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Toluidine Blue -A Review with a Case Report.

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ABSTRACT

There is various chair side investigatory techniques were used in detection of oral premalignant lesions out of which toluidine blue was a preferred technique which is used since 1963 by Reichart. It is a cost effective technique with high amount of sensitivity and specificity. Here we are presenting a review of literature with a case report.

Keywords: Toluidine blue, DNA, RNA, Dye, OSCC

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INTRODUCTION

Toluidine blue also known as tolonium chloride, is an acidophilic metachromatic dye which selectively stains acidic tissue components. Toluidine blue (TB) has an affinity for nucleic acids, and therefore binds to nuclear material of tissues with a high DNA and RNA content. Toluidine blue has been known for various medical applications since its discovery by William Henry Perkin in 1856. Toluidine blue has been extensively used as a vital stain for mucosal lesions and also has found applications in tissue sections to specifically stain certain components owing to its metachromatic property.

Case Report

A 35 year old female patient reported to the outpatient department of Balaji dental college & hospital with a chief complaint of swelling gums and mobile teeth for a period of 3 days. The patient gives history of similar complaint 3 years back which gradually progressed to reach the present stage. The patient reported to have the habit of placing snuff in the oral cavity for the past 14 years. Patient was moderately built and nourished and on general physical examination reveals no abnormality was detected. On intraoral examination a homogenous non-scrapable greyish white patch was present bilaterally on right and the left buccal mucosa. On the right mucosa a wrinkle greyish white patch was evident which measures around 4×1 cm in size and lesion appears to be raised and surrounding mucosa appears to be hyperpigmented. It extends anteriorly 2cm away from the commissure of the lip, posteriorly up to the retromolar region. On the left buccal mucosa an erythematous greyish white patch was present which appears to be irregular in shape with well defined borders. On palpation both the lesions on the right and left buccal mucosa were non tender and non scrapable. On examination the gingiva appears to be enlarged and deep pocket was evident in relation 45. A localized collection of pus was evident in relation to 45. Several teeth appear to be mobile and missing. Stains and calculus appear to be severe. With this we come to a provisional diagnosis of Chronic generalized periodontitis, inflammatory gingival enlargement with periodontal abscess in 45.



Figure 1



Figure 2



Figure 3



Figure 4

Other diagnostic conditions suspected are Erythroleukoplakia on the left buccal mucosa and leukoplakia relation to the right buccal mucosa. Routine blood examinations are found under normal limits. Toluidine blue staining was done and an incisional biopsy was performed. Histopathology report reveals parakeratinized stratified squamous epithelium with underlying connective tissue stroma. The epithelium show hyperkeratosis and shows

dysplastic features. Basal cell degeneration were seen in some areas. Connective tissue shows dense infiltrate of chronic inflammatory cells with melanin incontinence were seen in few areas. With this we have come to the final diagnosis of Leukoplakia with severe dysplasia.

REVIEW OF LITERATURE

Toluidine blue also known as toloum chloride is an acidophilic metachromatic dye which selectively stains acidic tissue components (sulfates, carboxylates, and phosphate radicals)[1]. Toluidine blue (TB) has an affinity for nucleic acids, and therefore binds to nuclear material of tissues with a high DNA and RNA content [2]. It is a member of the thiazine group and is partially soluble in both water and alcohol [3]. Toluidine blue has been known for various medical applications since its discovery by William Henry Perkin in 1856, after which it was primarily used by the dye industry. Also known as methylalanine or aminotoluene, it basically has 3 isoforms, namely, ortho-toluidine, para-toluidine, and meta-toluidine. Toluidine blue has been extensively used as a vital stain for mucosal lesions and also has found applications in tissue sections to specifically stain certain components owing to its metachromatic property. Vital staining is the staining of cells or tissues in the living state. The earliest technique developed by Paul Ehrlich in 1885 involved the immersion of freshly removed tissue in methylated blue. There are two techniques of vital staining, namely, intravital staining in the living body (in vivo) and supravital staining outside the body usually applied to slide preparation of detached cell. [4] TB was first applied for in vivo staining by Reichart in 1963 for uterine cervical carcinoma in situ [5]. During 1960s suggestion was made that TB may stain malignant epithelia of the mucous membrane in vivo, whereas normal tissue failed to retain the dye. TB detects relative rather than absolute differences between normal and malignant cells and tissue. They can be used as 1% or 2% oral rinse or an application either in aqueous form or as weak acid solution or of undefined formulation. Only about 5% of dye by weight is retained in oral cavity following expectoration[6].

Its use in vivo is based on the fact that dysplastic and neoplastic cells may contain quantitatively more nucleic acids than normal tissues. Also malignant epithelium may contain intracellular canals that are wider than the normal epithelium, which may facilitate penetration of the dye. [1]

Vital staining of the oral epithelium has been suggested as a means of surveillance in patients who are at a risk of developing oral cancer and for those who had confirmed neoplasms of other parts of aerodigestive tract.[6] TB has been used as a vital stain to highlight potentially malignant oral lesions and may identify early lesions, which could be missed out on clinical examination. Moreover, it can outline the full extent of dysplastic epithelium or carcinoma prior to excisions,[7] and can detect multicentric or second tumors and can help in the followup of patients with oral cancer.

It is useful in obtaining the marginal control of carcinoma and in selecting the biopsy sample site in premalignant lesions. Loss of heterozygosity may be detected in TB-stained lesions. TB-stained tissue may appear dark royal blue or pale royal blue color.[3] Majority of the dyes stain tissues in differing degrees of intensity of the same color,

however, certain tissue components, which in the presence of certain basic dyes of the coal tar group, will stain a color other than that of the dye. Such staining reaction is known as metachromasy and the tissue is said to exhibit metachromasia and the dye as a metachromatic dye.

Among the principal tissue components that exhibit metachromasia are mucin, cartilage, and mast cell granules. The dyes exhibiting metachromatic properties are mainly of thiazine group, thionine, TB, azure A, azure B, methyl violet, safranin, and acridine orange.[4] TB is one such dye, which has been used in tissue sections for identification of various cells.

Zhang et al. [8] suggested that staining intensity may provide important data due to binding of toluidine blue at sites of molecular changes that predict malignant risk, and it is reported that even weakly stained areas had significantly increased molecular alterations compared to toluidine blue negative samples [8, 9]. Gandolfo et al. [10] reported that all OSCC stained toluidine blue positive and that none of the OSCC lesions stained pale blue. However, Gray et al [11]. and Missmann et al. [12] showed that when equivocal staining was included with positive stain, the sensitivity of toluidine blue staining was as low as 40% and as high as 100%. If equivocal stained lesions were considered negative, the sensitivity varied from 100% to 81%^[11]. In another study, when equivocal staining was accepted as positive, the specificity of toluidine blue in OPLs was reported from 31- <50% [11, 13, 14, 15] and as high as 93% [11, 12, 13] These reports suggest the importance of classification of toluidine blue staining intensity as negative or positive (with malignant potential) prior to determination of the need to biopsy and to guide biopsy site selection [16].

In study, conducted by Paloma. et al[17] ;12 / 13 (92.3%) of the squamous cell carcinomas (SCC) had a positive test result. However, 56.3% of the dysplastic lesions were not detected by the dye test alone, as only seven of the 16 histologically diagnosed lesions were considered TP. Following histopathological evaluation, 12 cases of mild dysplasia were diagnosed. Six of them retained TB (TP), whereas six did not retain the dye (FN): only 50% of the mild dysplasias had a positive test result. Fifty percent of the moderate dysplasias also retained the dye: four were diagnosed out of which two were considered TP and two FN. In study conducted by Lewei Zhang. et al[18], monitored OPLs from 100 patients without any history of oral cancer for an average of 44 months in order to evaluate the association of toluidine blue status with clinicopathologic risk factors, molecular patterns (microsatellite analysis on seven chromosome arms: 3p, 9p, 4q, 8p, 11q, 13q, and 17p) and outcome. Toluidine blue–positive staining correlated with clinicopathologic risk factors and high-risk molecular risk patterns. Significantly, a >6-fold elevation in cancer risk was observed for toluidine blue– positive lesions, with positive retention of the dye present in 12 of the 15 lesions that later progressed to cancer (P = 0.0008). This association of toluidine blue status with risk factors and outcome was evident even when the analysis was restricted to OPLs with low-grade or no dysplasia. Our results suggest the potential use of toluidine blue in identifying high-risk OPLs.

In study performed by E. Allegra et al[19], stated regarding toluidine blue staining, 77.7% of the negative lesions were confirmed as histologically benign while 96.2% of the lesions, toluidine blue positive, were histologically pre-cancerous or cancerous lesions

The study performed by Sathish Kumar et al [20] comprised of 17 clinically suspicious cases of oral leukoplakia. The effect of toluidine blue staining and histopathological features were also studied, and concluded that the toluidine blue staining is a sensitive vital staining method for leukoplakia. It is the useful chair side diagnostic test with the sensitivity of 92% and specificity of 100%.

CONCLUSION

Toluidine Blue can be performed easier and it is cost effective which can be performed within 5 mins time with high sensitivity and specificity, however lesion stains with toluidine blue and/or meets the visual criteria for precancerous lesion, biopsy is still mandatory

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