

Efficacy of Oral Exfoliative Cytology in Evaluation of Oral Leukoplakia and Oral Squamous Cell Carcinoma

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Abstract

Background: Oral Squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity. Many OSCCs arise within regions that previously had premalignant lesion. Early diagnosis and prompt treatment of premalignant lesions offers the best hope of improving the prognosis in patients with OSCC. Oral exfoliative cytology is a simple, non invasive technique which can be use for early detection of oral premalignant and malignant lesions.

Aims & Objectives: The present study was carried out to find the sensitivity and specificity of oral exfoliative

cytology in oral leukoplakia and OSCC. We have also included the cytological features noticed in leukoplakia and OSCC.

Material & Methods: The study group consisted of 60 patients in total, out of which 30 patients were clinically suspicious cases of Oral Leukoplakia and 30 patients were of OSCC. Each patients underwent conventional exfoliative cytology using a wooden spatula followed by biopsy for all these cases for histopathological confirmation and comparison of results. Sensitivity, specificity, positive and negative predictive values were calculated.

Results: Sensitivity and specificity were 77% and 100%, respectively, and positive and negative predictive values were 100% and 24%, respectively.

Conclusion: The present study reveals the efficiency of oral exfoliative cytology for the diagnosis of oral leukoplakia and OSCC that can be used as an adjuvant with histopathological examination.

Key words: Oral exfoliative cytology, Oral leukoplakia Oral squamous cell carcinoma.

Introduction

Cancer is Latinized from the Greek word “Karkinos,” meaning crab, denoting how carcinoma extends its claws like a crab into the adjacent tissues. Cancer being a genetic disorder involves multiple alterations of the genome progressively accumulated during a protracted period. Its overall effect of which surpasses the inherent reparative ability of the cell. During its progression, visible physical changes take place at the cellular level (atypia) and at the resultant tissue level (dysplasia). The sum total of these physical and morphological alterations are of diagnostic and prognostic relevance and is designated as “precancerous” changes. These changes are ultimately involved in driving cells further along the path to neoplastic transformation.[1]

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy worldwide and accounting for 90% of malignancy of oral cavity.[2] In addition to increasing incidence, it also accounts for 50–70% of total cancer mortality which is very high when compared with other cancer related mortality.[3]

The development of oral cancer is a multistep process arising from preexisting potentially malignant lesions, leukoplakia being the most common precancer representing 85% of such lesions.[4] As a clinical entity, leukoplakia may have varied histological presentations,

ranging from mildly hyperkeratotic lesions to the lesions that exhibit severe dysplastic features.[1]

A key factor in the lack of improvement in prognosis over the years is the fact that a significant proportion of OSCC are not diagnosed or treated until they reach an advanced stage. The prognosis for patients with OSCC that is treated early is much better, with 5-year survival rates as high as 80%. In addition, the quality of life improves after early treatment, because cure can be achieved with less complex and less aggressive treatment than is necessary for advanced lesions. [5]

Early detection of oral cancer is one of the most efficient ways to reduce the high mortality from this disease because of the easy accessibility of the oral cavity. It can minimize morbidity by instituting timely treatment, associated with a severe loss of function, disfigurement, depression and poor quality of life.[6]

Oral exfoliative cytology is the microscopic examination of shed cells from an epithelial surface. It is a non invasive, simple sensitive staining technique which can be used as an adjuvant for biopsy or where the gold standard biopsy is not feasible or in mass screening.[7] It is implemented as a prediagnostic tool in potentially malignant disorders like leukoplakia and oral cancers chiefly oral squamous cell carcinoma.[8]

Even though oral exfoliative cytology has its own advantages it has not replaced biopsy due to its false positive and false negative results. [9]

The Papanicolaou (PAP) stain is regarded as the universal stain for cytological preparations. This special stain imparts a different color to the cytoplasm of epithelial cells based on their degree of cellular differentiation.

The principle of PAP staining lies in the fact that the dehydration and better clearing solutions help in causing cellular transparency. This detects the overlapped cells and their individual morphology, which otherwise would

be confused for bi or multinucleated cells. It also shows a stability of stain over long periods, stability of color and of course, the better reproducibility of results.[1]

The present study was carried out to find the sensitivity and specificity of oral exfoliative cytology in oral leukoplakia and OSCC. We have also included the cytological features noticed in leukoplakia and OSCC and also compared the cytologic and histopathological findings.

Materials and Methods

The study group consisted of 60 patients in the Department of Oral Pathology and microbiology, BIDSHPatna, Bihar. Out of 60 patients, 30 cases were of Oral leukoplakia (OL), 30 cases of OSCC.

Oral Exfoliative cytology procedure

With the consent of the patients, scrapings were made with wooden spatula moistened with normal saline after rinsing the oral cavity thoroughly with water and were smeared on middle third of two clean, dried plain glass slides. The smears were fixed immediately in 95% ethanol, followed by Papanicolaou stain.

All cytological smears were interpreted based on papanicolaou's classification of cytological smear.

Classification Of Papanicolaou: [7,10]

CLASS I : (Normal) indicate that only normal cells were observed.

CLASS II: (Atypical) indicate minor atypia but no evidence of malignant changes.

CLASS III: (Intermediate) the cells display wider atypia that may be suggestive of cancer, but they are not clear cut.

CLASS IV: (Suggestive of cancer) few epithelial cell with malignant character or many cells with border line characteristic.

CLASS V: (Positive cancer) cells that are obviously malignant

In oral dysplasia and OSCC, the exfoliative cytology would differ markedly from normal cytology in the following aspects:

- Hyperchromasia of nuclei
- Increased nuclear to cytoplasmic ratio
- Anisonucleosis and nuclear pleomorphism
- Irregularities of nuclear membrane
- Nuclear crowding
- Nuclear moulding, clumping and irregular distribution of chromatin
- Dyskeratosis
- Tadpole and strap cell [11]

There is no single, measurable or constant criterion that one can depend on to separate unhesitatingly one category from other. However, the following cellular feature variations are helpful in determining the stage of atypia with relative precision.[12]

Feature variations which decrease with increased severity of dysplasia include

- Cellular cohesion
- Amount of cytoplasm
- Degree of maturation

Feature variations which increase with increased severity of dysplasia include

- Mitosis
- Nuclear to cytoplasmic ratio
- Anisochromatism
- Nuclear membrane irregularities

Other feature variations with increased severity of dysplasia include

- Nuclear hypertrophy
- Anisokaryosis
- Hyperchromatism
- Nucleoli[12]

Biopsy

The incisional biopsies were also taken and fixed in 10% formalin and routinely processed and stained with hematoxylin-eosin stain for all the cases for histopathological confirmation and comparison of results.

The grading of dysplasia and squamous cell carcinoma was done based on the WHO criteria. On histopathological examination, the dysplasia was graded as mild, moderate, severe and carcinoma in situ, while squamous cell carcinoma was graded as well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma.

Results

Cytological finding

Out of 30 OL cases 10 cases were classified as Class I, 13 cases as Class II, 7 cases as Class III. Out of 30 OSCC, 7 cases were classified as Class II, 5 cases as Class III, 12 cases as Class IV, and 6 cases as Class V [Table 1] [Fig:1,2,3,4,5,6]

Histopathological findings

Out of 30 cases of OL 6 showed hyperkeratosis, 10 cases showed mild dysplasia, 6 cases showed moderate dysplasia, 4 cases showed severe dysplasia whereas 4 cases was carcinoma in situ. Out of 30 cases of OSCC 15 cases were diagnosed as WDSCC, 11 were MDSCC, and 4 were PDSCC. [Table 1]

Sensitivity & Specificity of Oral Exfoliative Cytology

Out of 60 cases including both OL and OSCC 43 cases were found to be positive both cytologically and histopathologically (**True positive**). 4 cases were negative on both histology and cytology (**True negative**). 13 cases were found to be positive on histology and negative on cytology (**False negative**), and not a single case was histologically negative and cytology positive (**False positive**) was found.

Sensitivity was 77% and Specificity was 100% Positive predictive value was 100%

Negative predictive value was 24% [Table 2]

Table 1: Comparison of cytological and histopathological findings in Leukoplakia and Squamous cell carcinoma.

Grade of Severity Dysplasia	Histopathology Diagnosis	Cytological Diagnosis				
		Class I	Class II	Class III	Class IV	Class V
Hyperkeratosis	6	4	2			
Mild Dysplasia	10	6	4			
Moderate Dysplasia	6		4	2		
Severe Dysplasia	4		1	3		
Ca- In Situ	4		2	2		
Oral Squamous Cell Carcinoma						
Well Differentiated	15		5	2	6	2
Moderately Differentiated	11		2	3	4	2
Poorly Differentiated	4				2	2

Table 2: Evaluation of cytopathology versus histopathology

True positive	43
False positive	0
True negative	7
False negative	13
Sensitivity	77%
Specificity	100%
Positive predictive value	100%
Negative predictive value	24%

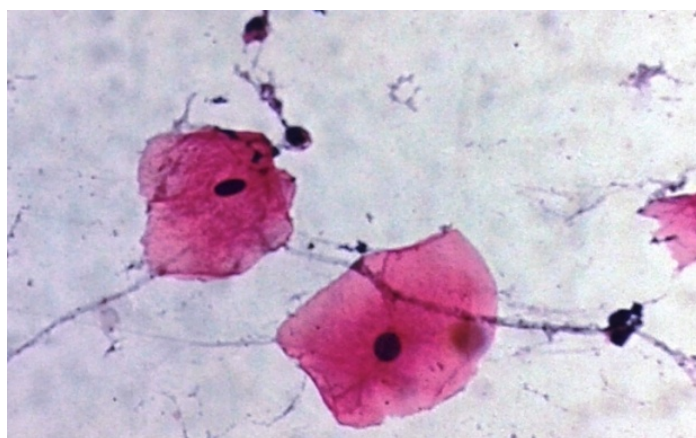


Figure: 1 Fig showing Papanicolaou stained smear of normal oral mucosa (PAP, x1000)

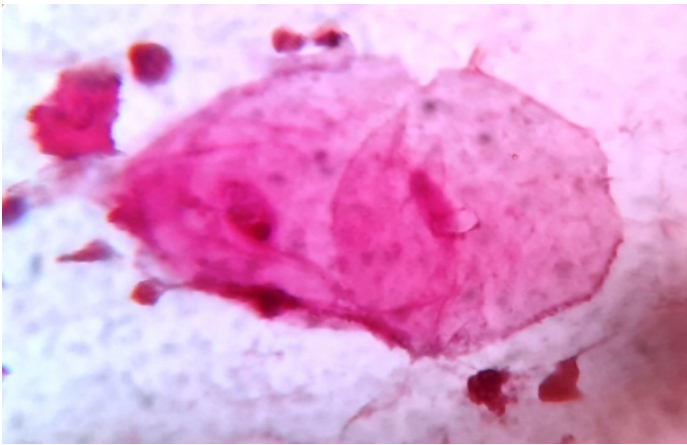


Figure 2: Fig showing atypical cells showing pleomorphic nucleus and eosinophilic cytoplasm and numerous keratohyaline granules. (PAP, x1000)

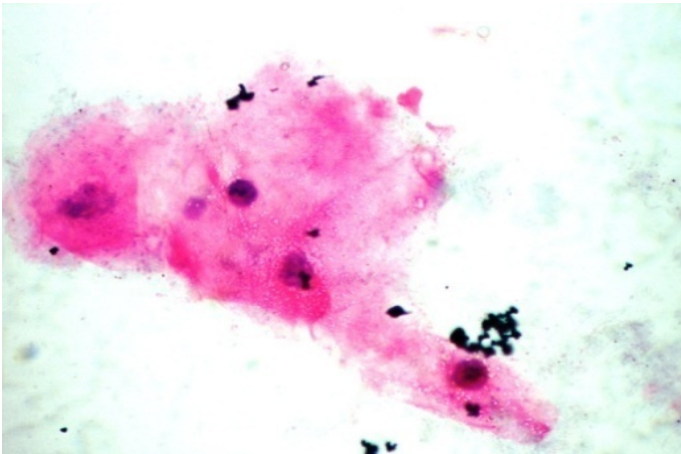


Figure: 3 Figure showing cells having pleomorphic and mild hyperchromatic nucleus (PAP, x1000)

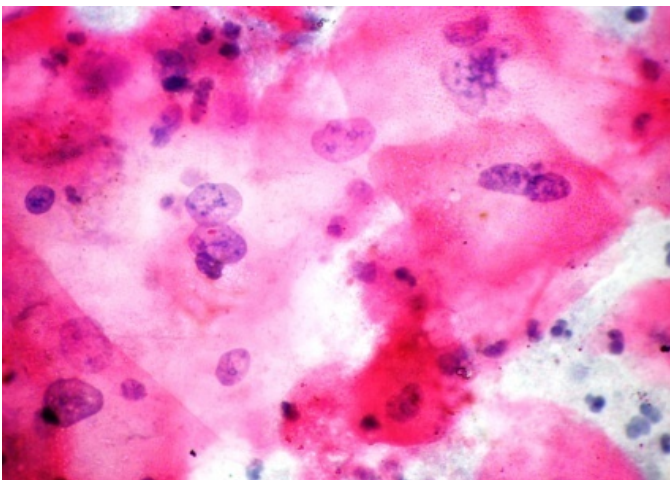


Figure 4 : Fig showing dysplastic cell showing nucleomegaly with prominent nucleoli having uneven chromatin distribution (PAP, x1000)

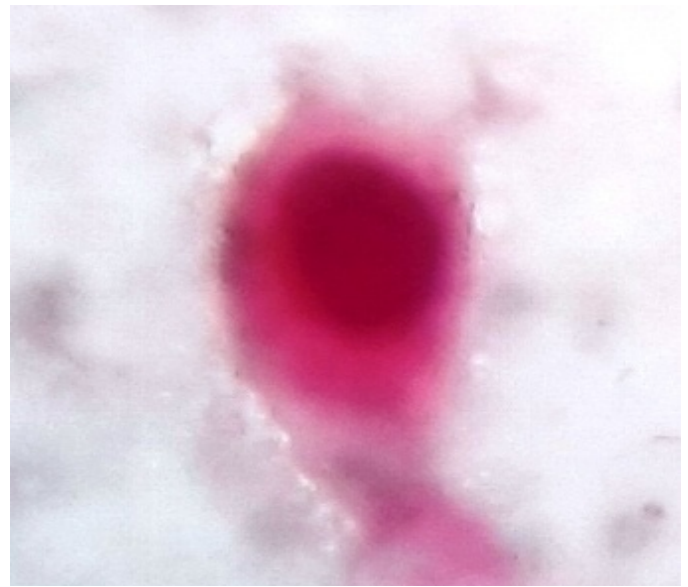


Figure :5 Fig showing neoplastic cells with high N/C ratio, mild chromatism. (PAP, x1000)

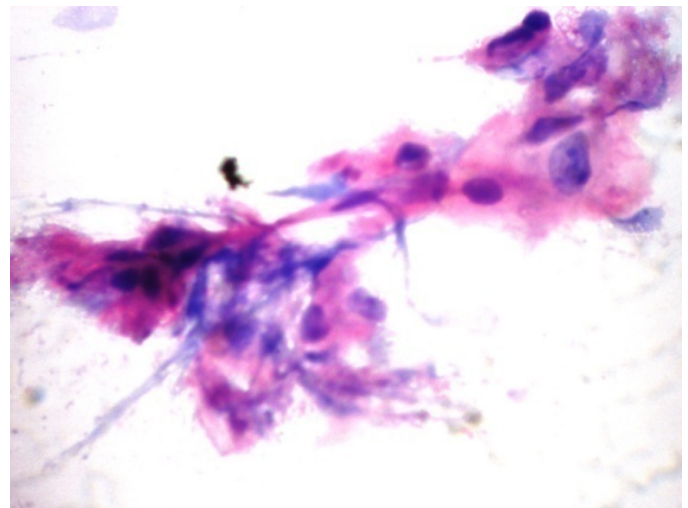


Figure 6: Fig showing dysplastic cells predominantly nuclear changes including an increase in size, slight irregular margins/smooth membranes; irregularly clumped coarse nuclear chromatin, hyperchromasia with indistinct nuclear membranes in few. (PAP, x1000)

Discussion

As per the normal physiology, the oral epithelium renews itself rapidly (probably every 2 weeks). The rationale of oral exfoliative cytology is based on this physiological process, examining cells that are desquamated or abraded from the surface of the oral mucosa.[1,13] Under normal conditions the cells of the epithelium strongly adhere to

each other. When the epithelium becomes the seat of malignant disease or of some benign conditions the cells may lose their cohesiveness so that the deeper cells may be exfoliated along with the superficial cells. Loss of cohesion, which favors exfoliation, permits the collection of cast off cells for microscopical examination.[14] The superficial epithelial cells do contain nuclei and alterations in these cells can serve as reliable indicators of dysplastic or neoplastic changes. [1,13]

The basic defect of the alteration of any cell begins at the molecular level triggering a series of reactions and thereby affecting the entire cell system and consequently its morphology. The general biological activity is reflected best in nucleus and functional activity is reflected in cytoplasm. [1,5]

Malignant cells show a significant increase in the nuclear area and diameter due to the increase in nuclear content required for replication and in such cells with increase activity the ability of the cell to form cytoplasm decreases. The amount of cytoplasm a malignant cell makes is decreased as compared to the amount of nucleoplasm in malignant cells, and therefore their nuclear dimensions increase and the cellular dimensions decrease.[2] Accordingly we found that there was a decrease in the cellular size and an increase in the nuclear size.

In our study, the sensitivity and specificity of exfoliative cytology in detecting dysplasia and oral squamous cell carcinoma were 77% and 100%, respectively, and positive and negative predictive values were 100% and 24%, respectively that was comparable with the study of Satish et al [7] where they found sensitivity 65% and specificity 100% regarding leukoplakia and sensitivity 75% and specificity 100% in squamous cell carcinoma. Vezhavendhan et al.[9] correlated cytologic and histopathologic in cancer diagnosis where they found cytological examination of oral cancer has 88% sensitivity

and 87% specificity. Also other study conducted by Babshet et al.[12] that was comparable with our study where they detected efficacy of oral brush cytology in the evaluation of the oral premalignant and malignant lesions and found 77% sensitivity and 100% specificity respectively, and positive and negative predictive values 100% and 38% respectively.

Despite the advantages of oral exfoliative cytology, it has certain disadvantages like inadequate sampling and false-negative results. The possible reason for cytological interpretation could be that the smears would have been taken from hyperkerotic areas or highly inflamed necrotic areas.[9] Ramesh et al also suggested that smears of the hyperkeratotic lesion would give only anucleated squames and this hyperkerotic superficial cell will mask the underlying dysplastic cells.[16]

Babshet et al.[12] discussed false-negative results and errors or pitfalls in cytopathological interpretation can be attributed to several factors like:

1. Sampling error: Smear obtained from a non-representative site may not show abnormal diagnostic cells. Therefore, a direct sampling of the site of the lesion should be performed which generally produces the maximum number of diagnostic cells.
2. Improper fixation: Air drying the smear or using a wrong fixative may produce artifacts and alterations in the cellular morphology.
3. Cytopreparation: Staining and processing errors.
4. Subjective errors by the inexperienced or careless cytopathologists. It is essential to screen the slide completely and mark the more characteristic cells. An effort should be made to identify every structure found in the smear and all cells should be analyzed.
5. Lack of clinical information may also lead to improper interpretation of the cytological smear.[12]

Conclusion

Cytological evaluation of oral cells is a non-invasive technique that is well accepted by the patient, and is therefore an attractive option for the early diagnosis of potentially malignant and malignant lesions of oral mucosa. . Early detection of these lesion promises to improve the survival and morbidity of patients suffering from these conditions. Oral exfoliative cytology is highly specific, however, relatively less sensitive. It has a high positive predictive value and a low negative predictive value. Inadequate cellularity and overlapping of cells are common reasons for unsatisfactory smears in conventional exfoliative cytology. Therefore improved accuracy is obtained by combining conventional oral exfoliative cytology with image analysis has been used to increase the sensitivity of the procedure, since these techniques are precise, objective & reproducible.

References

1. Verma R, Singh A, Badni M, Chandra A, Gupta S, Verma R. Evaluation of exfoliative cytology in the diagnosis of oral premalignant and malignant lesions: A cytomorphometric analysis. *Dent Res J* 2015;12:83-8.
2. Joshi PS, Kaijkar MS. Cytomorphometric Analysis of Oral Premalignant and Malignant Lesions Using Feulgen Stain and Exfoliative Brush Cytology. *J Interdiscipl Histopathol* 2013; 1(4): 204-211
3. Bhandari AP, Gadkari RU. Evaluation of role of cytodagnostic techniques in detection of oral premalignant and malignant lesions: Study of 50 cases. *Int J Oral Health Sci* 2015;5:21-9.
4. Khanna S, Purwar A, Singh NN, Sreedhar G, Singh S, Bhalla S. Cytogenetic biomonitoring of premalignant and malignant oral lesions by micronuclei assessment: A screening evaluation. *Eur J Gen Dent* 2014;3:46-52.
5. Mehrotra R, Gupta A, Singh M, Ibrahim R. Application of cytology and molecular biology in diagnosing premalignant or malignant oral lesions. *Molecular Cancer* 2006, 5:11
6. Jairajpuri ZS, Rana S, Hajela A, Jetley S. Toward early diagnosis of oral cancer: Diagnostic utility of cytomorphological features, a pilot study. *Natl J Maxillofac Surg* 2019;10:20-6.
7. Kumar S, Vezhavendhan N, Priya S. Role of Oral Exfoliative Cytology in Oral Leukoplakia and Squamous Cell Carcinoma. *IJCDS • FEBRUARY*, 2011. 2(1)
8. KUMARESAN GD, JAGANNATHAN N. EXFOLIATIVE CYTOLOGY – A PREDICTIVE DIAGNOSTIC TOOL. *Int J Pharm Pharm Sci*, Vol 6, Issue 5, 1-3
9. Vezhavendhan N, Shanthi V, Sathish KG, Balaji N, Sumathi MK. Cytologic and Histopathologic Correlation in Cancer Diagnosis. *Journal of Scientific Dentistry*, 1(2), 2011
10. Papanicolaou, G N and Traut H F : *Diagnosis of uterine cancer by the vaginal smear*. The commonwealth Fund, New York, 1943
11. Grey W, Kocjan G. *Diagnostic cytopathology*. 3rd Edition. Churchill Livingstone : Elsevier; 2010.
12. Babshet M, Nandimath K, Pervatikar SK, Naikmasur VG. Efficacy of oral brush cytology in the evaluation of the oral premalignant and malignant lesions. *J Cytol* 2011;28:165-72.
13. Khandelwal S, Solomon MC. Cytomorphological analysis of keratinocytes in oral smears from tobacco users and oral squamous cell carcinoma lesions — A histochemical approach. *Int J Oral Sci* 2010;2:45-52.
14. Nayar AK, Sivapathasundharam B. Cytomorphometric analysis of exfoliated cytology

normal buccal mucosa cells. IJDR, Vol.14, No.2, Apr-June, 2003.

15. Sivapathasundharam B, Kalasagar M. Yet another article on exfoliative cytology. J Oral Maxillofac Pathol 2004;8:54-7.
16. Ramesh T, Mendis BR, Ratnatunga N, Thattil RO. Cytomorphometric analysis of squames obtained from normal oral mucosa and lesions of oral leukoplakia and squamous cell carcinoma. J Oral Pathol Med 1998;27(2):83-6