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Review Article

Evaluation of tolonium chloride as an aid in diagnosis of dysplastic changes in oral mucosa among tobacco smokers

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ABSTRACT

Background & Objectives: To evaluate the clinical utility of Tolonium chloride as a diagnostic aid amongst high-risk patients i.e. patients with tobacco smoking habit, to identify potential areas that might proceed into cancer and which are not otherwise identified during clinical examination and thus guide to select the biopsy site.

Materials and Methods: A total of 180 individuals were selected for the study, Out of these 180 individuals, 95 individuals showed areas of stain retention in their oral mucosa and these 95 individuals constituted the study group. However, 62 individuals out of the study group were not willing to undergo biopsy. Only the remaining 32 individuals volunteered to participate in the study, from these volunteers' biopsy specimens were obtained involving both stained and unstained area. Which were considered separately, making a total of 64 specimens.

Results: The results of the present study showed sensitivity and specificity of 56.34% and 50.56% respectively for the Tolonium chloride mouth rinse to be used as a routine screening agent in detecting premalignant areas in otherwise apparent normal oral mucosa in high-risk group of people. In this study 0.403 P value shows insignificant correlation between the histopathological evaluation of the stained and the unstained areas.

Conclusion: Tolonium chloride rinse is more sensitive as compared to the clinical examination alone, in detecting dysplastic changes in clinically apparent normal oral mucosa, among tobacco smokers. Tolonium chloride vital staining cannot be used to delineate the biopsy margins. Tolonium chloride might be used as a diagnostic tool in the biopsy site selection. The procedure cannot be performed on routine basis for high-risk group of people.

1. Introduction

Most cases of oral cancer are associated with habits and are preceded by asymptomatic clinical lesions collectively referred to as oral potentially malignant disorder.[1] OPMD is a clinical diagnosis for which the histological diagnosis may be hyperplasia, hyperkeratosis, oral epithelial dysplasia (OED) or oral squamous cell carcinoma (OSCC). OED is characterized by cytological and architectural alterations reflecting the loss of normal maturation and stratification pattern of surface epithelium.[2,3]

Oral lesions are asymptomatic initially, highlighting the need for oral screening. Most dental professionals carefully examine the oral cavity and oropharynx during routine care and may do a brush biopsy of abnormal areas.[4] The lesions may appear as areas of erythroplakia or leukoplakia and may be exophytic or ulcerated. Cancers are often indurated and firm with a rolled border. As the lesions increase in size, pain, dysarthria, and dysphagia may result. Oral cancer includes a group of neoplasms affecting any region

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of the oral cavity, pharyngeal regions and salivary glands. However, this term tends to be used interchangeably with oral squamous cell carcinoma (OSCC), which represents the most frequent of all oral neoplasms. It is estimated that more of 90% of all oral neoplasms are OSCC.[5,6]

The pathogenesis of oral cancer is a multistep procedure, which starts with subtle chemical changes to histological transformation to morphologically evident lesions. At present there are several methods available for diagnosis of the disease at all the above-mentioned stages. Early detection in the asymptomatic stage greatly improves not only the rate of prognosis but also patients' quality of life as a consequence of less radical and therefore debilitating treatments. Many procedures that are available for early detection of oral cancer are either tedious or expensive. Biopsy is still the gold standard, but invasive. With the lack of thorough knowledge about the procedure and the risk factors associated with biopsy, there is a need for a simple, noninvasive procedure for detection of oral cancer especially at its earliest stages. In this regard many studies have proved the usefulness of Toluidine blue in the early detection of oral cancer.[7,8]

The lake of specificity amongst many of the studies was later hypothesized to be due to many of the impurities and other dyes that are generally found in generic Toluidine blue. The generic Toluidine blue is basically combination of Tolonium Chloride, other dyes and impurities amongst which only Tolonium Chloride, which is positively charged gets selectively retained in the mitochondria of cancer cells. Further research indicates that use of Tolonium Chloride alone is more specific in detection of cancer cells.

Till date very few studies have been conducted in this regard i.e. to study the efficacy of Tolonium chloride in detecting dysplastic changes in morphologically unaltered oral mucosa. This study was undertaken to define the clinical utility of Tolonium chloride as a diagnostic aid amongst high-risk patients i.e. patients with tobacco smoking habit, to identify potential areas that might proceed into cancer and which are not otherwise identified during clinical examination and thus guide to select the biopsy site.

2. Materials and Methods

The present study was conducted on the patients who visited department of oral medicine and radiology in the dental college. The ethical committee was informed about the study to obtain the clearance certificate. The patients with the history of tobacco use in any form were included in the initial screening of the study. All the patients were informed about the standard protocol of the study and the signed informed form was taken.

Subjects who took up stain in some part of oral mucosa and those indulged in tobacco habit, patients with apparent normal oral mucosa and those who were cooperative and capable to follow the instructions were included in the study. Those who were hypersensitive to Tolonium chloride, having habit other than smoking, pregnant or lactating mother, those who developed complications and with inability to rinse oral cavity were excluded from the study.

The study subjects were selected based on the inclusion and exclusion criteria. Total of 180 individuals were included in the study. The details of the patient's history and clinical examination were recorded along with a questionnaire about the tobacco smoking habit. The questionnaire was included to know the details of the type, frequency and duration of the smoking habit. The patients were asked to follow the rinsing protocol. A single stage rinse procedure was followed after ruling out any pathologically altered areas from the clinically apparent normal oral mucosa.

The oral rinsing protocol: Oral rinse protocol was followed.

1. **Step 1:** 20-second pre-rinse with 30ml of 1% acetic acid.
2. **Step 2:** 20-second water rinse.
3. **Step 3:** 20-second 10ml of the 0.5%Tolonium Chloride solution.
4. **Step 4:** 20-second post rinse with 30ml of 1% acetic acid.
5. **Step 5:** A final water rinse.

The areas, which took up the stain, were biopsied along with the adjacent unstained area with the standard procedure. The size of the biopsy specimen was not less than 1 cm in diameter, such that a minimum of 5 mm of unstained area was covered. The specimen was left immersed in formalin solution overnight. The next morning the specimen was removed from the solution, blot dried using a filter paper. The area of specimen, which had taken up the Tolonium chloride stain, was coated with India ink and left for drying in shade and humid condition for 2 hours. This procedure was followed to differentiate the stained area from the unstained area with respect to Tolonium chloride during microscopic evaluation. Such dried specimen was re-immersed in 10% formalin and referred to the Department of Oral Pathology for the histopathological evaluation.

The Statistical software namely SPSS 11.0 and Systat 8.0 were used for the analysis of the data. The Chi-Square Test/Fisher Exact Test was used to get the P value of the study (P</= 0.05), which signifies the correlation of the samples of the study.

3. Results

Total of 180 individuals were included in the study. All the included patients meet the inclusion and exclusion criteria. Out of these 180 individuals, 94 individuals showed areas of stain retention in their oral mucosa and these 94 individuals constituted the study group. However, 62 individuals out of

the study group were not willing to undergo biopsy due to the inherent fear or the myth. Only the remaining 32 individuals volunteered to participate in the study.

From these volunteers biopsy specimens were obtained involving both stained and unstained area (from the periphery of stained area). The unstained areas were considered separately, making a total of 64 specimens. The results obtained after the rinse protocol and histopathological evaluation were recorded as follows.

When the histopathology of the stained and unstained specimen was done, maximum cases were evaluated as Hyperplastic in both categories. No cases of severe dysplasia were found. When the statiscal evaluation was done no significant difference was noticed in the histopathological findings between the stained and unstained specimens. Detailed description of the histopathological evaluation is shown below. (Table 1) the result obtained after overall result evaluation is as follows (Table 2).

Table 1: Histopathology findings of stained and unstained specimen

Histopathological findings	Stained specimen	Unstained specimen	P value
Normal	4	8	0.54
Hyperplasia	16	14	0.69
Mild dysplsia	8	10	0.86
Moderate dysplasia	4	-	0.53

Table 2: Overall diagnostic statistics

After rinse protocol	Histopathological evaluation		Total
	Positive	Negative	
Positive	12	20	32
Negative	10	22	32
Total	22	42	64

4. Discussion

Toluidine blue is an easily available, economical, metachromatic dye known to bind DNA of dividing cells.[9] Toluidine blue staining is considered to be sensitive in identifying early oro-pharyngeal premalignant and malignant lesions.[10] It has previously been described to stain malignant and premalignant cells but not normal mucosa.[9,11]

Toludinr blue dye's has basic affinity for staining nucleic acids, both RNA and DNA. Cancerous and precancerous cells have a very high level of active RNA and DNA metabolism compared to healthy normal tissue and as a result are preferentially stained with Toluidine blue O. Tolonium chloride; a cationic dye commonly used in cytochemistry has affinity to nucleic acids, predominantly to RNA.[9,11]

Although several studieshave revealed the efficacy of Toluidine blue as a staining agent for detecting premalignant/malignant lesions of oral mucosa, the results were highly inconsistent. Most of the authors concluded that biopsy and histopathological evaluation of such lesions is mandatory. However a press release newswire in London by Zila Europe inc. mentioned that the failure of Toluidine blue as a perfect staining agent is due to the impurities and other dyes that are generally found in the generic Toluidine blue stain.[12] Most of the patients complained of an unpleasant taste of the rinse i.e. 1% acetic acid rinse and found the Tolonium chloride rinse to be tasteless.

The histopathological evaluation was done according to the protocol proposed by WHO 1978 for oral epithelial dysplasia with slight modification to the standard protocol with addition of hyperplasia as per suggestion of Joel B Epstein.[13]

Results showed that the areas with retained stain demonstrated a 37.50% probability of having areas with epithelial dysplasia. The negative predictive value, however, was high, indicating that areas that do not retain stain demonstrated a 68.75% probability of not having areas of epithelial dysplasia. The P value of 0.709 also showed insignificant correlation between the histopathological evaluation of the stained and the unstained areas, which signifies the unreliability of the procedure to be performed on routine basis for high-risk group of people.

There were total 12-dysplastic areas seen among 32-stained biopsy specimens and 16 others showed only basilar hyperplasia with/without hyperkeratosis. With the statistical evaluation of the study, the stain retention was not seen in correlation with the grade of dysplasia or the histopathological alteration, indicating that the stain retention is independent of dysplasia. 24 of the 32 biopsies, i.e., a total of 48 specimens (24 stained and 24 unstained) showed equivocal results with histopathological diagnosis i.e. there was no difference in the degree of histopathological interpretation between areas of stain uptake and unstained regions in the same patient, indicating the poor reliability of the Tolonium chloride rinse in delineating the biopsy margins as claimed by the previous studies.

Whereas in the present study (consisting of clinically apparent normal oral mucosa), the correlation of inflammatory/traumatic areas with dye retention other than dysplasia could not be appreciated, with 16 hyperplasias and 4 showing normal epithelium among the 32 stained specimens. The results of the present study indicate that the sensitivity of the Tolonium chloride rinse was found to be greater than that of the clinical examination alone. This finding is consistent with the results of similar studies conducted previously by different authors. In one of the

previous studies the Tolonium chloride rinse could not only identify clinically suspicious lesions by showing areas of greatest stain retention but also could identify lesions which were not clinically detectable, suggesting increased sensitivity of the rinse.

5. Conclusion

The Tolonium chloride mouth rinse was not found to be a reliable routine screening method but showed a greater sensitivity and poor specificity to that of the oral examination of the clinically apparent normal oral mucosa. Tolonium chloride rinse is more sensitive as compared to the clinical examination alone, in detecting dysplastic changes in clinically apparent normal oral mucosa, among tobacco smokers.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Dionne KR, Warnakulasuriya S, Zain RB, Cheong SC. Potentially malignant disorders of the oral cavity: Current practice and future directions in the clinic and laboratory. *Int J Cancer*. 2014;136:503–15.
2. Abidullah M, Kiran G, Gaddikeri K, Raghoji S. Leuloplakia-Review of a potentially malignant disorder. *J Clin Diagn Res*. 2014;8:1.
3. Sitheeque M, Ahmad Z, Saini R. Awareness of oral cancer and precancer among final year medical and dental students of Universiti Sains Malaysia (USM), Malaysia. *Arch Orofac Sci*. 2014;9:53–64.
4. Rethman MP, Carpenter W, Cohen EE, Epstein J, Evans CA, Flaitz CM, et al. Evidence-based clinical recommendations regarding screening for oral squamous cell carcinomas. *J Am Dent Assoc*. 2010;141:509–20.
5. Markopoulos AK. Current Aspects on Oral Squamous Cell Carcinoma. *Open Dent J*. 2012;6:126–30.
6. Orakzai GS, Nisa WU, Orakzai SH, Factors. Oral white lesions-histomorhological assessment and associated risk factors. *J Ayub Med Coll Abbottabad*. 2015;27:865–8.
7. Messadi DV. Diagnostic aids for detection of oral precancerous conditions. *Int J Oral Sci*. 2013;5(2):59–65.
8. da Conceição MGD, Figueiró AC, Luiza VL. Non-Invasive Methods for Early Diagnosis of Oral Cancer. In: Oral Diseases; 2019. doi:10.5772/intechopen.89280.
9. Panta P, Rich LJ, Seshadri M. Application. Oral Cancer Detection: Novel Strategies and Clinical Impact. In: Oral Cancer Screening. Springer International Publishing; 2018. p. 153.
10. 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:e187–91.
11. 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 Italica*. 2009;29:187.
12. Maini AP. Evaluation of efficacy of tolonium chloride as an aid in diagnosis of dysplastic changes in clinically apparent normal oral mucosa, among tobacco smokers; 2006.
13. Warnakulasuriya S, Johnson NW. Van der Waal I: Nomenclature and classification of potentially malignant disorders of the oral mucosa. *J Oral Pathol Med*. 2007;36:575–80.

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