Revisão

NON-INVASIVE TECHNIQUES IN THE CLINICAL DIAGNOSIS OF ORAL EPITHELIAL DYSPLASIA AND SQUAMOUS CELL CARCINOMA - THE ROLE OF TOLUIDINE BLUE

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RESUMO

A presença de displasia epitelial oral não pode ser estabelecida por suspeita clínica de lesão, sendo o exame histopatológico essencial para a obtenção do diagnóstico definitivo.

Atendendo que a biopsia é um método invasivo, a existência de outras técnicas menos invasivas e ao mesmo tempo tão fiáveis como a histopatologia deverão ser consideradas.

O azul de toluidina tem vindo a ser usado na detecção de lesões pré-neoplásicas e neoplásicas nas últimas décadas e o seu valor em termos de diagnóstico mantêm-se incerto, especialmente no que respeita à detecção de lesões com leve e moderado grau de displasia.

Este artigo tem como objectivo a revisão da literatura acerca do uso do azul de toluidina de forma a atestar da sua importância como método não-invasivo de diagnóstico de lesões pré-neoplásicas e neoplásicas.

PALAVRAS-CHAVE: displasia epitelial oral, carcinoma espino-celular, azul de toluidina

ABSTRACT

The presence of oral epithelial dysplasia cannot be established by clinical examination of suspicious lesions, and, therefore relies on biopsy followed by histopathological examination of the tissues.

Since biopsy is an invasive method, the existence of other diagnostic techniques, less invasive but at the same time as accurate as histopathologic examination, are of value.

Toluidine blue has been used in the detection of neoplastic and pre-neoplastic lesions for the past few decades and its diagnostic value still remains uncertain, especially in what concerns to the detection of mild and moderate dysplastic lesions.

The purpose of this article is to review the available literature on the use of toluidine blue in order to assess its relevance in the non-invasive diagnosis of oral malignancy and pre-malignancy.

KEY-WORDS: oral epithelial dysplasia, squamous cell carcinoma, toluidine blue

INTRODUCTION

The presence of oral epithelial dysplasia (OED) is the single most important predicting risk factor for the subsequent development of invasive neoplasia[4,14] and is defined as a lesion in which, at least part of the epithelium is replaced by cells showing various degrees of atypia. Oral cancer usually refers to oral squamous cell carcinoma (SCC) since it represents 95% of all oral malignancies[13], and, although relatively uncommon, with approximately 2000 new cases in the UK, has a significant morbidity and mortality[19].

It is well known that oral cancer is usually diagnosed when it becomes symptomatic[3,8].

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Delays in diagnosis are caused by both patients and practitioners together, and, in average, it takes about 10 weeks for an oral cancer to be diagnosed[21]. At this stage approximately two thirds of the patients will have already developed advanced disease with regional metastasis[3,8] and the prognosis for cure decreases by approximately one half if spread of the tumour has occurred[2]. Subsequently treatment requires surgery, radiotherapy and chemotherapy, alone or in combination. However, despite all the advances in treatment and in reconstructive surgery there has been no improvement in oral cancer prognosis for over four decades[6], and the mortality rate remains high, 40-50% survival at 5 years[19], depending on the studies.

The detection of early dysplastic lesions is desirable in order to provide treatment soon enough to prevent their progression to invasive neoplasia. Efforts should be made to reduce the delay in diagnosis, particularly by alerting the public to examine their mouths regularly and visit their dentist whenever an oral ulcer, white lesion, or other unusual complaint persists for more than 3 weeks[21].

RISK FACTORS

Apart from an extensive examination of the oral soft tissues, the clinician must be aware of the risk factors associated with the dysplastic transformation of the oral epithelial cells.

It is well established that tobacco and alcohol consumption are the two major risk factors for the development of oropharyngeal cancers [4,5,7,19]. The risk of having a dysplastic lesion is seven times that for non-smokers or ex-smokers of more than 10 years standing[6]. Furthermore, a recent study revealed that exclusive tobacco consumption is a more important risk factor for the development of OED than exclusive alcohol consumption[4]. It is also recognised that this risk depends on the number of cigarettes smoked per day and not on the type of tobacco consumed[1].

In what concerns to alcohol, and despite the fact that a correlation between the type of beverage consumed had been tried to be established[11], it is now recognised that this risk associated with each type of beverage is directly proportional to its alcohol content[7]. In conclusion, alcohol plays an important role in the development of OED when considered in conjunction with tobacco, as their combined consumption has a synergistic effect[1,4,18].

METHODS OF ASSESSMENT

Histopathological examination of a lesional tissue is the only reliable method to definitively diagnose oral cancer[19,20]. Despite this there is a demand for other less invasive techniques in order to help clinicians in the early diagnosis of oral cancer, particularly dysplastic lesions.

Exfoliative cytology is the microscopic examination of the cells from an epithelial surface, and has been principally used in screening for dysplasia and carcinoma of the uterine cervix[23]. Although oral exfoliative cytology fell from favour after the 1960's due to the subjective nature of its interpretation, the recent application of quantitative approaches and the differentiation of specific monoclonal antibodies has rekindled interest in this technique[15]. However the major drawback still remains that there is no single marker that is present in all oral cancers that is not present in normal or benign mucosal lesions, although the use of neural networks appears to be promising according to some authors[16]. At this moment the false-negative rate using exfoliative cytology is of approximately 30% [8].

Fluorescence photography is a non-invasive procedure that is also suggested to be of value as a diagnostic method for oral cancer. The basis for such affirmation relies on the fact that experimental cancers show a phenomenon of autofluorescence. This is explained by the presence of protoporphyrin and a trace of coproporphyrin in fluorescent materials, and the production of protoporphyrin by the growth of micro-organisms on the ulcerated tumour surface[17].

Another method for the assessment of OED is the vital staining of tissues with toluidine blue (TB).

TOLUIDINE BLUE

Mechanism of action

Toluidine blue is an acidophilic metachromatic dye of the thiazine group which selectively stains acidic tissue components (sulfates, carboxylates and phosphate radicals), thus staining DNA and RNA[3]. It is used in vivo because dysplastic and anaplastic cells may contain quantitatively more nucleic acids than normal tissues[3,8,12]. Also, malignant epithelium may contain wider intracellular canals, which may facilitate the penetration of the dye[3]. On the other hand, ulcerated and inflamed tissues are also rich in nucleic acids and therefore are expected to retain the dye[12] and this can lead to a misinterpretation of the results.

It was suggested that TB could be a carcinogen and that its repeated use for asseing "high risk" patients and oral premalignancies was associated with some risk[23]. However, in vivo studies on hamster cheek pouch suggested that TB was not carcinogenic itself[24] and appears to be systemically non-toxic and therefore safe in its use[19].

Clinical use

Toluidine blue can be applied using two different techniques. One is the direct application of the dye on the lesion using a cotton pellet[2,3] and the other is the use of a commercially available TB solution (Orascan", Zila Pharmaceuticals Inc.) as a mouthrinse[13,24]. The later probably easier to apply since it is available in a kit (Fig1) prepared to be used in the dental surgery and should be applied as follows:

-Rinse for about 20 seconds with the pre-rinse solution and after with water.

-After the pre-rinse patient will be asked to rinse and gargle with the staining solution for about 20 seconds without swallowing.

-Finally patient will be asked to rinse twice with the post-rinse solution, again for 20 seconds each time, followed by a final rinse with water.

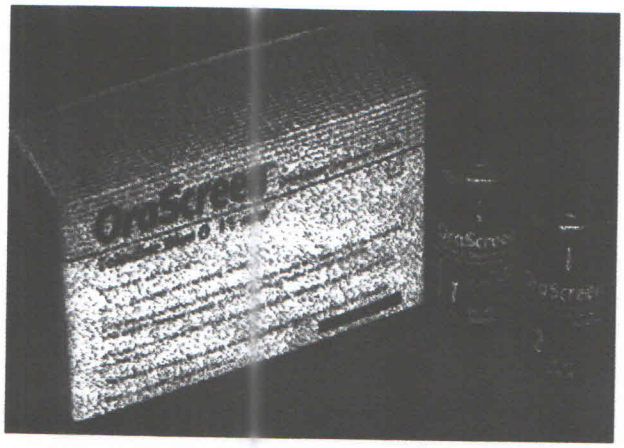

Fig. 1 - The OraScreen Kit

Clinical evaluation

The clinical identification of epithelial dysplasia and early SCC is often difficult because of the varied appearances of the lesions in the oral cavity which may confuse them with a number of similar appearing benign lesions[22]. Therefore, the use of tests such as the application of TB in the aforementioned lesions have been tried.

In order to assess the diagnostic value of TB solution in the detection of both SCC and OED we must be aware of notions such as sensitivity and specificity.

Sensitivity, also known as the true-positive rate, is the proportion of disease individuals who are correctly identified by a certain test, whereas specificity is the proportion of non-diseased individuals who are identified as having the disease by the same test[2].

The sensitivity of TB in the detection of carcinoma has been widely studied. Martin and co-workers[8] pointed a 42% false-negative rate for carcinoma *in situ*. A review of the literature made by Warnakulasuriya and Johnson[24] revealed that the sensitivity rates for TB in the detection oral carcinoma ranges from 86 to 100%. As a matter of fact, in a study performed by these authors in 102 individuals with unconfirmed oral mucosal lesions the sensitivity of the test for SCC was truly 100%. The same rate was obtained by Epstein and co-workers[2], while the sensitivity of the clinical evaluation was only 78%.

Controversy still exists concerning the efficacy of TB stain a clinical indicator of premalig-

nant lesions of the oral cavity, and a study with 11 patients revealed a false-negative rate of 58% for moderate and severe dysplasia[8]. Warnakulasuriya and Johnson[24] claimed that lesions with limited dysplasia or atypia do not stain consistently with the application of toluidine blue. In 1998, Miller and co-workers[12] used 24 Golden Syrian hamsters and induced dysplastic lesions on their buccal pouches with DMBA (Eastman Organic Chemicals, Rochester, N.Y.), and then applied TB on the hamster's pouches. The results showed that a 98.8% false-negative rate for moderate and severe dysplasia and a 82.6% false-negative rate for carcinoma *in situ* were observed. The authors suggested that TB is of little value in the detection of premalignant lesions in the hamster buccal pouch and raised the possibility that very early oral human premalignant lesions may similarly fail to retain the stain. However, other study showed that in 102 patients, 12 with no visually distinct oral lesions retained the dye in a discrete fashion and on biopsy 5 of these demonstrated some degree of dysplasia[24]. Nevertheless, these authors claimed that these findings alone may not justify stain-testing healthy people with clinically normal oral mucosa, particularly in low incidence countries.

Another problem found with the use TB solution is the occurrence of false-positive results with the staining procedure[2,13,24] wich contributes for the poor specificity rates obtained. Specificity rates can be as low as 62% [24]. Among the lesions that may retain the dye are the ones with inflammatory processes, such as ulcers and keratotic areas[9,19,24]. The false-positive rate and therefore the risk of inappropriate diagnostic results with this kind of lesions can be decreased by waiting 10-14 days before a second stain application (two stages technique)[9], allowing a reduction of the false-positive rates to 7 to 9% [10]. It is reported that TB may also stain the dorsal surface of the tongue, buccal mucosa with surface debris and/or keratin[9,19] and the posterior soft palate due to mechanical adherence rather than nuclear affinity[9].

CONCLUSIONS

In fact, the detection of dysplastic lesions is desirable in order to provide treatment early enough to prevent their progression to invasive neoplasia. Efforts should be made to reduce the delay in the diagnosis particularly by alerting the public to examine their mouths regularly and visit their dental practitioner if they have an oral ulcer, white lesion, or other unusual complaint that persists for more than three weeks [21].

Although the use of toluidine blue is of value it should be used carefully and by experienced clinicians. The two-stages technique will pick up most oral cancers [19] and it is a stain of value due to its sensitivity for SCC. However, its use for the detection of OED is limited specially for mild and moderate dysplastic lesions. On the other hand, it is reduced in specificity due to the potential of false positive results in benign lesions [3].

Toluidine blue may help in the determination of the most appropriate site for biopsy but cannot replace it. Nevertheless, a rational use of the solution in the everyday clinical practice may be of value.

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