Original Article

Comparative cytopathologic evaluation using acridine orange with Papanicolaou stain in psychoactive substances abusers with potentially malignant and malignant disorders

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Abstract

Introduction: Oral cancer is a major health problem and its early detection is advantageous for therapeutic purposes. According to available evidence, the risks of oral malignancies increase with the usage of tobacco and other psychoactive substances (PSs). The present study showed expression pattern of nuclear and cytoplasmic changes from normal individuals without habit to oral potentially malignant disorders (OPMD) and oral squamous cell carcinoma (OSCC) in PS abusers with the help of fluorescence acridine orange (AO) stain and Papanicolaou (PAP) stain.

Aim and Objectives: This study aimed to investigate and compare diagnostic efficacy of fluorescence microscopic evaluation of AO stain in cytological smears with PAP staining under light microscopy in PS abusers having oral potentially malignant and malignant lesions.

Materials and Methods: Oral smears from 120 individuals among which 40 from potentially malignant disorders, 40 from oral malignancy and 40 normal buccal mucosa smears were prepared. One set of smears was stained by AO staining and the other by PAP staining and examined under fluorescence and light microscope, respectively. The results of both the stainings were evaluated by grading cytology smears in class-I to class-V cytology.

Results: The AO fluorescence stain reliably demonstrated malignant cells based on the differential fluorescence. The efficacy of AO fluorescence stain was higher than PAP stain in screening of oral lesions suspicious of malignancy. The sensitivity of PAP staining and AO staining is 57.50% and 61.25%, respectively.

Conclusion: As compared to PAP staining method, fluorescent AO method is more effective in screening of OPMD and OSCC in PS abusers.

Keywords: Acridine orange stain, oral potentially malignant disorders, oral squamous cell carcinoma, Papanicolaou stain

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INTRODUCTION

The incidence of oral cancer has been rising in many countries in the world, including India.[1] Many oral squamous cell carcinomas (OSCCs) are preceded by clinically evident oral potentially malignant disorders (OPMD).[2] Squamous cell carcinoma of the oral cavity is a major cause of mortality in several developing countries, comprising 40%–50% of all malignancies in parts of India and southeast Asia. This high prevalence is in contrast to 2%–4% of the total malignancies in the developed western countries. In India, most tobacco-related oral malignancies preceded by a clinically distinctive premalignant stage.[3]

Habitual psychoactive substance (PS) use is defined as the repeated use of PS despite the knowledge of its negative health consequences. The common PS use in India includes alcohol, tobacco and areca nut.[4] Cigarettes, cigars, pipes and smokeless tobaccos (chewing tobacco and snuff) cause oral cancer. Available evidence suggests that the risks of oral diseases increase with greater use of tobacco.[5]

As oral cancer is estimated to be the sixth most common malignancy and represents approximately 5% of all malignant tumors involving the body, its early detection is desirable so that successful therapy can be carried out.[6] Recent advances in techniques for detecting oral premalignant lesions and OSCC have improved the chances of early diagnosis. The techniques used for detection of lesions include oral examination followed by biopsy procedure, toluidine blue staining and exfoliative cytology examination, among which exfoliative cytology is well accepted by the patients due to its noninvasive nature.[7]

Exfoliative cytology is a noninvasive diagnostic procedure, which is growing rapidly in importance as a tool of early diagnosis of cancer.[8] Early detection of PS abusers with potentially malignant lesions and malignant lesions in the oral cavity could be greatly improved through techniques that permit visualization of subtle cellular changes indicative of the neoplastic transformation process. One such technique is fluorescence microscopy.

Fluorescence microscopy has been applied to cytology using berberine sulphate, acid fuchsin and the acridine group of stains, which is based on the affinity of the basic fluorochrome dyes for the nucleic acids. The most prominent cytochemical property of a malignant cell is the great amount of nucleic acid within the cell. The application of fluorescence microscopy results in increased brilliance depending on the various degrees of polymerization of nucleic acid in malignant cells.[6,8]

AO is a histochemical fluorochrome with a selective affinity for nucleic acids. The DNA and RNA fluoresce yellow to whitish green and red, respectively. The differential fluorescence of the malignant and normal cells allows a comparison of the total concentration of nucleic acids in the various cells in the preparation. In addition, the morphological features of the cell are clearly visualized.[8-11]

The present study was designed to investigate and compare the diagnostic efficacy of fluorescence microscopic evaluation of acridine orange (AO) stain used in cytological smears with standard Papanicolaou (PAP) staining under light microscopy in PS abusers having potentially malignant and malignant lesions of the oral cavity.

MATERIALS AND METHODS

The study comprised the following groups:

One hundred and two participants were selected for the study after checking inclusion and exclusion criteria.

- Study Group I: 40 PS abusers with OPMD (OSMF, Oral Lichen Planus, Oral Leukoplakia)
- Study Group II: 40 PS abusers with OSCC
- Control Group: 40 participants without any clinically observable lesions and without any habit.

The present study included participants with an age range varying from 20 to 80 years and individuals without any habit and no observable lesion were considered in a control group, whereas participants with a history of PS abuse formed a study group. Patients with systemic or local diseases and with special health-care needs were excluded.

Samples of buccal mucosa smears for the control group and lesional mucosa for the study group were made by scraping with flat wooden sticks. The smears were fixed using biofix spray. One set of smears was stained by AO staining. Smears stained with AO were examined under a fluorescence microscope. The corresponding set of smears was stained with PAP stain using a rapid PAP stain kit. Smears with folded cells were not considered for evaluation. Results of all the groups of both stainings were evaluated by grading cytology smears into Class-I to Class-V cytology which is used for the evaluation of cytological smear routinely.[12]

The exfoliated malignant cells showed reddish brown to bright orange–red cytoplasmic fluorescence, whereas the normal exfoliated cells from the control group showed greenish cytoplasmic fluorescence [Figures 1-8].

RESULTS

Following observations were drawn from this study:

- We found that study group participants were more in the sixth decade of life followed by the fourth decade in case of the control group [Table 1]. Overall, there was male predominance in both the groups [Table 2]
- Among the study groups, maximum number of cases had habit of tobacco usage, followed by smoking habit [Graph 1]. More number of participants had PSs usage which was 1–3 times per day followed by 4–6 times per day [Graph 2]
- In Group-I, a greater number of cases were observed on the left buccal mucosa, followed by the right buccal mucosa and labial mucosa. In Group-II, a greater number of cases were observed on the tongue followed by the left buccal mucosa, right buccal mucosa, lower labial vestibule, palate and lower left retro-molar region [Table 3]
- The sensitivity of AO stain in the demonstration of malignant cells was found to be 61.25% as against 57.50% sensitivity of Papanicolaou stain [Tables 4 and 5].

DISCUSSION

Since oral cancer is a major life-threatening problem, its early detection is desirable so that successful therapy can be carried out.[6] Being noninvsive technique, exfoliative cytology is growing rapidly in importance as a tool of early diagnosis of oral cancer. AO fluorescence microscopy technique permits visualization of subtle cellular changes indicative of the neoplastic transformation process in potentially malignant and malignant lesions of the oral cavity.

As the risks of oral malignancies increase with greater use of tobacco and other PSs, the present study was

Table 1: Age-wise distribution

Age	Group I	Group II	Group III
21-30	8	7	6
31-40	14	5	10
41-50	6	9	8
51-60	8	11	10
61-70	2	6	4
71-80	2	2	2

Table 2: Gender-wise distribution

Sex	Group I	Group II	Group III
Male	38	30	33
Female	2	10	7

Table 3: Location-wise distribution

Area of lesion	Group I	Group II	Group III
Right buccal mucosa	10	6	19
Left buccal mucosa	16	11	20
Labial mucosa	2	0	0
Right and left buccal mucosa	10	0	0
Lower labial vestibule	2	4	1
Tongue	0	17	0
Hard and soft palate	0	1	0
Lower left retromolar region	0	1	0

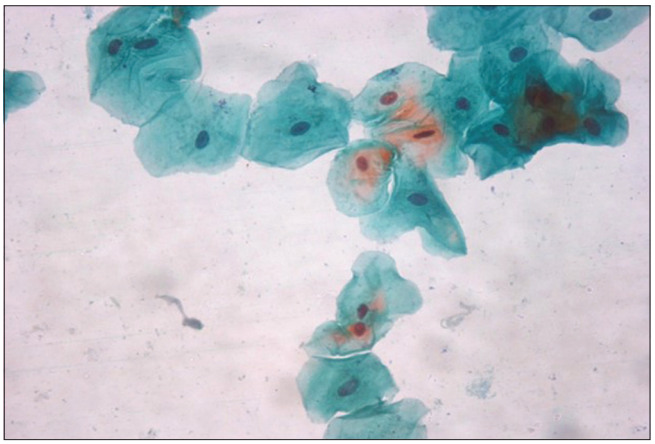


Figure 1: Photomicrograph showing Papanicolaou-stained Class I cytology

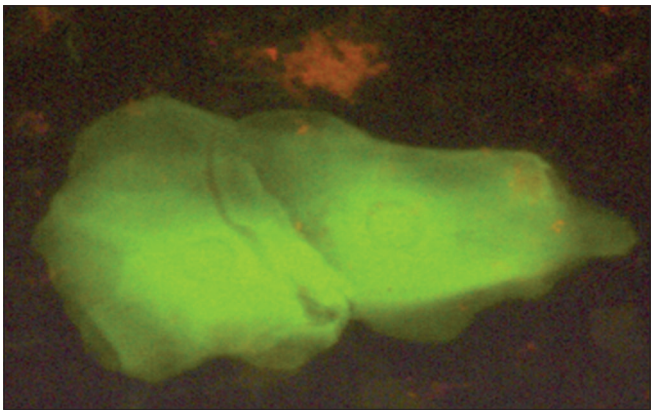


Figure 2: Photomicrograph showing acridine orange-stained Class I cytology

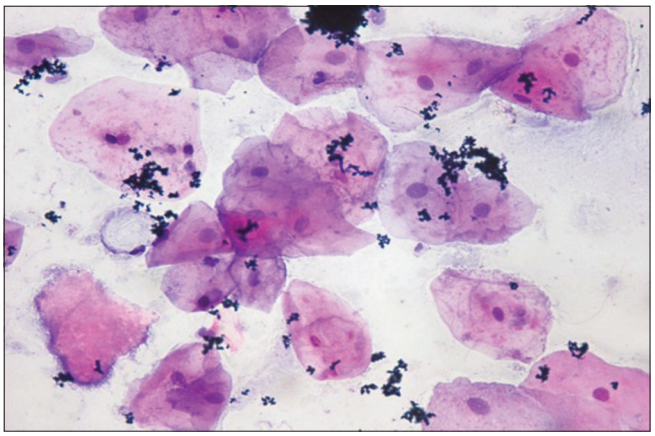


Figure 3: Photomicrograph showing Papanicolaou-stained Class II cytology

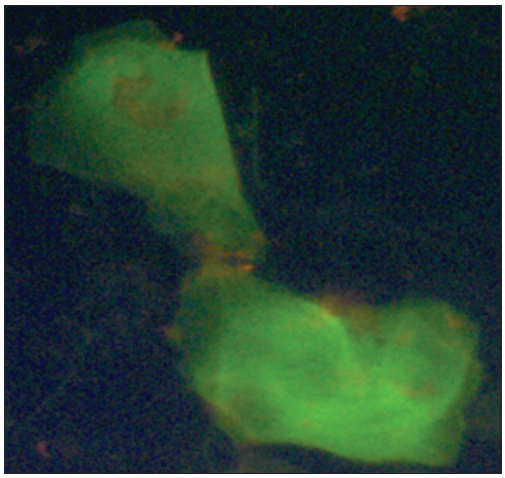


Figure 4: Photomicrograph showing acridine orange-stained Class II cytology

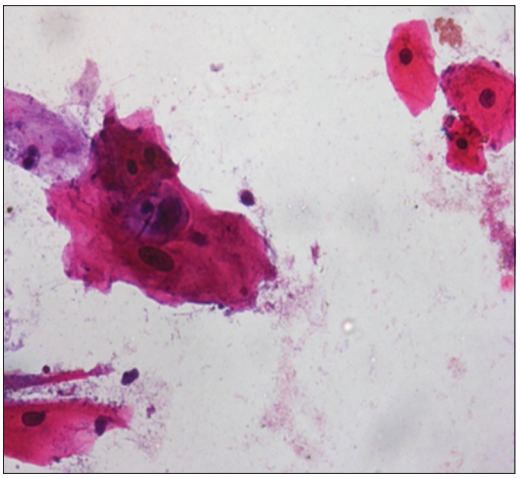


Figure 5: Photomicrograph showing Papanicolaou-stained Class III cytology

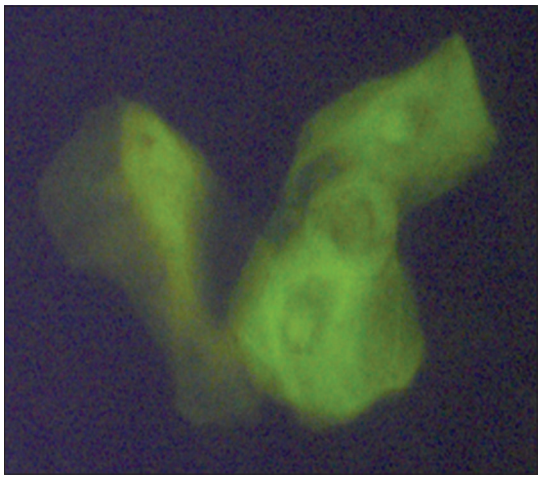


Figure 6: Photomicrograph showing acridine orange-stained Class III cytology

Table 4: Comparison of the results obtained using acridine orange and Papanicolaou stains in all the three groups

Staining methods	Groups	Positive result	Negative result	Total
PAP	Group-I	37	3	40
	Group-II	26	14	40
	Group-III	40	0	40
	Total	103	17	120
AO	Group-I	40	0	40
	Group-II	32	8	40
	Group-III	40	0	40
	Total	112	8	120

PAP: Papanicolaou, AO: Acridine orange

designed to observe the expression pattern of nuclear and cytoplasmic changes from normal patients without use of any PS to OPMD and OSCC in PS abusers with the help of fluorescence AO stain and PAP stain.

In the present study, a maximum number of cases were observed in the sixth decade followed by the fourth decade of life and is in accordance with the study conducted by

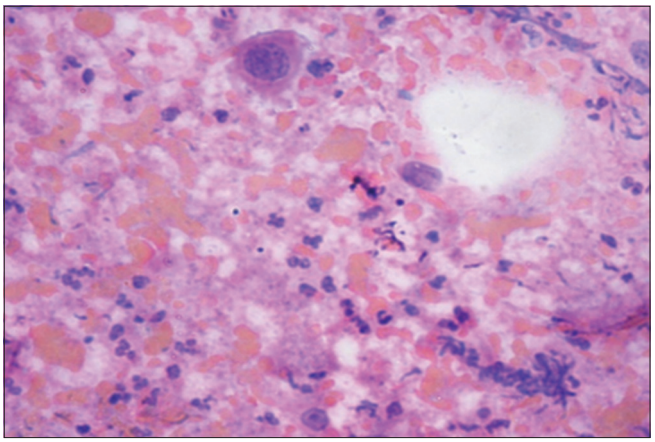


Figure 7: Photomicrograph showing Papanicolaou-stained Class IV cytology

Prakash *et al.* who found a maximum number of cases of oral cancer in the sixth and seventh decades of life.[6]

In the present study, a greater number of cases were observed on the left buccal mucosa which is in contrast with the study conducted by Prakash *et al.* who found gingivobuccal sulcus as the most common site of occurrence of oral malignancy.[6]

In this study, we compared PAP stain with AO stain in cytosmears of OPMDs and OSCC. The results of our study were in accordance with a study conducted by Aziz *et al.*, they found that AO stain proved better than PAP stain for visualizing nuclear details with its remarkably shorter staining time.[13]

The present study found that the sensitivity of AO and PAP was 61.25% and 57.50%, respectively, which is lower than that reported by Verma *et al.*, who found that the sensitivity of AO and PAP stain was 88.89% and 72.22%,

Table 5: Comparison between acridine orange and Papanicolaou stain

Stains	True positive	False positive	True negative	False negative	Number of cases	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
AO	49	0	40	31	120	61.25	100.00	100.00	56.34	74.17
PAP	46	0	40	34	120	57.50	100.00	100.00	54.05	71.67

PAP: Papanicolaou, AO: Acridine orange

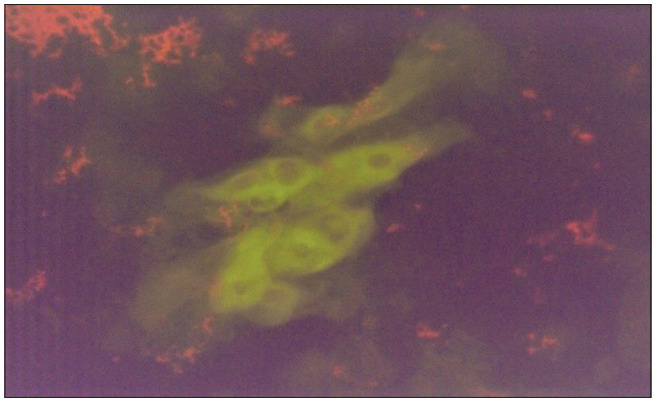
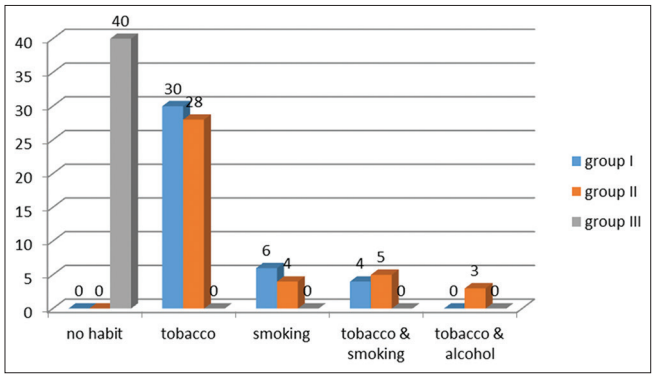
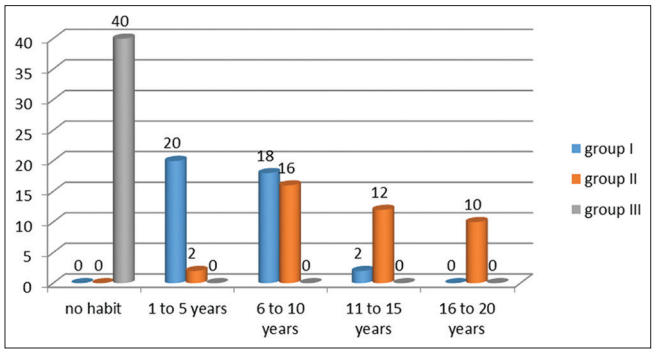


Figure 8: Photomicrograph showing acridine orange-stained Class IV cytology



Graph 1: Habit-wise distribution



Graph 2: Duration of habit-wise distribution

respectively[14] and also in accordance with the study conducted by Prakash *et al.* who found that the sensitivity of AO staining and PAP staining was 85% and 70%, respectively.[6]

The present study showed that the sensitivity of AO fluorescence microscopy is greater than the PAP staining method which is in accordance with the study conducted by Grubb and Crabbe who compared AO fluorescence microscopy with the PAP method, in cytological specimens of serous fluids, sputa and gastric washings. They observed that the sensitivity of AO fluorescence microscopy is greater in all the specimens while comparing with the PAP staining.[8]

Our results showed that the fluorescent AO stain was more reliable in demonstrating malignant cells in OPMDs and OSCC than the conventional PAP stain.

CONCLUSION

As compared to the PAP staining method, fluorescent AO method is more effective in screening of OPMD and OSCC in PS abusers. Hence, fluorescent AO can be a better option for the screening of carcinomas and it is especially helpful in the follow-up detection of recurrent carcinoma in previously treated cases.

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Conflicts of interest
There are no conflicts of interest.

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