Republic Of Iraq

Ministry Of Higher Education

And Scientific Research

University Of Baghdad

College Of Dentistry

Diagnostic Strategies For Early Detection Of Oral Squamous Cell Carcinoma

A Project Submitted to

The College of Dentistry, University of Baghdad
Department of Oral Diagnosis/ Oral Medicine Clinic in
Partial Fulfillment for the Bachelor of Dental Surgery

By :**Tabarak Majed Najm**

Supervised by:

Noor Saad Mohammed Ali

B.D.S., M.Sc.

APRIL, 2023

Certification of the Supervisor I certify that this project entitled "Dignostic Stratigies of early detaction of oral seqamous cell carcinoma" was prepared by the fifth-year student Tabarak Majed Najm under my supervision at the College of Dentistry/University of Baghdad in partial fulfilment of the graduation requirements for the Bachelor Degree in Dentistry.

Supervisor's name: Dr.Noor Saad Mohammed Ali Date: 2023,april

Acknowledgment

My gratitude to Dr. Raghad Abdulrazak Dean of College of , Dentistry\University of Baghdad

Dr. Bashar Hamed the Head of the Department of Oral Diagnosis and Medicine To all the professors and seniors in the Department My special thanks to Dr. Noor Saad for her supervision, teaching, guiding, and help in this review.

Dedication

With love, I dedicate my graduation research: To My father and my mother who thay always inspire and encourage me to do better,to my brothers and sisters who always stand with me and help me.To everyone who helped me achieve my goals. To everyone who has been stopped by the obstacles of the road and the difficulties of life from achieving his dreams.

TABLE OF CONTENTS

List Of Figures

List of Tables

Absrtact

Background

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer and one of the main causes of cancer-related death around the world. Besides, oral cancer causes facial disfigurement and morbidity.The risk factors include smoking and alcohol consumption, chronic inflammation, UV radiation (lip cancer), HPV and Candida infections, immunosuppression, and genetic predisposition. Besides, oral microbiome and inflammatory cells play essential roles in the malignant transformation of the oral mucosa.

The aim of present study

The purpose of this review was to provide an overview of the diagnostic and prognostic biomarkers in OSCC.

Conclusion

the importance of early detection along with different factors and techniques to detect oral cancer. The biomarkers were divided into three groups as follows: salivary biomarkers, circulating biomarkers, and tissue biomarkers,In this review article, salivary biomarkers along with the circulating and tissue biomarkers were reviewed. Besides, some detection .techniques were explained

Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer and one of the main causes of cancer-related death around the world. Besides, oral cancer causes facial disfigurement and morbidity (Bagan .(2010

The risk factors include smoking and alcohol consumption, chronic inflammation, UV radiation (lip cancer), HPV and Candida infections, immunosuppression, and genetic predisposition. Besides, oral microbiome and inflammatory cells play essential roles in the malignant transformation of the oral mucosa (Irani,et al,2020).

Oral cancer patients are still diagnosed at advanced stages. Lymph node metastasis (especially to cervical lymph nodes) is an important prognostic factor and a leading cause for cancer-related death globally (Irani,2009)

Early diagnosis is an attractive strategy to improve survival rates in patients. Cancer control strategies depend on early diagnosis and detection which rely on clinical assessment. The delay in diagnosis can be attributed to either the patient or clinician (Sujir,et al,2019)

Total delay is a period of time from the patient's first awareness of symptoms to the onset of treatment. In addition to patient's awareness, clinical presentations can aid the clinician (Gigliotti,et al 2020).

Delayed diagnosis has mostly been indicated in the cases of gingival and . buccal cancers (Bagan, 2019)

Screening programs such as mammography and Pap smear test are widely used for screening for breast cancer and cervical cancer; however, oral cancer is diagnosed only after progression of the disease. Therefore, conventional visual examination of the lesion under white light

illumination and palpation play pivotal roles in the diagnosis of highly susceptible lesions. Based on current knowledge, tissue biopsy and histological examinations are the gold standards for accurate diagnosis of oral dysplasia at an early stage of disease progression. However, non-invasive biomarkers are required (McRae,et al,2019)

According to the World Health Organization (WHO), lesions and conditions in the oral cavity, which may undergo malignant transformation, are referred to as oral potentially malignant disorders (OPMDs). It is proposed that 50% of oral cancers develop from precursor lesions. Hence, the early detection and proper management of precancerous lesions have great impacts on oral cancer prevention Starzyńska),2014).

AIM OF THE STUDY

The purpose of this review was to provide an overview of the diagnostic and prognostic biomarkers in OSCC.

CHAPTER ONE

Review of literature

1.1 Oral Sequamous cell carcinoma

1.1.1 Defention

Oral cancer is a malignant neoplasm which grows within the oral cavity. Approximately 90% of the oral cancers have a histological origin of squamous cells and as a result, this type of cancer is typically defined as OSCC. The OSCC has few levels of differentiation and a tendency for regional lymph node metastasis The oral cavity is very accessible during every clinical evaluation, but even to that fact, most of the OSCC are been diagnosed at a very critical and late stages. The main reasons for this situation are the lack of knowledge from the patient's side and of course from the doctor side who did not diagnose the condition properly. The late diagnosis drastically reduces the rate of survival although the broad possible methods of treatment (César Rivera,2015).

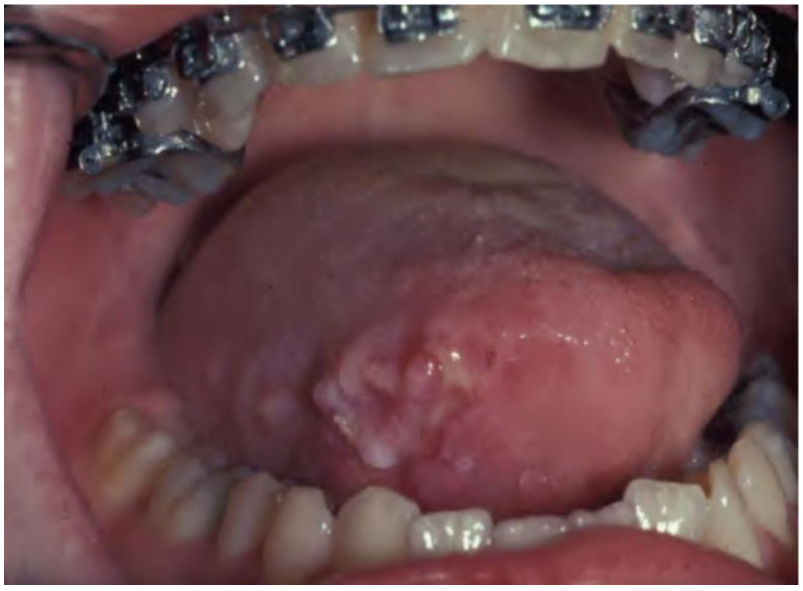

Figure 1:Indurated and ulcerated lesion of the right anterior tongue in a year-old girl, persisting after removal of orthodontic appliances, -15 proven to be squamous cell carcinoma on biopsy (Click et al,2021).

1.1.2 Epidemiology

Epidemiology of Oral SCCOral SCC more frequently affects men than women (M:F = 1.5:1) most probably because more men than women indulge in high-risk habits. The probability of developing oral SCC increases with the period of exposure to risk factors, and increasing age adds the further dimension of age-related mutagenic and epigenetic changes. In the USA the median age of diagnosis of oral SCC is 62 years. However, the incidence of oral SCC in persons under the age of 45 is increasing (Warnakulasuriya, 2009).

The reason for this is obscure.A number of conditions have been associated with an elevated risk of developing oral SCC including Li Fraumeni syndrome, Plummer-Vinson syndrome, Fanconi anemia, chemotherapy induced immunosuppression of organ transplantation, dyskeratosis congenita, xeroderma pigmentosum and discoid lupus erythematosus (Scully and Bagan, 2009).

In Western countries oral SCC affects the tongue in 20% - 40% of cases and the floor of the mouth in 15% - 20% of the cases, and together these sites account for about 50% of all cases of oral SCC . The gingivae, palate, retromolar area and the buccal and labial mucosa are oral sites less frequently affected (Warnakulasuriya 2009).

The ventral surface of the tongue and the floor of the mouth are the sites most commonly affected by SCC because they are lined by thin non-keratinised epithelium. Not only do carcinogens readily penetrate this thin epithelium to reach the progenitor cell compartment, butcarcinogens, particularly tobacco products and alcohol in solution, constantly accumulate in the floor of the mouth and bathe the tissues of the floor of the mouth and the ventrum of tongue (Neville and day ,2002)

.The mean 5-year survival rate of persons with oral SCC is about 50% with no gender difference; but black persons have a lower five year survival rate than persons of other races . Other socio-demographic factors such as age, potentially carcinogenic habits (using alcohol, tobacco, betel quid) or socio-economic status are not consistently related to survival rates (Bello,et al ,2010).

The stage of advancement of oral SCC at the time of diagnosis is the most important prognostic factor (Scully and Bagan, 2009). Oral SCC is most frequently diagnosed late in the course of the disease because affected persons fail to seek professional advice timeously, either because they do not understand the significance of early signs and symptoms, or because they are ignorant of the health implications (Warnakulasuriya, 2009).

1.1.3 Etiology

The incidence of oral cancer is age related, which may reflect time for the accumulation of genetic changes and duration of exposure to initiators and promoters. These include chemical and physical irritants, viruses, and hormonal effects. In addition, decreased immunologic surveillance over time may be another explanation for the age relation. Furthermore, immunosuppressed patients following solid organ and hematopoietic stem cell transplantations, patients treated with chemotherapy, and HIV patients have an increased risk. (Glick et al,2021).

1.1.4 Risk factors

The most significant risk factors for OSCC, with a rate of over 90% are the long term over consumption of alcohol and use of tobacco (Maleki,et al 2015)

Tobacco

Smoke of tobacco contains 3 groups of chemicals such as nitrosamines, benzopyrenes and aromatic amines which promotes cancer. Smokers have a 3 times higher risk for developing an OSCC compared to nonsmokers. In case of nonsmokers, the involuntary smoking as a result from the surrounding environment may increase the chance of developing OSCC in 87% compared to those who have not been in that environment. In addition, smoking not only reduce the immunity of the oral cavity, it also promotes gingivitis, periodontitis and of course OSCC (Maleki et al ,2015)

Alcohol

Is known as Ethanol and has a negative effect on the organism. This effect acts on the local level by allowing higher permeability into the oral mucosa, dissolving lipid particles of the epithelium and leading to epithelial atrophy in general. On the systemic level, it has a mutagenic effect which leads to a smaller salivary flow, decreased liver competence to deal with carcinogenic chemicals and eventually lead to impairment of the immunity system. This impairment results an increased risk for infections and new abnormal growth of tissue (Maleki et al ,2015)

Other risk factors Less common but still possible risk

factors may be insufficient dental hygiene, genetic tendency, chronic mechanical trauma by a sharp object such as a tooth or a denture, chewing of areca nut which occurs mostly in indo-asian populations, human papilloma virus (HPV) which according to the International Agency for Research on Cancer (IARC) the HPV16 is responsible for the cancers of the tonsils, pharynx and oral cavity while HPV18 is responsible for oral cancer . In addition, ultraviolet radiation (UV) which is mostly related to lip cancer, other viruses such as hepatitis C and

Epstein-barr virus (EBV) may be also related to OSCC (Carreras-Torras and Gay-Escoda,(2015).

1.1.5 Pathogenesis

Carcinogenesis is a genetic process that leads to a change in molecular function, cell morphology, and, ultimately, cellular behavior. Carcinogenesis is not limited to the epithelium but involves a complex epithelial, connective tissue, and immune function interaction. Major genes involved in OSCC include oncogenes and tumor suppressor genes (TSGs). Regulatory genetic molecules may be involved as well(Mishra,2013).

The genetic changes may be reflected in allelic loss or addition at chromosome regions corresponding to proto-oncogenes and TSGs, or epigenetic changes such as deoxyribonucleic acid (DNA) methylation or histone deacetylation. Extracellular enzymes, cell surface molecules, and immune function play a role in the development and spread of oral cancer; viruses and carcinogens are involved as well (González-Moles et al,2014). While the principal studies have been related to epithelial changes, some of the components listed can constitute a complex environment that suggests an epithelium-connective tissue theoretical model. For example, the interplay of extracellular enzymes, cell surface molecules, growth factors, and the immune system leads to epithelial–connective tissue interaction. According to this model, mucosal differentiation and maturation of epithelial cells represent an epithelial and connective tissue bidirectional process that may be invoved in carcinogenesis (Glick et al,2021).

1.1.6 Classification

The most significant and predictive factor which will determine the survival rate is the stage of the tumor during the diagnosis (Giovannacci et al,2016). The TNM classification is a worldwide known method of oral cancers staging which is used by healthcare practitioners such as doctors, researchers and cancer registration facilities (Pałasz et al,2017)(Huang &O'Sullivan ,2017).

The initials, T stands for tumor, N for lymph nodes and M for metastases, are based on the measurements of the disease prior to treatment (Liu et al,2016).

The main role is to provide an anatomical classification and to properly describe the development of the cancer.Specific description is the key for the selection of a correct method of treatment, the possible outcome and limitation for certain activites (Huang&O'Sullivan ,2017)

Table 1:Classification of oral sequamous cell carcinoma(omer ,2013)

Table 1: T—primary tumour.

TNM	FIGO
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Tumour 2 cm or less in greatest dimension
T2	Tumour more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumour more than 4 cm in greatest dimension
T4a (lip)	Tumour invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin (chin or nose)
T4a (oral cavity)	Tumour invades through cortical bone, into deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), maxillary sinus, or skin of face
T4b (lip and oral cavity)	Tumour invades masticator space, pterygoid plates, or skull base or encases internal carotid artery

Note: superficial erosion alone of bone/tooth socket by gingival primary is not sufficient to classify a tumour as T4.

Table 2: N—regional lymph nodes.

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis as specified in N2a, 2b, 2c below
N2a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension

Note: midline nodes are considered ipsilateral nodes.

Table 3: M—distant metastasis.

MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Table 4: Stage grouping.

Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1, T2	N1	M0
	T3	N0, N1	M0
Stage IVA	T1, T2, T3	N2	M0
	T4a	N0, N1, N2	M0
Stage IVB	Any T	N3	M0
	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

1.1.7 diagnosis

Diagnostic methods Diagnosis of a doubtful lesion most often

begin with the conventional oral examination, which includes clinical evaluation and palpation of the mucosa of the oral cavity under the lighting of the dental chair (Giovannacciet al,2016). The ability to make a diagnosis at an early stage of OSCC is very important in order to reduce the high rate of sickness and death among the patients (Mascitti et al,2018).

The most common methods used for diagnosis of PMDs and OSCC in an early stage are listed below (Carreras-Torras & Gay-Escoda ,2015).

Vital Staining Methods such as Toluidine blue (TB), Methylene blue staining, Rose bengal staining, Lugol's iodine staining. Staining with TB is a known method for the identification of premalignant and malignant lesions, which is recommended to be as part of the clinical evaluation of oral mucosal tissues, especially in high-risk patients. Those methods are not expensive, quite easy to apply and effective (Liu et al,2016). The staining implemented by various types of dyes over the mucosa in order to mark the neoplastic cells, cells with a high reproductive activity and to indicate the specific areas for examination and biopsy (Carreras-Torras & Gay-Escoda ,2015).

Light-based detection In order to identify oral PMDs and OSCC in their initial stage, several light-based devices have been developed (liu et al,2016]. Those specific devices can emit certain light which will reflect the abnormal tissue and improve the clinical evaluation (Carreras-Torras & Gay-Escoda ,2015).

Histological methods Incisional and/or excisional biopsy are the

most accurate diagnostic methods and together with the histopathological tests, remains as the most reliable methods for OSCC diagnosis (Carreras-Torras & Gay-Escoda ,2015)(Mascitti et al,2018).

Before the procedure of excisional biopsy, it is important that the margins and depth of the tissue will be verified of being a disease-free. Epithelial dysplasia is known as the most prognostic sign of any malignancy. WHO have defined that dysplasia may be classified as mild, moderate and severe .

Cytological methods Those are methods that use a microscope

in order to evaluate the cells which were obtained from smears, scraping and needle aspiration over various depts of the mucosa . The common findings are a typical mucosal lesion which at first sight looks normal, but the prepared specimen will present atypical cells. The cytological tests which have been taken from the oral cavity may help to identify and diagnose tissues with a high-risk or even malignancy (Carreras-Torras & Gay-Escoda ,2015).

Imaging diagnostic methods Those methods include local dental

radiographs, orthopantomogram (OPG), magnetic resonance imaging (MRI), computed tomography perfusion (CTP), C-arm CT, nuclear medicine such as single-photon emission computed tomography (SPECT), ultrasonography and combination of few methods such as PET, CT/ MRI and SPECT/CT (Carreras-Torras & Gay-Escoda ,2015)(Pałasz et al,2017).

1.2 detection technique for oral sequemous carcinoma

1.2.1 Salivary Biomarkers as Non-invasive Diagnostic Tools

Saliva analysis is a noninvasive and inexpensive tool for cancer diagnosis. Compared to blood or tissue samples, saliva has a few advantages including the ease of collection, transport, and processing)Wang et al,2017Additionally, biomarkers in saliva are diluted and .(easily available. Importantly, saliva has a direct contact with oral cancer) lesions, and hence is a suitable method for detecting oral cancerNair et al,2018).

Saliva is a biofluid that contains several proteins and factors, circulating and tissue derived cells, extracellular vesicles (EVs), DNA and RNA molecules, and different cytokines. A variety of salivary biomarkers can

be detected in patients with OSCC such as cell-free DNAs (cfDNAs), circulating tumor DNA (ctDNA), EVs, and miRNAs. Necrotic and apoptotic cells can release DNA/RNA molecules into body fluids during) physiologic and pathologic conditionsCristaldi et al,2019).

In physiological conditions, these molecules are phagocytic targets; however, they accumulate in the tissue microenvironment and biological fluids in cancers. It is believed that the increased number of apoptotic/necrotic cells in cancer patients is the underlying mechanism)Cristaldi et al,2019cfDNAs are short (70–200 bp) or long (up to 21 kb) .(fragments of double-stranded DNA. They can be detected in blood, saliva, plasma, urine, cerebrospinal fluid, and other bodily fluids. cfDNA is freely circulating DNA, but it does not necessarily originate from a tumor as it can be released from normal dying cells. Apoptotic tissue and hematological cells which release the DNA into the circulation are the major sources of cfDNA in a body fluid. In healthy individuals, cfDNA is found atlow levels because apoptotic cells and cfDNA are cleared very quickly. However, in chronic inflammation and cancer cases, clearance is inadequate; therefore, cfDNA can accumulate. In cancer patients, cfDNAs are found in tissue samples and reflect the genetic and epigenetic) alterationsFici,2019Circulating tumor DNAs (ctDNAs) are tumor- .(derived fragmented DNAs which are not associated with cancer cells. Several cancer characteristics such as cellular turnover, vascularity, and drug responses are related to ctDNA concentrations. Although ctDNA is mainly released into the bloodstream, it can be found in other body fluids including saliva. Interestingly, due to less dilution and contamination, the) analysis of ctDNA in saliva is highly sensitiveFici,2019).

miRNAs, small endogenous single-strand RNA molecules, are) dysregulated in many diseasesIrani et al,2019miRNAs can control .(

different events in cancers such as proliferation, differentiation,) apoptosis, survival, motility, invasion, and metastasisMaroof et al,2019Salivary miRNAs are the potential biomarkers for detection of .( ) oral cancerYoshizawa et al,2013).

For instance, down-regulation of salivary miR-139-5p has been detected) in early tongue cancerDuz et al,2016In a previously published study, .(salivary miR-31 level was evaluated in patients with OSCC and patients with oral verrucous leukoplakia. According to the results of this study, miR-31 level was significantly increased in patients with oral carcinoma at all clinical stages, compared to that in patients with oral verrucous) leukoplakiaLiu et al,2012).

A study carried out on saliva samples showed an elevated miR-21 expression level in the salivary samples of OLP, dysplastic OLP, and OSCC patients compared to those of control individuals. In addition, a significantly increased expression level of miR-31 was found in samples from dysplastic OLP and OSCC patients compared to those from healthy) controlsMehdipour et al,2019EVs are lipid bilayer-delimited particles .(that growth are naturally released from cells. EVs represent one of the intercellular communications found in tumor microenvironment (TME) and saliva. In saliva, cell-derived EVs contain some factors such as DNAs, RNAs, miRNAs, and proteins. Recent investigations have indicated that EVs may have essential roles in oral cancer . The most studied vesicles in tumor growth are micro-vesicles and exosomes)Cristaldi et al,2019 .(

Exosomes are small membrane vesicles, ranging from 40–150 nm, which are present in the TME. Exosomes contain proteins, lipids, mRNAs, miRNAs, and mitochondrialDNA. They are released from a variety of

cells into biological fluids such as saliva, urine, semen, amnioticfluid, cerebrospinal fluid, lymph, tears, and blood in physiologic and pathologic conditions. Salivary exosomal miRNAs are considered as diagnostic biomarkers for various malignancies, including OSCC (Wang et al,2014). For example, salivary exosomal miR-24-3p has been demonstrated as a potential biomarker for OSCC (He et al ,2020).

In addition, tumor-derived exosomes in saliva have divergent morphological and molecular characteristics. Therefore, they can be used in the early detection of precancerous lesions and malignant transformation (Cristaldi et al,2019). Exosomes also play essential roles in pro-metastatic niche formation, as well as bone marrow and lymph node metastases. Exosomes can be used as a predictive tool in cancer patients and to differentiate healthy individuals from cancer patients (Nair et al,2018).

It is suggested that exosomes contribute to tumorigenesis, invasion, and metastasis (Irani,2019). Besides, salivary exosomes contain elevated IgA level which has a significant role in the local immune response of the oral cavity (Cheshmi et al,2020). Dysregulation of some other factors and cytokines can be found in saliva. For example, a published paper revealed increased levels of salivary tumor necrosis factor alpha (TNF-α), a cell signaling protein, in patients with OSCC compared to healthy control subjects and patients with leukoplakia. The authors suggested the salivary TNF-α level as a tool for monitoring the malignant transformation of leukoplakia to OSCC (Deepthi et al,2019). An early investigation on salivary and serum IL-6 levels showed significant differences in oral premalignant lesions and oral cancer. IL-6 promotes cancer cell proliferation and involves in the inactivation of the p53 tumor suppressor gene (Dineshkumar et al,2016). Some data have shown significant

correlations between salivary and serum biomarkers. However, some others have shown that biomarkers may differ between blood and saliva. For instance, extracellular RNA biomarkers in the blood are different from those in the saliva. Standard techniques for saliva collection and processing criteria play essential roles in the reliability of the analysis (Oh et al,2020).

1.2.2Circulating Biomarkers

cfDNAs are released into the bloodstream. However, a detailed study which analyzed the plasma level of cfDNAs in 390 patients (90 potentially malignant lesions, 150 OSCCs, and 150 post-treatment OSCCs) and 150 healthy controls did not find any significant difference between the examined groups. The authors proposed that due to the rich lymphatic drainage of the oral mucosa, cfDNA does not enter the bloodstream (Babji et al,2019). Circulating DNA (ctDNA) can be detected in different forms such as free DNA, protein-bound complexes either as free circulating molecules or encapsulated in vesicles (apoptotic bodies, microvesicles, and exosomes). It is now clear that ctDNA has an active role in carcinogenesis. CtDNA has a short half-life of around 10 to 15 minutes. It is rapidly degraded by blood nucleases and eliminated by the liver, spleen, and kidneys (Bronkhorst et al,2019).

Circulating miRNAs or cell-free miRNAs, a class of short non-coding RNAs, are also considered as noninvasive cancer biomarkers. Interestingly, cell-free miRNAs are encapsulated in lipoprotein complexes, and hence are protected from endogenous RNase activity in body fluids (Zhao et al,2019) Circulating miRNA levels have been studied in patients with oral premalignant lesions and OSCC. For instance, the expression level of miR-21 was assessed in the serum of patients with oral submucous fibrosis (OSF) and OSCC. According to the results of

this study, a significant difference was found between OSF and OSCC patients in terms of miR-21 expression level. Additionally, there was a significant relationship between the expression of miR-21 and the clinical stages of OSCC patients (Singh et al,2018).

Data collected from a previous study has shown that up-regulation of plasma miR-10b can be used as an early detection marker for oral cancer (Lu et al ,2012). In a published study, circulating miR-196a and miR-196b were assessed in plasma samples of 53 healthy individuals, 16 pre-cancer patients, and 90 oral cancer patients. The results showed a significant distinction between normal and precancerous patients and between normal and cancer patients. The authors suggested that the combination of miR-196a and miR-196b may be a useful tool for the early detection of oral cancer (Lu et al,2012). Circulating expression levels of 3 miRNAs (miR-222-3p, miR-423-5p, and miR-150-5p) were indicated in patients with oral leukoplakia and OSCC in a published article. According to the findings of this study, miR-222-3p expression level was significantly down-regulated in leukoplakia patients compared to the normal and OSCC patients whereas miR-423-5p and miR150-5p expression levels were elevated in OSCC patients compared to the healthy individuals and leukoplakic patients. The authors suggested miR-222-3p, miR-423-5p, and miR-150-5p as potential biomarkers for the early diagnosis of OSCC and oral leukoplakia (Lu et al,2015).

A number of scientists have indicated that circulating tumor cells (CTCs) can also be used to diagnose cancer at an early stage. CTCs are rare epithelial cells shed from primary tumor into the vasculature. CTCs are found in 30%–40% of cancer patients. CTCs can be considered as prognostic markers (Hristozova et al,2011). Morphologically, CTCs are similar to the primary solid tumor cells and play crucial roles in

metastasis. Hence, it is important to identify patients with CTCs to predict metastasis at an early stage. CTCs have been detected in patients with head and neck SCC and correlate with lymph node metastasis (Hristozova et al,2011). CTCs undergo epithelial-mesenchymal transition (EMT), a crucial event in cancer development, to migrate to distant organs . In a previous study on OSCC patients, CTCs were detected in 12.5% of patients with OSCC. Surprisingly, significant correlations were found between CTCs and tumor size (Gröbe et al ,2015).

Circulating tumor microemboli (CTM) are clusters of tumor cells. It is proposed that CTM can be easily caught in narrow vessels than CTCs, which provide a new suitable environment for the survival of tumor cells (Irani, 2019,Anitha et al,2015). Circulating cytokines are also involved in malignant transformation and tumor growth. The role of cytokines has been evaluated in oral premalignant lesions. According to the results of a study, cytokines such as TNF-a, TGF-β1, and IL-6 have significant effects on the risk of development of precancerous lesions (Hsu et al,2014).

1.2.3 Tissue Biomarkers

Aberrant levels of miRNAs have been detected in oral samples. For instance, the expression of miR-375 was significantly decreased after progression from premalignant lesion to OSCC. It is suggested that miR-375 expression level is a useful tool to identify progressive premalignant lesions from non-progressive samples (Harrandah et al,2016).

Recent studies have shown that OSCC parental cells release exosomes with oncogenic markers which are able to influence the surrounding TME. In OSCC, exosomes are present in TME and can increase the expression of TGF-β, a key player signaling pathway in tumor progression (Lousada-

Fernandez et al,2018). TME consists of different cell types. Among them, cancer-associated fibroblasts (CAFs) have the capacity to transport miRNAs and proteins to cells by exosomes (Li et al,2018). CAF-derived exosomes enhance OSCC metastasis (Bovy et al ,2015 ,Wang et al ,2021).

In a previous study on oral leukoplakia and OSCC samples, the expression level of p53 and epithelial growth factor receptor (EGFR) increased as the lesion progressed from non-dysplastic lesions to moderate dysplastic lesions. The authors proposed that p53 and EGFR play critical roles in the progression of premalignant lesions to carcinomas (Singla et al,2018). Cytokeratins (CKs) are useful biomarkers for the assessment of histopathological progression of oral cancer. In a previously published paper, the expression of CK10-ab1 was assessed in keratinized squamous stratified epithelium. Sever expression of CK10-ab1 was indicated in the suprabasal layers of all specimens in normal and hyperplastic samples; however, CK10-ab1 disappeared gradually with the progression of malignant changes. Therefore, the expression of CK10-ab1 was mild in all poorly differentiated SCCs. The authors suggested CK10-ab1 as a predictable marker for the early detection of OSCC (Al-Jandan et al ,2018). A detailed investigation on CDK1 has demonstrated a higher expression level of CDK1 in OSCC samples. In this study, there was a significant correlation between the expression level of CDK1 and histological grade of OSCC. Hence, overexpression of CDK1 was found in high grade tumors (Chen et al ,2015). Moreover, cytokines and growth factors produced by inflammatory cells can be stained in tissue specimens. Increased expression levels of IL-1α, Il-1β, TGF-β, platelet-derived growth factor, and basic fibroblast growth factor have been found in both epithelium and underlying connective tissue of OSF samples (Haque et al ,1998). Besides, the expression of NF-κB has been detected in oral

premalignant and malignant lesions. A previous study has reported a statistically significant gradual increase of NF-κB cytoplasmic immunostaining score from normal mucosa to OSCC (Kamperos et al,2016). EMT markers can also be detected in oral potentially malignant disorders (OPMDs) s and cancer tissue samples. Expression levels of E-cadherin and vimentin, the most important EMT markers, were assessed in 64 OMPD tissue samples and 23 malignant cases of oral cavity using immunohistochemical (IHC). The results of this study showed a significantly reduced expression level of E-Cadherin in invasive carcinoma samples compared to dysplastic and carcinoma in situ cases. In this study, the expression level of vimentin was positively correlated with tumor progression (Akhtar et al,2019). Additionally, early detection of OSCC can rely on the identification of new biomarkers of extracellular matrix such as matrix metalloproteinases (MMPs) (Kumar and Hema ,2019).

Higher expression levels of MMP-1 and MMP-9 have been demonstrated in OMPDs which progressed to cancer (Jordan et al 2004).

Figure 1 represents a summary of the biomarkers which can be used for the early detection of OSCC.

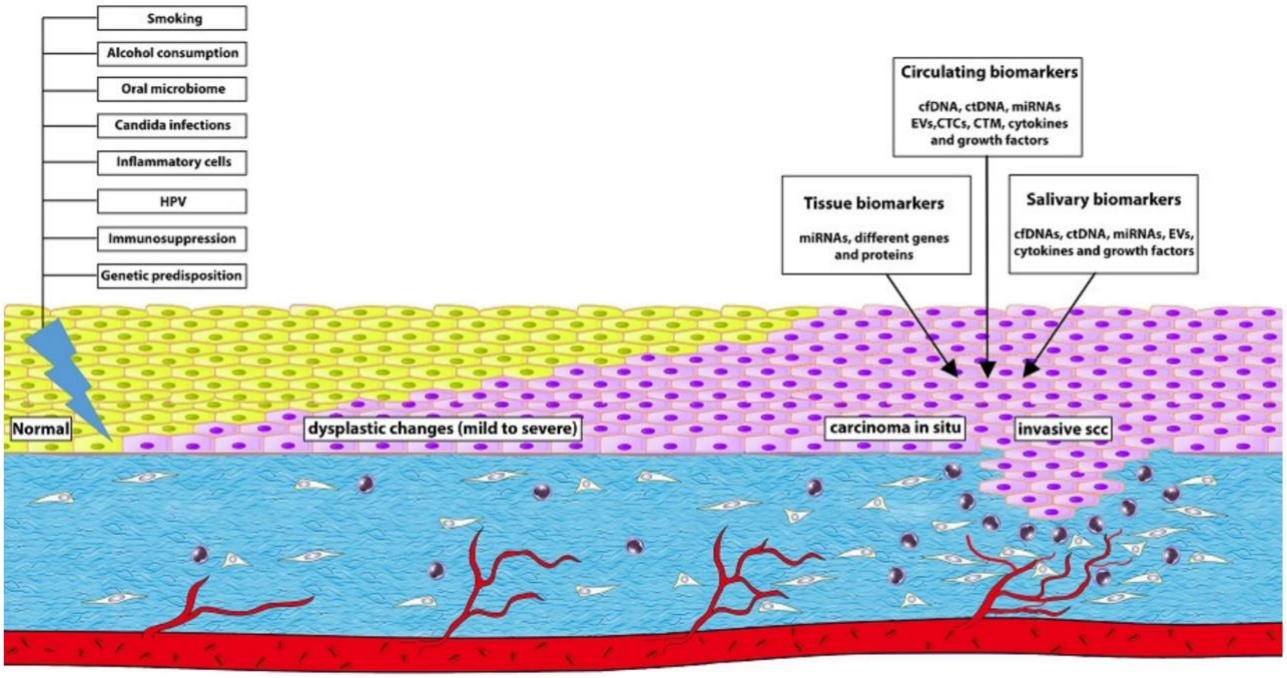

Figure 2: Overview of the major risk factors which promote oral dysplasia and develop oral cancer. Molecular biomarkers can aid in the early detection, monitoring and prognosis of oral cancer(Irani,2022).

1.2.4 Detection Techniques

As the early diagnosis of oral cancer is a key factor in improving the survival and quality of life, it is important to employ different detection techniques. In addition to tissue biopsy, some other helpful techniques are addressed below.

;(A) Brush biopsy obtains specimens from all three layersthe basal, intermediate, and superficial layers. A brush biopsy is a non-invasive and painless technique that facilitates cytological analysis. However, the problem is that the diagnosis needs to be confirmed by tissue biopsy; therefore, it delays the diagnosis.

(B) The fluorescence method (chemiluminescence)is also used for the early detection of precancerous and cancerous lesions. It is the direct visualization of oral cavity and has a good sensitivity to detect oral addition, this technique costs quite high (Cicciù et al,2019).

(C) Toluidine blue stains the cells with an increasedamount of DNA and broader intercellular canals. A previously published study has indicated that the sensitivity and specificity of toluidine blue test are 92.6% and 67.9%, respectively, and the overall diagnostic accuracyis 80%. The toluidine blue staining is simple, rapid, and noninvasive. In addition, the application of toluidine blue can reduce the number of biopsies (Vijayakumar et al 2019).

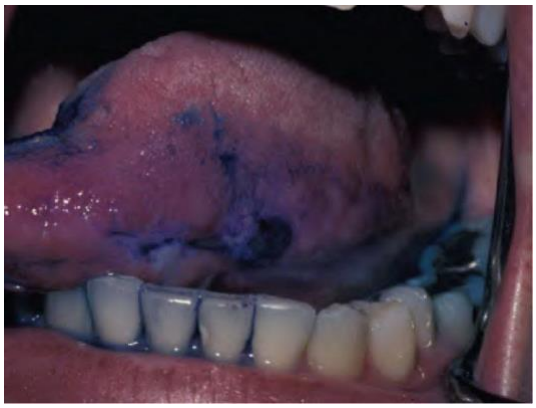

Figure 3: Irregular erythroleukoplakia, following application by toluidine blue. Inferior of lesion and superior stained site were biopsy-proven squamous cell carcinoma.Click et al,2)021)

(D) The methylene blue staining shows the increasedamount of DNA in potentially malignant cells. Accumulating data indicates that methylene blue has high sensitivity but low specificity (Lejoy et al,2016).

(E)Immunohistochemistry IHC uses antibodies to detect the location of proteins and other antigens in tissue samples. It is also easy to determine microvessel density by IHC (Cicciù et al,2019).Occult nodal metastasis may be unnoticed in the routine pathological examination but serial sectioning and immunohistochemistry with pan-cytokeratin markers can help in the early detection of micro-metastasis (Irani et al ,2015)

(F) Liquid biopsy is a non-invasive method to detectOSCC. In contrast to tissue biopsy, liquid biopsy is easy, less invasive, and more comfortable for patients. Liquid biopsy is also a helpful method for detecting HPV DNA.A previously published study has found a higher level of HPV-16/18 DNA (cfDNA) in the plasma of 14% of patients (Mazurek et al ,2015).

,(G) Proteomics, the large-scale study of proteinsinvestigates different proteins expressed in cells and tissues. A variety of tools can be used for proteomic analysis such as ELISA, PCR, Western Blot, and IHC (Madhura et al ,2020).

(H) For a successful result, some other methods arealso helpful. For example, Sanger sequencing, q-PCR-based methods, fluorescent assays, and chromatographic methods are powerful tools for cfDNA analysis (Babji et al,2019). miRNAs can be detected by microarray analysis, RT-qPCR, northern blotting, and in situ hybridization. Recently, nanomaterials such as gold nanoparticles, magnetic nanoparticles, silver nanoclusters, and quantumdots have been applied for miRNA detection (Ye et al ,2019). CTCs can be detected by telomerase activity, aptamer technology, and the CELLSEARCH system (Anitha et al,2015). A variety of techniques based on microfluidics such as Lab-on-a-chip, microfluidic digital PCR, microfluidic single-cell RT-PCR, digital microfluidics, and microfluidic co-culture technique based on 3D spheroids are useful in analyzing genetic mutations, TME, cell proliferation, cancer growth, and interactions between cancer cells and mesenchymalcells (Madhura et al ,2020). CTCs can be detected and isolated by telomerase activity, aptamer technology, the CELLSEARCH system, and microfluidic technologies (Anitha et al ,2015).

1.3 Therapy

TreatmentThe treatment of oral SCC generally requires the services of a multidisciplinary team (shah and Gil ,2009),(Lorch et al ,2009), the primary aim of treatment always being to eradicate the cancer, to prevent recurrence, and insofar as is possible to restore the form and function of the affected parts. The selection of a specific treatment modality is dictated by the nature of the carcinoma and by the general condition of the patient. Salient factors related to the carcinoma include the specific site affected, the clinical size, the extent of local invasion, histopathological features, regional lymphnode involvement and distant metastasis. Patient factors include age, general health status, a history of previously treated oral SCC and high-risk habits (shah and Gil,2009).A variety of modalities are available for the treatment of oral SCC. These include excision/resection, radio-therapy, systemic cytotoxic chemotherapy and blocking of epithelial growth factor receptor (EGF-R), or a combination of these, either concurrently or in an orderly sequence (Rapidis et al,2009),(Mazeron et al ,2009).Surgery is the preferred first line treatment of small, accessible oral SCCs. However, advanced-stage oral SCC is usually treated by a combined treatment program of surgery, chemotherapy, and radiotherapy (shah and Gil,2009),(Braakhuis et al,2011). In cases of recurrent oral SCC, EGF-R inhibitor coupled with chemoradiotherapy, is the first line of treatment (Lorch et al,2009).Surgical resection of oral carcinoma with tumour free margins of less than 5 mm may be followed by local recurrence and possibly by distant metastasis, and usually necessitates the administration of post-surgery chemoradiotherapy. The importance of the presence of dysplastic epithelium in post-resection carcinoma-free margins is of debatable importance, but it is not usually considered to be a strong indication for

further treatment (Braakhuis et al,2010).Twenty to thirty percent of cases of resection of oral SCC with adequate,wider than 5 mm, tumour-free margins as evidenced on histopathological examination will develop local or contiguous regional “recurrence” (Braakhuis et al,2002). There are two possible explanations for this highrate of recurrence. Firstly, some carcinomatous keratinocytes may have remained in the margins of the surgical wound, but because there were so few, they were not detected by histopathological examination; secondly, the large field of precancerized epithelium comprising precancerous keratinocytes at different stages of transformation from which the primary carcinoma developed, was not removed at the surgical procedure. Epithelium from a field of precancerization may appear normal microscopically, or it may be dysplastic. It may also appear normal microscopically, but nevertheless may harbour keratinocytes with cytogenetic alterations including loss of heterozygosity and p53 mutations (Braakhuis et al,2002),(Braakhuiset al,2010), or epigenetic changes in methylations of certain promoters of tumoursuppressor genes and DNA repair genes (Goldenberg et al,2004). Following acquisition of additional genetic alterations, either keratinocytes in the dysplastic epithelium or the genetically transformed keratinocytes may become cancerous giving rise to a new field carcinoma close to where the primary carcinoma had been excised (Braakhuis et al,2002),(Goldenberg et al, 2004), creating an impresssion of recurrence.Thus, the reappearance of SCC in the immediate or general vicinity of the primary oral SCC, may be a recurrence if the two carcinomata exhibit identical genetic profiles; may be a new field carcinoma from a subclone of cells within the field if the genetic profiles of the two cancers are similar, but not identical; or may be another primary carcinoma from a different clone within the same field of precancerization if the genetic profile of the two tumours are dissimilar

(Braakhuis et al,2002).It would be greatly advantageous if it were possible to treat a field of precancerized oral epithelium. However, as markers which predict with any degree of certainty progression of precancerized epithelium to SCC have not yet been identified, and as only 30% of patients with primary oral SCC will develop a second field tumour, any type of treatment of a precancerized field is likely to be harmful to those 70% of patients, who were not going to develop “local recurrence”.Although a precancerized field could be identified by molecular techniques or occasionally histologically, the problem is where to take tissue samples since molecularly precancerized fields are not clinically identifiable(Braakhuis et al,2004)

1.4 Prognosis

The most important factors influencing survival in patients with oral and oropharyngeal cancer are the presence of HPV and the stage of disease at diagnosis.(Douglas et al,2018). Unfortunately, the majority of oral cancers continue to be diagnosed at advanced stages, after becoming symptomatic. Cancers positive for HPV, particularly type 16, have a better prognosis compared to HPV-negative tumors.(Ang et al ,2010),(Fakhry et al,2008).This parameter is now critical to stratify the patient’s risk; however, HPV testing must not be considered in isolation, as there are important interactions with other parameters, such as tobacco and alcohol exposure. Additional prognostic factors for oral cancer include DOI, perineural invasion, differentiation level, lymphocytic infiltrate at interface, status of surgical margins, and ENE of cervical metastases.(Hirshberg et al,2007), There is rarely a second chance for a cure, as cure rates decline rapidly if the lesion is not successfully managed with initial therapy, and therefore the initial approach to therapy is critical. Locoregional causes of death from head and neck cancer may

be due to erosion of major vessels, erosion of the cranial base, nutritional compromise, cachexia, and secondary infection of the respiratory tract. The fact that the OS in younger patients is better reflects that a more complex medical background and comorbidities in older patients expose them to additional systemic complications and poorer outcome.(Glick et al,2021).

1.5 Prevention

Primary prevention has focused on tobacco as a major cause of upper aerodigestive tract cancers, and attention has been paid to strategies of tobacco cessation. Diet has been studied, with evidence supporting a diet rich in fresh fruits and vegetables in developing countries, but with less support in the developed world. Vitamins or nutritional supplements have not been shown to be effective, including no definitive studies investigating antioxidant supplementation. Without vaccination, almost all adults who have been sexually active are exposed to HPV at some point in their lives, usually before age 26. Population-level intervention for preventing associated oral/oropharyngeal cancer is aimed at elimination of HPV infection through vaccine.(Glick et al,2021).

CHAPTER TWO

Conclusion

1. Oral cancer is a malignant neoplasm which grows within the oral cavity The OSCC has few levels of differentiation and a tendency for regional lymph node metastasis

2. Epidemiology of Oral SCCOral SCC more frequently affects men than women (M:F = 1.5:1) oral SCC affects the tongue in 20% - 40% of cases and the floor of the mouth in 15% - 20% of the cases, and together these sites account for about 50% of all cases of oral SCC

3. The incidence of oral cancer is age related,which may reflect time for the accumulation of genetic changes and duration of exposure to initiators and promoters. These include chemical and physical irritants, viruses, and hormonal effects

4. Diagnostic methods Diagnosis of a doubtful lesion most often begin with the conventional oral examination ,Vital Staining Methods,Histological methods Incisional and/or excisional biopsy ,Cytological methods and Imaging diagnostic methods

5. Saliva analysis is a noninvasive and inexpensive tool for cancer diagnosis saliva has a direct contact with oral cancer lesions, and hence is a suitable method for) detecting oral cancer.

6. It is now clear that ctDNA has an active role in carcinogenesis.

7. It is suggested that miR-375 expression level is a useful tool to identify progressive premalignant lesions from non-progressive samples,Cytokeratins (CKs) are useful biomarkers for the assessment of histopathological progression of oral cancer.

8. As the early diagnosis of oral cancer is a key factor in improving the survival and quality of life, it is important to employ different detection techniques as brush biopsy ,The fluorescence method,Liquid biopsy and Proteomics.

9. The selection of a specific treatment modality is dictated by the nature of the carcinoma and by the general condition of the patient.advanced-stage oral SCC is usually treated by a combined treatment program of surgery, chemotherapy, and radiotherapy.

10. The most important factors influencing survival in patients with oral and oropharyngeal cancer are the presence of HPV and the stage of disease at diagnosis.

Referance

1. Akhtar K, Ara A, Siddiqui SA, Sherwani RK. Transition of immunohistochemical expression of E-cadherin and vimentin from premalignant to malignant lesions of oral cavity and oropharynx. Oman Med J. 2016.

2. Ali AA, Al-Jandan BA, Suresh CS. The importance of ctokeratins in the early detection of oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2018.

3. Anitha N, Jimson S, Masthan KM, Jacobina JJ. Circulating tumor cells in oral squamous cell carcinoma-an enigma or reality? J Pharm Bioallied Sci. 2015.

4. B. W. Neville and T. A. Day, "Oral Cancer and Precancerous Lesions," CA: A Cancer Journal for Clinicians, 2002

5. Babji D, Nayak R, Bhat K, Kotrashetti V. Cell-free tumor DNA: emerging reality in oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2019.

6. Bagan J, Sarrion G, Jimenez Y. Oral cancer: clinical features. Oral Oncology. 2010.

7. Bovy N, Blomme B, Frères P, Dederen S, Nivelles O, Lion M, et al. Endothelial exosomes contribute to the antitumor response during breast cancer neoadjuvant chemotherapy via microRNA transfer. Oncotarget. 2015.

8. Bronkhorst AJ, Ungerer V, Holdenrieder S. The emerging role of cell-free DNA as a molecular marker for cancer management. Biomol Detect Quantif. 2019.

9. C. Scully and J. Bagan, “Oral Squamous Cell Carcinoma Overview,” Oral Oncology, , 2009.

10. Carreras-Torras, C. and Gay-Escoda, C.. Techniques for early diagnosis of oral squamous cell carcinoma: Systematic review. Medicina Oral Patología Oral y Cirugia Bucal, pp.e305-e315,2015

11. Carreras-Torras, C. and Gay-Escoda. Techniques for early diagnosis of oral squamous cell carcinoma: Systematic review. Medicina Oral Patología Oral y Cirugia Bucal,2015.

12. César Rivera ,Essentials of oral cancer ,international journalof clinical &Experimmental pathalogy,2015

13. Chang YA, Weng SL, Yang SF, Chou CH, Huang WC, Tu SJ, et al. A three-microRNA signature as a potential biomarker for the early detection of oral cancer. Int J Mol Sci. 2018.

14. Chen X, Zhang FH, Chen QE, Wang YY, Wang YL, He JC, et al. The clinical significance of CDK1 expression in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2015.

15. Cheshmi B, Cheshomi H. Salivary exosomes: properties, medical applications, and isolation methods. Mol Biol Rep. 2020.

16. Cicciù M, Cervino G, Fiorillo L, D’Amico C, Oteri G, Troiano G, et al. Early diagnosis on oral and potentially oral malignant lesions: a systematic review on the VELscope® fluorescence method. Dent J (Basel). 2019.

17. Cristaldi M, Mauceri R, Di Fede O, Giuliana G, Campisi G, Panzarella V. Salivary biomarkers for oral squamous cell carcinoma

diagnosis and follow-up: current status and perspectives. Front Physiol. 2019.

18. Deepthi G, Nandan SR, Kulkarni PG. Salivary tumour necrosis factor-α as a biomarker in oral leukoplakia and oral squamous cell carcinoma. Asian Pac J Cancer Prev. 2019.

19. Dineshkumar T, Ashwini BK, Rameshkumar A, Rajashree P, Ramya R, Rajkumar K. Salivary and serum interleukin-6 levels in oral premalignant disorders and squamous cell carcinoma: diagnostic value and clinicopathologic correlations. Asian Pac J Cancer Prev. 2016.

20. Duz MB, Karatas OF, Guzel E, Turgut NF, Yilmaz M, Creighton CJ, et al. Identification of miR-139-5p as a saliva biomarker for tongue squamous cell carcinoma: a pilot study. Cell Oncol .(Dordr). 2016

21. Fici P. Cell-free DNA in the liquid biopsy context: role and differences between ctDNA and CTC marker in cancer management. Methods Mol Biol. 2019.

22. Gigliotti J, Madathil S, Makhoul N. Delays in oral cavity cancer. Int J Oral Maxillofac Surg. 2019.

23. Giovannacci, I., Vescovi, P., Manfredi, M. and Meleti, M. (2016). Non-invasive visual tools for diagnosis of oral cancer and dysplasia: A systematic review. Medicina Oral Patología Oraly Cirugia Bucal

24. González-Moles MA, Scully C, Ruiz-Ávila I, Plaza-Campillo JJ. The cancer stem cell hypothesis applied to oral carcinoma. oraloncology.2013

25. Gröbe A, Blessmann M, Hanken H, Friedrich RE, Schön G, Wikner J, et al. Prognostic relevance of circulating tumor cells in blood and disseminated tumor cells in bone marrow of patients with squamous cell carcinoma of the oral cavity. Clin Cancer Res. 2014.

26. Haque MF, Harris M, Meghji S, Barrett AW. Immunolocalization of cytokines and growth factors in oral submucous fibrosis. Cytokine. 1998.

27. Harrandah AM, Fitzpatrick SG, Smith MH, Wang D, Cohen DM, Chan EK. MicroRNA-375 as a biomarker for malignant transformation in oral lesions. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016.

28. He L, Ping F, Fan Z, Zhang C, Deng M, Cheng B, et al. Salivary exosomal miR-24-3p serves as a potential detective biomarker for oral squamous cell carcinoma screening. Biomed Pharmacother. 2020.

29. Hristozova T, Konschak R, Stromberger C, Fusi A, Liu Z, Weichert W, et al. The presence of circulating tumor cells (CTCs) correlates with lymph node metastasis in nonresectable squamous cell carcinoma of the head and neck region (SCCHN). Ann Oncol. 2011.

30. Hsu HJ, Yang YH, Shieh TY, Chen CH, Kao YH, Yang CF, et al. Role of cytokine gene (interferon-γ, transforming growth factor-β1, tumor necrosis factor-α, interleukin-6, and interleukin-10) polymorphisms in the risk of oral precancerous lesions in Taiwanese. Kaohsiung J Med Sci. 2014.

31. Huang, S. and O'Sullivan, B. Overview of the 8th Edition TNM Classification for Head and Neck Cancer. Current Treatment Options in Oncology 2017.

32. I.O. Bello, Y. Soini and T. Salo, “Prognostic Evaluation of Oral Tongue Cancer: Means, Markers and Perspectives (I),” Oral Oncology, 2010.

33. Irani S, Barati I, Badiei M. ,Periodontitis and oral cancer-current concepts of the etiopathogenesis, 2020.

34. Irani S, Dehghan A. Expression of vascular endothelialcadherin in mucoepidermoid carcinoma: role in cancer development. J Int Soc Prev Community Dent. 2017.

35. Irani S, Salajegheh A, Gopalan V, Smith RA, Lam AK. Expression profile of endothelin 1 and its receptor endothelin receptor endothelin receptor A in papillary thyroid carcinoma and their correlations with clinicopathologic characteristics. Ann Diagn Pathol,2014.

36. Irani S, Shokri G. The role of miR-143, miR-145, and miR-590 in expression levels of CD44 and vascular endothelial cadherin in oral squamous cell carcinoma. Middle East J Cancer. 2019.

37. Irani S. Emerging insights into the biology of metastasis: a review article. Iran J Basic Med Sci. 2019.

38. Irani S. Metastasis to head and neck area: a 16-year retrospective study. Am J Otolaryngol. 2011.

39. Irani S. New insights into oral cancer-risk factors and prevention: a review of literature. Int J Prev Med. 2020.

40. Jordan RC, Macabeo-Ong M, Shiboski CH, Dekker N, Ginzinger DG, Wong DT, et al. Overexpression of matrix metalloproteinase-1 and -9 mRNA is associated with progression of oral dysplasia to cancer. Clin Cancer Res. 2004.

41. Kamperos G, Nikitakis N, Sfakianou A, Avgoustidis D, Sklavounou-Andrikopoulou A. Expression of NF-κB and IL-6 in oral precancerous and cancerous lesions: an immunohistochemical study. Med Oral Patol Oral Cir Bucal. 2016.

42. Kumar KV, Hema KN. Extracellular matrix in invasion and metastasis of oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2019.

43. Lejoy A, Arpita R, Krishna B, Venkatesh N. Methylene blue as a diagnostic aid in the early detection of potentially malignant and malignant lesions of oral mucosa. Ethiop J Health Sci. 2016,

44. Li YY, Tao YW, Gao S, Li P, Zheng JM, Zhang SE, et al. Cancer-associated fibroblasts contribute to oral cancer cells proliferation and metastasis via exosome-mediated paracrine miR-34a-5p. EBioMedicine. 2018.

45. Liu CJ, Lin SC, Yang CC, Cheng HW, Chang KW. Exploiting salivary miR-31 as a clinical biomarker of oral squamous cell carcinoma.Head Neck. 2012.

46. Liu, D., Zhao, X., Zeng, X., Dan, H. and Chen, . Non-Invasive Techniques for Detection and Diagnosis of Oral Potentially Malignant Disorders. The Tohoku Journal of Experimental Medicine 2006

47. Lousada-Fernandez F, Rapado-Gonzalez O, Lopez-Cedrun JL, Lopez-Lopez R, Muinelo-Romay L, Suarez-Cunqueiro MM. Liquid biopsy in oral cancer. Int J Mol Sci. 2018.

48. Lu YC, Chang JT, Huang YC, Huang CC, Chen WH, Lee LY, et al. Combined determination of circulating miR-196a and miR196b levels

produces high sensitivity and specificity for early detection of oral cancer. Clin Biochem. 2015.

49. Lu YC, Chen YJ, Wang HM, Tsai CY, Chen WH, Huang YC, et al. Oncogenic function and early detection potential of miRNA10bin oral cancer as identified by microRNA profiling. Cancer Prev Res (Phila). 2012.

50. Madhura MG, Rao RS, Patil S, Fageeh HN, Alhazmi A and Awan KH. Advanced diagnostic aids for oral cancer. Dis Mon. 2020.

51. Maleki, D., Ghojazadeh, M., Mahmoudi, S., Mahmoudi, S., Pournaghi-Azar, F., Torab, A., Piri, R., Azami-Aghdash, S. and Naghavi-BehzadEpidemiology of Oral Cancer in Iran: a Systematic . ,Review. Asian Pacific Journal of Cancer Prevention2015.

52. Maroof H, Irani S, Arianna A, Vider J, Gopalan V, Lam AK. Interactions of vascular endothelial growth factor and p53 with miR-195 in thyroid carcinoma: possible therapeutic targets in aggressive thyroid cancers. Curr Cancer Drug Targets, 2019.

53. Mascitti, M., Orsini, G., Tosco, V., Monterubbianesi, R., Balercia, A., Putignano, A., Procaccini, M. and Santarelli, A. An Overview on Current Non-invasive Diagnostic Devices in Oral Oncology. Frontiers in Physiology,2018.

54. Mazurek AM, Rutkowski T, Fiszer-Kierzkowska A, Małusecka E, Składowski K. Assessment of the total cfDNA and HPV16/18 detection in plasma samples of head and neck squamous cell carcinoma patients. Oral Oncol. 2016.

55. McRae MP, Modak SS, Simmons GW, Trochesset DA, Kerr AR, Thornhill MH, et al. Point-of-care oral cytology tool for the screening

and assessment of potentially malignant oral lesions. Cancer Cytopathol. 2020

56. Mehdipour M, Shahidi M, Manifar S, Jafari S, Mashhadi Abbas F, Barati M, et al. Diagnostic and prognostic relevance of salivary microRNA-21, -125a, -31 and -200a levels in patients with oral lichen planus-a short report. Cell Oncol (Dordr),2018.

57. MIichal Glick , Martin s. Greenberg , Peter B. Lockhart and Stephen J. Challacombe , Burket oral medicine,211-224 ,2021

58. Mishra R. Cell cycle-regulatory cyclins and their deregulation in oral cancer. Oral Oncol.oraloncology.2013.

59. Nair S, Tang KD, Kenny L, Punyadeera C. Salivary exosomes as potential biomarkers in cancer. Oral Oncol. 2018.

60. Oh SY, Kang SM, Kang SH, Lee HJ, Kwon TG, Kim JW, et al. Potential salivary mRNA biomarkers for early detection of oral cancer. J Clin Med. 2020.

61. Pałasz, P., Adamski, Ł., Górska-Chrząstek, M., Starzyńska, A. and Studniarek, M. (. Contemporary Diagnostic Imaging of Oral Squamous Cell Carcinoma – A Review of Literature. Polish Journal of Radiology,2017.

62. Polioudaki H, Agelaki S, Chiotaki R, Politaki E, Mavroudis D, Matikas A, et al. Variable expression levels of keratin and vimentin reveal differential EMT status of circulating tumor cells and correlation with clinical characteristics and outcome of patients with metastatic breast cancer. BMC Cancer. 2015.

63. S. Warnakulasuriya, “Global Epidemiology of Oral and Oropharyngeal Cancer,” Oral Oncology 2009.

64. Singh P, Srivastava AN, Sharma R, Mateen S, Shukla B, Singh A, et al. Circulating microRNA-21 expression as a novel serum biomarker for oral sub-mucous fibrosis and oral squamous cell carcinoma. Asian Pac J Cancer Prev. 2018.

65. Singla S, Singla G, Zaheer S, Rawat DS, Mandal AK. Expression of p53, epidermal growth factor receptor, c-erbB2 in oral leukoplakias and oral squamous cell carcinomas. J Cancer Res Ther. 2018.

66. Starzyńska A, Pawłowska A, Renkielska D, Michajłowski I, Sobjanek M, Błażewicz I. Oral premalignant lesions: epidemiological and clinical analysis in the northern Polish population. Postepy Dermatol Alergol. 2014.

67. Sujir N, Ahmed J, Pai K, Denny C, Shenoy N. Challenges in early diagnosis of oral cancer: cases series. Acta Stomatol Croat. 2019.

68. Vijayakumar V, Reghunathan D, Edacheriyan B, Mukundan A. Role of toluidine blue staining in suspicious lesions of oral cavity and oropharynx. Indian J Otolaryngol Head Neck Surg. 2019.

69. Wang H, Hou L, Li A, Duan Y, Gao H, Song X. Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma. Biomed Res Int. 2014.

70. Wang X, Kaczor-Urbanowicz KE, Wong DT. Salivary biomarkers in cancer detection. Med Oncol. 2017.

71. Wang X, Wang X, Xu M, Sheng W. Effects of CAF-derived microRNA on tumor biology and clinical applications. Cancers (Basel). 2021.

72. Ye J, Xu M, Tian X, Cai S, Zeng S. Research advances in the detection of miRNA. J Pharm Anal. 2019.

73. Yoshizawa JM, Wong DT. Salivary microRNAs and oral cancer detection. Methods Mol Biol. 2013.

74. Zhao C, Sun X, Li L. Biogenesis and function of extracellular miRNAs. ExRNA. 2019.