoli, atypical of keratinocytes was well established. Abnormal mitosis informs and superabasal mitosis and abnormal form of mitosis(PAS stain).

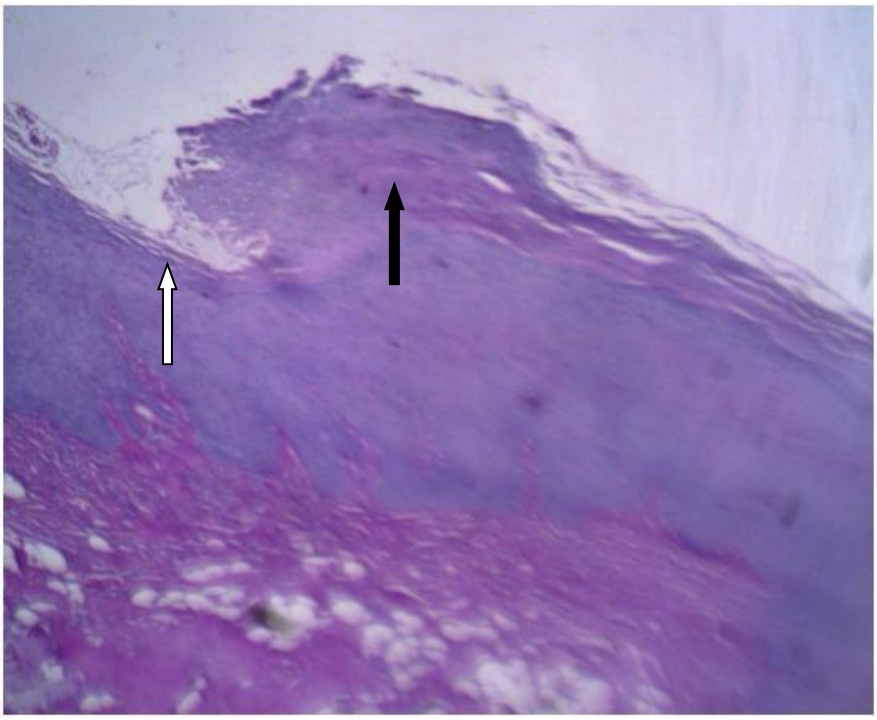

Figure 18: A photomicrograph of erythroplakia

This photomicrograph is showing hyperkeratosis (black arrow), atrophic epithelial area (the white arrow) (PAS stain).

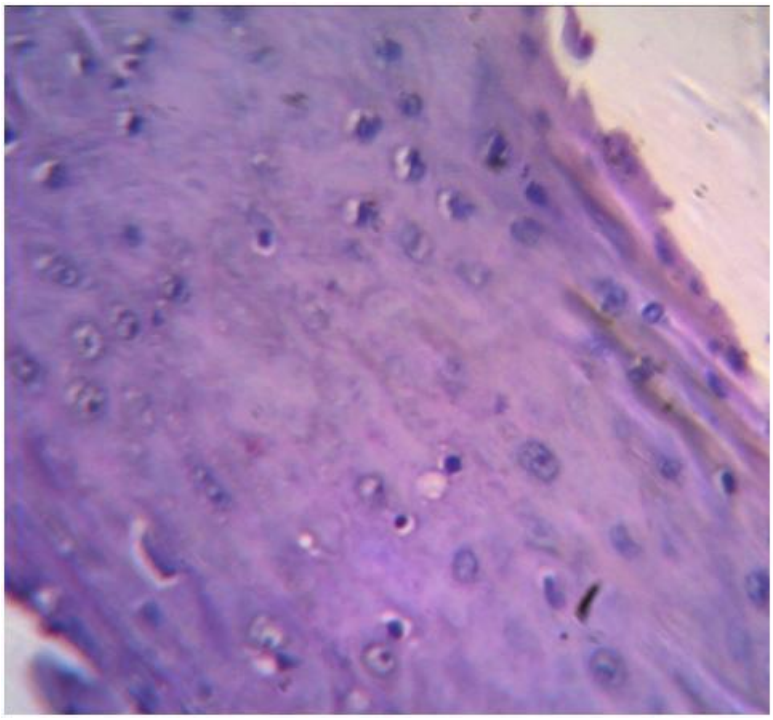

Figure 19: A photomicrograph of erythroplakia

A photomicrograph is showing nuclear and cellular pleomorphism with abnormal mitosis and hyperchromatic nucleoli in moderated epithelial dysplasia (PAS stain).

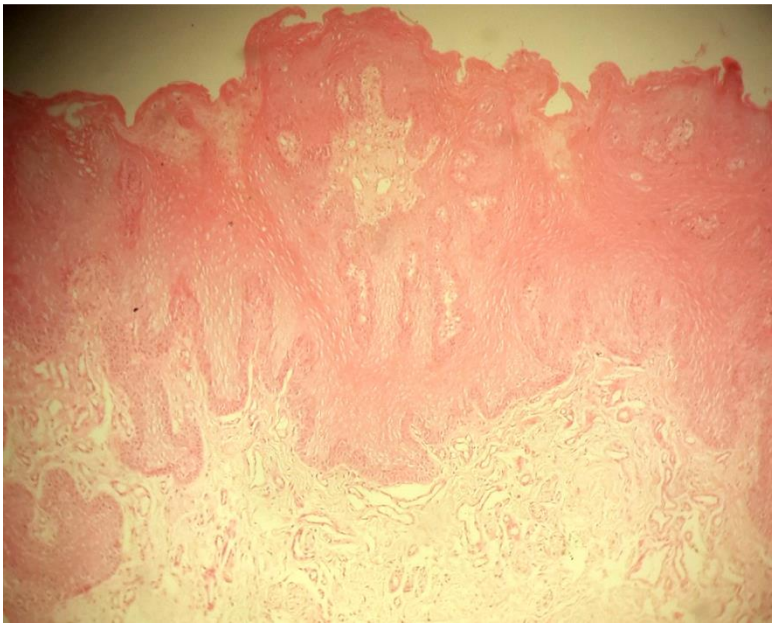

Figure 20: A photomicrograph of leukoplakia

A photomicrograph is showing drop-shape in papillary hyperplasia in moderated dysplasia with extensive pushing without local destruction to basement membrane (H & E stain).

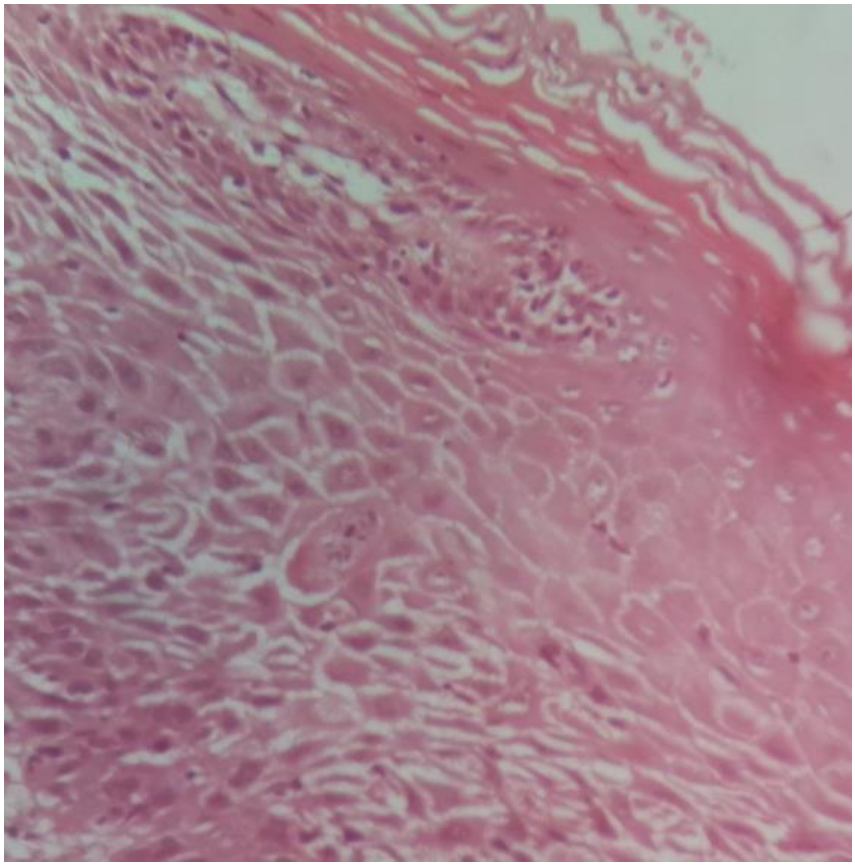

Figure 21: A photomicrograph of leukoplakia

A photomicrograph is showing sever dysplasia showing super basal mitosis, loss of intracellular adhesion, cellular atypia from top to bottom (H & E stain).

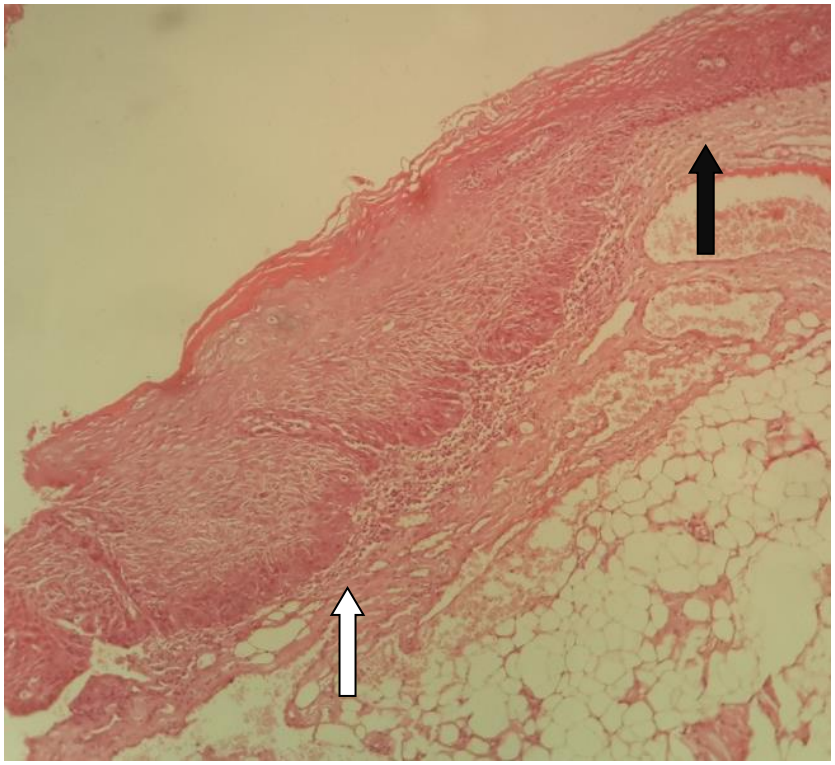

Figure 22: A photomicrograph of severe dyplasia in leukoplakia

Basilary hyperplasia with drop-shape rete ridge (white arrow). Normal epithelium (black arrow). (H & E stain).

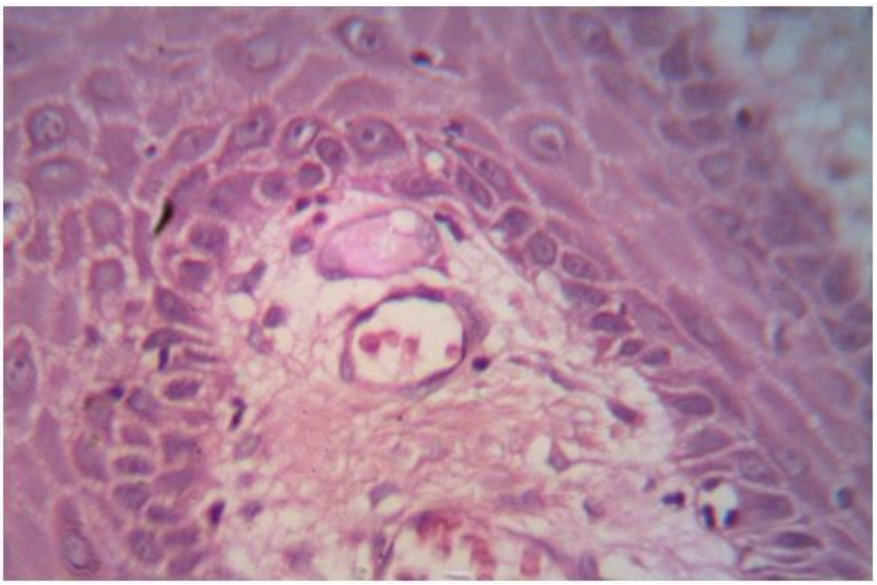

Figure 23: A photomicrograph of leukoplakia

A photomicrograph is showing epithelial dysplasia showing basal cell hyperplasia and drop- shape rete pegs(H & E).

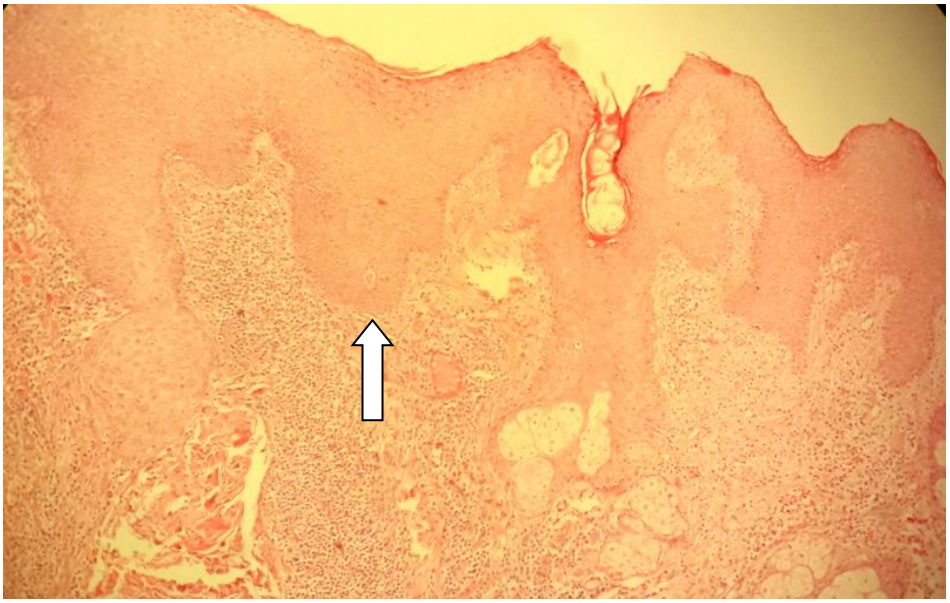

Figure 24: A photomicrograph of actinic cheilitis

The various histological picture as Basophilic change of submucosa, thickness of prickle cell layer with telangiectasia. The epithelial surface shows a thickened layer of Para keratin. There is irregular thickening of the spinous cell regions resulting in elongation of the rete ridges.

The rete ridges have a bulbous architecture in some areas (arrow) and the epithelium shows dysplastic changes with disruption of the normal maturation pattern (H & E stain,).

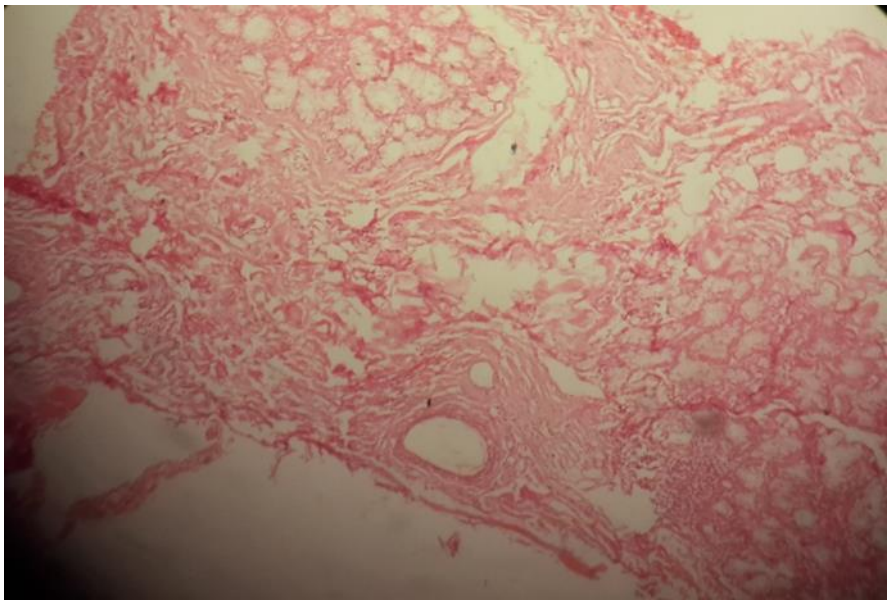

Figure 25: A photomicrograph of submucouse fibrosis

This photomicrograph is showing submucous fibrosis where there is an atrophy of epithelium and subjacent fibrosis, lamina propria is poorly vascularized and hyalinized, collagen fiber predominates in submucosa around blood vessels salivary glands and muscle, mild dysplasia occurred (H & E stain).

DISCUSSION

Chapter: 6 DISCUSSION

Oral cancer is a major worldwide public health problem. A delay in diagnosis and treatment was the key factor that has led to lack of improvement in prognosis of oral cancer over the years (Ho et al., 2013). In many instances, a significant proportion of oral squamous cell carcinomas develop from premalignant lesions and conditions (Banoczy and Csiba 1976). The identification of such lesions and conditions is very important in order to prevent malignant transformation (Brennan et al., 2007).

According to the findings of this study, PMOLs constitute about 4.7% of 1894 biopsies examined in this department in a 12 years period. This figure is almost similar to the findings of many previous studies done elsewhere in the world (Warnakulasuriya et al., Rao et al., in 1998, Lim et al., 2003, Hari et al., 2014, Suvarna and Manthapuri et al., 2018), but it was higher than those obtained from other studies (Bhonsle et al., 1976). These conflicting results indicate real regional variation PMOLs prevalence. In this study more than 49% of PMOLs had been detected in the first instance at the age of 35 to 55 years in an agreement with the findings of many previous studies, which stated that most of PMOLs occurred in the age group of 30 to 50 years (Saraswathi, 2006, (Pindborg, Reibel et al., 1985).

Although the incidence of oral cancer is indisputably higher in males, it seems that the biopsies from the suspected malignant or premalignant lesions are equal in number in both genders. This figure can be attributed to the large number of cases of OLP which is much higher in females and constituted the largest proportion of cases in this study, while there were more cases of leukoplakia in males. This reflects the higher prevalence of OLP among Libyan population than other PMOLs (Elmurtadi and Ingafou, 2017).

Most biopsies were taken from buccal mucosa which is presumably the most common site of involvement by PMOLs, followed by the labial mucosa, and the tongue. These results are in accordance with many previous studies (Bhurgri, 2005, Shyam et al., 2014, Suvarna et al., 2018).

The most commonly encountered potentially malignant lesions are leukoplakia and erythroplakia; these two entities put under the name of "oral potentially malignant disorders" that are highly associated with the presence of OED at first biopsy, while lesions of submucous fibrosis develop OED after being present for years (Woo 2019).

As more than 90% of all oral cancers are OSCCs, which is often preceded by a premalignant lesion (Camile et al., 2014). This study was conducted to update the current knowledge about some aspects of potentially malignant lesions and the role of candida in their transformation to malignancy. It is well accepted that all potentially malignant disorders do not progress to cancer but they have higher risk of malignant transformation unless diagnosed early and treated.

Although little information is available regarding the real prevalence of PMOLs in the general population, a commonly accepted prevalence of 1–5% has been reported (Van der Waal, 2014). (Warnakulasuriya 2001) conducted a meta-analysis and reported a malignant transformation rate of 10.5% among patients with histologically confirmed OED undergoing long-term follow-up. Genomic and proliferation markers are likely to be associated with histopathological parameters, but their relationship with the grading of dysplasia remains uncertain. They are potential biomarker candidates but their utility in prognosis of oral precancer deserves further study.

A significant amount of scientific literature has amassed on oral epithelial dysplasia relating to aspects of its diagnosis and management. However, the evidence base is weak as a result of the significant variability of published research. Poorly described study methods, variability in different oral epithelial dysplasia grading systems, inter- and intra-examiner variability causing issues of reliability, inadequate sample size, and inconsistent durations of follow-up are some of the methodological issues contributing to the failure to provide dependable information. Randomized clinical trials on the malignant transformation potential of oral epithelial dysplasia and its treatment outcomes are limited (Tilakaratne et al., 2019).

(Lumerman et al., 1995) reported that mild dysplasia as the more prevalent grade than severe and moderate dysplasia, with carcinoma in situ being the least (3.9%). In all other studies, mild dysplasia was more prevalent as compared to moderate or severe dysplasia. The probable reason for that could be late presentation of the disease due to patient's unawareness of symptoms and lack of concern for the disease (Singh et al., 2020)

The grade (Geetha et al., 2015) of epithelial dysplasia may not be proportional to the risk of malignant potential and clinical characteristics may complement therapeutic decisions. Molecular genetic changes found in oral epithelial dysplasia remain unclear and at present lack clinical significance.(Warnakulasuriya 2001). Most of the dysplastic changes in leukoplakia lesions in this study graded as mild dysplasia, whereas other

few lesion is moderate in accordance with the previously reported rates (Waldron and Shafer, 1975).

Proliferative verrucous leukoplakia is an uncommon variant of oral leukoplakia, occurring in less than 1% of adults, no data on its worldwide incidence. in this study two females and another male of more than 55 years was affected in buccal mucosa and lip in accordance with the findings of some previous studies (Van der Waal, 2008, Barns et al., 2005) and in contrast with others who reported higher incidence in the gingiva (Silverman et al., 1997, Fettig et al., 2000, Bagan et al., 2003). Treatment of oral precancer is largely based on histological grading of epithelial dysplasia, despite the fact that this estimation is subjective and therefore carries a low reproducibility (Warnakulasuriya 2001).

OLP is a fairly common mucocutaneous disease. (Leo et al., 2006, Elmurtadi and Ingafou, 2017) as it represented the largest bulk of cases in this study (68.8%) and 3.5% of the biopsied oral lesions in this firm. As this study was not designed to determine the prevalence of OLP, the reported prevalence from worldwide studies reflects considerable variation in its prevalence, which ranged from 0.15% to 3.8%, this reflects the local policy of the department of taking biopsy for any lesions suspected to be potentially malignant. Cases of OLP was surpluses all other types of PMOLs in this study because of its higher prevalence in general population.

Dysplastic changes seen in all cases of OLP in this study (mostly mild dysplasia) which confirms the idea that the dysplasia in OLP is only reactive to the ongoing inflammation in this disease which usually results in a reactive cellular atypia in the form of mild type of dysplasia.

Oral submucous fibrosis is commonly seen in individuals from India a subcontinent and Southeast Asia decedents, but it can be encountered all over the world due to high migration, and the discovery of additional etiological factors rather than betel nut which primary factor in the development of OSMF, but several factors contributing to it such as nutritional factors, vitamin deficiencies, hypersensitivity to many potential irritant(areca nut, dietary spices and tobacco) in addition to chronic consumption of chili pepper (Cox and Walker, 1996). The two cases reported in this study has no connotation to candidal hyphae. the scarce published data on the co-existence of fungal hyphae in biopsy-based studies of submucous fibrosis makes the comparison of the present data with other studies quite difficult.

Erythroplakia is rare in comparison with leukoplakia, but most of the discovered lesions represent severe dysplasia or malignancy (Waldron and Shafer, 1975). The common sites of involvement are the floor of the mouth, tongue, retromolar pad, and soft palate (Neville et al., 2002), in the present study one lesion detected in the buccal mucosa and the tongue. Although erythroplakia is seen less frequently than leukoplakia but it is more life threatening as it carries 90% of malignant transformation rate, so early detection and immediate surgical excision are recommended.

On histopathological evaluation in the present study the epithelial dysplasia in PMOLs, some degree of dysplasia was detected in almost all the slides whatever the type of PMOLs lesion in contrast with some other studies who reported a much lower figures of dysplastic changes in PMOLs (Manthapuri and Sanjeevareddygari, 2018).

Candidiasis is the most commonly encountered fungal infection, and oral candidiasis is often observed as a local opportunistic infection. In the oral cavity, the co-adhesion of *Candida albicans* with bacteria is crucial for its persistence, and a wide range of synergistic interactions with various oral species were described to enhance colonization in the host (Vila et al., 2020), (Wang 1991) has reported 8 cases of chronic hyperplastic oral candidiasis with epithelial dysplasia. Inflammatory infiltration was related to the amount of candida. The more candida gets into the epithelium, the heavier inflammation is. The association of Candida with various precancer and cancer lesions has been attributed to the carcinogenic compounds, like nitrosamines, N-nitrosobenzylmethylamine produced *Candida albicans* as it get adhered to epithelium. Furthermore, strains with high nitrosation potential were isolated from lesions with more advanced precancerous changes (Krogh.et al., 1987) ,(Krogh, 1990)

The role of fungal infection in malignant transformation has extensively been studied by many investigators and holds a promise as an indicator to predict malignant transformation, so this study was designed to figure the pattern of fungal hyphae existence in biopsies of patients with clinically diagnosed cases of premalignant lesions and conditions.

Recently, the interest in studying oral candidiasis has markedly increased, mainly due to its association with viral infection particularly with human immunodeficiency virus, but also because of its correlation with potentially malignant and malignant lesions of oral mucosa (Vuekovic et al., 2004).

In a study of 70 cases, there was significant association between degree of epithelial dysplasia with presence or absence of fungal hyphae in all the study groups, the

presence of fungal hyphae in potentially malignant lesions and conditions may prove to be a useful indicator in predicting malignant transformation (Hongal et al., 2015).

Oral Candida induces a variety of symptoms, such as oral mucosal inflammation manifesting as an uncomfortable feeling, pain, erythema, erosion, taste abnormalities, and hyperplasia of the oral mucosa. Candida overgrowth in the oral cavity may disseminate to distant organs. Therefore, in order to avoid the Sequelae of systemic candidiasis, oral candidiasis should be rapidly controlled(Yamamoto 2010).

The probable role of candida in oral carcinogenesis is unclear. It could be argued that the increased colonization and infestation associated with dysplasia are entirely coincidental, reflecting a change in the environmental conditions conducive to the proliferation of these ubiquitous commensals. If chronic candidal infection were to be an important co-factor in carcinogenesis, then many more patients with chronic mucocutaneous candidiasis syndromes should develop oral carcinomas.

The present study was carried out to look for any association between the presence of candida in number of potentially malignant lesions such as leukoplakia, lichen planus, erythroplakia, actinic cheilitis, candidial leukoplakia and submucous fibrosis but fails to demonstrate and valid evidence of an increased presence of *Candida albicans* that the level present in the unhealthy population.

Biopsy-based studies have reported candidal hyphae to constitute 7–50% in leukoplakia (Sitheeque and Samaranayake, 2003). In this study, from the total of 30 sections, candidial hyphae were detected in only 2 cases of candidal leukoplakia and verrucous leukoplakia, representing 6.66% of the cases, in contrast to the findings of some other studies which showed a higher prevalence of fungal hyphae in tissue sections of leukoplakia (Berret et al., 1998, Banoczy and Csiba, 1976, Silverman et al., 1984).

Some previous studies however, revealed that the prevalence of candidal hyphae in the biopsies of OLP lesions ranged between 0% and 17% of cases, with no apparent predilection for any clinical type of OLP (Holmstrup and Dabelsteen, 1974, Lundstrbm et al., 1984, Krogh et al., 1987, Hatchuel et al., 1990). Interestingly, however, clinical improvement of OLP has been reported by the use of antimycotic treatment (Lundstrom et al., 1984), and treatment with corticosteroids may predispose to candidiasis.

In this study, however, there the absence of candidal hyphae in OLP biopsies can be explained on the basis of the wide varieties of etiological factors that lead to OLP lesions such as genetic background, immunological dysregulation, sensitivity to dental

materials and certain drugs. Beside the variation study designs, techniques and geographic origins of the study groups in different countries.

The absence of fungal hyphae in the cases of submucous fibrosis (SMOF) of this study in comparison with the results obtained by (Kumar et al., 2009) who reported a prevalence of (33.3%) positive hyphae in 24 cases and in (Bhagyalaxmi et al., 2015) who reported 25% of 16 cases, clearly due to the very small number of patients with OSMF studied here.

The presumption that there are credible association between colonization of *Candida albicans* and manifestation of epithelial dysplasia was not substantiated in this study. However, the degree of epithelial dysplasia was significantly associated with presence or absence of fungal hyphae in all cases. Although almost all sections of PMOLs examined in this study revealing some degree of epithelial dysplasia so the existence of candidial hyphae in epithelium layers unnecessarily causes dysplasia, as epithelial dysplasia occurs the absence or presence of *Candida albicans*.

The discrepancy in percentage prevalence of fungal hyphae in tissue sections in our study when compared to various studies can be explained as follows. It is possible that overall detection rate of 5% represents an underestimate and there is abundant evidence that values of fungal infection as assessed by PAS staining are lower than those obtained by culture and using the PAS stain there is 13% chance of missing fungal infection, particularly if hyphae are inadequate or only one section is obtained and very old and expose to terrible environment factor (Roed-Petersen et al., 1970).

Histopathological examination is the quickest way of identifying those cases in which microorganisms have invaded the epithelium where they are more likely to be pathogenic. Non-invasive hyphae and yeasts may be detected in smears or grown in culture, but lost during histological processing (Cawson, 1968) producing a negative result on staining the section with PAS.

It is unconceivable that there are some limitations of this study such as the included number is small. The clinical data is retrospective and the study was not longitudinal to follow up the patient to exactly mark those case which transferred to malignancy. The study using light microscope findings. Whereas the contemporary methodology involving more advanced means of technology based on molecular biology, immunohistochemistry chromatography and digital imaging modalities.

6.1. CONCLUSIONS

This study confirmed the existence of some degree of epithelial dysplasia in almost all the studied 30 sections of PMOLs (mostly mild epithelial dysplasia), but failed to demonstrate the existence of candida in a large number of biopsies.

Although *Candida albicans* has been considered as an etiological factor for potentially malignant disorders, the pathogenesis of that is not clearly understood and is still a field under extensive research. As *Candida albicans* being a normal commensal in the oral cavity, its presence alone cannot be plamed for the etiology of PMOLs.

Malignant transformation of PMOLs has always been of a multifactorial etiology and not merely due to candidal reasons alone. Moreover, some authors substantiated that candidial hyphae does not penetrated deeper into prickle cell layers and may also beyond the junction between granular layer and prickle cell layer, so that any carcinogenic substance to be further away from the rest of the epithelium below and lamina propria, so it is unlikely to cause biological alteration in tissue.

6.2. RECOMMENDATIONS

Future research should include studies with large sample size and species isolation of Candida. Using more recent microbiological, cytological and histopathological technologies to given more precisely adulate and accurate result.

The presence of epithelial dysplasia is more important in predicting malignant development than the clinical characteristics of the lesion, so the histopathological biopsy should be taken immediately in any white or red non healing lesion.

Whether candida is present or absent in a specimen, the degree of epithelial dysplasia much more important predictor of malignant transformation and should be used as a guide for the most suitable treatment options through serial biopsies over the time.

APPENDIX

APPENDIX

Audit form for the candida and dysplasia study

Biopsy No. Date of biopsy obtained…….

Patient name. Age. sex. Nationality. site.

Diagnosis.: ……

Histopathological finding (H&E stain):

I- *Epithelium:*

-Hyperparakeratosis ( ), Hyper orthokeratosis ( ), Acanthosis ( ),Atrophy ( ) - Ulceration ( ).

-Epithelial rete ridges hyperplasia & elongation ( ), Degeneration of basal cells( )

-Subepithelial bullae ( ) Lymphocytic infiltration of basal region( ), Micro-abscesses ( ).

-Pseudoepitheliomatous hyperplasia ( ),Cellular atypia ( )

-Degree of dysplastic change: mild ( ), moderate ( ), severe ( ).

II- *Lamina properia:*

-Connective tissue: loosely CT ( ), dense CT ( ), hyalinization of subepithelial CT ( ).

-Vascularity: high ( ), low ( ), avascular ( ), perivascular inflammatory cells ( )

-Inflammatory cells infiltrate ( ).

-Type of inflammatory cells: lymphocytes ( ), plasma cells ( ), macrophages ( ) giant cells ( ), neutrophils ( ), eosinophils ( ),basophils ( ).

III- *Candida albicans* (PAS stain)

Candida albicans :present ( ) NO ( ).

-Location: superficial cell layer ( ), prickle cell layer ( ), Basal cell layer ( ).

-Type of candida: yeast (blastospore) ( ),hyphal form of *Candida albicans* ( ).

PROTOCOL OF STUDY

PROTOCOL OF STUDY

Clinical and Histopathological Correlation between Dysplastic Changes in Oral Potentially Malignant Lesions and Presence of *Candida albicans*

العلاقة السريرية والنسيجية بين التغيرات السابقة للاورام الفموية الخبيثة ووجود المبيضات البيضاء

Thesis proposal
By
Mahgub Abdulkareem Mahmoud
BDS (1986)

in partial fulfillment
of requirements for the degree of
MSc In Oral Pathology.

Supervisor: Professor Ali Mohamed Elmurtadi
Co-supervisor: Professor Mohamed Ingafou

Faculty of dentistry Benghazi University
(2017)

Contents

Introduction

A precancerous lesions is defined as morphologically altered tissue in which cancer is more likely to occur than in its normal counterpart,[1] on the other hand a precancerous or (premalignant) condition is a generalized disorder associated with a significantly increased risk of developing cancer. [2]

Precancerous lesions or conditions of the oral mucosa include Leukoplakia, Erythroplakia, lichen planus, oral submucous fibrosis and actinic cheilitis. [2] Leucoplakia has been studied for many years and it is commonly agreed upon as representing the most common pre-malignant oral mucosal lesion. Epithelial dysplasia has conventionally been the marker used to predict malignant potential in the Precancerous clinical lesion. [3,4] Recently, interest in the study of oral candidiasis has markedly increased mainly because of its association with potentially malignant lesions of oral mucosa. [5]

The involvement of *Candida albicans* in the aetiology or progression of leukoplakic lesions remains controversial, however some studies have been suggested that correlation [6].

Elaboration of nitrosamine compounds by some candidal biotypes may thus play a role in the carcinogenesis. [7] Many previous studies failed to find any significant association between Candida-positive cases and cellular atypia when, histological sections were stained by HE (haematoxylin-eosin) and PAS (Periodic Acid –Schiff) [8,9], other study findings were not statistically significant. More recent study by(Vekoviae.etal, 2004) revealed the presence of candida in Leucoplakia case(3/12 or 25%), and in lichen planus cases (4/9 or 44.4%), in which epithelial dysplasia diagnosed in a very small number of cases with Leucoplakia, was associated with Candidal infection (1/30 or 3.3%), the histological sections were stained with the HE method and stained with the PAS method, for the visualization of fungi [10].

Retrospective study on oral lichen planus to study the role of candida infection in its dysplastic changes by using PAS technique revealed that *Candida albicans* was positive in 5 cases (45.5%) of erosive OLP with dysplasia. [11]

Aims and objectives of the study

1-To study the epidemiological features of precancerous cases such as age, sex and their clinical presentation.

2- To examine the relationship between the presence of *Candida albicans* and the epithelial dysplasia in a number of precancerous lesions.

Material and Methods

Thirty biopsies of cases diagnosed clinically and confirmed histopatholgically as oral precancerous lesions will be obtained from the archives of the Oral Pathology department in faculty of dentistry Benghazi University.

The clinical data of the selected cases included demographic data such as age, sex and site will be noted from the patient's records (Appendix 1).

The examined tissue of all cases includes part of the adjacent normal oral mucosa, the diagnosis of the selected cases was done by experienced pathologist in the department.

Paraffin sections of all selected cases are stained by haematoxylin and eosin stain using standard methods.

Histochemical study

All the examined Section will be stained with PAS (Periodic Acid –Schiff) according to recommended technique ([12]) for clear visualization of the location and the prevalence of candidal hyphae. The collected data will be analysed by using statistical package for Social Sciences (SPSS).

Audit of the candida and dysplasia study

I- Histopathological finding (H&E stain):

Biopsy No..... Date of biopsy obtained..... Age..... sex.....

Nationality..... site.....

Diagnosis.....

II- Epithelium:

Hyper parakeratosis □, Hyper orthokeratosis □, Acanthosis □

Atrophy □ - Ulceration □. -Epithelial rete ridges hyperplasia & elongation □

Degeneration of basal cells □ -Subepithelial bullae □

Lymphocytic infiltration of basal region □, Micro abscesses □.

Pseudoepitheliomatous hyperplasia □, Cellular atypia □

Degree of dysplastic change: mild □, moderate □ severe □.

III- Lamina propria:

Connective tissue: loosely CT □, dense CT □,

hyalinization of subepithelial CT □.

Vascularity high □, low □, avascular □, perivascular inflammatory cells □

Inflammatory cells infiltrated □.

Type of inflammatory cells: lymphocytes □, plasma cells □, macrophages □ giant cells □, neutrophil □, eosinophil □,basophil □.

IV- *Candida albicans* (PAS stain)

Candida albicans:present □ NO □.

Location: superficial cell layer □, prickle cell layer □, Basal cell layer □.

Type of candida: yeast (blastospore) □ ,hyphal form of *Candida albicans* □.

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الملـــخص العربي

العلاقة السريرية والنسيجية بين التغيرات السابقة للاورام الفموية الخبيثة ووجود المبيضات البيضاء

أعداد

محجوب عبدالكريم محمود

تحت أشراف

أ.د علي محمد المرتضي

المشرف المساعد

أ.د محمد صالح إنقافو

الملـــخص العربي

الخلفية: تطور مفهوم نشوء سرطان الغشاء المخاطي للفم من خطوتين، بدءًا من وجود المبدئي لآفة (ما قبل الخبثية أوما قبل السرطانية) والتي تتطور لاحقًا إلى سرطان صريح هو مفهوم راسخ. فالآفات الحمراء مثل البقع الحمراء ، و طلاوة البقع الحمراء، و الآفات الثؤلولية والآفات التقرحية قد تمثل مخاطر أعلى من غيرها، في حين أن الطلاوة المتجانسة تحمل خطرًا أقل لخلل التنسج أو للورم الخبيث عند التشخيص. المبيضات البيضاء، هي عبارة عن كائن حي متعدد التمظهر يتكيف بشكل جيد مع مضيفه البشري بانتقاله من النوع المتعايش إلى النوع الممرض، أما دور المبيضات البيضاء كعامل مسبب محتمل للطلاوة ودورها المحتمل في التحولات الخبيثة لا يزال غير واضح.

الهدف: تهدف هذه الدراسة إلى النظر في السمات الوبائية للآفات المحتمل خبثهاومظهرها السريري وتحديد مدى انتشار الخيوط الفطرية في 30 عينة من الأنسجة المشخصة على انها لافات ما بل السرطان الخبيث.

المواد والأساليب: تم الحصول على البيانات السريرية لعدد 90 (4.7٪) من الحالات المشتبه بها سريريًا والمؤكدة تشريحًا من ما مجموعه 1894 خزعة اجريت في الفترة من 1998 إلى 2010 بالقسم بالإضافة الى عدد ثلاثون خزعة منها تم صبغها أولاً بالهيماتوكسيلين و الايوسين (H& E) أولاً ثم بصبغة باس (PAS) للبحث عن شعيرات المبيضات في هذه الانسجة.

النتائج: من بين 90 حالة، 62 (68.8٪) منها كانت OLP اشنات زاحفة، و (22.2%) 20 OLEP، (3.3%) 3 من VL و2 (2.2٪) من .OSMF في حين وجدت حالة واحدة من التهاب الشفة الشعاعي و OEP. و لم يتم العثور إلا على حالة واحدة فقط بها شعيرات المبيضات فى اللطاخ الأبيض لثؤلولي VL .

لوحظت تغيرات من نوع خلل التنسج بدرجات متفاوتة في جميع الخزعات تقريبًا باستثناء خزعة واحدة. كانت هذه التغيرات في خلل التنسج غالباً من نوعية خفيفة في 20 حالة، ومعتدلة في 6 حالات وشديدة في 3 خزعات. تم الكشف عن خيوط المبيضات في حالتين فقط حيث مثلت الخميرة (blastospore) ٪6.6من هذه الحالات.

المحصلة: لا تعتبر الواصلة الفطرية في الآفات والحالات الخبيثة مؤشرًا مفيدًا في توقع التحول الخبيث، بينما يعد وجود خلل التنسج الظهاري أكثر أهمية في التنبؤ بالتنسف الخبيث من الخصائص السريرية للآفة.