ORIGINAL ARTICLE

Acetic acid as an adjunct vital stain in diagnosis of tobacco-associated oral lesions: A pilot study

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ABSTRACT

Background: One of the most important risk factors for oral precancer and cancer in India is the use of tobacco. In chronic tobacco users, the mucosa may appear clinically healthy, however, changes are observed histologically. Screening of such tobacco users for an early diagnosis is, therefore, of paramount importance. Several adjunctive diagnostic modalities have been used in the past, but none has been conclusively validated as confirmative and cost-effective screening methodology. The aim of this study was to evaluate the use of 5% acetic acid as a vital staining agent in tobacco-associated oral lesions. **Materials and Methods:** The study subjects were divided into two groups. Group I (*n* = 40) subjects with a history of chronic tobacco use and clinically apparent normal mucosa. Group II (*n* = 40) subjects suspected of having oral cancer, 5% acetic acid was applied to the mucosa/lesions, followed by incisional biopsy for confirmatory diagnosis. **Results:** The sensitivity and specificity for Groups I and II were 97%, 50% and 95%, 60%, respectively. Positive predictive value (PPV) and negative predictive value (NPV) of Group I were 0.95 and 0.66. Group II showed PPV and NPV of 0.95 and 0.60. **Conclusion:** The results of this study suggest that acetic acid holds promise for future. Hence, further studies are needed to be undertaken on a large scale to assess its potential as a screening tool for high-risk individuals and oral cancer.

Key words: Acetic acid, oral cancer, precancer, tobacco, vital stain

INTRODUCTION

Oral cancer is a major health problem, being the most common cause of cancer-related death in men, that is, 83,000 new cases and 46,000 deaths yearly in India. Majority of oral cancers develop from oral premalignancies or are associated with it.[1] The concept of "early diagnosis leads to improved prognosis" is based on the fact that carcinoma develops over a long period of time, going through intermediate stages of different biological significance and the treatment at the pre-invasive stage offers the best prognosis.[2]

Despite numerous advances in treatment, the 5-year survival has remained approximately 50% for the last three decades. This poor prognosis is, likely due to advanced extent of the disease at the time of diagnosis, with over 60% of patients presenting in stages III and IV. The typically late diagnosis of oral cancer is ironic because the oral cavity is readily accessible for screening and visible changes in the mucosa are frequently associated with the development of the disease. The early changes are often so subtle that they probably go unnoticed by visual examination.[3]

The major risk factors for oral cancer and precancer in India are chronic tobacco and alcohol use.[4] In tobacco users, the mucosa may appear apparently healthy clinically; however,

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application of vital stains to such mucosae shows positivity and dysplastic changes on histological examination.[5] Over the years, numerous adjunctive diagnostic aids in early detection of oral precancer and cancer have been used. These include vital staining with toluidine blue, Lugol's iodine and light-based detection systems like ViziLite Plus, VELscope and histopathological methods such as oral cytology, oral brush biopsy, etc.[6]

In the past, 3–5% acetic acid was used as a vital stain for detection of cancer in developing countries. Sankaranarayanan *et al*. used 5% acetic acid for the detection of cervical cancer.[7] Further, Bhalang *et al*. used 5% acetic acid as a clinical marker for the detection of oral cancer.[8] Nevertheless, there is paucity of information related to the use of 5% acetic acid in detecting premalignant and malignant changes in clinically apparent normal mucosa of chronic tobacco users. Thus, this study aims to estimate the efficacy of 5% acetic acid as a vital staining agent in the detection of premalignant and malignant changes in clinically apparent normal mucosa of chronic tobacco users and to detect oral cancer. This information would potentially be useful in identifying a clinically apparent normal mucosa, which could turn malignant in due course of time with continued tobacco use, thus, facilitating early diagnosis.

MATERIALS AND METHODS

The study sample was derived from the patients who presented to the Outpatient Department of our institution. The study was carried out after explaining and obtaining written informed consent. Protocol of the study was reviewed and approved by the Institutional Ethical Committee. Clinical examination was carried out by an oral diagnosis specialist. A total of 80 patients were screened and divided into two groups. Group I comprised 40 subjects with a history of chronic tobacco use (minimum of 5 years with the frequency being 5–10 times/day) and on clinical examination had apparently normal mucosa. Group II comprised 40 subjects with lesions suspected of having oral cancer. A detailed case history, conventional oral examination and photographs of the patients were taken.

Five percent acetic acid was freshly prepared every time before use. A piece of gauze soaked with the acetic acid was applied to a cleaned and dried mucosa/lesion. For larger and generalized lesions, subjects were asked to rinse the mouth with the same. After 2 min, the lesion was photographed again. The effect of vital stain was recorded as positive if the lesion changed its color to opaque white [Figures 1a and b and 2a and b] and negative if no change or change to transparent white was observed. The results were recorded by two trained oral pathologists who were blinded to the clinical examination results. Incisional biopsy was advised; more than one biopsy was obtained in the case of more than one positively stained area. A total of 43 biopsy specimens were obtained from Group I and 46 from Group II. The biopsy specimens were fixed using 10% formalin for 24 h, conventionally processed, stained with hematoxylin and eosin and subjected to histopathological evaluation.

Statistical analysis

Descriptive analysis was used for the evaluation of sensitivity, specificity, positive predictive values and negative predictive values (PPV and NPV).

RESULTS

Group I: Of the 40 subjects, 43 biopsy specimens were obtained. All the patients were males ranging from 28 to 70 years in age with tobacco chewing habit. Occasional alcohol consumption was reported in 70% patients and 30% of patients reported both betel quid and alcohol habit. Duration of the habit ranged from 8 to 25 years. Histopathological

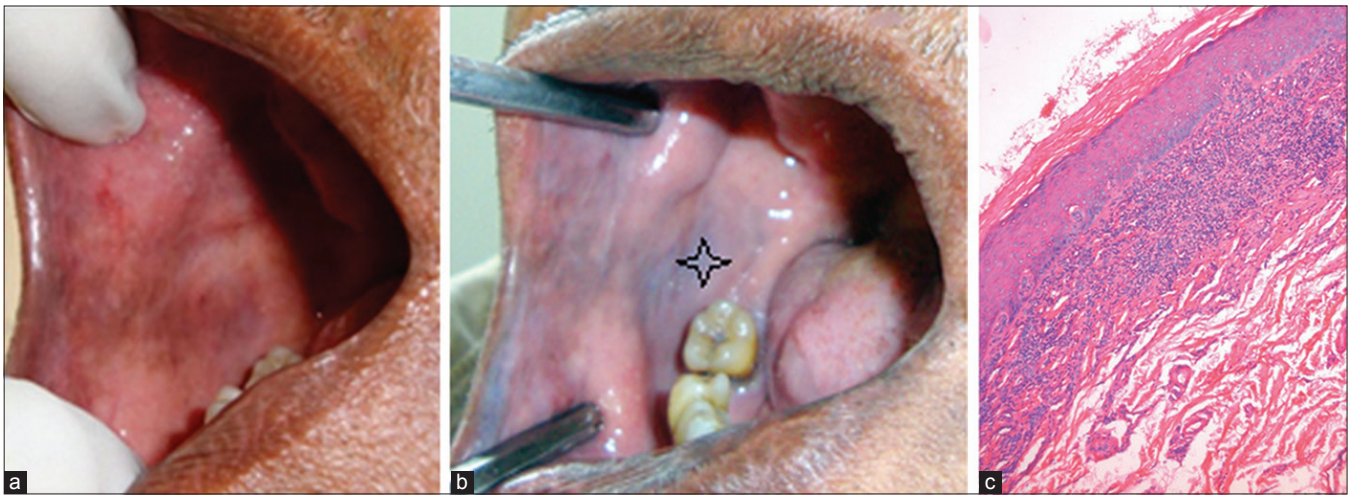

Figure 1: (a) Group I cases: Before application of the vital stain. (b) After application of vital stain, asterisk showing biopsy site. (c) Histopathology showing hyperkeratosis (H&E stain, ×100)

evaluation showed hyperkeratosis in 51% (n = 22), mild dysplasia in 40% (n = 17) and mild inflammatory changes in 9% (n = 4). Hyperkeratosis [Figure 1c] and mild dysplasia were considered histopathologically positive whereas mild inflammatory changes were considered as negative. The sensitivity and specificity were 97% and 50%. PPV and NPV were 0.95 and 0.66, respectively [Table 1].

Group II: A total of 40 subjects with oral lesions were screened, from whom 46 specimens were obtained. In this group, majority were females 65% (n = 26). The age of the subjects ranged from 35 to 68 years. Majority (66%) were tobacco chewers and alcoholics, whereas 34% were betel quid chewers. Duration of the habit ranged from 10 to 33 years. 41 biopsies of Group II patients were histopathologically positive [Table 2]. The principal diagnosis was squamous cell carcinoma (n = 40) with 68% (n = 26) females and 32% (n = 12) males. Histopathological evaluation showed WDSCC in 48% (n = 22) [Figure 2c], MDSCC in 33% (n = 15), early invasive carcinoma in 6% (n = 3), moderate dysplasia in 2% (n = 1) and no evidence of malignancy (inflammatory/reactive lesions) in 11% (n = 5), i.e. false positive results. The sensitivity and specificity were 95% and 60%. PPV and NPV were 0.95 and 0.60, respectively [Table 2].

DISCUSSION

Oral cancer is usually diagnosed when it becomes symptomatic and by this stage approximately two-third of patients would have already developed advanced disease with regional metastasis.[1] The key in reducing the morbidity and mortality is to detect precancerous or cancerous lesions at their most incipient stage. This can be achieved by development and use of diagnostic aids that could aid the general dentist or dental specialist to identify more readily or assess persistent oral lesions of uncertain biologic significance. Several chair side diagnostic methods are tried in the past; of them vital staining is one amongst the popular diagnostic method.[6] It was first used for detecting cervical dysplasia and carcinoma *in situ*. Niebel and Chomet were the pioneers who used dye material to detect oral cancer in 1964.[9] Very limited research has been done in patients with high-risk factors (such as tobacco, alcohol and betel quid) for early premalignant changes. Hence, this study was conducted in tobacco users; with apparently normal mucosa and suspected of having oral cancer using 5% acetic acid as the vital staining agent.

Chronic use of tobacco and its contact with oral mucosa sets in subtle changes at the cellular level in an otherwise clinically normal mucosa. Acetic acid causes osmolar changes in the cells resulting in outward movement of water. Thus, leading to dehydration and finally resulting in the collapse of the cell membrane around abnormal and enlarged nucleus along with coagulation of cellular proteins. This reduces the transparency of epithelium and the lesion appears white.[10,11]

In the present study, the proportion of female patients with oral cancer in our study was higher which could be probably because of the high rate of betel quid consumption by females in this area. Jayant and Notani noted that incidence rates for oral cancer in females were much higher in India than in other registries.[12] Buccal mucosa constituted the most

Table 1: Efficiency of acetic acid in group I

Acetic acid vital test (clinical)	HPD		Total
	Positive*	Negative$^{\infty}$	
Positive	38	02	40
Negative	01	02	03
Total	39	04	43

Sensitivity: 97%, Specificity: 50%, PPV: 0.95 and NPV: 0.66. *HPD positive: Hyperkeratosis, epithelial dysplasia, $^{\infty}$HPD negative: Normal mucosa, inflammatory changes. PPV: Positive predictive value, NPV: Negative predictive value, HPD: Histopathological diagnosis

Table 2: Efficiency of acetic acid in group II

Acetic acid vital test (clinical)	HPD		Total
	Positive*	Negative$^{\alpha}$	
Positive	39	02	41
Negative	02	03	05
Total	41	05	46

Sensitivity: 95%, Specificity: 60%, PPV: 0.95 and NPV: 0.60. *HPD positive: Early invasive carcinoma, squamous cell carcinoma epithelial dysplasia, $^{\alpha}$HPD negative: No evidence of malignancy (inflammatory/reactive lesions). PPV: Positive predictive value, NPV: Negative predictive value, HPD: Histopathological diagnosis

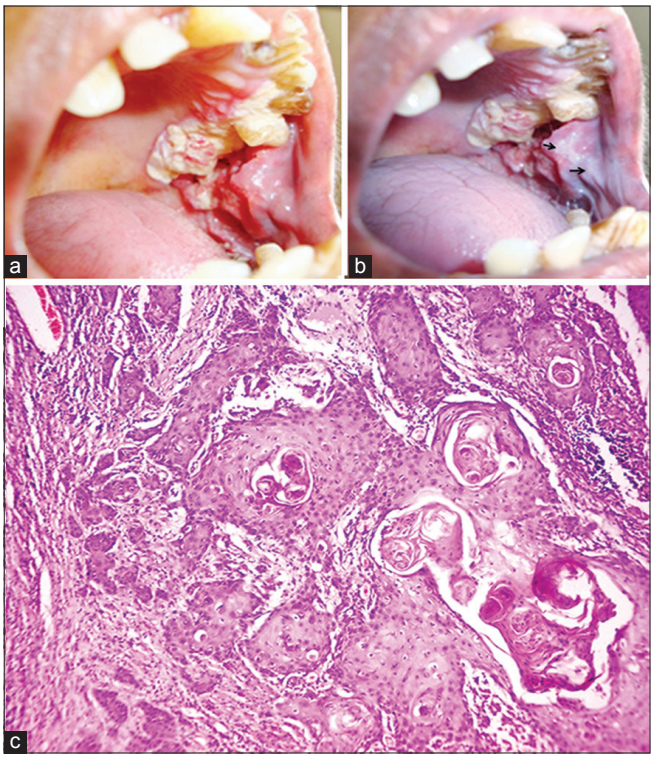

Figure 2: (a) Group 2 cases, before application of the vital stain. (b) After application of vital stain asterisk showing biopsy site. (c) Histopathology showing well-differentiated squamous cell carcinoma (H&E stain, ×100)

common site of oral cancer that is, 60% ($n = 24$), followed by lower gingivobuccal comple × 37.5% ($n = 15$) and dorsum of tongue 2.5% ($n = 1$). This is consistent with a study performed by Prabhu and Daftary who found that buccal mucosa and alveolus are the most common areas for oral cancer in the Indian scenario.[13] Findings of the present study are different from western observations where carcinomas most commonly occur in the floor of the mouth.[14]

Two cases showed false positivity, which could be attributed to inflammation, which increases the permeability of the cell membrane. In two patients, certain areas apart from the lesional tissue showed positivity. Histopathological diagnosis showed features of early invasive carcinoma and moderate dysplasia. This could be explained on the basis of field cancerization where field changes are evident in the mucosa distant to the primary lesion, increasing the chances of the second primary tumor.[15]

In Group I, (apparently normal mucosa of chronic tobacco users) hyperkeratosis and mild dysplasia can be due to constant irritation caused by tobacco and its contents. It is suggestive of slow mucosal alteration that occurs before the actual manifestation of the clinical lesion.[16] Previously, it had been reported that toluidine blue when used as a vital stain in tobacco users with apparently normal appearing mucosa histopathologically showed hyperkeratosis with mild dysplasia in 5% cases ($n = 38$).[5] The present observation supports the conclusion of Braakhuis *et al*. that genetically altered cells gradually proliferate and expand into a noninvasive field that is vulnerable to further genomic damage. This field, despite being macroscopically undetectable, is fertile ground for the evolution of premalignant lesions and eventually invasive cancer.[17] Malignant transformation of oral leukoplakia has been reported to be up to 43%, early intervention and diagnosis of high-risk patients may contribute toward reducing the mortality and morbidity.[18] To the best of our knowledge, this is the first study done on the apparently normal mucosa of chronic tobacco users using *in vivo* application of the acetic acid vital stain. The significant relationship found between clinical examination using 5% acetic acid and histopathological diagnosis confirmed that acetic acid reacted better with tissues that had turned dysplastic or malignant than the normal tissue.

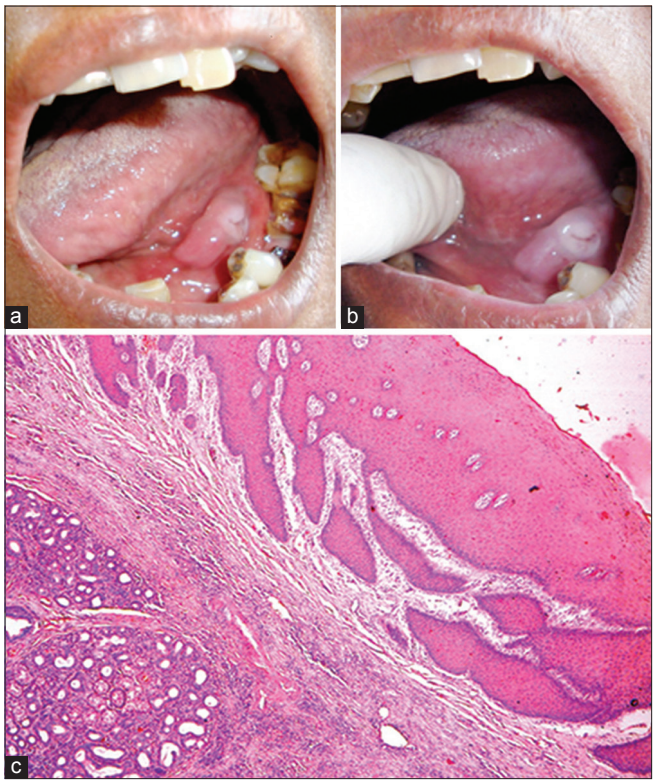

Figure 3: (a) Group 2 cases, before application of the vital stain. (b) Vital stain showing positivity. (c) Histopathology showing inflammatory hyperplasia (false positive) (H&E stain, ×100)

The sensitivity and specificity of the present study for Group I and Group II were 97% and 50%; and 95% and 60%, respectively. Bhalang *et al*. observed sensitivity and specificity of 83.3% and 84.21% on using household vinegar (5% acetic acid) for detection of oral cancer.[8] Studies of cervical cancer screening in India and western countries have shown sensitivity ranging from 49% to 92% and specificity from 49% to 90%.[10,19] In comparison to toluidine blue, the sensitivity and specificity ranges from 77%–100% to 44–93%.[20,21]

The advantages of using 5% acetic acid as vital stain are its simple procedure, convenient preparation method, no undue mucosal coloring as seen with agents such as toluidine blue, Lugol's iodine, etc., *In vivo* application does not result in any appreciable artifacts in the specimens on histopathological examination. It could be used for mass screening because of its cost effectiveness.

This study, however, has few limitations like positivity and negativity of vital staining being subjective and the exclusion of the precancerous group. Clinical discrimination in the staining intensity between keratotic, inflammatory [Figure 3a-c], malignant or potentially malignant disorders of the oral mucosa is also difficult. Because of small sample size, the PPV and sensitivity were remarkably high. To know the accurate sensitivity and specificity, it should be tried in large-scale oral screening of high-risk patients.

CONCLUSION

The results of this study suggest that 5% acetic acid holds a promising future as a screening tool in high risk individuals and oral cancer patients. It should be further evaluated on a larger population to get a more thorough insight into the benefits and limitations of using the same.

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Conflicts of interest

There are no conflicts of interest.

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