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Identification of Potentially Malignant Oral Lesions

Potansiyel Malign Oral Lezyonların Belirlenmesi

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ABSTRACT

Oral cancer has reached its magnificent height with newer victims being added everyday into its registry. Conditions that predispose to cancer must be identified at its earliest. Diagnostic aids that are currently employed, identify lesions with morphological alteration at a stage where frank malignancy has already set in. There seems to be a great urgency to identify novel biomarkers to diagnose the malignant potential in these habit-associated lesions and prevent the ever-increasing number of oral cancer cases. The following review addresses the current diagnostic aids used, with its shortcomings and future trends in proteomics, which should become a reality.

Key words: Oral cancer, potentially malignant disorders, biomarkers.

ÖZ

Ağız kanseri sıklığı her gün eklenen yeni hastalar ile çok yüksek bir insidansa ulaşmıştır. Kansere neden olan koşullar en erken zamanda belirlenmelidir. Halihazırda kullanılan teşhis araçları, lezyonları morfolojik değişikliğe uğratan ve frank malignitenin önceden belirlendiği bir aşamada tanımlamayı amaçlamaktadır. Alışkanlıkla ilişkili lezyonların malign potansiyelini teşhis etmek ve giderek artan sayıda oral kanser vakalarını önlemek için yeni biyolojik belirteçleri belirlemek büyük aciliyet arz etmektedir. Bu yazıda kullanılan mevcut teşhis araçları, eksiklikleri ve gelecekte gerçek olabilecek proteomik yönelik eğilimler ile ele alınmıştır.

Anahtar kelimeler: Oral kanser, potansiyel malign hastalıklar, biyobelirteçler.

Introduction

Cancer has emerged as a modern day global plague with newer victims being added everyday. More than 90% of the malignancies involving head and neck area belong to oral

squamous cell carcinoma (OSCC) group. Its ever increasing incidence worldwide is around 5,00,000 new cases every year, thus creating a significant worldwide health problem[1].

American Cancer Society has reported over 40,000 new cases of OSCC in the year 2012 in the United States alone with tobacco use and alcohol consumption as the main risk factors. OSCC constitutes the largest group of malignancies in India, with an incidence rate of up to 30-40%, which being very high poses a threat to the developing country. The National Cancer Registry of the Indian Council of Medical Research (ICMR) has confirmed the fact that oral cancer makes up most of the bulk of cancer in India[2,3].

Most of the patients are diagnosed when the disease has moved to an advanced stage, with metastasis to regional lymph nodes and invasion to deeper structures[4,5]. This can be attributed to delay from the patients side, inability to reach health centers, especially in rural areas where there is inaccessibility to health aids and advanced diagnostic equipment, insufficient patient awareness, asymptomatic stage of the condition and sometimes also due to the inability to diagnose the patient at these very initial asymptomatic stages where, there is just a mucosal change with increased risk of malignant conversion[6 7]. These potentially malignant conditions like leukoplakia and oral submucous fibrosis (OSMF) can be diagnosed at an early stage and intercepted before the condition turns malignant. There seems to be a great urgency to identify novel biomarkers to diagnose the malignant potential in these habit-associated lesions and prevent the ever-increasing number of oral cancer cases. The following review article discusses about the current diagnostic aids and the need for newer ones in diagnosing potentially malignant conditions.

Mucosal Changes Preceding Oral Cancer

The initially described terms by the WHO (World Health Organization) namely 'pre malignant lesion' and pre-malignant condition', saw a drastic re-naming process into 'potentially malignant disorders' by the 2005 WHO committee[8]. This was done so as to convey that all these disorders had the potential in them to convert into malignancy. Some of these potentially malignant oral disorders are habit associated, some are stress and autoimmune related and some others have a hereditary component.

Leukoplakia, erythroplakia, palatal lesion of reverse cigarette smoking, oral submucous fibrosis (OSMF), oral lichen planus, discoid lupus erythematosus, epidermolysis bullosa and

dyskeratosis congenita are the disorders that are termed potentially malignant by the 2005 working group of WHO[9].

The term 'leukoplakia' was first used, to describe white changes on the tongue of patients diagnosed with syphilis. The worldwide prevalence of oral leukoplakia is about 2%; with males affected more than females[10]. In India, cases of leukoplakia are more prevalent in some states, than the others. This could be due to the cultural differences and gutka or tobacco chewing habits. Gujarat has the highest number of cases of leukoplakia, followed by Andhra Pradesh[11]. Various etiologic factors have been described to explain the occurrence of this white patch or plaque, including chronic friction, electro-galvanic shock due to dissimilar opposing restorative materials, ultraviolet radiation, and tobacco use and alcohol consumption. Smoked tobacco is the forerunner in the list, with smokers being six times more prone to develop the lesion than those that don't smoke, with alcohol synergizing the effect. Human papilloma virus (HPV) could have a role, with few studies supporting it[12,13].

Various studies conducted have shown that between 16% and 62% of OSCCs are associated with or which are preceded by clinically detectable potentially malignant disorders (PMD's) such as oral leukoplakia and oral submucous fibrosis[14,15]. The annual malignant transformation rate of oral leukoplakia is between 0.1 to 17%[16]. The differences in the rate of malignant transformation in different parts of the world are probably due to differences in ethnic, environmental factors and lifestyle risk factors, such as different tobacco and dietary habits. Follow-up studies have shown that between 0.13% and 36.4% of oral leukoplakia's may transform into oral cancer after they have been watched for 1 to 11 years[15,17]. Various risk factors for the malignant transformation of oral leukoplakic lesions that are habit associated have been put forward. They are female gender, specific sites in the oral cavity such as lateral aspects of the tongue, floor of the mouth and soft palate, non-homogenous type of lesion, Candidal colonization of the lesion and time duration since the condition first appeared[18].

Erythroplakia is a rare lesion and mainly seen in older people, with an incidence of 0.02% - 0.83%. In smokers and alcohol abusers, these red velvety areas may show severe epithelial dysplasia or may have features of carcinoma in situ[19].

Oral submucous fibrosis (OSMF) as the name suggests is fibrosis of the sub mucosal region of the oral cavity and sometimes the pharynx associated with vesiculations, ulcerations, burning sensation on eating spicy food to eventually decreased elasticity of the mucosa, with

functional disability causing general debilitation of the patient. OSMF is seen most commonly among the younger generation in South Asia, with over five million cases in India[20,21].

Areca nut chewing is the main cause for this condition. Other factors implicated in the aetiology are chillies, genetic causes, immunological causes and nutritional deficiency. Paymaster was the first to notice the association of OSMF in patients diagnosed with oral squamous cell carcinoma. Increased malignant transformation of oral submucous fibrosis has been observed over long-term follow up[22]. As observed in a South Indian study, 2.3% of patients had a malignant transformation with OSMF over 10 years follow-up. Another 15 years follow-up demonstrated a malignant transformation rate of 4.5%. Another 17 year follow up showed a malignant transformation rate of 7.6%[23-25].

Oral lichen planus is a T-cell mediated autoimmune disorder affecting middle-aged women[26,27]. Some retrospective studies state that they have a malignant potential. There is less than 1% risk of malignant change. Dysplasia is sometimes seen in the ulcerative anerosive types[28-32].

Palatal lesions in reverse cigar smokers are induced due to smoking with the lit end of the cigarette inside the mouth. There is alteration of the mucosa of the palate, with slight increase in potential for malignant transformation[33]. Another tobacco-related change of the oral mucous membrane occurs due to the use of chewable form of tobacco. These lesions are commonly seen in the vestibule region. Epithelial dysplasia if found in such cases in mostly mild. The lesion is reversible with discontinuation of the habit[34].

Diagnosis of Potentially Malignant Oral Lesions

Screening for oral cancer is mainly done using clinical methods. As morphologically altered tissue can be detected easily in the oral cavity due to its visibility and accessibility, it can be subjected to a variety of chair side investigations including vital staining, tissue auto florescence etc., enabling the clinician to pick out the person with suspected lesions and subject to further laboratory tests. Oral visual screening can thus reduce cancer related fatalities worldwide either by preventing their transformation to a malignant one or by down staging the disease[35].

The most commonly employed method is using normal light to identify changes in the oral cavity. However, histologic changes that occur in small areas may be missed in areas that

appear normal clinically and although systematic studies have been undertaken to assess the value of clinical examination, it lacked standardized diagnostic criteria[36,37].

As most of the oral cancers occur in areas which is easy to self examine, like buccal mucosa, gingiva, hard palate, tongue, floor of the mouth, a lot of authors have suggested that high-risk cases with tobacco and alcohol abuse be taught to do a self examination of the mouth, but the efficacy in properly self diagnosing is yet to be established and proven[38,39].

Biopsy and histopathology are considered to be the gold standard for grading the dysplastic changes in potentially malignant oral disorders. But, as it is an invasive technique, it cannot be used in all cases and cannot be used in multiple sites in the same patient, in order to just rule out changes. As the entire oral mucosa is exposed to the insult of tobacco and alcohol, errors could be made in picking up the ideal spot, thus missing the diagnosis entirely.

Exfoliative cytology is a non-invasive method to diagnose clinically suspicious lesions. However, plenty of false positives and negatives have been reported[40,41]. Oral CDx brush biopsy is another non-invasive method that used a computed assisted system and when a lesion shows dysplastic changes, a traditional biopsy is done so as to confirm the diagnosis[42]. Toluidine blue is a vital dye that stains areas with increased mitotic activity. It has been used as an adjunct to identify abnormal areas, prior to biopsy[43,44]. Studies have been done suggesting that toluidine blue is a great tool to detect oral cancers, but not dysplasia[45].

Differences in absorption and refraction of light from tissues, is the basis of light-detection systems. It is broadly divided into two types: Chemiluminisence and tissue fluorescence imaging. In chemiluminisence, normal cells show a bluish color, whereas abnormal tissue appears white[46-48]. Tissue fluorescence imaging involves exposing the tissue in doubt to a light source of particular wavelength. Abnormal tissues don't fluoresce and appear darker than the surrounding normal healthy tissue[49].

Optical coherence tomography (OCT) also known as "optical biopsy" shows promise as a non-invasive tool to visualize oral mucosa and detect abnormalities up to 1 mm depth, although issues with special resolution have been noticed and a higher resolution OCT is required[50].

Confocal reflectance microscopy is another likely contender in non- invasive diagnostics. It is a device for diagnosing and monitoring various oral pathologies, including early detection of cancers. The images received resemble histology sections and they do not require any invasive

extraction of tissue sample or staining. But due to the large rigid probes, some areas are difficult to visualize and access to all parts of the oral cavity are limited. Moreover, motion artefacts are seen due to the poor stability of soft tissue intra orally[51-53].

Future of Diagnosis

Having described the current methods of identification of potentially malignant oral disorders, visual screening methods just tells us if the altered tissue has a potential for malignant transformation, but it does not tell us what the chances are that the altered tissue will convert into a sure state of malignancy. This is where the role of molecular biomarkers comes to play. Every type of cell has a unique molecular character, which can be measured and quantified, indicative of a normal physiologic process, or it even can be a cell's response to any treatment. This unique characteristic is called a biomarker.

Biomarkers can be divided into pathological or imaging biomarkers based on modality of investigation. Based on the biomolecule used, they can be divided into DNA biomarkers, RNA biomarkers, protein biomarkers and glyco biomarkers. Based on state of the disease process they could be prediction, detection, diagnosis or prognosis biomarkers[54]. Diagnostic and prognostic biomarkers help oncologists to formulate a treatment plan and enable them to know the outcomes of those treatments; in other words, they can have tailor-made treatment protocols for individual patients.

Saliva as a Diagnostic Fluid

Saliva is an aqueous bio-fluid, an exocrine secretion, which contains secretions not only from the salivary glands but also from the gingival crevicular fluid. Saliva comprises of a wide range of components and is one of the most non-invasive diagnostic fluids of the body, which can be utilized for biomarker detection. It consists of 99% water, containing electrolytes, proteins, enzymes, immunoglobulins, antimicrobial factors, mucosal glycoproteins, traces of albumin, peptides, glucose, urea and ammonia[55].

One of the greatest advantage of saliva over blood as a diagnostic fluid is that it can be collected non-invasively; moreover, repeated samples can be collected without any harm, it does not need any special equipment for collection and storage, does not coagulate like blood and is an advantageous diagnostic fluid for patients in whom blood drawing can be difficult. Also, oral tissues are constantly bathed with saliva, and this makes saliva a good diagnostic

fluid. Saliva can be used for diagnosis of systemic diseases, because it contains serum constituents. These constituents are derived from the vasculature of the salivary glands and gingival crevicular fluid (GCF).

Most often, diagnosticians are criticized for using saliva as a diagnostic fluid, because biomarkers are said to be in very low quantities. But since this fluid bathes the oral lesion, it makes it more appropriate than blood for diagnosis. Moreover, advances in the field of "omics", has allowed even very small quantities of substances to be identified and classified. Thus, the inferior status of saliva is rapidly vanishing[56].

Proteins and polypeptides in saliva constitute a significant portion of the mix, and play an important role in carrying on the functions of saliva. So far, over 2000 proteins and peptides have been found in human saliva[57]. The most abundant proteins are α-amylase, lactoferrin, secretory-IgA, albumin, cystatins, hystatins, lysozymes, proline rich proteins, statherin and transferrin all of which together account for more than 98% of the total salivary proteins[58,59]. Overexpressed telomerase, matrix metallopeptidase-9 and 2, Salivary IL-8 are all seen in excess in oral malignancies[60]. In oral premalignant lesions and oral cancers, promoter hypermethylation is used as a potential biomarker for early detection of primary and relapsing oral squamous cell carcinoma. There has been a strong association of cyclin D1 gene amplification with poor prognosis in OSCC. Increased Ki67 marker levels and increased transferrin and decreased miRNA levels are all indicated in OSCC[61,62].

The main aim and ultimate goal of using biomarkers for detection, is allowing the diagnosis to be done at a point where the malignant change has just set in and the treatment is to be a sure success.

Future with Proteomics

Marc Wilkins coined the terms "proteomics" and "proteome" way back in 1900's. Although they do reflect the words "genomics" and "genome", they talk about the protein expression profile of a cell and ultimately within the body, under defined conditions. The human genome with its vast array of genes, expresses many proteins, which function in a specific way under specific condition. Proteomics not only aims to identify all the proteins in a cell but also to map it out.

There are different types of proteomics, and can be described as expression proteomics, structural and functional proteomics. Expression proteomics studies about the protein expression in a particular sample and compares that with other samples in order to identify the expression of disease-specific proteins. Structural proteomics identifies all the proteins and plots out the assembly inside a protein complex and identifies where they are located, and describes all protein-protein interactions. In Functional proteomics, the proteins are studied and characterized and important information regarding signaling of proteins with subsequent health and disease mechanisms are learned[63].

The typical identification of a specific protein relating to health or disease can be divided into three stages. Firstly, the proteins in a sample are separated and isolated. This is done either by using one-dimensional gel electrophoresis (1-DE) or two dimensional gel electrophoresis (2-DE). These methods have specific indications. They cannot be used in all situations, like in case of hydrophobic proteins or proteins of extreme pH. Moreover only single samples can be analyzed at a time and is a tedious process. To overcome gel electrophoreses and to enable representation of greater number of proteins in a mixed sample, various other methods of breaking the protein down into peptides and subjecting it to various purification procedures like liquid chromatography, capillary electrophoresis, reverse phase chromatography and isotope-coded affinity tags (ICAT) are followed[64].

The acquisition of protein structural information for the purposes of protein identification and characterization is done either by Edman sequencing or using the mass spectrometry. Once the protein structural information is obtained from Edman sequencing or mass spectrometry, it must be identified from the database search engine. Once identified, slight modifications in the expression parameters of the proteome, which could be due to various reasons like drugs, tobacco or alcohol, will change the protein pattern and change its expression within a cell, which will cause a modification in the way the cell functions can be identified. All these modifications are challenges that are posed while identifying the protein expressions. Although, once these protein expressions are identified, they can be switched on or off to prevent the manifestation of the disease completely[65].

Conclusion

Oral cancer is a burden on the society. Potentially malignant disorders need to be identified and followed up closely. The use of proteomics will advance the diagnosis and treatment of

various oral malignancies. Correct identification of their malignant potential may help in early diagnosis of cancer and down staging of the dreadful condition. The "gold standard" of biopsy and histopathology has to change to better and non-invasive identification of biomarkers. Studies need to be conducted in identification of valuable biomarkers not only in the established cases of malignancy, but also in the tissues that show a potential to transform, in order to give a better intervention, hopefully delaying the progression if not completely halting it.

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