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Comparative Evaluation Of Toluidine Blue Vital Staining And Degree Of Epithelial Atypia In Oral Potentially Malignant Disorders, Oral Squamous Cell Carcinoma, And Normal Oral Mucosa : An In Vitro Study

Busaidurai S[1], Aravindha Babu N[2], Masthan KMK[3], Rajesh E[4]
[1]Post Graduate Student, Department of Oral Pathology and Microbiology, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research (BIHER), Bharath University, Chennai, Tamil Nadu, India.
[2]Professor & Head, Department of Oral Pathology and Microbiology, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research (BIHER), Bharath University, Chennai, Tamil Nadu, India.
[3]Professor, Department of Oral Pathology and Microbiology, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research (BIHER), Bharath University, Chennai, Tamil Nadu, India.
[4]Professor, Department of Oral Pathology and Microbiology, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research (BIHER), Bharath University, Chennai, Tamil Nadu, India.

Abstract
Background: *Oral cancer is the most common cancer in India with an incidence of 12.6 per 100,000 population with 50-70% of mortality. Oral Squamous cell carcinoma can be treated successfully on early diagnosis.*
Aims: *To compare the staining intensity of toluidine blue with degree of atypia in Potentially Malignant disorders and Squamous Cell Carcinoma. To evaluate the cellular atypia in patients with habits without lesion.*
Settings and Design: *The study was designed as a case-control study among patients visiting dental outpatient department. The study group comprises of two groups I and II. Group I consist of 156 patients with oral potentially malignant disorders (OPMDs) and Oral Squamous cell carcinoma (OSCC). Group II consists of 68 patients with habits but without clinically visible lesions.*
Materials and Methods: *The two groups were clinically examined and categorized. Toludine blue staining followed by exfoliative cytology were taken.*
Result: *Chi square test revealed significant correlation between cytologic finding and toluidine blue stain intensity (P<0.001). Sensitivity and specificity of toluidine blue staining showed 42% and 87%.*
Conclusion: *Cytological diagnosis using Toluidine blue staining techniques may be useful in early detection and is a relatively inexpensive chairside method. It has been emphasized as anon invasive adjunct for the diagnosis of oral potentially malignant disorders and oral cancers.*
Keywords: *Toluidine blue, Oral Potentially malignant disorders, Oral Squamous cell carcinoma, atypia, dysplasia*

INTRODUCTION

Cytology is the study of cells that reflects an attractive technique for the early diagnosis of oral potentially malignant lesions. Normal epithelium undergoes continual exfoliation, the deeper layer of the cells are strongly adherent to each other. When the epithelium becomes the site of benign and malignant conditions, the cells may lose their attachment so that the deeper cells may be shed off along with the superficial cells during cytology.[1]

Toluidine blue is based on the principle of metachromasia. In vivo toluidine blue stains the deoxyribonucleic acid and helps to identify dysplastic epithelium and clinically appears as royal blue areas while in normal tissues fail to retain the dye. In addition, malignant epithelium may contain intracellular canals that are wider so the penetration of the dye is more than normal epithelium.[2] It is useful in high risk population for early diagnosis and aids in selecting the best site for biopsy. The test is sensitive, simple, non-invasive and highly cost-effective procedure proving beneficial for developing countries like India.[3]

In India, oral cancer is one of the most common cancers with every 4 in 10 cancers being oral Cancer. The oral cancer prevalence is primarily high because of tobacco consumption in the form of gutka, Betel quid, snuff or mishri. India comprises 40 per cent of the world's smokers.[4] Worldwide, oral cancer accounts for 2% - 4% of all cancer cases. In India the incidence of head and neck cancer is variable, with some areas showing rates of cancer among the highest in the world and other areas with rates comparable to the United States. Oral and pharyngeal cancers are highest in the area of Ahmedabad in West India.[5] The percentage of 5 year survival for patients with OSCC varies from 40-50%.[6] Exfoliative cytology offers a simple and non-invasive procedure as an adjunct to biopsy.

MATERIALS AND METHODS

The study group comprises of two groups I and II. Group I consist of 156 patients with oral potentially malignant disorders (OPMDs) and Oral Squamous cell carcinoma (OSCC). Group II consists of 68 patients with habits but without lesions.

METHODOLOGY

Using aseptic precautions, Toluidine blue stain was applied over the lesion following standard protocol. The intensity of stain uptake was photographed. Then, the smears were obtained with the help of a moistened wooden spatula by gently scraping the oral mucosa. The exfoliated cells were transferred onto the glass slide, from the spatula with firm pressure and the smear was made with the help of another slide keeping it perpendicular to the first and spreading it in a smooth gliding manner to obtain a uniformly spread smear. Two smears were made for each case. All the slides were fixed in 95% alcohol for minimum of one hour and slides are stained using Papanicoloau staining method. The degree of dysplasia was graded using standard grading system by two senior oral pathologists. Degree of dysplasia was correlated with the toluidine blue staining intensity. Cytological features were evaluated using ProgRes image analyzing software and compared between the groups. The results obtained were subjected to statistical analysis using chi square test.

RESULTS

The age group in our study spanned from 20 to 70 years, with highest incidence in the age group of 41-50 years age group.Out of the total 224 patients 69.6 % were GROUP I and 30.4% were GROUP II. Tobacco chewing (in any form) was the most common
addiction either alone or concomitant with other addictions with 80% patients addicted to it. Tongue was the commonest site of presentation of these lesions over this site.Out of the total 224 patients 92.9% were males and 7.1% were females.Among the study sample, a highest 23.1 % had OSMF. This was followed by 16.7% patients who had leukoplakia, 15.4 % who had OSCC and 14 % who had Erythroplakia.

Among total number of cases that showed intense toluidine blue staining, 62.5 % belonged to class V category, suggesting they were positive for cancer. 16.7% belonged to class IV category which was suggestive of cancer. Intense toluidine blue staining was seen only in very few cases belonging to class III, class II and class I category with a percentage of 11.1, 8.3 and 0.8 respectively. Moderate toluidine blue staining was seen in 37.5 % cases belonging to class IV category and 28.1% belonging to class V category (Table 1,2). 16.7 % cases had moderate staining intensity and belonged to each class III and class II categories. Only 9.2 % class I cases had taken up moderate toluidine blue staining intensity. Among the cases that showed mild intensity of toluidine blue staining 52.8% belonged to class III category, 50 % to class II category and 42.5 % to class I category.A 37.5 % class IV cases had mild toluidine blue staining intensity, while only 3.1 % of class V cases showed mild toluidine blue staining intensity. Among the cases that had not taken up toluidine blue stain, 47.5% belonged to class I category, while 25%, 19.4%, 8.3% and 6.2% belonged to class II, class III,class IV and class V respectively. Chi square test revealed significant correlation between cytologic finding and toluidine blue stain intensity ($P< 0.001$). The clinical findings showed 40.5% of lesions in buccal mucosa and 15.8% of lesions in tongue had mild intensity stain with toluidine blue, 17.6% of lesions in buccal mucosa and 5.3% of lesions in tongue had moderate

intensity stain with toluidine blue and 10.2% of lesions in buccal mucosa and 47.4% of lesions in tongue had intense intensity stain with toluidine blue. However; 31.7% lesions in buccal mucosa and 31.6% of lesions in tongue had not stained with toluidine blue (Table 3). Chi square test revealed significant correlation between site and toluidine blue stain intensity is < 0.001 (Table 4).

Out of 37 cases with Leukoplakia, 56.8% showed mild toluidine blue staining intensity, 29.7% showed moderate intensity, 10.8% showed no toluidine blue staining and 2.7% showed intense toluidine blue staining.

Out of 31 cases with erythroplakia, 51.6% showed mild toluidine blue staining intensity, 25.8% showed moderate intensity, 12.9% showed no toluidine blue staining and 9.7% showed intense toluidine blue staining. Out of 51 cases with osmf, 58.8% showed mild intensity staining, 23.5% showed no toluidine blue staining, 9.8% had moderate intensity staining and 7.8 % had intense toluidine blue staining. Out of 34 cases with SCC, 64.7% showed intense toluidine blue stain 26.5% showed moderate intensity, 5.9% showed mild intensity and. 2.9% showed no toluidine blue staining intensity. Among total number of 68 cases with no lesion, 70.6% had no toluidine blue staining intensity, 23.5% had mild toluidine blue staining intensity and 5.9 % had moderate toluidine blue staining intensity. Chi square test revealed significant correlation between clinical diagnosis and toluidine blue intensity is of < 0.001(Table 5,6).

DISCUSSION

Exfoliative cytology is a painless, non – invasive procedure, and is very well accepted by patients as it causes little discomfort. Whilst oral exfoliative cytology is popular as an oral cancer screening tool, its potential as a diagnostic mode is still challenged. There is an increasing need to refine exfoliative cytology techniques for it to be utilized as a diagnostic test.[7,8] Thereby, an attempt was made to identify cytologic features of keratinocytes that could signify an impending malignant transformation.

In the present study, we have used the technique of exfoliative cytology to examine and study the cytologic features of cells from normal oral mucosa, cells from patients having tobacco habits with no clinically visible lesion and with clinically apparent lesions. Cytological features were evaluated using ProgRes image analyzing software and compared between the groups. The results obtained were subjected to statistical analysis using chi square test.

In the present study, we have studied comparison of cytological finding with toluidine blue stain intensity to be statistically significant (P < 0.001). These findings correlated with previous study done by Rahman F et al (2012)[8] The specificity and sensitivity for oral squamous cell carcinoma and oral premalignant lesions detection of 1% toluidine blue was 81.35% and 66.67%, respectively, while cytology attained 77.97% and 70.37%, respectively. Sharma N et al (2011) compared the results of exfoliative cytology with toluidine blue in diagnosing dysplasia in leukoplakia,it showed 50% sensitivity and 83.3 % specificity.[9] In our study the sensitivity and specificity of toluidine blue staining showed 42% and 87% when compared to cytological finding where it showed 41% sensitivity and 49% specificity. Our study confirms that the toluidine blue vital staining procedure is effective in diagnosing borderline lesions that may transform into early squamous cell carcinoma.

CONCLUSION

Toluidine blue staining serves as a valuable adjunct to the diagnostic process and is required for the diagnosis and treatment plan of various disorders related to oral cavity. The method has good sensitivity with a very low false negative rate. However, clinical examination and histopathology remain the “gold standard” for the detection of oral cancer. Toluidine Blue may provide valid information regarding lesion margins, accelerate the decision to biopsy, guide biopsy site selection and treatment of oral premalignant and malignant lesions. This study states the importance of early recognition of cellular alterations for identification of individuals who require early intervention even in the absence of visible changes of mucosal surface. Hence, cytological diagnosis using other non-invasive techniques may be useful in early detection and it has been emphasized as a method of reducing the morbidity and mortality associated with oral cancers.

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INTENSITY * cytological findings Cross tabulation								
			cyto findings					Total
			CLASS I (Normal)	CLASS II (Atypical)	CLASS III (Indeterminate)	CLASS IV (Suggestive of cancer)	CLASS V (Positive for cancer)	
Intensity	No Stain	Count	57	3	7	2	2	71
		% within cyto findings	47.5%	25.0%	19.4%	8.3%	6.2%	31.7%
	Mild	Count	51	6	19	9	1	86
		% within cyto findings	42.5%	50.0%	52.8%	37.5%	3.1%	38.4%
	Moderate	Count	11	2	6	9	9	37
		% within cyto findings	9.2%	16.7%	16.7%	37.5%	28.1%	16.5%
	Intense	Count	1	1	4	4	20	30
		% within cyto findings	0.8%	8.3%	11.1%	16.7%	62.5%	13.4%
Total		Count	120	12	36	24	32	224
		% within cyto findings	100.0%	100.0%	100.0%	100.0%	100.0%	100.0 %

Table 1: Distribution of study subjects by Toluidine Blue Intensity with Cytological diagnosis

Chi-Square Tests			
	Value	df	P VALUE (significant if <0.05)

Pearson Chi-Square	22.249	3	**<0.001**
N of Valid Cases	224		

Table 2: Statistical Analysis of Toluidine Blue Intensity with Cytological diagnosis

Crosstab								
			clinical finding					Total
			No Lesion	Leukoplakia	Erythroplakia	OSMF	SCC	
Intensity	No Stain	Count	48	4	4	12	1	69
		% within cl find	70.6%	10.8%	12.9%	23.5%	2.9%	31.2%
	Mild	Count	16	21	16	30	2	85
		% within cl find	23.5%	56.8%	51.6%	58.8%	5.9%	38.5%
	Moderate	Count	4	11	8	5	9	37
		% within cl find	5.9%	29.7%	25.8%	9.8%	26.5%	16.7%
	Intense	Count	0	1	3	4	22	30
		% within cl find	0.0%	2.7%	9.7%	7.8%	64.7%	13.6%
Total		Count	68	37	31	51	34	221
		% within cl find	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 3: Distribution of study subjects by Toluidine Blue Intensity with Clinical findings

Chi-Square Tests			
	Value	df	P VALUE (significant if <0.05)
Pearson Chi-Square	168.077	12	**<0.001**
N of Valid Cases	221		

Table 4 : Statistical analysis of Toluidine Blue Intensity With Cytological diagnosis

Crosstab								
			cl find					Total
			No Lesion	Leukoplakia	Erythroplakia	OSMF	SCC	
Cyto findings	CLASS I (Normal)	Count	62	22	12	22	0	118
		% within cl find	91.2%	59.5%	38.7%	43.1%	0.0%	53.4%
	CLASS II (Atypical)	Count	5	2	1	2	2	12
		% within cl find	7.4%	5.4%	3.2%	3.9%	5.9%	5.4%
	CLASS III (Indeterminate)	Count	1	5	11	18	0	35
		% within cl find	1.5%	13.5%	35.5%	35.3%	0.0%	15.8%
		Count	0	7	4	8	5	24

	CLASS IV (Suggestive of cancer)	% within cl find	0.0%	18.9%	12.9%	15.7%	14.7%	10.9%
	CLASS V (Positive for cancer)	Count	0	1	3	1	27	32
		% within cl find	0.0%	2.7%	9.7%	2.0%	79.4%	14.5%
Total		Count	68	37	31	51	34	221
		% within cl find	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 5: Distribution of study subjects by Toluidine Blue Intensity with Cytological diagnosis

Chi-Square Tests			
	Value	Df	P VALUE (significant if <0.05)
Pearson Chi-Square	203.612	16	**<0.001**
N of Valid Cases	221		

Table 6: Distribution of study subjects by Toluidine Blue Intensity with Clinical findings