Original Article

Toward early diagnosis of oral cancer: Diagnostic utility of cytomorphological features, a pilot study

ABSTRACT

Introduction: Early detection of oral cancer is one of the most efficient ways to reduce the high mortality from this disease because of the ready accessibility of the oral cavity. We need to devise urgent diagnostic tools to detect early oral premalignant and malignant lesions.

Aim: The aim of the present study was to grade the oral lesions in an attempt toward developing a novel cytological grading system. Further, morphometric analysis of cellular parameters was also performed to compare their significance in differentiating benign from malignant lesions.

Materials and Methods: The present study was conducted at a tertiary care hospital catering to the low socioeconomic population. Patients presenting in the various Out Patient Departments with suspicious oral lesions were evaluated by cytology in the Department of Pathology.

Results: A total of 72 patients were evaluated with a mean age of 43.54 ± 10.35 years. The involvement of the buccal mucosa was the most common site of oral lesions. Cytologically, the lesions were graded according to the oral/oro-pharyngeal cytology grading system into grades A to F. Cyto-morphometric analysis showed an increasing trend in mean nuclear diameter from benign to malignant cases while the mean cytoplasmic diameter decreased, value of $P < 0.05$ was observed indicating a statistically significant difference between the two groups.

Conclusions: Cytological features of pleomorphism are a unique feature in oral carcinoma reflecting intracellular alterations in cells. Grading of lesions according to cytological characteristics can be helpful in standardizing the reporting of the oral lesion. However, our study was restricted by limited data; we emphasize more extensive studies to assess the usefulness and applicability of such a grading system. We also conclude that the use of cytomorphometry can improve the diagnostic reliability of exfoliative cytology.

Keywords: Cytology, early detection, morphometry, oral cancer

INTRODUCTION

Oral cancer is one of the 10 most common cancers as stated by the World Health Organization localized predominantly to the tongue however may also occur on the floor of the mouth, gingiva, lip, and palate.[1] In India, oral cancer is a major health problem, accounting for 30%–40% of all cancers diagnosed.[2]

Early detection of oral cancer is one of the most efficient ways to reduce the high mortality from this disease because of the ready 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.[3] Currently, prognostic evaluation and treatment planning for oral squamous cell carcinoma (OSCC) is based on clinical staging and histological grading which in turn are based on subjective evaluation of parameters and are therefore often not sufficiently reproducible.[4]

An urgent need to devise critical diagnostic tools for the early detection of oral potentially malignant and malignant lesions that are practical, noninvasive and easily performed is

Zeeba Shamim Jairajpuri, Safia Rana, Apoorva Hajela[1], Sujata Jetley

Departments of Pathology, Hamdard Institute of Medical Sciences and Research, [1]Hamdard Institute of Medical Sciences and Research, New Delhi, India

Address for correspondence: Dr. Sujata Jetley, Department of Pathology, Hamdard Institute of Medical Sciences and Research, Jamia Hamdard, New Delhi - 110 062, India.
E-mail: sujatajetley@gmail.com.com

Received: 11-03-2017, **Revised:** 27-12-2018, **Accepted:** 08-01-2019

Access this article online	
Website: www.njms.in	Quick Response Code
DOI: 10.4103/njms.NJMS_12_17	

How to cite this article: 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.

felt.[3] The Bethesda method of classification represents one of the great success stories in cervical cytology however little interest exists for the adoption of a unique grading system in oral cytology.[5] This general lack of interest in oral cytology can probably be due to a high percentage of false-negative diagnoses[6,7] attributed to variation in technical quality, cellularity of oral smears and inadequate sampling methods.[5]

Finding carcinomas of innocuous appearance, at curable and early stage has been aided by computer-assisted analysis of exfoliative cytology material that adds to the clinical examination in the differentiation of premalignant and malignant alterations of oral lesions. Cytomorphometric evaluation of oral exfoliative cytology can serve as a useful diagnostic screening for the early detection of oral cancer,[8] which promises to improve the survival and morbidity of these patients. Cytological study of oral cells is a nonaggressive technique well accepted by the patient, therefore an attractive option for the early diagnosis of oral lesions, including epithelial atypia and squamous cell carcinoma.[9]

The aim of the present study was to grade the oral lesions in an attempt toward developing a novel cytological grading system. Further, morphometric analysis of cellular parameters was also performed to compare their significance in differentiating benign from malignant lesions.

MATERIALS AND METHODS

The present study was a cross-sectional study conducted at a tertiary care hospital. Our hospital mainly caters to a predominant population of low socioeconomic status residing in the nearby localities. A total of 72 patients presenting in the various Out Patient Departments with suspicious oral lesions including leukoplakia patches, ulcer/growth were evaluated. Informed consent in the language they understood, was obtained from all patients. Patient details and other relevant history was taken. Part of the data was submitted under the Student Term Studentship program of ICMR.

The samples were collected by a wooden spatula or cytology brush wherever available. The harvested material was transferred on to a glass slide taking care that minimum damage to cells is incurred. Fine-needle aspiration (FNA) cytology was done for palpable lesions (deep mucosal lesions) not amenable to oral exfoliative cytology. The smears were manually stained by hematoxylin and eosin and papanicolaou stain in the cytology laboratory of the Department of Pathology. The cytopathological evaluation was done by pathologists at the hospital; the lesions were characterized and graded according to Table 1 wherever possible.[10]

Table 1: Oral/oropharyngeal cytology grading system

Grading system
Specimen adequacy
Adequate for evaluation (note the presence of basal/parabasal cells)
Inadequate for evaluation (specify reasons, for example, obscuring elements, unlabeled or broken slides)
General categorization
Normal
Reactive
Atypical - probably reactive/low grade including LSIL
Atypical-probably high grade
HSIL
Invasive squamous cell carcinoma
Other neoplasms: Specify

LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion

Further, the H and E stained smears were subjected to morphometric analysis after taking images, by computer-based image analytic (Motic image analyzing) system with a ×40 objective lens. Cytoplasmic diameter (CD), nuclear diameter (ND) cytoplasmic area (CA), and nuclear area (NA) were assessed. Nuclear-cytoplasmic ratio (N: C) was calculated. Patients belonging to all ethnicities, cultural groups were included. Those with oral lesions with/without history of tobacco use and those agreeing to give consent were part the study. Patients who did not give consent were excluded from the study. Relevant institutional ethical clearance was taken. Relevant statistical analysis was performed on the acquired data.

RESULTS

The present study was conducted in the Department of Pathology, a total of 72 patients were evaluated of which, 56 (77.8%) were male and 16 (22.2%) were female. Age-wise distribution of the patients showed a maximum number of 28 (38.9%) cases, in the age group of 41–50 years with a mean age of 43.54 ± 10.35 years, followed by the age group of 31–40 years with 22 (30.6%) patients [Figure 1].

A detailed history and clinical examination of the oral cavity were carried out in each case. Site of the lesion was identified and relevant history regarding tobacco abuse was elicited. We found that the buccal mucosa was the most common site of involvement in oral lesions (22,30.5%) followed by the gingiva (20,27.8%), and then tongue (16,22.2%). Most of the patients in this study were adults with a history of tobacco usage in its various forms. Consumption of either smoking or smokeless tobacco was seen along with 9.8% of patients showing consumption of both. However, we also noted that 6.9% of patients did not give a history of any type of tobacco abuse [Table 2].

Out of the 72 cases, 83.3% (60 samples) met the adequacy criteria [as mentioned in Table 1] whereas 16.7% (12 samples) were inadequate predominantly due to obscuring elements such as necrosis and blood. Cytological evaluation of the smears was done and the lesions were graded according to the oral/oro-pharyngeal cytology grading system[10] [Table 1]. The grading of the lesions was done on the basis of cytological features as stated below:[5,10]

The lesions were graded from A to F on cytological evaluation, majority of the cases were in the category D (23.8%), i.e., atypical probably high grade [Table 3].

The smears were subjected to cytomorphometric analysis and the findings are depicted in Table 4. An increasing trend was noticed in mean ND from benign to atypical and malignant cases whereas the mean CD decreased from benign to malignant cases. The mean N: C ratio also showed an increase from Grade A through to Grade F, the *P* value (using Chi-square test) for the mean N: C ratio between benign (Grades A and B) and atypical changes (Grades C, D, E, F) was <0.05, indicating a significant difference between the two groups.

Biopsy for histopathological evaluation was available in 35 out of 60 adequate samples and concordant correlation to cytological findings was observed in these cases

Table 2: Distribution of cases according to site of lesion and tobacco abuse

Location	Number of cases (%)	Smoking	Smokeless	Both	None
Buccal mucosa	22 (30.5)	12	5	2	3
Gingival sulcus	20 (27.8)	7	13	-	-
Tongue	16 (22.2)	7	6	2	1
Palate	8 (11.1)	5	1	2	-
Angle of mouth	2 (2.8)	1	-	1	-
Lip	2 (2.8)	1	1	-	-
Floor of mouth	2 (2.8)	1	-	-	1
Total	72	34	26	7	5

Table 3: Distribution of cases according to grade of lesion

Nature of lesion	Grade	Number of cases (%)
Normal	A	7 (11.6)
Reactive	B	10 (16.7)
Atypical - low grade	C	8 (13.3)
Atypical - high grade	D	16 (26.7)
HSIL	E	9 (15.0)
Invasive SCC	F	10 (16.7)

SCC: Squamous cell carcinoma, HSIL: High grade squamous intraepithelial lesion

DISCUSSION

In India, oral cancer is a major health problem, in high-risk countries such as Sri Lanka, India, Pakistan, and Bangladesh, oral cancer is the most common cancer in men, and may contribute up to 25% of all new cases of cancer.[2,11]

Early detection of oral cancer is one of the most efficient ways to reduce the high mortality from this disease. Oral cancer may disguise itself and appear as a benign lesion in its early stages, patients usually remain unaware and report to a physician at a late stage, at which point often invasive treatment plan needs to be undertaken.[12] Hence, an urgent need to devise critical diagnostic tools for the early detection of oral malignant as well as potentially malignant lesions that are practical, noninvasive and can be easily performed is felt.[3]

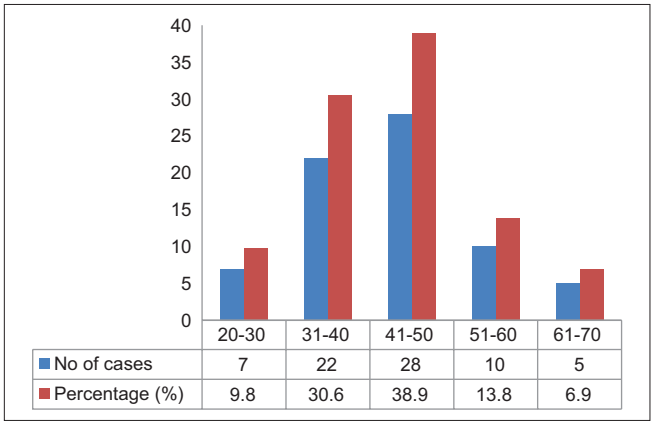

Figure 1: Distribution of cases according to age

Table 4: Cyto-morphometric analysis of squamous cells in various grades of lesions of the oral cavity

Grade	Mean±SD						
	ND (μm)	NA (μm²)	NP (μm)	CD (μm)	CA (μm²)	CP (μm)	N:C ratio
A	15.77±2.29	198.45±58.40	40.82±7.84	61.55±12.91	3074.84±1216.38	174.6±35.46	0.075
B	13.44±2.79	141.31±66.15	38.07±10.21	48.81±10.86	1951.66±793.69	158.92±40.67	0.086
C	14.95±0.42	176.16±9.40	37.21±2.87	52.23±6.67	2168.84±529.49	143.65±47.93	0.08
D	18.53±4.01	281.07±130.37	52.67±8.72	31.42±8.12	821.36±441.77	88.58±16.69	0.342
E	22.56±9.17	321.08±95.89	58.7±1.95	36.7±17.84	1258.50±1336.57	105.86±34.20	0.422
F	22.63±4.51	416.72±170.29	58.24±11.73	29.36±4.41	691.03±197.63	88.89±12.09	0.654

ND: Nuclear diameter, NA: Nuclear area, NP: Nuclear perimeter, CD: Cytoplasmic diameter, CA: Cytoplasmic area, CP: Cytoplasmic perimeter, SD: Standard deviation, N: C: Nuclear to cytoplasmic ratio

A total of 72 cases were included in the study with mean age of 43.5 ± 10.35 years, this was in concordance with other authors;[13] however, the mean age observed was <50.9 ± 8.70 years reported in the literature.[14,15] Age is an important factor during carcinogenesis in general and triggers in the field of cytological pleomorphism in oral carcinoma. The recorded age of the patients in the study ranged from 20 to 68 years. Oral cancer is the most common cancer in men in high-risk countries.[11] A strong male predominance in majority of the studies has been well documented in literature, they vary from 60%,[16] 77%,[13] 74%[17] to 77.8% in the present study.

All forms of tobacco are known to cause oral cancer, evidenced by increased risks associated with greater amounts or longer duration of usage and the consistency of the findings for oral cancer across cultures.[18] Smoking has been shown to be related to many pathologies, ranging from harmless and reversible lesions, to oral cancer.[14,19] In the present study, the pattern of consumption of tobacco of either smoking (47.2%) or smokeless (36.1%) or both (9.8%) was seen [Table 2]. The preferred form of *tobacco abuse* being bidi, cigarette, and gutka. Site of localization of lesions varies according to the type of tobacco use. Localization to buccal mucosa followed by the tongue and alveolus has been reported by Jamadar *et al*.,[16] a similar trend was observed in the our study also.

Cytological evaluation of oral cells is a nonaggressive technique, well accepted by the patient and an important tool in the assessment of oral lesions. It can be used for the early identification of recurrent pre malignant and malignant lesions, particularly when supplemented by an adequate image analysis method.[20,21] Early detection of these lesion promises to improve the survival and morbidity of patients suffering from these conditions. Miller *et al*. were the first to study the cytology of the oral epithelium and concluded that epithelial alterations in oral mucosal cells serve as reliable indicators for dysplastic or neoplastic changes.[22] Many investigators have used or continue to use a three-tiered oral cytologic grading system on adequate samples.[23,24] Feldman *et al*. have documented FNA results in one of four categories: unsatisfactory, negative, suspicious, or positive for malignancy.[23] In a prospective multicenter study to determine sensitivity and specificity of oral brush biopsy for the detection of precancerous and cancerous lesions of the oral mucosa, Sciubba reported results as positive, atypical or negative.[25] Oral cytopathology has broad potential to fill the diagnostic gap that currently challenges the early detection of oral lesions, including epithelial atypia and squamous cell carcinoma.[26] However, due to a high percentage of false-negative diagnosis[7] attributed to variation in technical quality and cellularity of oral smears as well as the use of inadequate sampling procedures, there has been limited interest in the development of a grading system similar to Bethesda classification.[5] Lack of a standard method for reporting oral cytology adversely affects the proper management of patients oral lesions. Afrogheh *et al*. proposed an oral cytological grading method [Table 1] in an attempt to standardize reporting,[10] we adopted this to categorize our samples [Table 5]. About 83.3% of the samples in our study met the adequacy criteria while 16.7% were inadequate predominantly due to obscuring elements such as necrosis and blood. This was in concordance with 80% adequacy reported by Al Bahrani, 20% inadequate samples were attributed to technical errors.[14] Inadequate cellularity and overlapping of cells are common reasons for unsatisfactory smears in conventional exfoliative cytology, image analysis along with oral exfoliative cytology has been used to increase the specificity and sensitivity of the procedure, for early diagnosis of oral cancers, since these techniques are precise, objective, and reproducible.[27]

The smears obtained by exfoliative cytology can be analyzed not only qualitatively but quantitatively also thus serving as a useful adjunct.[28,29] With advancements in this field of application of these techniques to smears from oral lesions,

Table 5: Cytological features of different grades of the oral lesion

Grade	Cytological features
A [Figure 2]	Normal: Predominant population of intermediate and superficial squamous cells with pyknotic nuclei, abundant eosinophilic cytoplasm
B [Figure 3]	Reactive: Cells with mature cytoplasm with mild nuclear enlargement and a slight elevation of N:C. Nuclear outlines usually smooth and hyperchromasia not evident. Numerous acute inflammatory cells also seen. May be seen in inflammatory/infective, repair or radiation induced oral cytological changes
C [Figure 4]	Atypical probably reactive/low grade (atypical-RL): Cells with predominantly nuclear changes including an increase in size, slight irregular margins/smooth membranes, mild hyperchromasia. Nucleoli are absent. May be seen in reactive cells and low grade squamous intraepithelial lesion
D [Figure 5]	Atypical probably high grade (atypical-H): Comprise of cells with markedly enlarged nucleus, decreased cytoplasm with attendant increase in N:C ratio. Hyperchromatic nuclei and irregular nuclear membranes seen, nucleoli were absent
E [Figure 6]	High-grade squamous intraepithelial lesion: Single as well as streaming sheets/syncytial clusters seen markedly enlarged and hyperchromatic nuclei with scant dense cytoplasm and very high N: C ratio seen. Irregular nuclear contour present
F [Figure 7]	Invasive squamous cell carcinoma: Comprised of cells in clusters, syncytial groups with pronounced nuclear alterations including marked variation in nuclear shape, size, irregularities of nuclear membrane, prominent nucleoli. Chromatin irregular and coarsely clumped. Occasional keratin pearls were seen along with bizarre cells with spindling/tadpole or with long cytoplasmic projections

N:C: Nuclear to cytoplasmic ratio

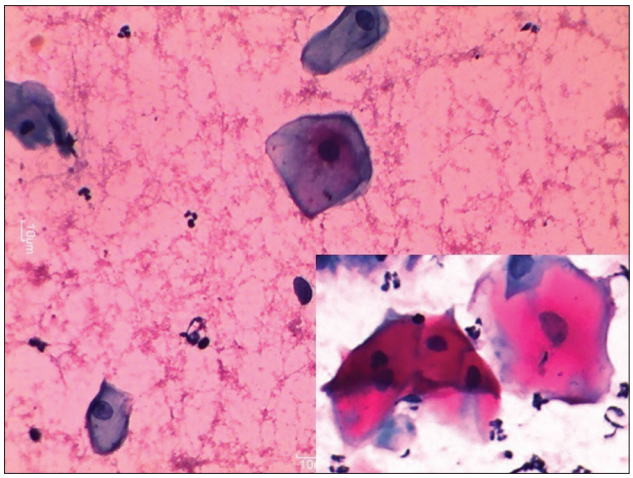

Figure 2: Microphotograph showing normal Grade A: Lesions with a predominant population of intermediate and superficial squamous cells with pyknotic nuclei, abundant eosinophilic cytoplasm (H and E, ×10, inset × 40)

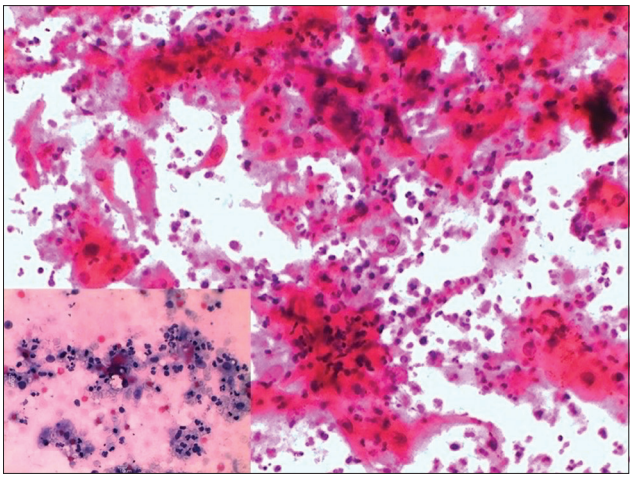

Figure 3: Microphotograph showing reactive Grade B: Lesions, cells with mild nuclear enlargement and a slight elevation of nuclear to cytoplasmic ratio (N:C). Numerous acute inflammatory cells also seen (H and E, ×5, inset: Pap, ×10)

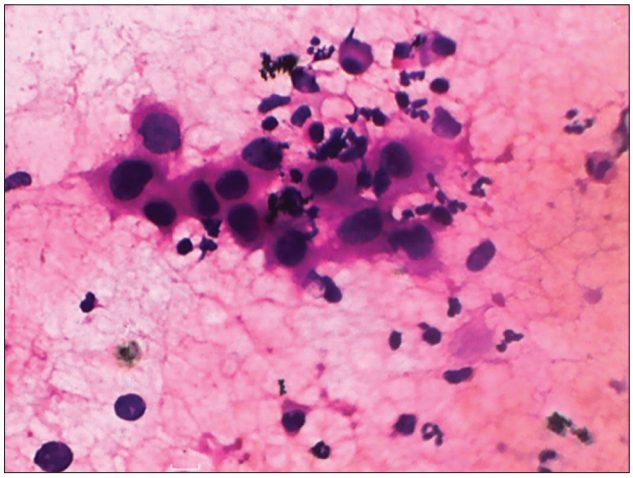

Figure 4: Microphotograph showing atypical probably reactive/low grade (atypical-RL) Grade C: Cells with predominantly nuclear changes including an increase in size, slight irregular margins/smooth membranes, mild hyperchromasia (H and E, ×40)

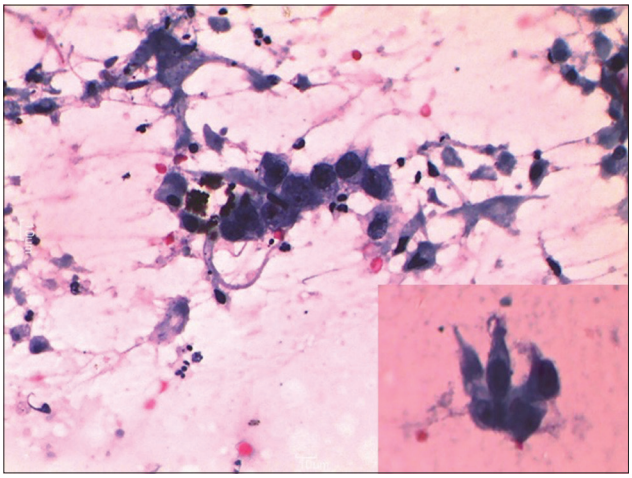

Figure 5: Microphotograph showing atypical probably high grade (atypical-H) Grade D: Cells with markedly enlarged nucleus, decreased cytoplasm with attendant increase in N: C ratio (Pap, ×10, Inset × 40)

the diagnostic value of oral cytology is hoped to improve.[30] Objective measurements of quantitative parameters such as nuclear size, cell size, nuclear-to-cytoplasmic ratio, nuclear shape, nuclear discontinuity can be evaluated collectively to confirm the diagnosis accurately; their usefulness is in their reproducibility. They form an important adjunct in making cytopathological diagnosis.[30] Recently, more reliable quantitative techniques such as cytomorphometry and the use of computer-assisted image analysis, interobserver variation are expected to be reduced.[9,30,31] Ogden *et al*. suggested that quantitative techniques, based on the evaluation of parameters such as NA, cytoplasmic area (CA), and nucleus-to-cytoplasm area ratio (NA/CA), may increase the sensitivity of exfoliative cytology for the early diagnosis of oral cancers, since these techniques are precise, objective, and reproducible.[32]

Quantitative cytomorphometric assessment has shown measurable changes in cells obtained from malignant and premalignant lesions. In a study by Cowpe *et al*. estimation of NA/CA using the planimeter method in smears demonstrated that exfoliative cytology is capable of detecting malignant changes.[33] These parameters, especially NA and N/C ratio, have been shown to provide meaningful results in the diagnosis of oral lesions.[30] The progressive reduction in cellular diameter shows that the reduction in cell size could be an early indication of malignant change.[34] An increase in the ND has been attributed to increased DNA content of the nucleus with resultant increase in nuclear size as compared to the cytoplasm.[33] The present study demonstrated quantitative alterations in the cellular parameters in the various grades as compared to Grade A [Table 3]. A significant quantitative alterations in

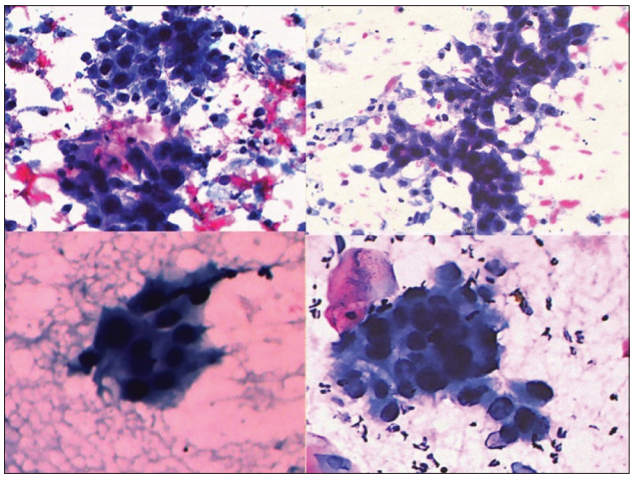

Figure 6: Microphotograph showing high-grade squamous intraepithelial lesion Grade E: Cells with markedly enlarged and hyperchromatic nuclei with scant dense cytoplasm and very high N: C ratio seen (H and E, Pap, ×40)

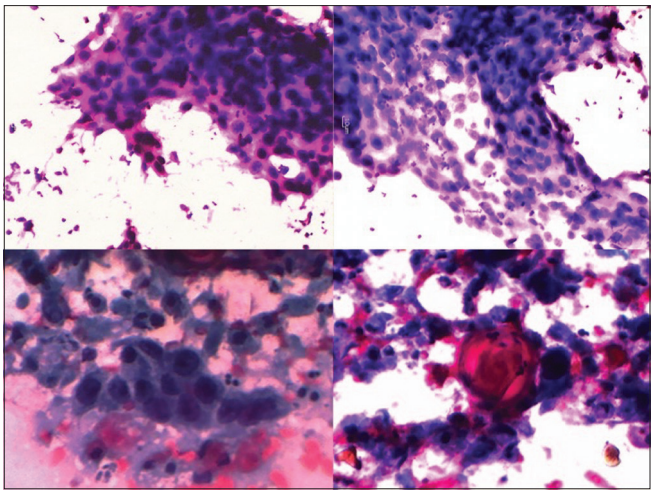

Figure 7: Microphotograph showing invasive squamous cell carcinoma Grade F: Comprised of cells in clusters, syncytial groups with pronounced nuclear alterations including marked variation in nuclear shape, size, irregularities of nuclear membrane, prominent nucleoli (H and E, Pap × 10, ×40)

the form of decreased cellular diameter increased ND and increase in the ratio of nuclear to cellular diameter in the various groups, compared to normal has been reported by Hande and Chaudhary.[13] Authors suggest that malignant transformation generally show a reduction in CA before the reduction in NA, they also suggested that samples of healthy mucosa from the same patient provide the best control.[34] Ramaesh *et al*. reported that CD was highest in normal mucosa, lower in dysplastic lesions, and lowest in SCCs. By contrast, ND was lowest in normal mucosa, higher in dysplastic lesions, and highest in SCCs.[35] Camilleri and Smith reported that one of the consistent findings during progression from benign to a state of malignancy is an increased nuclear to cytoplasmic ratio.[36] Authors report that the N: C ratio has the advantage of relating nuclear to cytoplasmic volume and possibly represents the significant changes that occur in the cell, more accurately at a morphological level.[19] A significant increase in N:C ratio was observed in our study in concordance with other authors who suggest a decreasing cellular diameter from normal mucosa to dysplastic lesions and lowest in OSCC while ND was lowest in normal mucosa, higher in dysplastic lesions, and highest in OSCC.[12,27,35] Reduction in cell area remains an early indication of dysplastic change and that tissues undergoing malignant transformation typically show a reduction in the cell areas.[34] During the transformation of normal tissue to premalignancy or malignancy, cellular changes occur at the molecular level before clinical changes become evident. Recent advances in molecular methodology along with the practical advantages of exfoliative cytology can be useful for early detection of potentially malignant oral lesions.[37] Identification of high-risk oral premalignant lesions and intervention at premalignant stages could constitute one of the keys in reducing the mortality, morbidity, and cost of treatment associated with OSCC.[11] Hence, cytological features with morphometric evaluation are of value in monitoring clinically suspect lesions as well as serve as a useful diagnostic adjunct for early detection of malignancy.

CONCLUSIONS

Exfoliative cytology is a simple, noninvasive technique, which allows collection of intact cells from different layers in the epithelium for microscopical examination and quantitative evaluation. Cytological features of pleomorphism are a unique feature in oral carcinoma reflecting intracellular alterations in cells. Grading of lesions according to cytological characteristics can be helpful in standardizing the reporting of the oral lesion. However, our study was restricted by limited data, we emphasize more extensive studies to assess the usefulness and applicability of such a grading system. We also conclude that use of cytomorphometry can improve the diagnostic reliability of exfoliative cytology; application of quantitative techniques to oral cytology smears is an invaluable parameter of the early recognition of cellular alterations for identification of individuals who require early intervention.

Acknowledgement

The authors would like to thank ICMR for grant of STS project.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Halder A, Chakraborty T, Mandal K, Gure PK, Das S, Roychowdhury R. Comparative study of exfoliated oral mucosal cells micronuclei frequency in normal, precancerous and malignant epithelium. Int J Hum Genet 2004;4:257-60.
2. Mohanta A, Mohanty PK, Parida G. Cytomorphometric analysis of keratinized round cells in human oral carcinoma. J Cytol 2015;32:107-12.
3. Mehrotra R, Gupta DK. Exciting new advances in oral cancer diagnosis: Avenues to early detection. Head Neck Oncol 2011;3:33.
4. Nandini DB, Subramanyam RV. Nuclear features in oral squamous cell carcinoma: A computer-assisted microscopic study. J Oral Maxillofac Pathol 2011;15:177-81.
5. Mehrotra R, editor. Oral Cytology: A Concise Guide. DOI 10.1007/978-1-4614-5221-8-3, New York: Springer Science + Business Media; 2013.
6. Nichols ML, Quinn FB Jr., Schnadig VJ, Zaharopoulos P, Hokanson JA, Des Jardins L, *et al.* Interobserver variability in the interpretation of brush cytologic studies from head and neck lesions. Arch Otolaryngol Head Neck Surg 1991;117:1350-5.
7. Kaugars GE, Silverman S Jr., Ray AK, Page DG, Abbey LM, Burns JC, *et al.* The use of exfoliative cytology for the early diagnosis of oral cancers: Is there a role for it in education and private practice? J Cancer Educ 1998;13:85-9.
8. Mulki S, Shetty P, Pai P. Cytomorphological analysis in oral squamous cell carcinoma lesions and normal controls using rub and rinse technique. Clin Cancer Invest J 2014;3:38-42.
9. Mehrotra R, Gupta A, Singh M, Ibrahim R. Application of cytology and molecular biology in diagnosing premalignant or malignant oral lesions. Mol Cancer 2006;5:11.
10. Afrogheh A, Wright CA, Sellars SL, Wetter J, Pelser A, Schubert PT, *et al.* An evaluation of the Shandon Papspin liquid-based oral test using a novel cytologic scoring system. Oral Surg Oral Med Oral Pathol Oral Radiol 2012;113:799-807.
11. Ferlay J, Pisani P, Parkin DM. Globocan 2002. In: Cancer Incidence, Mortality and Prevalence Worldwide: IARC Cancer Base (2002 estimates). Lyon: IARC Press; 2004.
12. Joshi PS, Kaijkar MS. Cytomorphometric analysis of oral premalignant and malignant lesions using feulgen stain and exfoliative brush cytology. Interdiscipl Histopathol 2013;1:204-11.
13. Hande AH, Chaudhary MS. Cytomorphometric analysis of buccal mucosa of tobacco chewers. Rom J Morphol Embryol 2010;51:527-32.
14. Al Bahrani AJ. Evaluation of the cytological changes of oral mucosal cells in smokers by using exfoliative Pap Stain. MDJ 2013;10:124-9.
15. Fontes PC, Corrêa GH, Issa JS, Brandão AA, Almeida JD. Comparison of exfoliative pap stain and AgNOR counts of the tongue in smokers and nonsmokers. Head Neck Pathol 2008;2:157-62.
16. Jamadar S, Narayan TV, Shreedhar B, Mohanty L, Shenoy S. Comparative study of various grading systems in oral squamous cell carcinoma and their value in predicting lymph node metastasis. Indian J Dent Res 2014;25:357-63.
17. Mulki S, Shetty P, Pai P. Oral rinse-based cytology and conventional exfoliative cytology: A comparative study. J Cancer Res Ther 2015;11:129-35.
18. Winn DM. Tobacco use and oral disease. J Dent Educ 2001;65:306-12.
19. Goregen M, Akgul HM, Gundoğdu C. The cytomorphological analysis of buccal mucosa cells in smokers. Turk J Med Sci 2011;41:205-10.
20. Moralis A, Kunkel M, Reichert TE, Kosmehl H, Driemel O. Identification of a recurrent oral squamous cell carcinoma by brush cytology. Mund Kiefer Gesichtschir 2007;11:355-8.
21. Remmerbach TW, Meyer-Ebrecht D, Aach T, Würflinger T, Bell AA, Schneider TE, *et al.* Toward a multimodal cell analysis of brush biopsies for the early detection of oral cancer. Cancer 2009;117:228-35.
22. Miller SC, Soberman A, Stahl SS. A study of the cornification of the oral mucosa of young male adults. J Dent Res 1951;30:4-11.
23. Feldman PS, Kaplan MJ, Johns ME, Cantrell RW. Fine-needle aspiration in squamous cell carcinoma of the head and neck. Arch Otolaryngol 1983;109:735-42.
24. Scher RL, Oostingh PE, Levine PA, Cantrell RW, Feldman PS. Role of fine needle aspiration in the diagnosis of lesions of the oral cavity, oropharynx, and nasopharynx. Cancer 1988;62:2602-6.
25. Sciubba JJ. Improving detection of precancerous and cancerous oral lesions. Computer-assisted analysis of the oral brush biopsy. U.S. collaborative OralCDx study group. J Am Dent Assoc 1999;130:1445-57.
26. Patton LL, Epstein JB, Kerr AR. Adjunctive techniques for oral cancer examination and lesion diagnosis: A systematic review of the literature. J Am Dent Assoc 2008;139:896-905.
27. Shaila M, Shetty P, Pai P. A new approach to exfoliative cytology: A comparative cytomorphometric study. Indian J Cancer 2016;53:193-8.
28. Einstein TB, Sivapathasundharam B. Cytomorphometric analysis of the buccal mucosa of tobacco users. Indian J Dent Res 2005;16:42-6.
29. Verma R, Singh A, Badni M, Chandra A, Gupta S, Verma R, *et al.* Evaluation of exfoliative cytology in the diagnosis of oral premalignant and malignant lesions: A cytomorphometric analysis. Dent Res J (Isfahan) 2015;12:83-8.
30. Rajesh SB, Reddy SB, Ramamurthy TK, Srinivas K, Patil S. Cytomorphometric analysis of obtained squames obtained from normaloral mucosa and lesions of oral submucous fibrosis. J Indian Acad Oral Med Radiol 2012;24:200-5.
31. Cowpe JG, Green MW, Ogden GR. Quantitative cytology of oral smears. A comparison of two methods of measurement. Anal Quant Cytol Histol 1991;13:11-5.
32. Ogden GR, Cowpe JG, Wight AJ. Oral exfoliative cytology: Review of methods of assessment. J Oral Pathol Med 1997;26:201-5.
33. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of normal oral squames: An age, site and sex-related survey. J R Soc Med 1985;78:995-1004.
34. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of abnormal oral mucosal smears. J R Soc Med 1988;81:509-13.
35. Ramaesh 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;27:83-6.
36. Camilleri GE, Smith CJ. Exfoliative cytology of the early lesions of experimental oral cancer in the hamster. Arch Oral Biol 1965;10:465-70.
37. Chitturi RT, Rathinam E, Santo R, Yoithapprabhunath TR. The role of exfoliative cytology and molecular biology in oral potentially malignant disorders. J Health Res Rev 2017;4:43-6.