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Research Article

QUANTITATIVE EXFOLIATIVE CYTOLOGY IN DIAGNOSING PREMALIGNANT OR MALIGNANT ORAL LESIONS USING CYTOMORPHOMETRY

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A B S T R A C T

Aims and Objectives: Tumors are distinguished from normal tissues by their pronounced variability of cellular and nuclear dimensions. Therefore the quantitative parameters such as cytoplasmic diameter, nuclear diameter and cytoplasmic to nuclear ratio in the exfoliated cells may be an indicator to assess whether the cells are malignant or not. Exfoliative cytology is a simple and non-invasive diagnostic technique that could be used for early detection of oral premalignant and malignant lesions.

The aim of this study was to evaluate the quantitative changes in nuclear diameter (ND), cytoplasmic diameter (CD) and nuclear/cytoplasmic ratio (N/C) in the cytological smears of premalignant and malignant lesions by comparing with the cytological smears of normal healthy individuals.

Materials and Methods: Oral exfoliated cells from 10 cases of each histologically proven Oral Leukoplakia, Oral Submucous fibrosis and Oral Squamous Cell Carcinoma and ten controls with healthy mucosa were taken and stained with H&E stain were evaluated for cytoplasmic diameters, nuclear diameters and nuclear/cytoplasmic ratios (N/C) using Image J analysis system. The data were evaluated using statistical methods, namely't' test and analysis of variance.

Results: The result showed that there was a significant increase in the cell diameter, nuclear diameter and nuclear to cytoplasmic ratio of all the study groups when compared to the control group.

Conclusion: Oral exfoliative cytological techniques could possibly be a noninvasive alternative diagnostic tool for diagnosing oral premalignant and malignant lesions.

INTRODUCTION

Oral cancer is the most common cancer and constitutes a major health problem in India and other developing countries, representing one of the leading cause of death. It accounts for almost 40% of all the cancers in India (1). A significant proportion of oral squamous cell carcinomas develop from premalignant lesions such as leukoplakia and oral submucous fibrosis. The lack of improvement in prognosis of oral squamous cell carcinoma is due to diagnostic delay caused by either patients or by health care workers. The patients with oral squamous cell carcinoma who were treated early has a better prognosis with 5 year survival rate as high as 80% and also there is an improved quality of life in early treatment for only less aggressive treatment is necessary in early stages. Though histological examination of tissue remains the gold standard for diagnosis and identification of oral lesions, oral cytology is suggested to be useful in detection of early carcinoma or premalignant lesions in asymptomatic patients with innocent appearing lesions

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The exfoliative cytology technique is a noninvasive method for initial and early diagnosis of cancers, as an adjunct to clinical examination. According to Sampaio Hde *et al*, exfoliative cytology has been in use since the 1960s and 1970s, as a cancer evaluation and diagnostic technique, with acceptable sensitivity and specificity.(2) Cytology can be used as a diagnostic technique in detecting early changes of diseases even in the absence of clinical manifestations. This technique is advantageous, for it's easy, fast implementation, adequate diagnostic value and non-invasiveness. It is painless, with a low-cost, and reproducibility. (3,4)

MATERIALS AND METHODS

Our study population constituted ten cases for each of the study groups along with ten cases of control group. The study group constituted histologically diagnosed ten cases of each oral leukoplakia, oral submucous fibrosis that were histologically benign and oral squames cell carcinoma. These were catergorized as group 1,2 and 3 respectively. The control group constituted healthy patients, who did not have any lesions.

Smear preparation

The smears of the oral squames were obtained from both the study and control groups with a sterile wooden spatula. The smear was fixed in 95% ethyl alcohol for a minimum period of 15 minutes and stained using the H&E method.

Morphometric analysis

Totally 100 cells of each smear were measured in both horizontal and vertical axis and an average of the two was taken. The cell diameter and nuclear diameter were measured by superimposing the eyepiece graticule on the stained cells in 100X objective. Only clearly defined cells were measured avoiding the clumped and folded cells. The nuclear to cytoplasmic ratio was deduced from the above values.

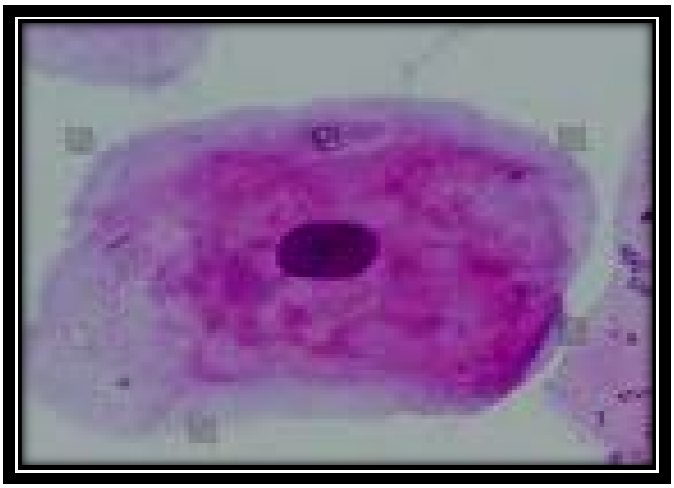

Figure 1 Figure demonstrating the contrast edited image of the smear.

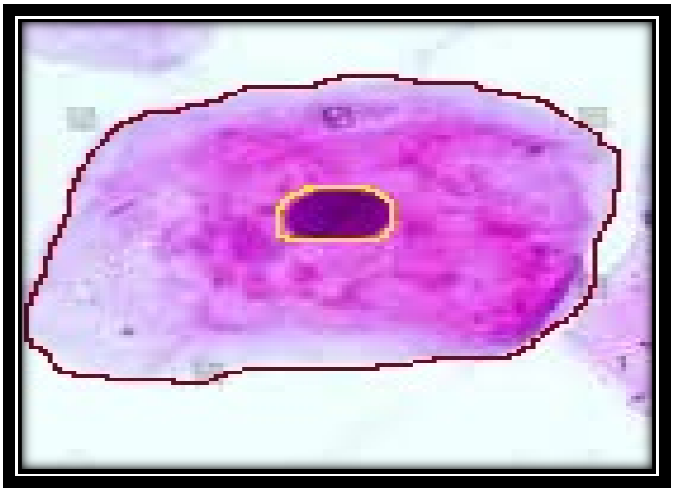

Figure 2 Figure demonstrating the edited image of the smear in MS paint.

Figure 3 Ocular micrometer disc.

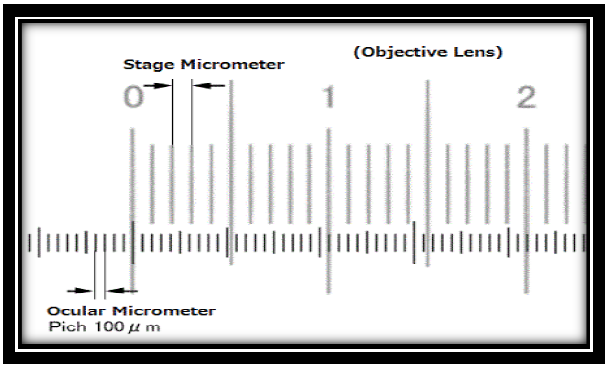

Figure 4 Figure demonstrating the picture of mounted ocular micrometer

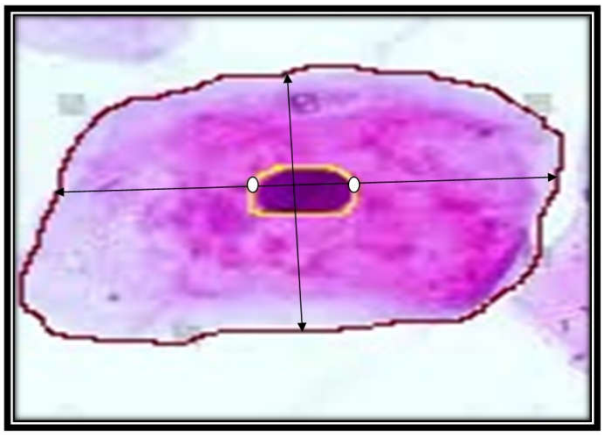

Figure 5 Figure demonstrating the measurement of long and short cell diameter and nuclear diameter.

Statistical Analysis

The differences in the mean values of cell diameter and nuclear diameter and the nuclear to cytoplasmic ratio between the 2 different groups was identified using 't' test. The collected datas were analyzed using SPSS package.

RESULTS

There is a significant reduction in the mean cytoplasmic diameter in all the study groups when compared to the control group and a significant increase in the nuclear diameter and nuclear to cytoplasmic ratio in all the study groups when compared to the control group.

Table 1 Comparison of cell diameter, Nuclear diameter and nuclear to cytoplasmic ratio of OSMF with control

	Group	N	Mean	SD	t	p
Cell Diameter	Control	10	5.57	0.76	5.12	< 0.001**
	Oral submucous fibrosis	10	9.17	2.09		
Nuclear Diameter	Control	10	42.04	5.56	3.98	0.001**
	Oral submucous fibrosis	10	28.32	9.38		
Nuclear / cytoplasmic ratio	Control	10	0.13	0.02	6.23	< 0.001**
	Oral submucous fibrosis	10	0.35	0.11		

**Significant at 1%

Table 2 Comparison of cell diameter, Nuclear diameter and nuclear to cytoplasmic ratio of oral Leukoplakia with control.

	Group	N	Mean	SD	T	p
Cell Diameter	Control	10	5.57	0.76	5.31	< 0.001**
	Leukoplakia	10	9.05	1.92		
Nuclear Diameter	Control	10	42.04	5.56	1.88	0.076
	Leukoplakia	10	31.66	16.53		
Nuclear / cytoplasmic ratio	Control	10	0.13	0.02	5.66	< 0.001**
	Leukoplakia	10	0.33	0.11		

**Significant at 1%

Table 3 Comparison of cell diameter, nuclear diameter and nuclear to cytoplasmic ratio of oral squames cell carcinoma with the control.

	Group	N	Mean	SD	t	p
Cell Diameter	Control	10	5.57	0.76	6.76	< 0.001**
	Oral squamous cell carcinoma	10	10.69	2.27		
Nuclear Diameter	Control	10	42.04	5.56	11.23	< 0.001**
	Oral squamous cell carcinoma	10	19.11	3.27		
Nuclear / cytoplasmic ratio	Control	10	0.13	0.02	8.18	< 0.001**
	Oral squamous cell carcinoma	10	0.58	0.17		

**Significant at 1%

Table 4 Comparison of cell diameter, nuclear diameter and nuclear to cytoplasmic ratio of all the parameters with the control.

		N	Mean	SD	ANOVA	p
Nuclear Diameter	Control	10	5.57	0.76	13.60	< 0.001**
	Oral submucous fibrosis	10	9.17	2.09		
	Leukoplakia	10	9.05	1.92		
	Oral squamous cell carcinoma	10	10.69	2.27		
	Total	40	8.62	2.60		
Cell Diameter	Control	10	42.04	5.56	8.89	< 0.001**
	Oral submucous fibrosis	10	28.32	9.38		
	Leukoplakia	10	31.66	16.53		
	Oral squamous cell carcinoma	10	19.11	3.27		
	Total	40	30.28	12.72		
Nuclear / cytoplasmic ratio	Control	10	0.13	0.02	24.82	< 0.001**
	Oral submucous fibrosis	10	0.35	0.11		
	Leukoplakia	10	0.33	0.11		
	Oral squamous cell carcinoma	10	0.58	0.17		
	Total	40	0.35	0.20		

DISCUSSION

The normal oral epithelium is a stratified squamous epithelium. The inner layer is the basal cell layer, then lies the prickle cell layer and then the surface cornified layer. The granular layer will be more if there is overlying keratin. ND decreases and CD increases from the basal layer to the superficial layers. The basal cell nucleus is relatively larger. The prickle cells are larger than the basal cells but the nucleus is smaller than the basal cells. As the epithelium matures, the physiologic activity of the nucleus decreases and the nucleus condenses towards the surface. As the cells cornify, the nucleus entirely disappears.[5,6] 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. The superficial epithelial cells do contain nuclei and alterations in these cells can serve as reliable indicators of dysplastic or neoplastic changes. (7)

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. (8)

In recent years, there has been increasing interest in the role of exfoliative cytological technique as a screening aid for the oral malignancy and pre-malignancies.

In oral smears, malignant cells are characterized by large nucleus with hyperchromatism and an increase in the nuclear-to-cytoplasmic ratio.[9]

The NA, NCD ratio, and nuclear CyA ratio values were found to have increased from normal subjects to tobacco users without any lesion to leukoplakia with the highest value in oral SCC.cytomorphometric analysis of exfoliated cells has also been suggested as a key approach to define and identify the cellular and nuclear changes in cytological smears. Computer-assisted image analysis techniques are faster, more accurate, and more reproducible. In this regard authors, such as Ogden *et al* suggested that this computer-assisted analysis of images formed by a microscope may increase the sensitivity of exfoliative cytology for early diagnosis of oral cancers, since these techniques are precise, objective, and reproducible. (10)
With reference to cytoplasmic changes, Cowpe *et al* found that tissues undergoing malignant transformation typically shows a reduction in CA before the reduction in NA. (11) This may be due to the fact that with increased activity, the maturing ability of cytoplasm diminishes. Besides, the cell makes less amount of cytoplasm in relation to the nucleoplasm. In our study there was a significant reduction of CA of all the study groups when compared to the control group.

Similar results has been obtained by Khandelwal and Solomon.[already men] In their study, oral smears from 60 cases with 20 cases each of normal mucosa, mucosa of tobacco users and OSCC showed a mean value of 1377.30 ± 76.68, 1120 ± 70.44 and 783.62 ± 53.33 respectively. The results revealed significantly different CA among the groups. Also, the studies done by Cowpe *et al* and Ogden *et al* described similar findings. (11,12,13)

With reference to nuclear area The increase in the nuclear size may be related to an increase in the nuclear contents required for replication. The study of Weigum *et al*, in their study of 41 cases, the mean NA of normal mucosa, dysplasia and SCC was 63.4 ± 11, 149 ± 23 and 165 ± 46 respectively. Here, the NA was significantly increased in dysplastic and SCC cytologic smears versus normal mucosa ($P < 0.001$). (14). Similarly, Khandelwal and Solomon in their study found that the mean NA was higher in OSCC lesions when compared with those from the mucosa of tobacco users and normal mucosa(15). In another study conducted by Goregen *et al*, on smokers and non-smokers, the authors observed a 16.5% increase in the NA value of smokers over non-smokers. In our study there was a significant increase in the nuclear area of all the study groups when compared to the control group. This increase in NA can be attributed to a cellular adaptation that depends on smoking. This adaptive change in the cell nucleus tends to be dysplastic.(16)

With reference to Nuclear to cytoplasmic ration Regarding the NA/CA ratio in the present study, the smears from normal oral mucosa, OLD and OSCC showed a mean value of 0.043 ± 0.004, 0.100 ± 0.008, 0.181 ± 0.015 respectively. Comparing the mean NA/CA ratio of three groups, there is a significantly different NA/CA ratio among the groups ($P < 0.001$). This may be due to significant elevation in mean NA and a significant reduction in mean CA.

A significant increase in mean NA/CA ratio had also been observed in the studies of Cowpe *et al*.[Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of abnormal oral mucosal smears. J R Soc Med. 1988;81:509-13. [PMC

free article] [PubMed] and Khandelwal and Solomon.[already men] On the contrary, Diniz-Freitas *et al*.[Diniz-Freitas M, García-García A, Crespo-Abelleira A, Martins-Carneiro JL, Gándara-Rey JM. Applications of exfoliative cytology in the diagnosis of oral cancer. Med Oral. 2004;9:355-61. [PubMed]] studied 10 healthy subjects and 10 patients with oral carcinoma and concluded that neither CA and NA nor NA/CA ratio differ between the oral mucosa of oral carcinoma patients and healthy patient. This may be due to the small sample size, difference in methodology employed, improper site selection and inclusion of only superficial cells.

Therefore to conclude Cytomorphometric evaluation of oral squames cells can serve as a useful diagnostic adjunct for early detection of oral cancer. It may also aid in establishing the prognosis of a dysplastic lesion.

CONCLUSION

Exfoliative cytology is a simple, noninvasive technique that could be used in the diagnosis of oral leukoplakia and oral SCC. Quantitative parameters such as morphometry are reproducible and eliminate the observer bias as it is obtained by software analysis and hence, improves the accuracy in the diagnosis of these lesions.

Results of the present study suggest that oral SCC and oral leukoplakia produce definite cytomorphometric changes. Hence, the cytomorphometric analysis of oral mucosal cells in leukoplakia and oral SCC could be used as an adjunct diagnostic tool in the detection of these lesions.

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