

Original Research Article

DOI: <http://dx.doi.org/10.18203/issn.2454-5929.ijohns20194931>

The study of use of toluidine blue as an adjunctive tool to clinical examination in early diagnosis of clinically suspicious oral premalignant and malignant lesions: a study of fifty cases

Himanshu Chhagan Bayad, Sanjeev Bhagat, Dimple Sahni*, Navneet Kaur,
Ravinder Singh, Dinesh Kumar Sharma, Baldev Singh

Department of ENT, Government Medical College, Patiala, Punjab, India

Received: 03 July 2019

Revised: 04 September 2019

Accepted: 09 September 2019

*Correspondence:

Dr. Dimple Sahni,

E-mail: sawhneygirish@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Oral carcinoma is among the most prevalent malignancies of head and neck region and is often diagnosed in the advanced stage with significant morbidity and treatment cost. Thus, there is a need for early detection of oral premalignant and malignant lesions. Toluidine blue staining can be used for early detection of these lesions.

Methods: The study included 50 patients with clinically suspicious oral premalignant and malignant lesions. These lesions were subjected to toluidine blue staining and biopsy. Diagnoses were confirmed by histopathological examination.

Results: Sensitivity and specificity of toluidine blue for oral premalignant lesions was 92.30% and 80% respectively with the positive predictive value of 92.30%, negative predictive value of 80% and accuracy of 88.88%. Sensitivity and specificity of toluidine blue for oral malignant lesions was 96.30% and 80% respectively with the positive predictive value of 96.30%, negative predictive value of 80% and accuracy of 93.75%.

Conclusions: The simplicity of toluidine blue staining and its accuracy suggest that it can be a useful adjunctive tool to diagnosis of oral lesions. Results should be carefully evaluated and correlated with clinical findings and histopathological diagnosis.

Keywords: Toluidine blue, Leukoplakia, Erythroplakia, Squamous cell carcinoma, Early detection, Adjunctive tool

INTRODUCTION

Oral cancer is usually defined as squamous cell carcinoma of lips, oral cavity and oropharynx. In the world, 1.6% of all malignancies are comprised of oral cancers; males in the age group of 40-50 years and belonging to lower socioeconomic strata are most commonly affected. The rate of diagnosis of late stage oral cancers in men in their 4th and 5th decade has increased from 3.6 to 8.4 per 100000.¹ Though there are many advanced surgical techniques and treatment

options, the 5-year survival rate is still ~40-50%.^{2,3} There is a strong association between habitual cigarette smoking and alcohol use and oral premalignant lesions and oral malignant lesions.

There are 3 types of premalignant lesions that are common in Punjab which are leukoplakia, erythroplakia and lichen planus. These lesions have greater potential for malignant transformation than other oral lesions. Their early detection is crucial for improving the survival rate and reducing morbidity, disfigurement, function loss, treatment duration, and hospital costs.

Oral malignant lesions mainly include squamous cell carcinoma of lips, tongue and floor of mouth, buccal mucosa, gingivobuccal complex and hard palate. Oral malignancies are generally at the advanced stage at the time of diagnosis. At an early stage, oral squamous cell carcinoma is often curable and treatment remains inexpensive.⁴

Early stages are difficult to detect as sometimes lesions may not be palpable and changes in color are not very different from the surrounding mucosa. The main difficulty is when and from where the biopsy should be taken. In ~16% of cases of leukoplakia, malignancy was found on histopathology.^{5,6} Thus, identifying clinically suspicious or undetectable lesions has gained importance whereby diagnosis can be confirmed by biopsy at an earlier stage.

The strategy should be targeted towards the development and use of easily available diagnostic aids that could help clinicians more readily identify or assess persistent oral lesions of uncertain biologic significance. The toluidine blue test is based on the principle that dysplastic cells contain quantitatively more nucleic acids and a dysplastic epithelium also has some loss of cohesion. These features help the penetration of acidophilic toluidine blue through the epithelium resulting in retention of the dye in malignant cells with increased DNA and RNA, whereas normal mucosa doesn't retain the dye.⁷ Toluidine blue test forms cheaper and more apt modality of choice, considering easy availability, limited resources and widespread need because of the higher incidence of oral premalignant lesions and oral malignant lesions in the region of Punjab. Hence, this study was conducted to evaluate the use of toluidine blue as an adjunctive tool to clinical examination in the early diagnosis of oral premalignant lesions and oral malignant lesions.

METHODS

The present study was conducted on 50 patients with oral lesions who visited in OPD in the department of Otolaryngology- Head and Neck Surgery, Government Medical College and Rajindra Hospital, Patiala from 2014-2016. This study included patients with age group of 20 to 80 years with clinically suspicious premalignant and malignant lesions of the oral cavity, irrespective of site and stage. Patients with history of head and neck irradiation and acute trauma in oral cavity were excluded from the study.

A detailed history of the symptoms and their duration was taken. Family history and history of alcohol, tobacco and other substance abuse was also taken. Examination of the oral cavity and oropharynx, IDL examination, laryngeal endoscopy, anterior rhinoscopy, posterior rhinoscopy, ear examination and general physical examination of each patient was done. Routine investigations such as complete haemogram, ECG and complete urine examination were done.

2% acetic acid was applied with cotton bud for 20 seconds on the lesions and then rinsed with water. 1% toluidine blue was applied with a cotton bud for 10-20 seconds and was decolorized with 2% acetic acid using a cotton bud for 20-30 seconds. Dark blue stain was considered as positive for lesions suspicious of malignancy, light blue retention was considered as positive for premalignant lesions unless proved otherwise by biopsy and the lesions without any retention of stain was considered as negative (Figures 1A, 1B, 2A and 2B).



Figure 1 (A): Oral premalignant lesion.



Figure 1 (B): Oral premalignant lesion post toluidine blue staining.



Figure 2 (A): Oral malignant lesion.

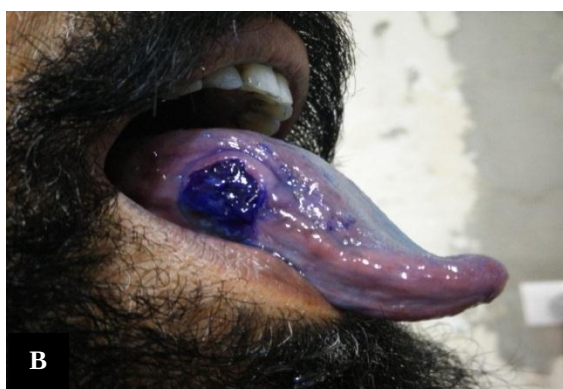


Figure 2 (B): Oral malignant lesion post toluidine blue staining.

Biopsy site was selected on the basis of clinical appearance and dye retention and in the sites where no retention of the stain occurred, clinical judgment directed the biopsy. Histopathological grading for premalignant lesions was performed as per the pathologic features, which was grouped into leukoplakia, erythroplakia and lichen planus. Oral malignancies were graded into well-differentiated (Grade 1), moderately differentiated (Grade 2) and poorly differentiated (Grade 3) squamous cell carcinoma.

Diagnostic validity tests that are sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and diagnostic accuracy (DA) was

performed to determine the utility of the test results for predicting the various condition of the disease. Software Program for Statistical Significance-20.0 software was used for statistical analysis. P value <0.05 was considered significant and p<0.01 was considered highly significant. Written and informed consent from each of the patients was taken for the study.

RESULTS

In our study, maximum incidence of oral premalignant lesions was seen from 4th to 6th decade with peak in 5th decade. Whereas oral malignant lesions were seen in 4th to 6th decade with peak in 6th decade (Table1). Males were predominant in the study, male female ratio being 1.4:1 and 5.2:1 for oral premalignant and malignant lesions respectively. Among oral premalignant lesions, maximum numbers of cases were recorded amongst unemployed followed by labourers. In oral malignant lesions, most of cases were amongst labourers followed by unemployed (Table 2).

Out of 50 cases, clinically suspicious oral premalignant and malignant lesions were 19 and 31 respectively. Whitish patch was the most common presenting symptom amongst clinically suspicious oral premalignant lesions (Table 3).

Table 1: Age distribution in clinically suspicious oral premalignant and malignant lesions.

Age in years	Clinically suspicious oral premalignant lesions (n=19)						Clinically suspicious oral malignant lesions (n=31)	
	Leukoplakia (n=12)		Erythroplakia (n=3)		Lichen planus (n=4)			
	N	%	N	%	N	%	N	%
21-30	2	16.67	0	0	0	0	0	0
31-40	1	8.33	0	0	1	25	5	16.13
41-50	6	50	2	66.67	3	75	6	19.35
51-60	3	25	1	33.33	0	0	11	35.48
61-70	0	0	0	0	0	0	5	16.13
71-80	0	0	0	0	0	0	4	12.90
Total	12	100	3	100	4	100	31	100
Mean age	41±6.50		48.33±5.77		42.25±6.50		55.94±12.74	

Table 2: Occupations in clinically suspicious oral premalignant and malignant lesions.

Occupation	Clinically suspicious oral premalignant lesions			Malignant lesions
	Leukoplakia N (%)	Erythroplakia N (%)	Lichen planus N (%)	
Farmer	1 (8.33)	0 (0%)	0 (0%)	3 (9.68)
Government servant	2 (16.67)	0 (0)	1 (25)	2 (6.45)
Unemployed	5 (41.67)	1 (33.33)	2 (50)	5 (16.13)
Labourer	2 (16.67)	2 (66.67)	0 (0)	16 (51.61)
Private job	2 (16.67)	0(0)	1 (25)	5 (16.13)
Total	12 (100)	3 (100)	4 (100)	31 (100)

Table 3: Presenting symptoms in clinically suspicious oral premalignant lesions.

Presenting symptoms	Clinically suspicious premalignant lesions (n=19)					
	Leukoplakia (n=12)		Erythroplakia (n=3)		Lichen planus (n=4)	
	N	%	N	%	N	%
Patch (whitish or reddish)	12	100	3	100	0	0
Mucosal discoloration	0	0	0	0	4	100
Ulceration	0	0	1	33	0	0

Table 4: Presenting symptoms in clinically suspicious malignant lesion.

Presenting symptoms	Clinically suspicious malignant lesion (n=31)	
	N	%
Dysphagia	5	16.67
Trismus	6	20
Ulceration	29	96.67
Pain in oral cavity	15	50
Mass in oral cavity	14	46.67
Lingual paresthesia	1	3.33
Loose teeth	6	20
Infraorbital paresthesia	1	3.33
Neck swelling	13	43.33
Otalgia	4	13.33

Table 5: Subsite involvement in clinically suspicious oral premalignant and malignant lesions.

Subsite	Clinically suspicious malignant lesion (n=31)		Clinically suspicious oral premalignant lesion (n=19)					
			Leukoplakia		Erythroplakia		Lichen planus	
	N	%	N	%	N	%	N	%
Lips (upper or lower)	2	6.67	0	0	0	0	0	0
Oral tongue ventral	0	0	1	8.33	0	0	0	0
Oral tongue dorsal	1	3.33	0	0	0	0	0	0
Oral tongue lateral	5	16.67	2	16.67	0	0	0	0
Mandibular gingiva	3	10	0	0	0	0	0	0
Maxillary gingiva	2	6.67	0	0	0	0	0	0
Buccal mucosa	11	35.48	9	75	3	100	4	100
Floor of mouth	2	6.67	0	0	0	0	0	0
Hard palate	1	3.33	0	0	0	0	0	0
Retromolar trigone	4	13.33	0	0	0	0	0	0

Table 6: Toluidine blue staining clinically suspicious oral premalignant and malignant lesions.

Clinically suspicious oral lesions	Toluidine blue staining	
	Positive	Negative
Premalignant (n=19)	14	5
Malignant (n=31)	26	5
Total (n=50)	40	10

Table 7: HPE of clinically suspicious oral premalignant and malignant lesions.

HPE		Total	
		Frequency	%
Oral premalignant lesions (with dysplasia) (n=15)	Leukoplakia	10	20
	Erythroplakia	2	4
	Lichen planus	3	6

Continued.

HPE		Total	
		Frequency	%
Oral malignant lesion (n=27)	Carcinoma in situ	1	2
	Well differentiated	11	22
	Moderately differentiated	12	24
	Poorly differentiated	3	6
Benign lesion		8	16

Table 8: Analysis of toluidine blue staining and HPE for oral premalignant and malignant lesions.

Lesion on histopathological examination		Toluidine blue staining			Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy	X2 (p value)
		Positive	Negative	Total						
Premalignant lesion	Positive	12	1	13	92.30	80	92.30	80	88.88	7.63 (0.014) Sig
	Negative	1	4	5						
Malignant lesion	Positive	26	1	27	96.30	80	96.30	80	93.75	8.63 (0.010) Sig
	Negative	1	4	5						

In clinically suspicious oral premalignant lesions, most common site involved in leukoplakia was buccal mucosa (73.33%) followed by oral tongue lateral surface (16.67%) and oral tongue ventral surface (8.33%). The most common of subsite of lichen planus and erythroplakia was buccal mucosa (100%) (Table 5). Overall in oral premalignant lesions, most common habit was smoking (52.63%), followed by alcohol use (21%) and tobacco use (in other form) (15.8%).

Amongst total 50 patients, in clinically suspicious oral premalignant lesions (n=19), 14 cases were TB staining positive and 5 cases were TB staining negative (Table 6). On histopathology, among oral premalignant lesions, dysplasia was seen in 15 cases (30%) (Table 7). Toluidine blue staining in oral premalignant lesions showed sensitivity of 92.30%, specificity of 80%, positive predictive value of 92.30%, negative predictive value of 80% and accuracy of 88.88% (Table 8).

In oral malignant lesions, ulceration (96.67%) followed by mass in oral cavity (46.67%) were the most common symptoms (Table 4). The most common of subsite of oral malignant lesions was buccal mucosa (35.48%) followed by oral tongue lateral (16.67%) and retromolar trigone (13.33%) (Table 5). In oral malignant lesions, the smoking was present in 70% of patients while alcohol use and tobacco use (in other form) was present in 43.33% and 36.67% respectively. Overall, smoking and alcoholism was present simultaneously in 15 cases (30%). Maximum number of cases of oral malignant lesions belonged to stage III (26%) followed by stage II (12%), stage I (8%), stage IV (6%) and stage 0 (2%). In clinically suspicious oral malignant lesions, TB staining positive and negative cases were 26 and 5 respectively (Table 6). Among oral malignant lesions, all were squamous cell carcinoma, of which, well differentiated

were 12 (24%), moderately differentiated were 12 (24%) and poorly differentiated were 3 (6%). Out of 50 cases, 8 (16%) cases were found to be benign (Table 7). Toluidine blue staining in oral malignant lesions shows sensitivity of 96.30%, specificity of 80%, positive predictive value of 96.30%, negative predictive value of 80% and accuracy of 93.75% (Table 8).

DISCUSSION

Toluidine blue is a vital dye that stains nucleic acids and abnormal tissues. Staining with toluidine blue has been used as an adjunctive tool for detection of oral premalignant and malignant lesions since decades. In our study, overall mean age for clinically suspicious oral premalignant lesions is 43.86 years, which was comparable with study done by Upadhyay et al who reported mean age to be 53.83 years.⁸ Among clinically suspicious oral malignant lesions, mean age at presentation was 55.94 years, which was congruent with study by Lekawale et al who reported the mean age of 58.6 years.⁹ Overall gender distribution (male to female ratio) in oral premalignant lesions was 1.4:1, which was in concordance with studies by Petkowicz et al and Starzynska et al, both reporting male to female ratio as 1:1.^{10,11} Among oral malignant lesions, male to female ratio was 5.2:1. This was comparable with a study by Iype et al reporting ratio of 6.7:1 and a study by Dewan et al reporting ratio of 5.77:1.^{12,13} Higher proportions of drinkers and/or smokers amongst males than females explains the male preponderance in oral premalignant lesions and oral malignant lesions. Oral premalignant lesions and oral malignant lesions were highly prevalent in cases of labourers and unemployed. This can be attributed to the low socio-economic status, lack of education and awareness regarding personal oral hygiene

and oral health. Due to lack of education there is high tendency towards substance abuse.¹⁴

In our study, in leukoplakia, the most common presenting symptom was whitish patch. In erythroplakia, the most common presenting symptom is reddish discoloration and ulceration was present in 33% of cases. In all patients of lichen planus, the most common presenting symptom was mucosal discoloration. As only one symptom is present in most of the cases of oral premalignant lesions, they are ignored more often than the lesions debilitating the patients such as pain and ulceration. We found that in clinically suspicious oral malignant lesions, the most common presenting symptom is ulceration (96.67%), followed by mass in oral cavity (46.67%) and neck swelling (43.33%). Sharma et al reported oral ulcer (76%) to be most common symptom followed by pain (43%) and growth (33%).¹⁵ Oliveira et al reported most common symptom as ulcer (87.9%) followed by pain (70.4%).¹⁶

In our study, in leukoplakia, most common site affected was buccal mucosa (73.33%), followed by lateral surface of tongue (16.67%) and ventral surface of tongue (8.33%). In all cases of erythroplakia and lichen planus, lesions were present in buccal mucosa. Lee et al and Pallagatti et al also found buccal mucosa (65.7% and 57.5%, respectively) as the most common site for oral premalignant lesions.^{17,18} In the present study, amongst clinically suspicious oral malignant lesions (n=31), most common site affected was buccal mucosa (35.48%), followed by oral tongue lateral surface (16.67%) and retromolar trigone (13.33%). Gaphor et al and Hosagadde et al also reported that buccal mucosa (37.5% and 32.86%) is most prevalent site for oral malignant lesions.^{18,19} Habit of keeping tobacco or other substances in buccal region is quite common in India which could be the reason for involvement of buccal mucosa in most oral premalignant lesions and oral malignant lesions.

Among oral premalignant lesions, we found that most common habit was smoking (52.63%), followed by alcohol use (21%) and tobacco use in other form (15.8%). Pallagatti et al reported smoking as the most common habit (70%), followed by alcohol use (26%).¹⁸ In oral malignant lesions, most common habit was tobacco smoking (70%), followed by alcohol use (43.33%), tobacco use in other form (36.67%) and simultaneous alcohol with tobacco use (30%). It is comparable with study by Prasan et al reporting most common habits as smoking and alcohol (44%, each).²⁰ Simultaneous use of alcohol and tobacco results in synergistic carcinogenic effect due to dehydrating effect of alcohol on cell membranes. It enhances penetrating ability of tobacco associated carcinogen into tissues. Also, alcoholics suffer from nutritional deficiencies and lack the antioxidants which prevent carcinogenesis.¹⁴

In oral malignant lesions, clinically, maximum number of cases belonged to stage III (26%), followed by stage I

(18%), stage II (12%), stage IV (6%) and stage 0 (2%). Shenoy et al reported most oral malignant lesions cases with stage III (82.37%), followed by stage II (11.53%) and stage IV (6.10%).²² Oral squamous cell carcinoma is associated with high morbidity due to late treatment. However, if it is diagnosed early, disease becomes curable and treatment becomes inexpensive.

In present study, in clinically suspicious oral premalignant lesions, 14 cases were toluidine blue staining positive and 5 cases were negative. In clinically suspicious oral malignant lesions, toluidine blue staining positive cases were 26 and negative cases were 5. These lesions were then subjected to biopsy and histopathology. On histopathology, among 50 cases, dysplasia was seen in 30% cases, carcinoma in situ was seen in 2% cases, 22% cases were well differentiated, 24% cases were moderately differentiated, 6% cases were poorly differentiated and 16% cases were benign. Ramasamy et al found well differentiated carcinoma in 36%, moderately differentiated carcinoma in 33% and poorly differentiated carcinoma in 16.2% cases.²³ Histopathological examination is the gold standard investigation for confirming the diagnosis of oral premalignant and malignant lesions. Prognosis of oral SCC depends on histopathological staging. Response to oncosurgical clearance and radiotherapy is good in well differentiated SCC and poor in poorly differentiated squamous cell carcinoma.²⁴

For oral premalignant lesions (dysplastic lesions), toluidine blue staining showed sensitivity of 92.30%, specificity of 80%, positive predictive value of 92.30%, negative predictive value of 80% and diagnostic accuracy of 88.88%. Our study is comparable with a study by Allegra et al, who reported that toluidine blue staining in oral premalignant and malignant lesions showed sensitivity of 96.20%, specificity of 77.7%, positive predictive value of 86.60% and negative predictive value of 93.30%.²⁴ Pallagatti et al reported sensitivity and specificity of toluidine blue staining in oral premalignant lesions to be 95% and 71.45% respectively, whereas positive predictive value of 84.6% and negative predictive value of 90.9%. The diagnostic accuracy was reported to be 86.48%.¹⁸ Our study is not in concordance with a study by Desai et al, who found that the sensitivity for the detection of dysplastic lesions by toluidine blue staining to be 69.77%.²⁵ Another study by Sinha et al, reported the overall specificity of toluidine blue staining in identifying dysplastic lesions to be 50%.²⁶

In our study for clinically suspicious oral malignant lesions, the sensitivity for toluidine blue staining was 96.30%, specificity was 80%, positive predictive value was 96.30%, negative predictive value was 80% and diagnostic accuracy was 93.75%. Our study is congruent with a study by Sapna et al who found that toluidine blue staining showed sensitivity and specificity to be 92% and 82% respectively. Their study reported positive predictive value of 93% and negative predictive value of

79%.²⁷ Vashisht et al also reported that sensitivity and specificity of toluidine blue was 86.36% and 76.9% respectively. The positive predictive value and negative predictive value were 86.36% and 76.9%.²⁸ The present study is not in concordance with study conducted by Onofre et al, who reported sensitivity and specificity for toluidine blue in oral malignant lesions to be 100% and 65% respectively.²⁹ Rodriguez et al reported the sensitivity and specificity for detection of oral malignant/premalignant lesions to be 65.5% and 73.3% respectively.⁷

Several factors played role for difference in the sensitivity and specificity. Firstly, irrespective of malignancy status, traumatic and ulcerative lesions tend to retain the dye yielding high number of false positive results. Secondly, highly keratotic lesions restrict the penetration of the dye to reach depth at which cellular changes take place, resulting in false negative results.³⁰ Also, misinterpretation of the intensity of color post toluidine blue staining can result in observer bias.³¹ Guo et al reported that a group of false positive lesions with allelic losses occurring at loss of heterozygosity, warranting further molecular study of false positive lesions.³² We followed the guidelines given by Mashberg et al regarding how to decrease the false positive cases.³³ All suspicious inflammatory or chronic traumatic lesions were given treatment for two weeks and re-evaluated and stained after 2 weeks. If the stain is retained, they were deemed to be positive. The lesions that stained faintly were histopathologically negative in most cases.

CONCLUSION

Toluidine blue staining along with clinical examination serves as an outdoor procedure for screening of oral cancer. It is also helpful in the patients who don't give consent for the incisional biopsy. Toluidine blue staining gives the information regarding the margins of lesion; accentuate the decision to biopsy, guides the biopsy site selection for early diagnosis and treatment of oral premalignant and malignant lesions.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Scully C, Bagan JVS. Scott- Brown's Otorhinolaryngology, Head and Neck Surgery. In: Gleeson M, ed. 7th ed. London: Hodder Arnold; 2008.
2. Jemal A, Thimas A, Murray T, Thun M. Cancer statistics, 2002. CA Cancer J Clin. 2002;52:181-2.
3. Woolgar JA, Scott J, Vaughan ED, Brown JS, West CR, Rogers S. Survival, metastasis and recurrence of oral cancer in relation to pathological features. Ann R Coll Surg Engl. 1995;46:518-39.
4. Hegde MC, Kamath PM, Shreedharan S, Dannana NK, Raju RM. Supravital staining: it's role in detecting early malignancies. Indian J Otolaryngol Head Neck Surg. 2006;58:31-4.
5. Banoczy J, Sugar L. Longitudinal studies on oral leukoplakia. J Oral Pathol. 1972;1:265-9.
6. Chiesa F, Tradati N, Sala L, Costa L, Podrecca S, Barachi P, et al. Follow-up of oral leukoplakia after carbon dioxide laser surgery. Arch Otolaryngol Head Neck Surg. 1990;116:177-80.
7. Rodriguez PC, Lapiedra RC, Gomez GE, Martinez SL, Warnakulasuriya S. The use of toluidine blue in the detection of premalignant and malignant oral lesions. J Oral Pathol Med. 2011;40:300-4.
8. Upadhyay J, Rao NN, Upadhyay RB, Agarwal P. Reliability of toluidine blue vital staining in detection of potentially malignant oral lesions-time to reconsider. Asian Pacific J Cancer Prev. 2011;12:1757-60.
9. Lekawale H, Raikwar KR, Ghodke M, Gawande P, Deshmukh V, Garde JB. Postoperative morbidity in squamous cell carcinoma of oral cavity. Ind J App Res. 2016;6(12):528-31.
10. Petkowicz B, Skiba M, Tomaszewski T, Wysokinska-Miszczuk J. Leukoplakia in clinical and epidemiological aspect- analysis of cases. Dent Med Probl. 2004;41:635-41.
11. Starzynska A, Pawłowska A, Renkielska D, Michajłowski I, Sobjanek M, Błazewicz I. Oral premalignant lesions: epidemiological and clinical analysis in the northern Polish population. Postepy Dermatol Alergol. 2014;31:341-50.
12. Iype E M, Pandey M, Mathew A, Thomas G, Sebastian P, Nair M K. Oral cancer among patients under the age of 35 years. J Postgrad Med. 2001;47:171.
13. Dewan AK, Dabas SK, Pradhan T, Mehta S, Dewan A, Sinha R. Squamous cell carcinoma of the superior Gingivobuccal sulcus: An 11- year Institutional experience of 203 cases. Jpn J Clin Oncol. 2014;44(9):807-11.
14. Liu L, Kumar SKS, Sedghizadeh PP, Jayakar AN, Shuler CF. Oral squamous cell carcinoma incidence by subsite among diverse racial and ethnic population in California. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105(4):470-80.
15. Sharma RG, Bang B, Verma H, Mehta JM. Profile of oral squamous cell cancer in a tertiary level medical college hospital: a 10 year study. Indian J Surg Oncol. 2012;3(3):250-4.
16. Oliveira MLC, Wagner VP, Sant'ana Filho M, Carrard VC, Hugo FN, Martins MD. A 10-year analysis of the oral squamous cell carcinoma profile in patients from public health centers in Uruguay. Braz Oral Res. 2015;29:1-8.
17. Lee JJ, Hong WK, Hittelman WN, Mao L, Lotan R, Shin DM, et al. Predicting cancer development in oral leukoplakia: ten years of translational research. Clin Cancer Res. 2000;6:1702-10.

18. Pallagatti S, Sheikh S, Aggarwal A, Gupta D, Singh R, Handa R, et al. Toluidine blue staining as an adjunctive tool for early diagnosis of dysplastic changes in the oral mucosa. *J Clin Exp Dent.* 2013;5(4):e187-91.
19. Gaphor SM, Sabri ZA. Prevalence of oral premalignant and malignant Lesions among referred Kurdish patients Attending Department of Oral and Maxillofacial in Sulaimani Teaching Hospital. *IOSR J Dent Med Sci.* 2014;13(2):32-6.
20. Hosagadde S, Dabholkar J, Virmani N. A clinicopathological study of oral potentially malignant disorders. *J Head Neck Physicians Surg* 2016;4:29-34.
21. Prasan D. Clinicopathological study of oral cancers. *IOSR J Dent Med Sci.* 2015;14(6):35-8.
22. Shenoy R, Devrukhkar V, Chaudhari, Sharma BK, Sapre SB, Chikhale A. Demographic and clinical profile of oral squamous cell carcinoma patients: A retrospective study. *Indian J Cancer.* 2012;49:21-6.
23. Ramasamy P, Sivapatham S. Assessment of factors associated with early and late diagnosis of buccal mucosa carcinoma. *J App Pharm Sci.* 2016;6(8):79-82.
24. Allegra E, Lombardo N, Puzzo L, Garozzo A. The usefulness of toluidine staining as a diagnostic tool for precancerous and cancerous oropharyngeal and oral cavity lesions. *Acta Otorhinolaryngol Ital.* 2009;29:187-90.
25. Desai VD, Narang P. Utility of toluidine blue in the detection of oral epithelial dysplasia: a diagnostic adjunct. *Ind J Pub Heath Res Dev.* 2015;6(1):82-5.
26. Sinha PK, Patidar M, Raj V, Chandra S, Agarwal N. Clinical and histological evaluation of toluidine blue positive and negative stained oral mucosal lesions. *J Oral Med Oral Surg Oral Pathol Oral Radiol.* 2016;2(3):120-4.
27. Sapna M, Rupnarayan R, Azeem MSM. Efficacy of toluidine blue and brush biopsy in oral lesions. *Int J Oral Maxillofac Pathol.* 2013;4(1):228.
28. Vashisht N, Ravikiran A, Samatha Y, Rao PC, Naik R, Vashisht D. Chemiluminescence and toluidine blue as diagnostic tools for detecting early stages of oral cancer: an in-vivo Study. *J Clin Diagn Res.* 2014;8:35-8.
29. Onofre MA, Sposto MR, Navarro CM. Reliability of toluidine blue application in the detection of oral epithelial dysplasia and in situ and invasive squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2001;91:535-40.
30. Rosenberg D, Cretin S. Use of meta-analysis to evaluate toluidine chloride in oral cancer screening. *Oral Surg Oral Med Oral Pathol.* 1989;67(5):621-7.
31. Gandolfo S, Pentenero M, Broccoletti R, Pagano M, Carrozzo M, Scully C. Toluidine blue uptake in potentially malignant oral lesions in vivo: clinical and histological assessment. *Oral Oncol.* 2006;42(1):89-95.
32. Guo Z, Yamaguchi K, Sanchez-Cespedes M, Westra WH, Koch WM, Sidranski D. Allelic losses in OraTest-directed biopsies of patients with prior upper aerodigestive tract malignancy. *Clin Cancer Res.* 2001;7(7):1963-8.
33. Mashberg A. Final evaluation of toluidine chloride rinse for screening of high risk patients with asymptomatic squamous carcinoma. *J Am Dent Assoc.* 1983;106:319-23.

Cite this article as: Bayad HC, Bhagat S, Sahni D, Kaur N, Singh R, Sharma DK, et al. The study of use of toluidine blue as an adjunctive tool to clinical examination in early diagnosis of clinically suspicious oral premalignant and malignant lesions: a study of fifty cases. *Int J Otorhinolaryngol Head Neck Surg* 2019;5:1585-92.