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Application of 5% acetic acid as a diagnostic adjunct in the detection of oral potentially malignant disorders

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Abstract

Background: Commercial vinegar (acetic acid) can be a good and cost-effective screening tool for Oral Potentially Malignant Disorders (OPMDs) in resource constraint countries.

Objectives: To assess the sensitivity, specificity, and accuracy of 5% acetic acid for the detection of epithelial dysplasia in OPMDs.

Methods: This study was an analytical cross-sectional study done to assess the diagnostic accuracy. It was done in hospital and field settings from, 2017 January to 2019 February, after ethical approval from IRC of BPKIHS. Convenience sampling technique was used and 114 individuals (57 participants with OPMDs and 57 participants with normal oral mucosa) were included. Acetic acid was applied and allowed to be in contact for one minute. A positive finding (acetowhitening) was designated in a lesion that changed its colour to opaque white, while a negative finding was designated for the lesion that showed no change. This was followed by biopsy and histopathology. Patients who were undergoing extraction at the community camps with normal oral mucosa were included as participants without target condition (OPMD). Sensitivity, specificity, and accuracy were calculated for 5% acetic acid in detecting oral epithelial dysplasia.

Results: Total number of patients included in the study was 114. Among the total sample, 54 had dysplasia on histopathological evaluation, 60 did not have dysplasia. Fifty-five patients had positive acetowhitening and 59 patients had negative acetowhitening reaction. The sensitivity, specificity, and accuracy were, 0.759, 0.766, and 0.763 respectively.

Conclusions: The sensitivity, specificity, and accuracy of commercially available vinegar in detecting oral epithelial dysplasia are good.

Key words: Acetic acid; Dysplasia; Mouth; Precancerous conditions; Sensitivity; Specificity.

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INTRODUCTION

Oral potentially malignant disorders (OPMDs) include a variety of lesions and conditions characterised by an increased risk of malignant transformation to oral squamous cell carcinoma (OSCC).[1] In this era, where oral habits are common, prevalence of OPMDs are very high, especially in developing country like ours.[2,3] It is extensively stated that OSCC is the most common cancer in oral cavity (90% of all oral cancers).[4] The financial resources, infrastructure, and technical expertise required to develop and maintain a cytologic screening program are beyond reach for most developing countries. Thus, non-cytologic methods for screening are explored.[3] The most widely used chemical agents is toluidine blue.[5] A newer agent, 3-5% acetic acid (household vinegar), being a cost-effective method, is investigated for detection of cervical cancer.[3] Since the anatomy and the types of cancer found in the oral cavity and cervix are comparable, acetic acid can be explored

in oral cancer as well. Studies assessing the effectiveness of 5% acetic acid for OPMDs have not been conducted widely.[6]

The objective of the study was to investigate the use of 5% acetic acid in the detection of OPMDs by assessing its sensitivity, specificity and accuracy for the diagnosis of OPMDs.

METHODOLOGY

This study was an analytical cross-sectional study, to assess the diagnostic accuracy of acetic acid in detecting oral epithelial dysplasia. The study was done in the Department of Oral Medicine and Radiology, in collaboration with departments of Public Health Dentistry, Pathology and Oral Pathology, B. P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Sunsari, Nepal. The duration of the study was 25 months (2017 January to 2019 February) after ethical approval.

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The ethical approval for this study was obtained from institutional review committee (IRC) of BPKIHS (Ref. 177/073/074-IRC).

For selection of sample, convenience sampling technique was used. Sample size was initially estimated as 120, 60 participants with OPMD (this was the average number of cases in the Department of Oral Medicine and Radiology with histopathological diagnosis of oral potentially malignant disorders, in the previous three years) and 60 participants without target condition (OPMD) (on the basis of 1:1 ratio for cases and controls).

The inclusion criteria were a provisional diagnosis of any of OPMDs for cases and participants without OPMDs for controls. For participants without target condition, cases clinically free from OPMDs were included. All the patients with the target condition were included from the outpatient section while patients without the target condition were included from community health camps. All the participants were above the age of 18 years. The exclusion criteria were cases with clinical evidence of inflammation and patients not willing to take part in the study.

Following diagnostic criteria was used to diagnose a case of oral lichen planus (OLP), oral leukoplakia, erythroleukoplakia, and oral submucous fibrosis (OSF):

A case was considered as oral lichen planus when there was multifocal symmetric distribution of white and red lesions exhibiting one or more of the following forms: reticular/papular, atrophic (erythematous), erosive (ulcerative), plaque, bullous. Lesions not exclusively localised to the sites of smokeless tobacco placement, adjacent to and in contact with dental restorations. Lesions' onset not co-relating to initiation of any medicine or cinnamon-containing products.[7]

Oral leukoplakias a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer.[8]

Erythroleukoplakia is leukoplakia of the oral mucosa that is clinically characterised by the presence of erythematous or red and white patches.[9]

Oral submucous fibrosis is diagnosed based on clinical criteria of mucosal blanching, burning, hardening and presence of characteristic fibrous bands and associated with gradual inability to open the mouth.[10]

The armamentarium used were cotton swab, punch biopsy kit, hydrogen, peroxide, cotton swab, commercial vinegar (5.25% acetic acid), betadine solution, 2.5 ml syringe, 2% lidocaine solution, tweezer, punch biopsy kit, and 10% formalin solution. A particular brand of commercial vinegar was used because the percentage of acetic acid in that brand of vinegar was closest to the required range, that is, 3% to 5%.

A semi-structured proforma with relevant socio-demographic profile and details of the examination findings were filled in, information sheet was provided and written informed consent was taken. Confidentiality and privacy of the patients were maintained.

The suspected lesions were examined for location, size morphological, and surface characteristics of suspected lesion. Patients were asked to rinse the mouth with water and hydrogen peroxide was applied to remove all the debris. For a safe intraoral application, 1% hydrogen peroxide was used, by diluting 3% hydrogen peroxide to 1%. This was done by mixing two parts of water with one part of 3% hydrogen peroxide. Hydrogen peroxide was applied intraorally for a duration of one minute. Acetic acid (5%) was applied with cotton swab all over the lesion and surrounding area and allowed to be in contact with the lesion for one min. Then patient was asked to rinse with water to remove the excess acetic acid. The oral cavity was re-examined for any colour change and the findings were noted. A positive finding was designated

for the lesion that changed its colour to opaque white, that is, acetowhitening (Figure 1), while a negative finding was designated for the lesion that showed no colour change (Figure 2). This was followed by punch biopsy with a 5-6 mm punch and the specimen was then sent for histopathological examination for presence or absence of dysplasia and the histopathological diagnosis.

The biopsy for participants without target condition was obtained from the patients who were undergoing extraction at the community camps, following the acetic acid test. In this group (control group), biopsy was taken from the gingiva around the teeth being extracted which looked clinically normal. The acetic acid application procedure was same for both the groups. For the patients without target condition, verbal consent was obtained for utilising gingival tissue of the tooth being extracted for histopathological examination. Patients were informed that the tissue will be used for a clinical study and patient will have no additional harm or drawback apart from those seen after extraction. All the clinical examinations, application of acetic acid and punch biopsy were performed by a single examiner.

Data were entered in Microsoft Excel sheet and transferred to Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 11.5 (SPSS Inc., Chicago, Ill., USA).

Sensitivity, specificity and accuracy in detecting oral epithelial dysplasia were calculated for 5% acetic acid (commercial vinegar) by using the two-by-two table.

RESULTS

Though initially the sample size was estimated to be 120, due to six tissue samples being inappropriate for histopathological evaluation, the total number of samples was reduced to 114. The remaining six tissue samples were inappropriate because they did not have an adequate tissue depth. The total numbers of male in this study were 65 (57%) and the total numbers of female were 49 (43%). Among the participants with target condition (57), there were 46 males (80.70%) and 11 females (19.30%). The overall mean age was 43.1 ± 16.414 (Mean ± SD) years while the mean age among patients with OPMD was 37.67 ± 15.743 years.

Among the patients with OPMD, 29 patients (50.87%) presented with restricted mouth opening, 15 cases (26.31%) had white patch in mouth, 10 cases (17.54%) had burning sensation, three cases (5.26%) had wound and one (1.75%) had growth inside mouth as a chief complaint.

The personal habit history which was most predominant in these cases were consumption of commercial products of arecanut and tobacco (gutkha®, zarda®, chewing tobacco) in 24 (42.10%) patients. Eighteen (31.57%) patients had the habit of areca nut and paan,10 patients (17.54%) had the habit of taking cigarette, alcohol and khaini, two patients (3.50%) used areca nut alone. Three patients (5.26%) had no deleterious habit.

The various clinical diagnoses that was present in patients with suspected lesions of OPMDs have been tabulated (Table 1).

Among the various histopathological diagnoses, majority of the cases were OSF (32 patients, 56.14%) followed by OLP (11 patients, 19.30%) and mild dysplasia (6 patients, 10.53%) (Figure 3).

Among the total 114 patients, 54 (47.37%) had dysplasia on histopathological evaluation, 47 in OPMD group and seven in the control group. Out of total patients, 60 (52.63%) did not have any evidence of dysplasia (10 in OPMD group and 50 in control group). When the acetic acid test was done and acetowhitening was observed, 55 (48.24%) patients had positive acetowhitening reaction, that is, white colour change (total of 48 patients in the group with target condition and a total of seven in the group without target condition) and 59 (51.75%) patients had negative acetowhitening reaction, that is, no white colour change (total of nine patients in the group with the target condition and a total of 50 patients in the group without target condition) (Table 2).

None of the patients participating in the study showed any adverse events after the application of acetic acid.

The sensitivity, specificity, and accuracy for acetic acid as a diagnostic adjunct was estimated to be 0.759, 0.766, and 0.763 respectively at a 95% confidence interval (CI).

The positive predictive value was 0.745 and the negative predictive value was 0.779.

The odds ratio for developing acetowhitening reaction on acetic acid application in lesions with dysplasia was 10.36.

Also, the relationship between the results of OPMD examination using 5% acetic acid and the results of histopathological evaluation for dysplasia revealed significant association ($p < 0.001$).

Table 1: Distribution of clinical diagnosis of the patients

Clinical diagnosis	Frequency (percent)
Oral submucous fibrosis	32 (56.14)
Leukoplakia	13 (22.8)
Oral lichen planus	11 (19.29)
Erythroleukoplakia	1 (1.75)

Table 2: Total patient distribution according to colour change and dysplasia, n (%)

Present		Dysplasia		Total
		Absent		
Colour change on acetic acid	Positive	41 (74.54)	14 (25.45)	55 (100)
	Negative	13 (22.03)	46 (77.97)	59 (100)
Total		**54 (47.36)**	**60 (52.63)**	**114 (100)**

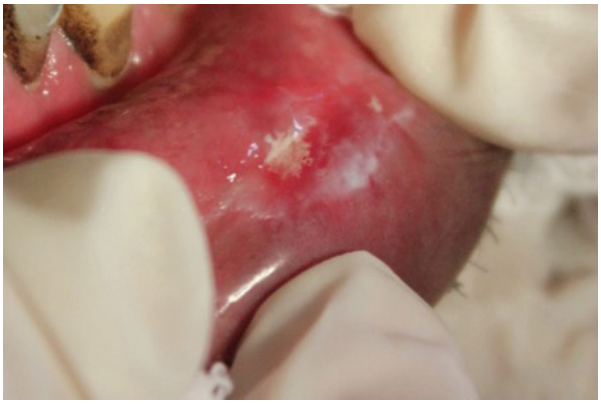

Figure 1a

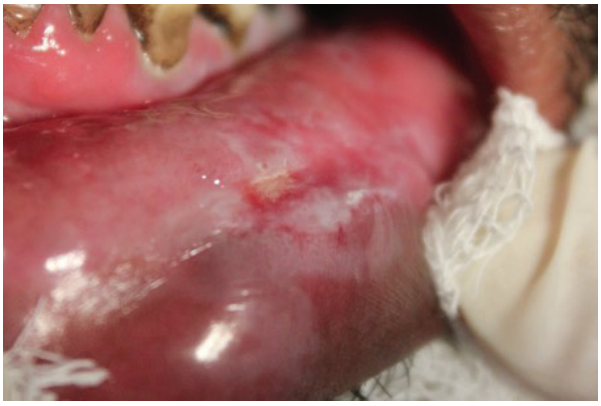

Figure 1b

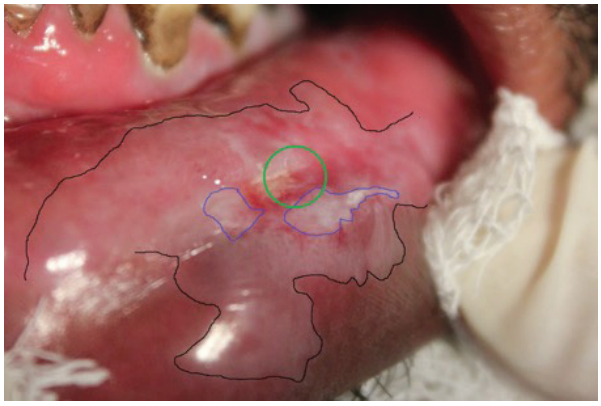

Figure 1c

Figure 1: Positive acetowhitening reaction a: prior to application of acetic acid, b: after the application of acetic acid, c: black outline showing the area of positive acetowhitening reaction, blue outline showing the previously present white plaque and green circle showing the biopsy site

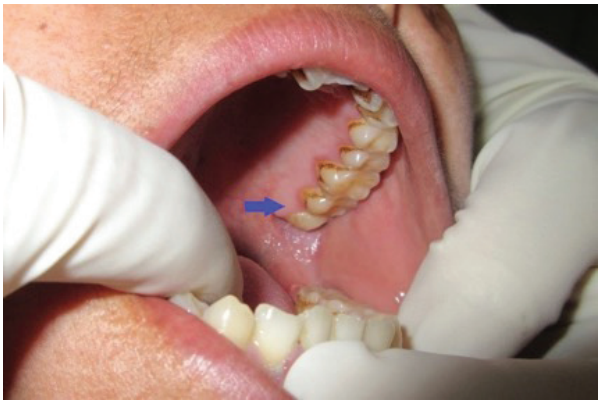

Figure 2a

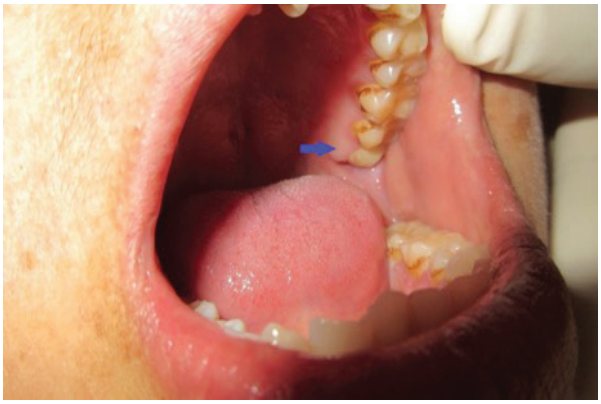

Figure 2b

Figure 2: Negative acetowhitening reaction (blue arrow) in a normal oral mucosa a: prior to, b: after the application of acetic acid

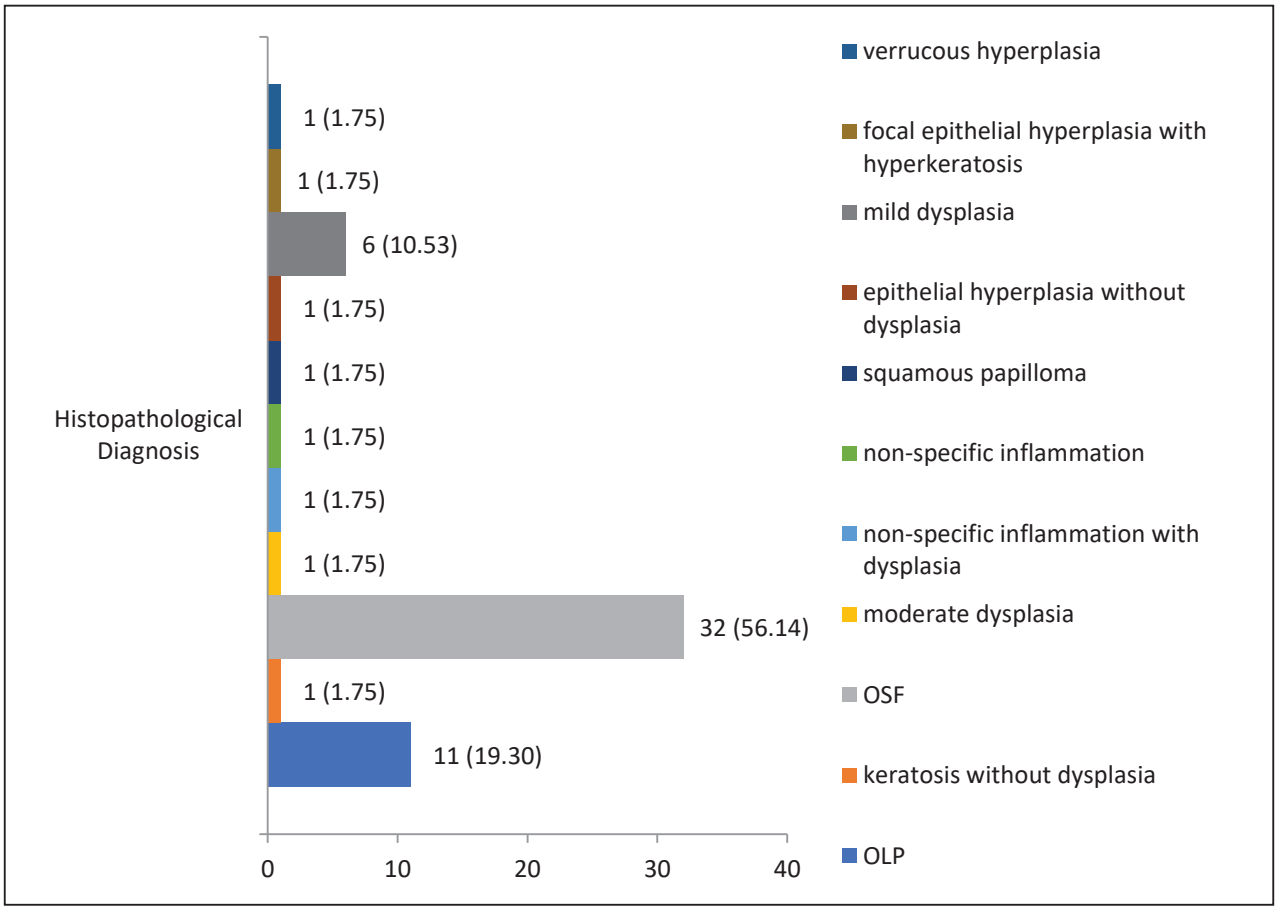

Figure 3: Distribution of histopathological diagnoses in participants with target condition, n (%)

DISCUSSION

Acetic acid has been widely used in detection of cervical dysplasia.[11-14] Unfortunately, there is a relative paucity in the number of studies done to detect oral potentially malignant disorders (OPMDs) or oral cancer. Few studies have been done to evaluate its application for oral cancer.[6,15]

Every person at any age can develop cancer, however the risk of developing cancer increases with age.[16] If people

live longer, it is more likely for a sporadic mutation to occur in their genome, which leads to genetic alterations and finally malignant phenotype. Among the genders, OPMDs show a predilection for males,[16,17] similar was the result in this study. This could be due to the increased habitual use of tobacco and alcohol among men, especially in countries with patriarchy system.

The mean age of patients with OPMDs in this study was 37.67 ± 15.743 years, which is a little earlier as the reported average age of the population affected with OPMDs. Studies have shown that average age of population affected with OPMDs is between 50-69 years, occurring earlier than oral cancer (five years earlier on an average). However, it is also documented that 1- 5% of OPMDs affect the younger age group of around 30 years. This may be due to the fact that various extrinsic and intrinsic etiological factors are now more prevalent in today's younger population.[18] This finding can be rationalised by the fact that in the current study majority of the cases were that of OSF, and in case of OSF the predominant population group that is involved is the younger group, often less than 35 years.[19,20]

Various attempts have been made to clinically highlight dysplastic areas before biopsy but they have not proven to be absolutely reliable. It will be helpful where there is widespread "field change" such as seen in patients at high risk for oral squamous cell carcinoma. In the study conducted by Bhalang et al. to detect oral cancer by the use of acetic acid, the sensitivity, specificity, and accuracy of using acetic acid for oral cancer examination were 83.33%, 84.21%, and 83.64%, respectively.[10] Likewise, in the study by Vinuth et al., the sensitivity and specificity for subjects with a history of chronic tobacco use and clinically apparent normal mucosa were 97% and 50%and for subjects suspected of having oral cancer were 95% and 60% respectively.[15] The difference in the result of these studies could be attributed to the nature of the lesion as oral cancer has higher degree of acetowhitening than OPMDs. Also, few cases with clinically undetectable signs of inflammation having histopathological evidence of inflammation gave a false positive result in the current study.

One important pattern, which was noted in the current study, was that there were seven cases of OSF where there was blanching and because of those blanched mucosa acetowhitening reaction (that is, white colour change) could not be appreciated well. With this finding we could infer that those cases may not have actually given negative acetowhitening result, but actually were not apparent due to blanching of the mucosa.

One other importance of using acetic acid, as a diagnostic adjunct in clinical and community setting is that biopsy is not possible in every diagnostic setup of remote areas. If we can generate some evidence regarding the use of acetic acid then it would be beneficial to the clinician from diagnostic point of view and also to the patient, as they will not have additional economic and psychological burden.

Another diagnostic adjunct which is widely used both as a vital staining in living tissues and as a special stain is Toluidine blue owing to its metachromatic properties. Toluidine blue has been widely used in vivo to identify dysplasia and carcinoma of the oral cavity owing to its metachromatic property.[21] Studies assessing toluidine blue have shown a sensitivity and specificity ranging from 93.5-97.8% and 73.3-92.9% respectively, for detection of oral cancer and potentially malignant disorder. The recorded sensitivity for oral dysplasia alone is comparatively less ranging from 42-87%.[22-25] Though the above-mentioned results look better for toluidine blue when compared with acetic acid application, the easy availability and ease of preparation and application could be potential factors, a rural clinician would choose acetic acid over toluidine blue.

The odds ratio of 10.36 also shows that there is high chance of detection of epithelial dysplasia by the use of 5% acetic acid (a commercial agent, vinegar). When comparing this ratio with the odds of clinical oral examination in detecting oral dysplasia and OSCC, this ratio is higher. The odds ratio for clinical oral examination has been reported to be 6.1 in the study conducted by Epstein et al.[26]

This diagnostic adjunct appears to be highly applicable and useful for clinical, rural and remote community settings of low and middle income countries. The challenges of resource constrains is mitigated with this simple, economical, easily available, non-invasive and minimal side effect agent.

Potential observer bias or exposure suspicion bias, due to single investigator for all clinical examination and evaluation, could have added some limitation to this study. For future similar studies, blinding of the investigator is recommended.

CONCLUSION

The sensitivity, specificity, and accuracy of commercially available vinegar in detecting oral epithelial dysplasia were found to be good. Thus, the authors conclude that

there is some evidence to support the use of 5% acetic acid (commercial vinegar) as an adjunct to clinical oral examination for OPMDs. Randomised controlled trial on larger sample size is recommended to accurately generalise the findings of this study.

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