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Review article

Evaluation of Adjunctive Diagnostıc Tools for the Detection of Oral Potentially Malignant Disorders and Malignant Lesions

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Abstract

Oral cancer defines squamous cell carcinoma of the lip, oral cavity and oropharynx which is a growing serious problem around the world. Despite advances in treatment, the 5-year survival among oral cancer patients has remained approximately 50% and due to the vast majority cases are diagnosed in advanced stages the survival rates are poor. In the past decade adjunctive techniques have been developed to enhance oral mucosal examinations and facilitate the distinction between oral benign lesions, oral potentially malignant disorders (PMDs) and oral malignant lesions. The purpose of this review is to analyse indications, effectiveness, advantages and disadvantages of the available diagnostic tests and techniques helped for the detection of oral PMDs and malignant lesions.

Keywords: Oral Cancer; Early Detection; Adjunctive Diagnostic Tools

Introduction

Oral cancer defines squamous cell carcinoma (SCC) of the lip, oral cavity and oropharynx. In many cancer reports oral cancer and pharynx cancer are gathered in the same group. Each year about 264,000 oral cavity cancer and 136,000 pharynx cancer are diagnosed worldwide. Oral cancer is the sixth leading cancer by incidence worldwide, and the seventh most common malignant tumor group throughout Europe [1-3].

Although epidemiological data related to oral cancer is limited in Turkey; according to the cancer statistics of Turkey Ministry of Health, in 2009 the incidence of the oral cancer in men (in 100,000) was 6.4, whereas the incidence in women (in 100,000) was 2.8 [4].

Despite advances in the treatment, the 5-year survival rate of oral cancer patients is about 50%, and this poor prognosis is explained by; oral cancer patients' second primary tumors rate (3-7% per year) is higher than all other types of malignancy and the vast majority of cases (60%) are identified at a advanced stage [1].

Early detection of oral cancer is one of the primary goals of public health. Dentists play a major role in the early diagnosis of PMDs and malignant lesions of the oral region, the identification of high-risk group of patients and ensuring public awareness on the importance of screening of oral cavity. Each dentist should do conventional oral examination (visual inspection and palpation) including the oral mucosa, tongue and lips for the oral cancer screening, under sufficient light. Although the visual inspection is a standard technique used for oral cancer screening for many years, its value in early detection of PMDs and malignant lesions is still a matter of debate. In the past decade, various adjunctive techniques are emerged to improve the inspection of the oral mucosa, to determine benign lesions, PMDs and malignant lesions of the oral region and to ease to differentiate these lesions [5-7].

The purpose of this review is to examine the indications,

effectiveness, advantages and disadvantages of diagnostic tests and techniques available; which help for detection oral PMDs and malignant lesions.

1-Toluidine Blue:

Toluidine Blue (TB) has been used for many years in the detection of mucosal abnormalities of the oral cavity and cervix. TB is a cationic metachromatic dye, which stains deoxyribonucleic acids in vivo and/or nucleic acids that are retained in dysplastic epithelium's intracellular spaces and clinically appear as royal blue areas. TB's working principle is based on the involvement of more nucleic acids of dysplastic cells numerously and some loss of cohesion of dysplastic epithelium. These features make TB easier to penetrate epithelium and dye retention takes place in cancer cells conversely of healthy cells [6-9].

For oral administration TB is prepared in 1% concentration. 100 ml of 1% TB includes 1 gm of TB powder, 10 ml of acetic acid, 4.19 ml of absolute alcohol and 86 ml of distilled water, its pH is generally adjusted to 4.5. The implementation of the technique starts with rinsing the mouth with water twice for 20 seconds for the removal of debris. After then, for elimination of the poor quality of saliva, the mouth is rinsed with 1% acetic acid for 20 seconds. Following that, if a mucosal lesion is seen, 1% TB is applied to the area with a cotton swab for 20 seconds, if any mucosal lesion is not seen; mouth is rinsed with 1% TB for 20 seconds. For the reduction of the dye which is held mechanically, mouth is rinsed with 1% acetic acid for two more times and finally with water [10]. After this process painted areas are assessed by the examination of oral cavity.

The relationship between the intensity of the color observed after TB application and the severity of dysplasia has been a subject of debate among researchers. Most researchers interpret dark royal blue staining as positive, light blue coloring as suspect, and no staining as negative [9]. Despite some researchers defense the acceptance of pale blue staining as positive[6], when the literature is examined, it has been reported that to accept light blue staining as positive decreases TB sensitivity from 100% to 40%, and TB specificity from 93% to 31%, and similar results have been shown by many various researchers [11,12].

TB is used as an adjunctive tool for diagnosis of oral PMDs and malignant lesions for the first time by Niebel and Chomet [13], these researchers suggested that, the verification of the clinically suspicious lesions as neoplastic, the delineation of the borders of the PMDs/malignant lesions, and the detection of the tumors which cannot be seen with the naked eye are possible with TB.

In 1987, the efficacy of detection of oral SCC by TB was evaluated in a meta-analysis study, in which TB sensitivity was reported to range between 93.5% and 97.8%; whereas TB specificity was ranged between 73.3% to 92.9% [14]. Epstein et al [15], stated that using TB for screening the recurrency within the patients who treated before because of upper respiratory-digestive cancer, increases sensitivity in detecting neoplasm based on only visual clinical examination, from 26.6% to 96.7%.

In the study of Barrellier et al [16], in which the clinical examination and the TB effectiveness were evaluated in the detection of new lesions within the patients who had an oral cancer history and were followed at regular intervals; it was reported that carcinomas were detected in 6 of 32 lesions that clinically invisible but had positive TB; and the sensitivity and specificity of TB was found as 92% and 42% respectively. Whereas in the study of Pallagatti et al [8] the sensitivity and the specificity of TB in detecting oral PMDs and malignant lesions were reported as 95% and 71.4%, and it was deduced that TB would confirm whether the lesions were potentially malignant or malignant, or can eliminate the existing doubts, and may decrease the number of biopsies to be performed. In another study in which patients with oral mucosal lesions were included, TB's effectiveness was investigated. According to the biopsy results SCC was detected in 12 of 30 TB positive lesions, and dysplasia was observed in 5 of them. 2 weeks later when the 13 false-positive lesions re-evaluated, it was found that lesions were not monitored clinically and TB tests were negative. In this study, it was concluded that, TB's false positive rates could be reduced with a second assessment done in 2 weeks and additively it is an important screening tool for patients at high risk for oral cancer [10]. Likewise Masberg [17] reported that , false positive results can be decreased to 8.5% with a repeated application of TB in asymptomatic lesions after 10-14 days.

False positive can arise as a result of the binding of TB to the nucleic acids that released in mucosal ulceration, granulation tissue or inflammatory lesions [18]. However, unlike malignant lesions, the blue appearance generated after application TB to the traumatic/benign lesions, does not stay for a long time on the tissue [19]. Yet sometimes staining are seen not full extent of the traumatic/ulcerated lesion (or related benign lesions) but only at its periphery [20].

TB application is a simple, quick, cheap and high sensitive technique as well as it allows to determine the lesion's borders, accelerates the decision of biopsy and allows to the proper selection of the biopsy site. Although it is considered to be an appropriate diagnostic aid to assist clinicians in performing oral cancer screening in large populations; it has disadvantages such as; increased false positive results in some lesions and showing toxicity for fibroblasts when swallowed [7,9,10].

2-Ligol's Iodine:

Lugol's iodine is a vital stain which was first used by Schiller for

identification of cervical cancer of the uterus [21]. Hereupon this technique has been employed for the upper gastrointestinal tract [22,23]. A number of studies have reported that vital staining with Lugol iodine is a beneficial method for detecting the extent of epithelial dysplasia surrounding oral or esophageal carcinoma [24,25]. In a comparative study, Epstein et al [26] investigated the efficacy of TB and Lugol's iodine both in combination and separately in 59 patients with oral PMDs and malignant diseases. They deduced that Lugol's iodine has less sensitivity in the identification of oral malignant and dysplastic lesions but it has greater specificity. Also recently several authors suggested that the Lugol's iodine might have great potential for oral PMDs and oral cancer screening [27,28].

Due to iodine's glycophilic character after the application of Lugol's iodine; iodine reacts with glycogen in the cytoplasm. The reaction results in uptake of iodine in normal glycogen-containing epithelium then a mahogany brown or black colur change is visualized. Because the areas of dysplasia or carcinoma lack of the normal glycogen content, they don't stain and appear as mustard-yellow or saffron-coloured [27,29].

Although Lugol's iodine is an inexpensive, easy and safe technique; in the oral cavity, efficacy of this staining method is restricted to non-keratinized mucosa hence for the other keratinized mucosal areas other techniques should be used for the detection of early carcinoma and designate its margins [27,30].

3-Oral Exfoliative Cytology:

Oral exfoliative cytology (OEC); is an adjuvant technique for the diagnosis of the superficial oral lesions, including fungal infections, cancer, viral diseases or vesiculobullous dermatoses. OEC determines the characteristics of the cells that flake off naturally or artificially from the oral mucosa. This method which is painless, non-invasive, simple and has high patient acceptability; is particularly useful in the evaluation of the different regions of large lesions and the identification of suitable areas for incisional biopsy [5,31].

The computer-assisted analysis of the oral brush biopsy, also known as OralCDx, was introduced in 1999 (OralCDx Laboratories® Inc., Suffern, New York, USA). This system is designed for the evaluation of oral lesions which dysplasia and cancer suspicion is low due to their clinical features and which biopsy can not be performed. Oral CDx brush biopsy results are classified into three categories, namely, 'negative' the absence of any epithelial abnormality, 'atypical' the presence of abnormal epithelial changes, 'positive' the existence of certain evidence for epithelial dysplasia or carcinoma. A biopsy must be obtained from atypical or positive resulted lesions with conventional method, since Oral CDx does not provide a definitive diagnosis [1,5,32].

Oral brush biopsy showed encouraging results in the evaluation of oral precancerous lesions in several studies. In a prospective, multicenter study of Sciubba et al [33]; the sensitivity of oral brush biopsy (Oral CDx) in the detection of oral PMDs/malignant lesions was recorded as 100% and the specificity was ranged between 92.9% and 100%. In the study of Scheifele et al [34], in which patients with leukoplakia, oral lichen planus and oral SCC were included; both conventional biopsy and brush biopsy (oral CDx) were obtained from all lesions. As a result of the study oral CDx's sensitivity and specificity was reported as 92.3% and 94.3% respectively. However, in this study the inclusion of two clinical groups (oral lichen planus and oral SCC), in which the use of oral brush biopsy were not recommended, makes the study controversial.

Casparis et al [35] also investigated the efficacy of oral CDx brush biopsy in 263 lesions with suspected malignancy; and conventional biopsies were also taken from the lesions that had oral CDx brush biopsy results as positive (n = 7) and atypical (n = 29). In this study, the sensitivity and specificity of Oral CDx were reported as 90%, and 44.1%.

In another research that compared oral brush biopsy and surgical biopsy in patients with clinically minimally suspicious oral lesions; it was reported that 27 oral lesions revealed histopathologic evidence of dysplasia or carcinoma, 26 of these lesions were also identified with the oral brush biopsy (sensitivity 95% - 96.3%); 52 oral lesions did not reveal any histopathologic evidence of dysplasia or carcinoma and brush biopsy reported 47 of these as "negative" and 5 of these as "atypical (specificity 90.4% - 95%). In this study, it was concluded that, the oral brush biopsy is an effective tool for the detection of minimally suspicious oral PMDs and malignant lesions (32).

4-Flow cytometry:

In recent years, DNA imaging cytometry system has started to be used which helps cytological diagnosis of oral mucosal lesions and allows early diagnosis of malignant transformation of squamous epithelial cells. This system is used to identify DNA aneuploidy, which is an internationally recognized marker, indicating the neoplastic changes in cells [36].

In Maraki et al's [36] study, which investigated the diagnostic accuracy of DNA imaging cytometry in patients with suspected oral lesions; in a leukoplakia case without dysplasia, in response to detection of severe dysplasia in its OEC and aneuploidy in its DNA cytometry, more detailed histopathological examination was re-performed and the lesion was diagnosed as severe dysplasia. Also in a case of erythroplakia, which had severe dysplasia in its oral cytology and showed DNA aneuploidy, carcinoma in situ was developed in this lesion after one year, despite the histopathological result was mild dysplasia. The researchers concluded that DNA cytometry is

a non-invasive method and it shows high sensitivity (100%) and specificity (97.4%) in the early diagnosis of oral epithelial malignancies.

Remmerbach et al [37] evaluated the effectiveness of DNA imaging cytometry for early diagnosis of tumor cells. They reported the sensitivity and specificity of this method as 98.2%, 100% respectively. In another study same researchers also suggested that, the presence of DNA aneuploidy pointed to malignant transformation 1-15 months prior to histopathologic examination [38].

DNA imaging cytometry shows promise for the future due to its adavtanges like non-invasiveness, painless and high patient acceptability, as well as reducing the surgical biopsy requirements and demonstrating high sensitivity and specificity for early diagnosis of oral malignancies.

5- Light-Based Detection Systems:

a- Chemiluminescence

Chemiluminescence is the condition of emission of light which generated by a chemical reaction. It has been used in the examination of cervical mucosa for years because of its ability to detect PMDs and malignant lesions. Due to oral and cervical SCC's clinical appearance similarities, this technology recently was adapted for use in the oral cavity. Currently it is marketed under the names with ViziLite® (Zila Pharmaceuticals, Phoenix, AZ, USA in the past; Tolmar Inc., Lincolnshire, IL, USA, at the present), ViziLite® Plus and MicroLux™/DL (AdDent Inc., Danbury, CT, USA) [1,39].

Vizilite® system was approved for use in the United States by the Food and Drug Administration since November 2001. This system is used in conjunction with conventional head and neck examination to identify the cancer lesions at an early stage. ViziLite® kit contains 1% acetic acid solution, disposable chemiluminescent light capsule, a retractor, and the manufacturer's instructions. In addition to these; TB is also available in Vizilite Plus® product kit [40-42].

Clinical application begins with the patient's 1 minute acetic acid mouth wash to remove glycoprotein barrier on the oral mucosa and afterwards drying the mucosa gently. If the ViziLite Plus® system will be used, then TB should be applied to the area. To activate the chemiluminescent light capsule, which consisted of a flexible plastic shell containing acetylsalicylic acid outer, and a fragile vial containing hydrogen peroxide inner, the capsule should be twisted and the inner bottle broken, thereby chemicals react and produce a blue-white color light in 430 to 580 nm wavelength for 10 minutes. Oral mucosa is examined under this blue-white light source in the dark. While normal epithelial cells appear lightly bluish by absorbing light; the light is reflected by abnormal cells with a higher nucleus cytoplasm ratio or by epithelium with hyperkeratinization and/or with a significant inflammation, which causes acetowhite appearance with brighter, more marked borders [1,6,40,41].

In the literature it is observed that, very different results were obtained from the studies investigating the ViziLite® and ViziLite Plus®'s efficacy on the detection of oral PMDs and malignant lesions. Even though the sensitivity of Vizilite® was reported as 100% in the studies of Ram et al [42] and Farah et al [43], the specificity rates were indicated as quite low, 14.2% and 0%, respectively. Additionally, all of the dysplasia and oral SCC lesions in both studies were stated to be noticed easily in a conventional examination, which made by inspection. In the study of Epstein et al [44] two lesions, which were not detected in routine oral examination, but with ViziLite® system, one of them was reported as oral SCC after the histopathological examination. Besides in this study all severe dysplasia, carcinoma in situ and SCC lesions were identified with ViziLite® system. However Awan et al [45] reported the sensitivity and specificity of ViziLite® system for the detection of a dysplastic lesion as 77.3% and 27.8%, respectively. Additionally researchers deduced that this system is not a reliable diagnostic tool for red lesions since it can only detect 50% of the erythroplakia cases in the study. These findings are consistent with the results of the other studies [44,46] which were made previously and also support the hypothesis that chemiluminescence systems can detect keratotic lesions more than the red lesions such as erythroplakia, in which the chance to encounter dysplasia is more.

Some writers advocate that ViziLite® plus system with TB would increase the specificity in the diagnosis of oral PMDs and malignant lesions [39]. Mojsa et al [47] investigated the effectiveness of the Vizilite® plus system as a diagnostic aid in the early detection of oral PMDs and cancer lesions. In this study TB showed 100% sensitivity in the detection of dysplastic lesions and one SCC lesion. When the lesions were examined with chemiluminescence; aceto-white appearance was observed in all degrees of dysplasia but the sensitivity of Vizilite® plus system to distinguish mild to moderate dysplasia were not discussed.

Whereas in Mehrota's [48] work, it was reported that with Vizilite® Plus system examination one oral SCC and two dysplastic lesions did not show aceto-white appearance; and the diagnosis of these lesions could be made after the incisional biopsy. More importantly, all three lesions were detected during visual clinical examination and the investigators stated that chemiluminescence cannot identify any new lesion, and this system cannot provide any benefit in the diagnosis of oral cancer.

Recently another systems; MicroLux™/DL and Orascoptic DK that have basic features of Vizilite®, have been introduced into

the market. These systems consist of battery-powered light emitting diode (LED) transilluminator with an autoclavable light guide that produces diffused light [6]. There is no investigation ever made in the literature about Orascoptic DK system, and since there are no sufficient publications which evaluate MicroLux™/DL system, it is not possible to compare the efficiencies of ViziLite® system with MicroLux™/DL and/or Orascoptic DK systems in correctly. In the study of McIntosh et al [49], the sensitivity, the specificity and the positive predictive value of MicroLux™/DL system in the detection of oral PMDs and malignant lesions were reported as respectively 77.8%, 70.7% and 36.8%. Researchers concluded that MicroLux™/DL system increases the visibility of lesions but it is insufficient to distinguish inflammatory, traumatic and malignant lesions. Ibrahim and et al [50] stated the sensitivity, the specificity and positive predictive value of MicroLux™/DL system as 100%, 32.4% and 17.9%, when compared with the gold standard biopsy results. Although MicroLux™/DL paved the way for detecting the lesions and uncovered new lesions compared to conventional oral examination, it did not alter the provisional clinical diagnosis or the biopsy site and also TB did not increase the effectiveness of this system.

The chemiluminescence systems have the advantages like non-invasiveness, easiness in using and having a high patient acceptability; but also they have significant disadvantages such as high costs, showing low specificity in the diagnosis of oral malignant lesions and still not having a strong evidence supporting its effectiveness as an adjunctive diagnostic tool in oral cancer screening which makes the routine use of chemiluminescence systems questionable [51].

b- Fluorescent Imaging Systems

Fluorescence imaging systems which have been developed to use in cancer screening, cause the autofluorescence of cellular fluorophores by stimulating tissue with a certain wavelight. Tissue fluorescence in the oral cavity can be affected from the metabolic activity, structural changes, the amount of hemoglobin in the tissue, vessel dilatation and possible tissue inflammation; and these cellular differences change the fluorophore concentration which influences scattering and absorption of light in the tissue, thereby causes changes in color that can be observed visually [1,6].

Velscope® (Visually Enhanced Lesion Scope, LED Dental Inc., Canada) is a narrow emission tissue fluorescence system designed by LED Medical Diagnostics in association with British Columbia Cancer Agency scientists; which detects fluorescence loss in visible and non-visible high-risk oral lesions by applying direct fluorescence. Velscope® system consists of a source of light that emits a wavelength of 400-460 nm and a selective (narrow band) filter hand tool enabling a direct imaging. While under this light, normal oral mucosa emits pale green color autofluorescence; due to decreased level of autofluorescence abnormal or suspicious tissues appear dark brown to black by comparison to the surrounding healthy tissue [1,40].

Researchers who support VELscope® system suggest that the lesions which can not be identified with conventional clinical examination can be determined with this system. Although British Columbia Cancer Agency [40] was reported that VELscope® system shows a specificity of 100% and a sensitivity of 98% for discriminating normal tissue from severe dysplasia, carcinoma in situ and invasive carcinoma, using histology as the gold standard; some researchers advocate that in the existence of considerably inflamed lesions false positive results are possible and the use of VELscope® system alone may cause failure to identify regions of dysplasia [52].

The researches that evaluated the effectiveness of VELscope® in detection of oral PMDs and malignant lesions, reported a wide range of sensitivity (30-98%) and specificity (15.3-100%) [53-58]. The variety of these sensitivity and specificity rates can be explained with the differenceness of the patient groups who were included and the centers where these studies carried out.

In a cross-sectional study, VELscope® and ViziLite® with TB were tested to ascertain their strength to aid in the decision-making process regarding whether additional evaluation of a clinically innocuous lesion was required. The study results showed that use of VELscope® or ViziLite® conjunction with a conventional examination for lesions clinically innocuous was not advantageous in determining dysplasia or cancer [48].

In Petruzzi's work autofluorescence and TB were compared for the detection of oral dysplasia and SCC in clinically suspicious lesions along with conventional examination. From the results of study, it was deduced that autofluorescence and TB are both sensitive but not specific in oral SCC and dysplasia diagnosis [59].

As a result, despite VELscope® is thought to be effective in differentiation between normal and abnormal tissue, there is no evidence showing the effectiveness in distinguishing between different types of abnormal tissue (like benign or potentially malignant). On the other hand, there is evidence that it would be more useful if VELscope® is used in specialized centers rather than in routine dental practices [60].

Conclusion

Although many adjunctive diagnostic tools have recently been introduced to assist in the early detection of oral PMDs and malignancies, there is no conclusive evidence for any technique or technology that increases the sensitivity and/or specificity in the oral cancer screening. Nowadays, tissue biopsy with

histological assessment remains the "gold standard" in the diagnosis of oral cancer. Also it can be concluded that available studies have shown promising results, but robust evidence to support the results of the technologies enable the diagnosis of PMDs earlier than conventional oral inspection is still lacking. Therefore there is a need for well-designed clinical trials to evaluate the numerous emerging adjunctive diagnostic tools and their benefits. In addition with increasing public awareness in the early detection of oral cancer, the morbidity and the mortality of this challenging disease can be reduced by the development of reliable adjunctive diagnostic tools to determine the lesions that can not be detected by conventional oral examination.

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