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Original Research Article

Toluidine blue: As an adjuvant screening tool

Rayees Ahmad Sheikh[1,*], Altaf Hussain Chalkoo[1], Bashir Ahmad Wani[1]

[1] *Dept. of Oral Medicine and Radiology, Government Dental College and Hospital, Srinagar, Jammu & Kashmir, India*

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ABSTRACT

Early detection and preventing the progression of potentially malignant disorders (PMDs) help in decreasing the incidence and improving the survival of those who develop oral cancer. The content of DNA and RNA is more in dysplasia and in situ carcinoma than the normal surrounding oral epithelium, the use of in vivo staining, by means of toluidine blue dye, is based on the fact that it is an acidophilic dye that selectively stains acidic tissue components such as DNA and RNA. Toluidine blue staining is considered to be sensitive in identifying early oral and oropharyngeal premalignant and malignant lesions. The results of the clinical evaluation, the toluidine blue test and histology, were compared in order to calculate the sensitivity (true-positivity) and specificity (true-negatives). According to the clinical examination, sensitivity was 53% while for toluidine blue staining, it reached 88.4% (p = 0.0007). Specificity was 76% for the clinical examination and 73.6% for toluidine blue staining (p = 0.79). The positive predictive value for clinical examination was 78.9% and 82% for toluidine blue staining (p = 0.85). The negative predictive value for clinical examination was 50% and 82.3% for toluidine blue staining (p = 0.0073). Our observations suggest that toluidine blue can act as a helpful adjuvant for biopsy in clinically suspicious lesions. So that toluidine blue negative lesions need not to be subjected to biopsies thus saving time and resourses. We conclude, toluidine blue stain could be a useful aid for clinically suspicious lesions in order to establish whether the lesions are at high risk of progression to malignancy and to contribute to an early diagnosis of oral and oropharyngeal cancer. Further studies with larger sample sizes have to be done to make the use of toluidine blue more widespread.

1. Introduction

Squamous cell carcinoma of oral cavity (OSCC) is the 8^{th} most common cancer in the world and it is among the three most common types of cancer in South and Central Asian countries.[1] Although there have been many advances in diagnostic armamentarium leading to early diagnosis and management of oral and oropharyngeal squamous cell carcinoma (SCC), the 5-year survival rate still remains ~40-50%.[2,3] Oral cancer, however, is usually detected at an advanced stage two-thirds of the patients present at advanced stage of the disease), only after it has become symptomatic due to secondary infection or invasion of surrounding tissues. This leads to a difficulty and hence a compromised management and having a high rate of morbidity and mortality. Detection at an early stage, rapid diagnosis and aggressive management of oral pre-malignant lesions are important to reduce the chances of malignancy and improve the survival rate and quality of life (QoL).[4]

Oral potentially malignant and premalignant lesions being precursors of OSCC need to be managed aggressively for early diagnosis and intervention as it is very critical for prevention of carcinoma in oral cavity.[5] Precancerous lesions are asymptomatic and are difficult to detect due

* *Corresponding author.*
E-mail address: shkrays@gmail.com (R. A. Sheikh).

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to a high likelihood of false positive rates making their early detection far from being easy or straightforward.[6] The problem is to determine the site of the biopsy to be taken from suspected lesions, which is most likely to be malignant and this depends on the clinician's ability to clinically differentiate premalignant lesions from reactive and inflammatory diseases of oral cavity.[7] Histopathology of the lesion in question continues to be used as the Gold standard test for diagnosing OSCC. An early detection of oral cancer results in the best outcome as it is often curable, inexpensive to treat, and affords better quality of life. The Detection of the oral potentially malignant lesions at the earliest stage, especially in high-risk groups like tobacco users, beetle quid chewers etc, is of utmost importance to prevent further morbidity and mortality, as they show a high rate of progression and transformation to malignancy, of up to 17% within a mean of 7 years after diagnosis.[8] The diagnosis of premalignant lesions may be very difficult because often, a white patch or plaque, that appears clinically as leukoplakia, it is difficult to define it as another disorder either inflammatory or reactive.[9] Oral cancers are preceded by clinically visible changes in the oral mucosa, usually in the form of white or red patch or ulcers, is an established fact by researchers. Detecting the oral mucosa changes at an early stage and establishing a final diagnosis and thus preventing the progression and conversion of such potentially malignant disorders (PMDs) help in decreasing the incidence of oral cancer and thereby improving the survival of those who develop oral squamous cell carcinoma. The precancerous lesions are usually asymptomatic and there is a lack of education and public awareness about the signs and symptoms among general masses. There is also a lack of knowledge in healthcare providers for early detection of precancerous lesions, that is believed to be primarily responsible for the delay in early and timely identification of the PMDs.[10] Lots of research has been going on for developing diagnostic techniques to support clinical examinations, aiming to improve early detection of oral and oropharyngeal cancer and thereby decreasing the associated morbidity and mortality. The DNA and RNA content is more in dysplastic epithelial cells and in situ carcinoma than the normal surrounding oral epithelium, the use of toluidine blue as in vivo staining, is based on the fact that it is an acidophilic dye having a high tendency to selectively stain acidic tissue components such as DNA and RNA of dysplastic cells in potentially malignant lesions. The use of toluidine blue as in vivo staining is considered to be sensitive in identifying and diagnosing early oral and oropharyngeal premalignant and malignant lesions.[4] The research literature on toluidine blue staining shows that it is a chair side, practical, rapid, inexpensive, and an effective adjunct diagnostic tool for the identification of various potentially malignant lesions.[11] We conducted the study to evaluate, usefullness of in vivo staining by toluidine blue to identify clinically suspecious oral and oropharyngeal premalignant and malignant lesions and to compare the clinical and histological evaluation with toluidine blue stain.

2. Aim of Study

The aim of this study was to determine the usefulness of toluidine blue to identify clinically suspicious oral and oropharyngeal premalignant and malignant lesions.

3. Objective

1. The main objective of this study was to determine whether TB application would be helpful in the diagnosis of oral malignancies and dysplastic lesions.
2. To compare the effectiveness of toluidine blue with clinical examination in detecting suspecious oral and oropharyngeal premalignant and malignant lesions.
3. To determine the effectiveness of toluidine blue as a screening tool for potentially malignant lesions.

4. Materials and Methods

This study was conducted in the Department of Oral Medicine and Radiology at Govt. Dental College and Hospital Srinagar, J&K. The study was conducted from January 2020 to December 2020. The study focuses on 45 oral mucosa lesions from 45 patients. Out of 45 patients 26 were male and 19 were female, the mean age of the patients was 55±5.5 years, range 42-72. The location of the lesion was buccal mucosa in 18 subjects, floor of the mouth, Alveolar mucosa, gingival and hard or soft palate in 13 subjects, tongue in 12 subjects,and Oropharynx in 2 subjects. After properly explaining the study to the patients a written consent was taken from them for the study and after that the toluidine blue staining and biopsy were performed on the patients. The study subjects were made to rinse their oral cavity properly with water for 20 s and to remove remaining debris,that could be mechanically retained rinsing with 1% acetic acid for 20 s was done. After cleaning the oral cavity patients were asked to rinse with Toluidine blue (1% W/W) for 20 s and then again 1% acetic acid was used as oral rinse for 20 s to eliminate mechanically retained stain. Lesions that showed dark blue staining with toluidine blue were considered to be positive, while those that stained light or did not take up the stain were considered negative for potentially malignant lesion. An incisional biopsy was taken under local anesthesia; all specimens were labelled with a number in ascending order and in a separate book, for each specimen, the clinical examination and the result of the toluidine blue staining were reported.

The pathologist was not informed regarding the clinical or staining evaluation of each sample.

4.1. Statistical methods

Statistical software's SPSS (Version 20.0) and Microsoft Excel were used to carry out the statistical analysis of data. Chi-squared analysis was used for comparison and for the purposes of determining the sensitivity and specificity information for toluidine blue. The data were presented by tables. $P < 0.05$ was considered statistically significant.

5. Results

Table 1: Showing location of lesion in the patients

Location	Frequency (n)	Percentage (%)
Tongue	12	26.66%
Alveolar mucosa,gingival, floor of mouth	13	28.88%
Buccal mucosa	18	40%
Oropharynx	2	4.44%

Table 2: Clinical examination and histological correlation

Histology	Clinically Benign (%)	Clinically suspected (%)
Benign	13(50)	4(21)
Dysplasia	11(42.1)	7(36.7)
Carcinoma	2(7.6)	8(42)
Total	26	19

Table 3: Toluidine blue examination and histological correlation

Histology	Toluidine blue negative (%)	Toluidine blue positive (%)
Benign	14(82.3)	5(17.8)
Dysplasia	3(17.5)	12(42.7)
Carcinoma	0(0)	11(39.2)
Total	17	28

Table 4: Histopathologic evaluation compared with clinical examination

Clinical Examination	Histologically Positive	Histologicaly Negative
Positive	15	4
Negative	13	13

Table 5: Histopathologic evaluation compared with toluidine blue staining

Toluidine Examination	Histologically Positive	Histologicaly Negative
Positive	23	5
Negative	3	14

Out of the 45 lesions that were recorded 26 (57%) were categorized as clinically benign, 19(42.3%) were categorized as suspicious lesions. After histopathological evaluation 17 (37.7%) were categorized as benign lesions (like hyperkeratosis, hyperparakeratosis, papillomatosis) and 28 (62.2%) were categorized precancerous or cancerous lesions. Among the 26 lesions that were categorized as benign after clinical evaluation 13 were found histologically benign. Of the 19 lesions that were categorized as clinically suspicious 15 were confirmed histologically as precancerous or cancerous. Table 2 gives the correlation between clinical examinations and histological results. 28 (62.2%) out of the total 45 lesions showed dark blue staining and 17 (37.7%) lesions were negative for toluidine blue staining. Histology of the 17 lesions, that were negative for toluidine blue staining showed 14 (82.3%) were benign whereas from the 28 lesions, that were positive for toluidine blue staining 23 (96.3%) were histologically found as premalignant or malignant. Table 3 presents the correlation between toluidine blue staining and histology results. The sensitivity (true-positivity) and specificity (true-negatives) was calculated from Tables 4 and 5. With clinical examination, sensitivity was 53% while for toluidine blue staining, it reached 88.4%, the results were statistically significant ($p = 0.0007$). Specificity was 76% and 73.6% for the clinical examination and toluidine blue staining respectively ($p = 0.79$). The positive predictive value was 78.9% and 82% for clinical examination and toluidine blue staining respectively ($p = 0.85$). The negative predictive value for clinical examination was 50% and 82.3% for toluidine blue staining ($p = 0.0073$).

6. Discussion

In the present study the sensitivity and specificity for clinical examination was 53% and 76% while as for toluidine blue the sensitivity increased to 88% and specificity was 73%. The results are consistent with the studies conducted by Pallagatti et al.,[12] Allegra et al.,[4] Kumbhare and Taralekar,[13] Rahman et al.,[14] and Parakh, et al.[15] In the study conducted by Pallagatti et al., they included patients with suspected lesions only without using any control group and their study the sensitivity and specificity was 95% and 71.45%, respectively. In the study that was conducted by Allegra et al., they compared the patients for clinical and histological results after toluidine blue staining of the lesions and they found results for sensitivity and specificity were 96.2% and 77.7%, respectively, which is at par with our study. Kumbhare and Taralekar conducted a study in which they compared Vizi Lite and toluidine blue staining. They included lesions that were suspected as potentially malignant in their study, and they found sensitivity 87% and specificity 81% for toluidine blue staining. Rahman et al. conducted a study on potentially malignant lesions and in their study they compared exfoliative cytology and toluidine blue staining of the lesions and found the sensitivity and specificity to be 81.35% and 66.67%, respectively.

In the study of Singh and Shukla the sensitivity and specificity at 97.8% and 100% respectively was higher than all the previous conducted studies, their results were significantly higher than the results of our study. The higher sensitivity and specificity in their study could possibly be due to selection bias. Parakh, et al. conducted a study for identifying the most suitable site from which a biopsy can be taken, that would provide the best histologic results and be most helpful of potentially malignant lesions and found the sensitivity and specificity at 88.89% and 74.19%, respectively.

The results of our study however were contradictory to the studies conducted by Cancela-Rodriguez et al.[16] Ramanathan et al.,[17] Awan et al.[18] The study conducted by Cancela-Rodriguez et al. included lesions that were precancerous and cancerous and found the sensitivity of 65.5% and specificity of 73.3% for toluidine blue staining. In the study conducted by Cancela-Rodriguez et al. the sensitivity of toluidine blue staining was much less than the results shown by our study, probably as they used both cancerous and precancerous cases in their study and the initial diagnosis of carcinoma was made clinically. Ramanathan et al. compared Vizi Lite with toluidine blue for assessment of high risk oral mucosal lesions and found the sensitivity of toluidine blue staining to be 55.5% which is significantly less than that of our study, while the specificity of toluidine blue staining was 91.6% which is significantly more than that of our study. This is probably as they used longer toluidine blue staining time than that of our study. In the study that was conducted by Awan et al. the sensitivity and specificity of toluidine blue staining was significantly lower than that found in our study. The reason for the lower sensitivity and specificity could probably be because they included frictional keratosis patients in their study which are not part of potentially malignant lesions, thus affecting the results of their study. Chemiluminescence and toluidine blue staining as noninvasive methods for early detection of oral cancer and found sensitivity and specificity to be 57% and 44% respectively.

Our observations suggest that toluidine blue can act as a helpful adjuvant for biopsy in clinically suspicious lesions. So that toluidine blue negative lesions need not to be subjected to biopsies thus saving time and resourses.

7. Conclusion

We conclude, toluidine blue staining could be a useful chairside and economic adjuvant diagnostic aid for clinically suspicious lesions of oral cavity, to establish whether the lesions are at high risk of progression to malignancy and thus be helpful to contribute to an early diagnosis of oral and oropharyngeal cancer. Further studies with larger sample sizes have to be done to make the use of toluidine blue more widespread.

8. Source of Funding

None.

9. Conflict of Interest

None.

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Author biography

Rayees Ahmad Sheikh, Post Graduate Student

Altaf Hussain Chalkoo, Professor and HOD

Bashir Ahmad Wani, Senior Resident

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