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ORIGINAL RESEARCH

Efficacy of toluidine blue, lugol's iodine and acetic acid for detecting oral lesions of Leukoplakia– A cross-sectional study

[1]Dr. Devashree Shukla, [2]Dr. Chandresh Shukla, [3]Dr. Kaushal Pati Tripathi, [4]Dr. Dilraj Singh, [5]Dr. Ayushi Sharma, [6]Dr. Sommyta Kathal

[1]Assistant Professor, Department of Dentistry, LN Medical College and JK Hospital, Bhopal, Madhya Pradesh, India
[2]Reader, Department of Orthodontics and Dentofacial Orthopaedics, Peoples College of Dentistry and Research Centre,Bhopal, Madhya Pradesh, India
[3]Senior Resident, Faculty of Dental Science Institute of Medical Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India
[4]Senior Lecturer, Department of Oral Medicine Radiology, Ideas Dental College, Gwalior, Madhya Pradesh, India
[5]Post Graduate student, Department of Public Health Dentistry, People's College of Dental Science & Research Centre, Bhopal, Madhya Pradesh
[6]M.D.S. Pediatric & Preventive Dentistry, Indore, Madhya Pradesh, India

Correspondence:
Dr. Devashree Shukla
Assistant Professor, Department of Dentistry, LN Medical College and JK Hospital, Bhopal, Madhya Pradesh, India

ABSTRACT

Background:The life expectancy and prognosis of patients suffering from oral cancer will be considerably improved if oral cancer is detected and treated early. To augment clinical examination and improve diagnosis, adjunctive diagnostic aids such as vital staining have been introduced.

Aim:The present research examined the diagnostic efficacy of acetic acid (2%), lugol's iodine (3% dilution), and toluidine blue (1%), to diagnose oral leukoplakia.

Materials and Methods:A cross-sectional study was undertaken at a hospital with 45 randomly selected subjects who had clinically verified cases of oral leukoplakia. Acetic acid, lugol's iodine, and toluidine blue were applied in respective order on oral lesions. The results of each staining were then compared to the clinical and histopathologic diagnoses. A control group was made up of cases diagnosed with epithelial hyperplasia. Numbers and percentages are used to present the information. To compare the effects of toluidine blue application, toluidine blue, and acetic acid, the Chi square test was utilised. In terms of its utility in predicting the dysplastic character of the lesion, the sensitivity and specificity test was used to determine diagnostic efficiency and reliability.

Results:Toluidine blue staining and lugol's iodine staining both had a sensitivity of 91.59 percent, while the former had a specificity of 23.33 percent and the latter had a specificity of 12.22 percent. The sensitivity of the acetic acid test was 58.25 percent and the specificity was 34.44 percent. There was no statistically significant difference between the staining with three agents and the histopathologic variations in multiple comparisons.

Conclusion: When compared to acetic acid, toluidine blue staining and lugol's iodine staining demonstrated high sensitivity in identifying oral leukoplakia. All three modalities employed had a low specificity.

Keywords: Lugols iodine, oral leukoplakia, oral lichen planus, sensitivity and specificity, toluidine blue

INTRODUCTION

Oral cancer (OC) is a major public health concern around the world, particularly in Southeast Asian countries. In India, OC is the most common cancer in men and the third most common cancer in women, indicating a huge public health issue that accounts for up to 40% of all malignancies. Oral Squamous Cell Carcinoma (OSCC) is the most frequent cancer of the mouth, accounting for over 90% of all oral cancers.The majority of OSCC are preceded by early lesions known as potentially malignant diseases (PMD). Oral Leukoplakia (OL), Erosive Lichen Planus (ELP), and Oral Submucous Fibrosis (OSMF) are three prevalent oral PMDs.[1]

Early diagnosis and treatment of these precursor lesions by clinicians can considerably enhance the survival rate and, ultimately, the prognosis of such individuals. Using serum, tissue, and salivary samples, many studies have been conducted to elucidate the role of reactive oxygen species (ROS) and endogenous antioxidants as early diagnostic markers for oral leukoplakia.[2]

Periodic clinical examination of the oral cavity, on the other hand, remains the gold standard for early identification of PMD. In order to improve the diagnosis of PMD and early malignant lesions, supplementary diagnostic aids such as vital staining have been developed to support clinical evaluation. Toluidine blue (TBS), acetic acid (AA), and Lugol's iodine are often used in vital staining (LIS).[3]

The current study looked at the diagnostic efficacy of acetic acid (2%), lugol's iodine (3% dilution), and toluidine blue (1%), all of which are used to diagnose oral leukoplakia.

MATERIALS AND METHODS

STUDY DESIGN

A hospital-based, cross sectional study was conducted.

SAMPLE CHARACTERISTICS

The study included a random sample of 45 people with clinically suspect PMD, specifically homogeneous oral leukoplakia. The Vanderwaal et al. diagnostic criteria for OL were followed (Van der waal et al. 2009). [17] All patients with a clinically established lesion of oral leukoplakia had a history of tobacco use, whether it was in the form of smoking or smokeless tobacco. Patients with PMD lesions who had had therapy in the previous 6 months, as well as those with associated chronic co-morbid systemic disease or dye allergies, were excluded from the study.

STUDY PROTOCOL

Patients were initially screened for oral leukoplakia. For patients with positive evidence of these lesions, a complete history was taken, with an emphasis on bad behaviours, and a comprehensive clinical examination was performed. Subjects who decided to participate in the study willingly were asked to complete an informed consent form. After that, each subject was tested for an allergic reaction to vital stain dye using a skin scratch test. Every participant was given a "chit" with a code showing the sort of stain to be applied after a satisfactory reaction. On the first day, each patient received AA solution and LIS application to their suspected lesions. The following day, the individuals were treated to TBS. Pre and post images were taken to analyse the stains.

Different examiners were involved in the screening and recruiting of patients, as well as the stain application technique, to prevent bias. The identification of lesion, application, and

assessment of the lesion following vital staining were all standardised across all examiners in the study. Following the conclusion of the staining technique and histological confirmation, all individuals received appropriate treatment, which included tobacco cessation counselling for those who had this harmful habit.

To lower the false positive rate, all lesions that stained positive were re-stained after 2 weeks. Clinical judgement guided the biopsy of lesions that showed negative staining, and the specimens were sent for histopathological investigation. The results of staining were compared to clinical and histopathologic diagnoses.

DATA ANALYSIS

The Statistical Package for Social Sciences (SPSS) version 21.0 software was used to analyse the obtained data. The TBS, LIS, and AA stains were compared using the Chi-square test. Sensitivity and specificity test equations were used to calculate diagnostic efficiency. The Kruskal Wallis test was used to make multiple comparisons of staining with three agents and histopathologic variations at a significance level of $P \leq 0.05$.

RESULTS

In this study, vital staining was used to assess 45 potentially malignant Oral leukoplakia diseases. Only epithelial hyperplasia was seen histopathologically in 31.1 percent of the patients. 14.4 percent of the patients had mild dysplastic characteristics, whereas 51 percent had significant epithelial dysplasia. Only 7.8% of oral leukoplakia cases [Table 1] had significant dysplastic characteristics. TBS and LIS both stained positive in all cases of mild and severe epithelial dysplastic cases, however both failed to detect three cases of intermediate dysplastic epithelial dysplasia. TBS staining produced false positive results in 8 cases of epithelial hyperplasia, whereas LIS produced 9 false positive results. Only three cases of mild dysplasia, eleven cases of moderate dysplasia, and two cases of severe dysplasia had positive AA findings. On the AA application, however, false positive results were only seen in seven cases.

Based on these data, the sensitivity of both TBS and LIS staining was calculated to be 91.59 percent, with the former test having a specificity of 23.33 percent and the latter having a specificity of 12.22 percent. [Table 2] The AA test had a sensitivity of 58.25 percent and a specificity of 34.44 percent. There was no statistically significant difference between the proportion of TBS stained with LIS and the percentage of AA stained with LIS ($P > 0.05$). However, when the proportion of TBS staining was compared to the percentage of AA staining, there was a statistically significant difference (P 0.05) [Tables 3-5]. Using the Kruskal Wallis test, several comparisons of staining with three agents and histopathologic variants were made, but no statistically significant difference ($P > 0.05$) was found.

Table 1: Showing frequency distribution of histopathology grading.

Histopathological Grading	Frequency- (%)
Hyperplasia	31
Mild dysplasia	14.4
Moderate dysplasia	51
Severe dysplasia	7.8

Table 2: Showing Sensitivity and Specificity calculated for each vital staining technique

	True positive (n)	False negative (n)	False positive (n)	True negative (n)	Sensitivity (%)	Specificity (%)
Toulidine blue	20	3	8	3	91.59	23.33
Lugol's Iodine	20	3	9	2	91.59	12.22
Acetic acid	13	10	7	4	58.25	34.44

Table 3: Showing comparison (cross tabulation) between Toluidine blue and Lugol's iodine and the difference in the percentage of staining was not statistically significant. (p>0.05)

Toluidine Blue Lugol's Iodine	Positive (+) (%)	Negative (-) n (%)	Likelihood ratio	Df	P
Positive (+)	89.6%	12.6%	0.919	1.00	0.452
Negative (-)	100%	0			

$\chi 2$ =Chi square test; df=degree of freedom; * P<0.05

Table 4: Showing comparison (cross tabulation) between Lugol's iodine and Acetic acid in the difference in the percentage of staining was not statistically significant. (p>0.05)

Lugol's Iodine Acetic Acid	Positive (+) (%)	Negative (-) n (%)	Likelihood ratio	Df	P
Positive (+)	56.8%	45.4%	3.196	2	0.081
Negative (-)	100%	0			

$\chi 2$ =Chi square test; df=degree of freedom; * P<0.05

Table 5: Showing comparison (cross tabulation) between Toluidine blue and Acetic acid and the difference in the percentage of staining was statistically significant. (p<0.05).

Toluidine Blue Acetic Acid	Positive (+) (%)	Negative (-) n (%)	Likelihood ratio	Df	P
Positive (+)	58.8%	43.4%	3.19	1	0.028*
Negative (-)	76%	26%			

$\chi 2$ =Chi square test; df=degree of freedom; * P<0.05

DISCUSSION

On histological grading, 31.1 percent of 45 premalignant lesions were hyperplasia, 14.4% were mild dysplasia, 16 were moderate dysplasia, and 3 were severe dysplasia in our study. TBS and LIS had a sensitivity of 91.59 percent in our investigation, indicating that they can detect real positive lesions. TBS, on the other hand, had a specificity of 23.33 percent, indicating that it is ineffective in detecting real negative lesions, whereas LIS had a specificity of 12.22 percent. There was no discernible change in the percentage of positive and negative staining between TBS and LIS staining.[4,5]

In their study, Epstein et al. found that TBS had 92.5 percent sensitivity and 63.2 percent specificity, whereas LIS had 87.5 percent sensitivity and 84.2 percent specificity.Despite the fact that the tests' sensitivity was consistent with our findings, the specificity of TBS and LIS was extremely high when compared to our findings. This discrepancy in TBS selectivity could be related to false positive staining of inflammatory lesions, which is the most prevalent TBS side effect. [20] Glycogen levels may be lowered even in epithelial hyperplasia cases, which served as a control group, resulting in more false positive findings

linked with LIS. Glycogen concentration fluctuates with keratinization throughout the oral mucosa. There is also a scepticism about the relationship between inflammation and glycogen levels.[6,7]

Warnakulasuriya and Newell Johnson (1996) used a 1% modified TBS stain to study 39 PMD with epithelial dysplasia and found that it had a sensitivity and specificity of 79.5 percent and 62 percent, respectively. The sensitivity was similar to what we found in our investigation, but the specificity was higher. They claimed that TBS staining is a valuable tool in the surveillance of high-risk individuals, and that it also has a high sensitivity for detecting early PMD.[8]

In the current investigation, AA application had a sensitivity of 58.25 and a sensitivity of 34.44. Inefficiency in diagnosing true positive cases is reflected in the decrease in sensitivity.The reason for this could be the non-keratinized/less keratinized lesions in the study group, because the acetowhite look that shows dysplasia when acetic acid is applied is mostly connected to the reversible coagulation of protein content in epithelium. AA application, on the other hand, had a higher specificity than TBS and LIS. This could be due to the lower keratin level in the epithelial hyperplasia group, which served as the study's control. Surprisingly, there was no significant difference between AA and LIS in terms of staining percentage (positive and negative).Despite this, there was a substantial difference in the proportion of staining when TBS was compared to AA.[9]

A study by Bhalang et al. looked at the diagnostic accuracy of 5% AA for PMD diagnosis. The sensitivity and specificity were respectively 83.33 percent and 84.21 percent. Both contradicted our findings, which could be due to the different clinical presentations of the lesions we included in the research group. As a result of our findings, we believe the staining procedures we used, particularly TBS and LIS, are helpful in detecting positive instances. False positives are, of course, less concerning in a clinical situation than false negatives, because every positive result should be followed up with a biopsy.[10,11]

The study's sample size was limited in different staining groups and even in the category of histopathological grades. The diagnosed dysplastic lesion received no further evaluation. An analysis like this would have backed up the findings of the current study.

CONCLUSION

When compared to acetic acid, toluidine blue staining and lugol's iodine staining demonstrated high sensitivity in identifying oral leukoplakia. All three modalities employed had a low specificity.

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