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Cytological Evaluation of Premalignant and Malignant Oral Lesions by Oral Brush Cytology

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Context - *Oral brush cytology is easy, chair side non invasive, painless test that can be used to evaluate any suspicious lesion including common small white and red oral lesions to rule out dysplasia. The use of brush cytology without computer-assisted analysis using toothbrush is less expensive and may have applications in resource-challenged areas and could be a risk-free method of evaluating oral lesions.*

Aims -*The present study was aimed at assessing the reliability of oral brush cytology in the detection of potentially malignant disorders and malignant lesions of the oral cavity in terms of sensitivity and specificity when compared with biopsy examination.*

Material and method –*The study was conducted on fifty patients with oral lesions. All patients underwent oral brush cytology using conventional tooth brush followed by punch biopsy. The cytological diagnosis was compared with histopathological diagnosis using Mann Whitney U test.*

Results - *The sensitivity, specificity, positive and negative predictive values of brush cytology in detecting dysplasia and oral squamous cell carcinoma were 84.21%, 83.33%, 94.12% and 62.5% respectively. Application of Mann-Whitney U test showed that there was no significant difference (p value > 0.05)*

between histopathology and brush cytology in assessing both premalignant and malignant oral lesions.

Conclusion - *The cytological study of oral cavity cells is simple, rapid and relatively painless: it is thus well accepted by patients and suitable for routine application in population screening programmes, for early analysis of suspect lesions, and for pre and post-treatment monitoring of confirmed malignant lesions.*
Key words: *brush cytology, malignant lesions, oral lesions, potentially malignant disorders*

INTRODUCTION

Cancer of the oral cavity is the sixth most common malignancy reported worldwide and one with the highest mortality rate among all malignancies [1]. In India, oral cancer represents a major health problem accounting for up to 40% of all cancers.

Use of the areca or betel nut in many cultures is a major etiological factor in Asian countries. The development of the oral squamous cell carcinoma (OSCC) is a multistep process that requires the accumulation of multiple genetic alterations usually preceded by detectable mucosal changes, most often leukoplakias and erythroplakias. The clinical appearance of oral precancerous lesions and their degree of epithelial dysplasia suggests their malignant potential[2].

The survival of the patients with OSCC has not improved in the last 30 years and still shows a five year survival rate lower than 50% [3]. A fundamental factor responsible for the bad prognosis in oral cancer is its diagnosis in advanced stages and thus late treatment [4]. Early detection of a premalignant or cancerous oral lesion will improve the survival and the morbidity associated with the treatment [5]. The prognosis in an early diagnosed and treated OSCC is very good, with a mean survival rate of more than 80%.

Clinical examination and histopathological studies of biopsied material are the classical diagnostic methods used for precancerous and cancerous oral lesions. There is an urgent need to devise critical noninvasivediagnostic tools for early detection of oral dysplasia and malignancy and which can be easily performed in an out-patient set-up.

It has been shown in many studies that the brush cytology is an adequate procedure because of its ease in sampling and the quality of the oral cytologic sample is satisfactory [6], [7].

The brush cytology collects cells from the full thickness of the oral epithelium. It is a chair-side, easy to perform, non invasive, painless test that can be used to evaluate any suspicious lesion including common small white and red oral lesions to rule out dysplasia.

In this study, brush cytology of potentially malignant disorders and malignant lesions of oral mucosa was performed using a conventional tooth brush and subjected to cytopathological examination. Brush cytology results were compared with that of conventional punch biopsy results.

MATERIAL AND METHOD

The present study was conducted on fifty patients with oral lesions presented in the ENT Outpatient Department and evaluated under Cytology section of Pathology Department, RNT Medical College, Udaipur.

A detailed history of the patient in terms of duration, progress, associated symptoms and any treatment received for the lesion was obtained. An emphasis was given on the history of any adverse habit, if present, like tobacco or quid or gutkha chewing. An oral brush cytological examination was done in each case, followed by punch biopsy.

A commercially available medium or hard nylon disposable toothbrush was used.The tooth brush was used to obtain a complete transepithelial biopsy with minimal discomfort, without using any local anaesthetic agent.

Using moderate pressure, the brush was repeatedly brushed in one direction over the entire lesion many times until pinpoint bleeding was obtained, signalling entry into lamina propria and thus obtaining epithelial cells through the full thickness of the epithelium [8].

The material from the brush was spread on the middle third of two clean, dried glass slides. The smears were dried ,fixed immediately with 95% isopropyl alcohol and stained with May Grunwald and Giemsa (MGG) stain. (fig 1,3)

Smears having at least 30 well preserved cells from deep epithelial layers were considered adequate.

Cytological smears were interpreted based on the following parameters:

- Cohesiveness of cell clusters
- Enlarged nuclei
- Variation in nuclear size and shape
- Nuclear membrane irregularity
- Nucleocytoplasmic ratio
- Normal/ abnormal mitosis
- No. of nuclei, binucleation or multinucleation
- Keratinisation
- Hyperchromatism and chromatin pattern

Smears showing dysplasia were graded as mild, moderate and severe on the basis of cytological criteria [7]. (fig 2,4)

Punch biopsies of the lesions were taken and histopathological diagnosis was made. An effort was made to correlate cytological findings with histopathological diagnosis to find out the sensitivity, specificity and usefulness of brush cytology in oral pre-malignant and malignant lesions. Statistical analysis of results was done to predict the significance of brush cytology in various oral lesions. The data were entered in personal computer and analysed by using MS Excel SPSS Ver. 16.0, Epi Info Ver. 6.0.

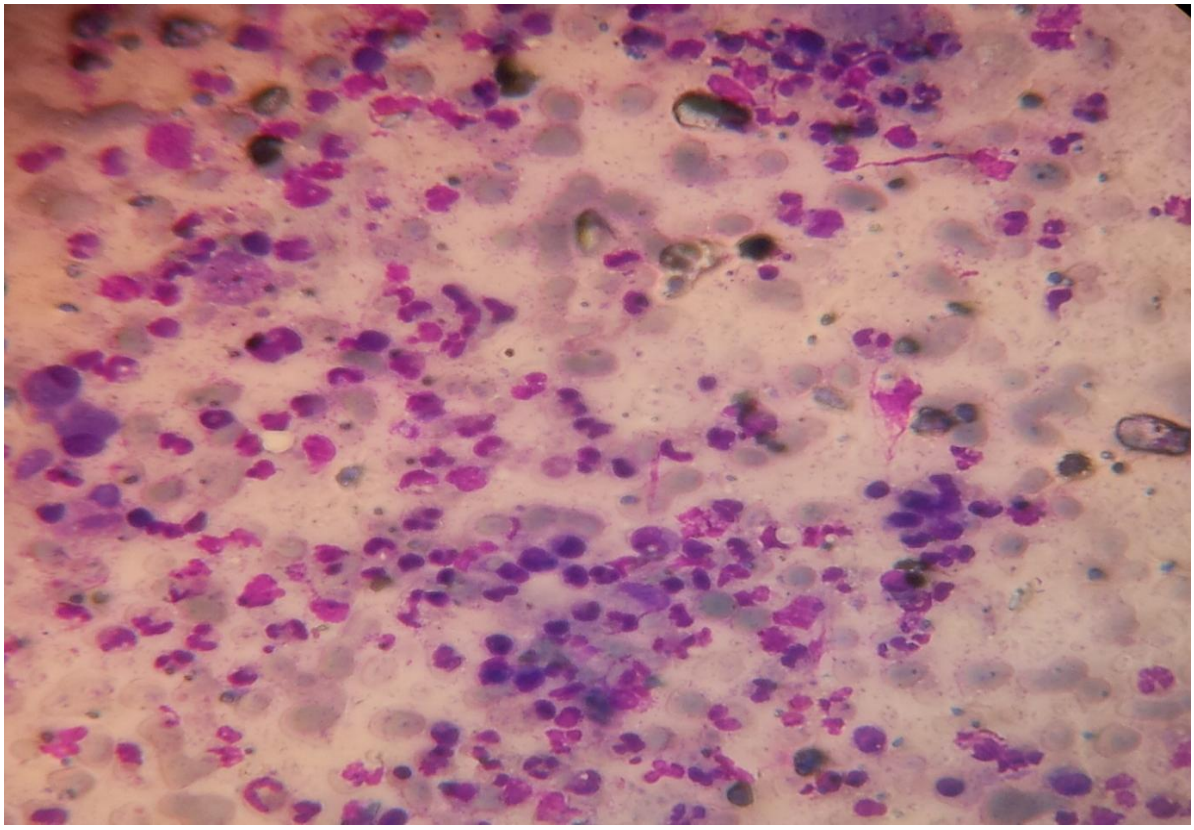

Fig 1: Inflammatory Oral Lesion: Normal Squamous Cells With Neutrophils and Macrophages (MGG X400)

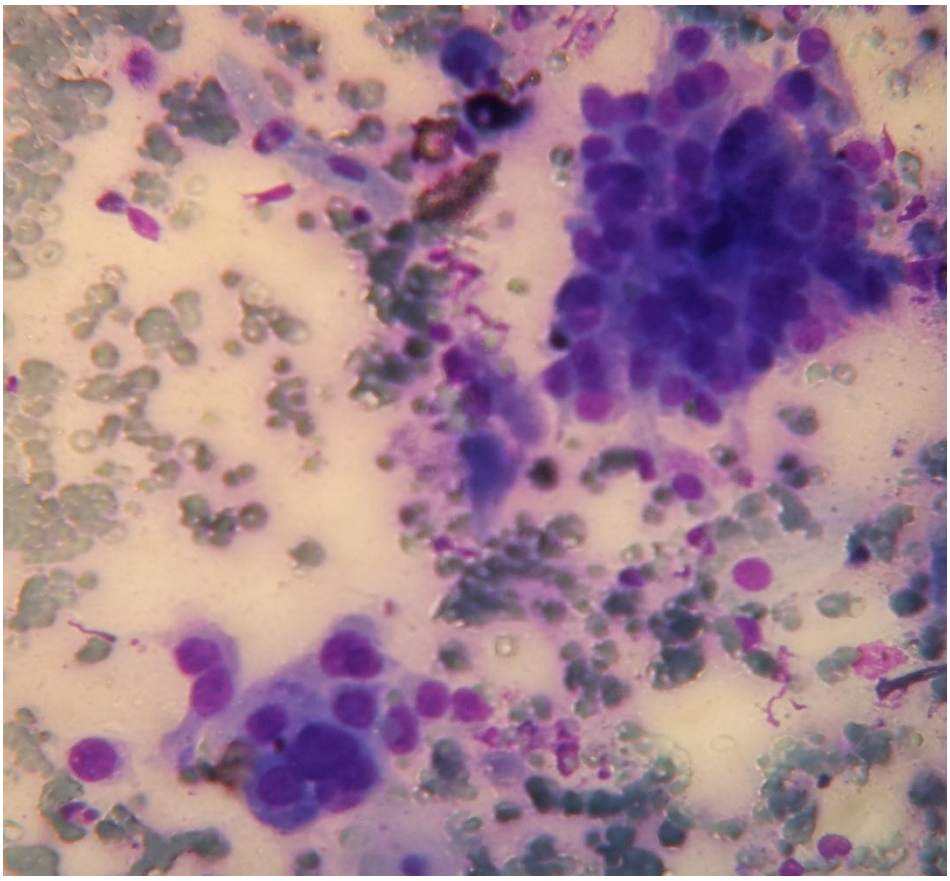

Fig 2: Malignant Oral Lesion: Clusters Of Malignant Squamous Cells Showing Nuclear Pleomorphism, Scanty Cytoplasm And Increased Nucleocytoplasmic Ratio (MGG X400)

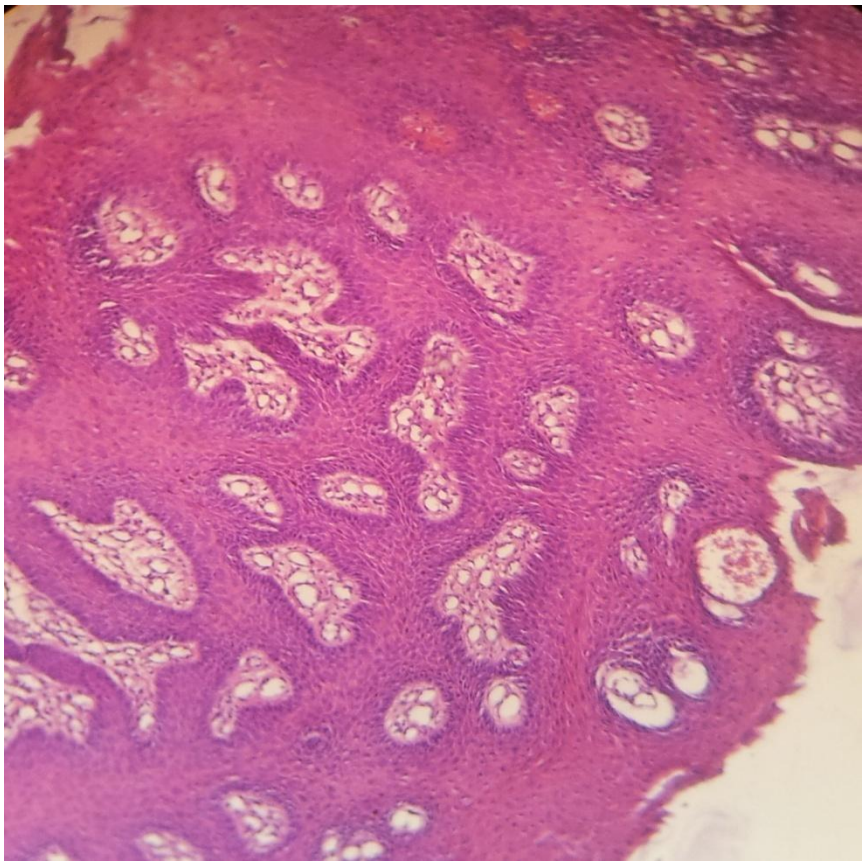

Fig 3: Hyperplastic Oral Lesion Showing Squamous Hyperplasia Of Epithelium (H & E X 100)

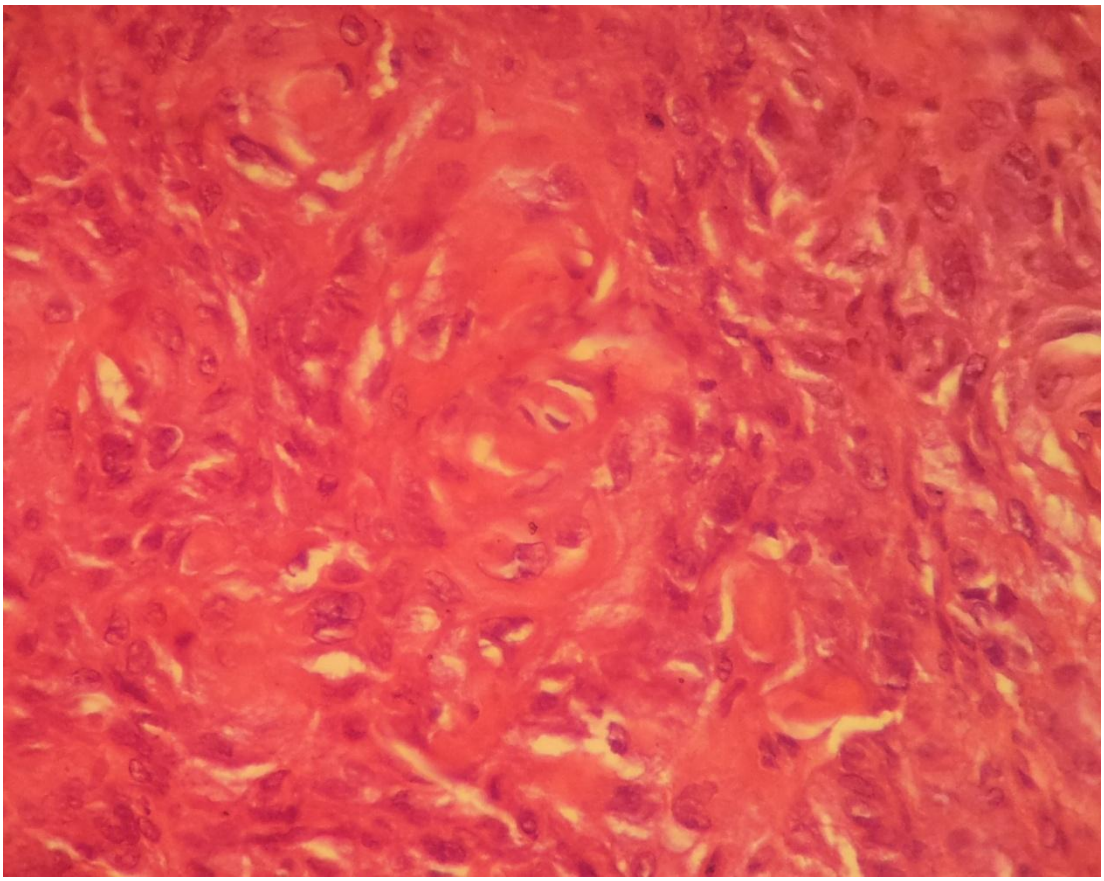

Fig 4: Squamous Cell Carcinoma: Anaplastic Squamous Cells With Keratin Pearls (H & E X 400)

RESULTS

Oral brush cytology was carried out on fifty patients with oral lesions.Most of the patients presented with ulcer, white patch or growth over the tongue, inner side of buccal mucosa, lip or hard palate with duration of 15 days to 5 years. However, some of the patients also presented with difficulty in chewing, swallowing and opening of mouth.

Out of the 50 patients studied, maximum numbers of patients affectedwere in the 3rd and 4th decade of life, with male: female ratio was 2.5:1. Benign oral lesions were most frequently found in the age group 31 to 40 years (38.88%) whereas malignant oral lesions were common in 61 to 70 years of age group (28.12%).

In our studymost commonly affected site was tongue followed by buccal mucosa, hard palate, lip and floor of the mouth.Risk factors like tobacco chewing and smoking was observed to be equally prevalent in both male (61.1%, 27.7%) and female (35.7%, 28.57%) patients.Tobacco chewing and smoking was found to be almost equally responsible for both benign as well as malignant lesions whereas other habits (like Quid, Gutkha, and Panetc) were more commonly associated with the development of malignant lesions.

On brush cytology malignant lesions were diagnosed in 64% of cases (varying grades of squamous cell carcinoma), epithelial dysplasia in 6% cases whereas benign lesionsaccounts for 30% of cases. (Table 1)

Punch biopsy was performed in all 50 cases. On histopathological examination malignancy was detected in 37 cases, dysplasia in 1 case and hyperplasia and inflammatory lesions were seen in 13 cases. Amongst malignant lesions MDSCC was more prevalent (42%) than WDSCC (32%). However, no case of PDSCC was found in our study. (Table 2)

Exact correlation between the cytological and histopathological diagnosis was found in 84% of the cases while it differed in 16% of the cases.

Malignant oral lesion diagnosed on cytological examination shows 100% diagnostic accuracy and correlate well with the histopathological diagnosis without any false positive results.

Out of the 15 cases of benign oral lesions diagnosed cytologically, 10 were confirmed benign,4 as malignant and 1 as dysplastic on histopathological examination.

However, the 3 dysplastic lesions diagnosed cytologically none was proved histologically. Out of these 3 lesions, one was diagnosed as malignant and rest two were diagnosed as inflammatory and hyperplastic. (Table 3)

	Diagnosis	**No. of Cases**	**Percentage**
1	Inflammatory and Hyperplastic	15	30%
2	Dysplastic	3	6%
3	Malignant	32	64%
		50	100%

Table 1: Oral Brush Cytology Results In The Present Study

	Diagnosis	**No. of Cases**	**Percentage**
A	Benign		
	Inflammatory and Hyperplastic	12	24%
B	Dysplasia	1	2%
C	Malignant		
	Well Differentiated Squamous Cell Carcinoma (WDSCC)	16	32%
	Moderately Differentiated Squamous Cell Carcinoma (MDSCC)	21	42%
	Poorly Differentiated Squamous Cell Carcinoma (PDSCC)	0	0
		50	100%

Table 2: Incidence Of Different Oral Lesions On Histopathological Examination

	Cytological		Histological					Diagnostic Accuracy
	Diagnosis	No. of Cases	Inflammatory and Hyperplastic	Dysplasia	Malignant			
					WDSCC	MDSCS	PDSCC	
1	Inflammatory And Hyperplastic	15	10	1	2	2	0	66.66%
2	Dysplasia	3	2	0	1	0	0	0%
3	Malignant	32	0	0	13	19	0	100%
		50	12	1.	16	21	0	

Table 3: Correlation Of Cytological Versus Histopathological Diagnosis

DISCUSSION

Incidence of oral cancers and oral pre-malignant lesions is very high in India as compared with western population. Though scalpel biopsy followed by histopathology is considered as gold standard in diagnosing these lesions, it may not be feasible to do scalpel biopsy in all suspected cases (the patient maybe medically compromised or may refuse to undergo scalpel biopsy). In such cases, brush cytology may offer an attractive alternative.

Brush cytology is an advantageous diagnostic procedure because it is non-invasive, relatively painless and inexpensive, and requires a minimum of technical skills. It is thus well accepted by patients and suitable for routine application in population screening programmes, for early analysis of suspect lesions, and for pre and post-treatment monitoring of confirmed malignant lesions. The procedure may be repeated if required without much inconvenience to the patients. Further, the procedure can be undertaken as an outdoor workup even in remote peripheral centres where proper sophisticated and diagnostic facilities are not available.

This study was undertaken to compare the two techniques in terms of sensitivity and specificity, ease of the technique and acceptance of the procedure by the patients. Statistical analysis in our study revealed 32 cases as true positive, 2 as false positive, 6 as false negative and 10 as true negative, resulting in sensitivity and specificity of 84.21% and 83.33%, and positive and negative predictive values were 94.12% and 62.5%, respectively.

The false-negative results and errors in cytopathological interpretation can be attributed to several factors like:

1. Sampling error
2. Improper fixation
3. Cytopreparation: Staining and processing errors.
4. Subjective errors.
5. Lack of clinical information may also lead to improper interpretation of the cytological smear [9].

In our study, it was hypothesized that there was no difference between histopathology and brush cytology and level of significance of 5% was set. On application of Mann Whitney U test in the present study, U value was 1135.500.On further evaluation Z and P value were -0.978 and 0.328 respectively. Application of Mann-Whitney *U* test showed *P* more than 0.05

suggesting there was no significant difference between histopathology and brush cytology in assessing both premalignant and malignant lesions.

The results were compared with the study performed by Babshet *et* al [7] on 60 cases which included both premalignant and malignant lesions. 37 cases were found to be positive and seven to be negative on both histology and brush cytology. Eleven cases were found to be false negative on brush cytology, but no false positive case was found giving sensitivity and specificity as 77% and 100%, with positive and negative predictive values as 100% and 38%, respectively. However, application of Mann Whitney U test showed p value more than 0.05 suggesting there is no significant difference between histopathology and brush cytology in assessing both premalignant and malignant lesions which is similar to our study.

A similar study carried out by Driemel *et al*.[10] evaluated the performance of oral brush biopsies using standard morphological analysis and HE staining for detecting oral squamous-cell carcinomas and their respective precursor lesions, found comparable sensitivity and specificity of 79% and 93%, respectively, and positive predictive value of 88% but with a higher negative predictive value of 88%.

Scheifele *et al* [11] suggested that the main reason for the use of oral brush cytology is not to find a substitute for scalpel biopsy, but rather to take advantage of a first-level test that is able to identify dysplastic cells.

Looking to all the above mentioned advantages of oral brush cytology in the diagnosis of oral lesions, it is recommended that all oral lesions should be properly investigated by simple cytology procedures rather directly going for biopsy examination. Cytologically diagnosed cases of malignancy need not undergo histopathological confirmation and adequate complete excision with a wide safer margin of normal tissue can be done at the first place.

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