ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Cytomorphometric Analysis of Basal Cells: An Objective Mean for the Diagnosis of Oral leukoplakia and Oral Submucous Fibrosis.

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ABSTRACT

The histopathological diagnosis of Oral leukoplakia (OL) and Oral Submucous Fibrosis (OSMF) is based on the subjective evaluation of morphological anomalies within the lesional tissue. Interpretation of dysplasia varies from one pathologist to another. This subjectivity has turned the interest toward applying computer-assisted morphometry to investigate the cellular and nuclear changes in correlation with the histological behaviour of the lesions. To measure the cellular area (CA) and nuclear area (NA) in OL, OSMF and Normal Oral Mucosa (NOM). 2.To measure the nuclear-cytoplasmic ratio (NCR) in basal layer of OL, OSMF and NOM. 3.To compare and correlate the measurements in OL, OSMF and NOM. 45 cases were included in the study with 20 cases of OL, 20 cases of OSMF and 5 cases of NOM. Basal cells were subjected to Morphometric analysis with image analyser software. The cellular and nuclear area were measured and the nuclear-cytoplasmic ratio was calculated in ten fields. Measurements were tabulated and subjected to statistical analysis. The CA & NA in OL, OSMF are larger compared to NOM with a significant P value of 0.015 and 0.40. The Post Hoc Analysis showed a significant difference of 0.023 between CA of OL and OSMF and a significant value of 0.005 between OSMF and NOM. NCR is increased in OL and OSMF when compared to NOM with a significant P value of 0.009 and Post Hoc analysis revealed significant difference of 0.008 between OL & OSMF and between OSMF and NOM. The significant values obtained in the results suggest that histomorphometry can allow objective information to be acquired and can discriminate between the lesions when the diagnosis is ambiguous.

Keywords: leukoplakia, OSMF, cellular area, nuclear area, dysplasia.

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ISSN: 0975-8585

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, progressive, scarring premalignant condition characterized by juxta-epithelial inflammatory reaction and progressive fibrosis of the lamina propria [1]. In Indian subcontinent the habitual chewing of areca nut is most prevalent due to which it constitutes its largest group of malignancy with an incidence rate as high as 30-40% [2].

Oral leukoplakia (OL) is a premalignant lesion that cannot be characterized clinically or pathologically as any other disease.[2] Various studies undertaken in the literature suggest that it has a malignant transformation potential of 4%. Specific clinical subtypes have a higher malignant rate [3].

The histopathological diagnosis of OL and OSMF is based on the subjective evaluation of morphological anomalies within the lesional tissue.[3] The prominence given by pathologists to particular histopathological features differ, which results in variation in the clarification of dysplasia among the pathologists.[3] With the advancement of technology, there is availability of increasingly versatile and user friendly softwares which give rise to much reliable quantitative technique like cytomorphometry to invalidate the inter observer variation [4].

The present study was conducted to analyze the morphological features like cell area (CA), nuclear area (NA) and nuclear-cytoplasmic ratio (N:C) of basal cells in oral submucous fibrosis, leukoplakia and normal oral mucosa and to compare and correlate the measurement between the same. The study consisted a total of 45 cases which included 20 cases of oral submucous fibrosis, 20 cases of leukoplakia and 5 cases of normal oral mucosa (control).

MATERIALS AND METHODS

This retrospective morphometric study was conducted on histopathologically diagnosed hematoxylin and eosin stained sections retrieved from the archives of Department of Oral and Maxillofacial Pathology and Microbiology, Rajarajeshwari Dental College and Hospital, Bangalore.

The study group included 20 hematoxylin and eosin stained slides of histopathologically confirmed cases of leukoplakia and oral submucous fibrosis each. The control group included normal oral mucosa sections.

Morphometric technique

In each case, images of 10 different fields were captured under high power magnification (40×), with the help of a penta-head research microscope. The images were classified, transferred and stored in the computer. In each field, 10 most representative cells were selected. The selected cells were the basal layer cells with distinct cellular and nuclear outlines. Areas of artifacts and cells overlapping were avoided.

In each cell, cell area, nuclear area and nuclear-cytoplasmic ratio were measured.

Cell area and nuclear area

To measure the cell area and nuclear area, the cell and nuclear outlines were traced with a digitizing pen stylus on the screen. The software directly converted the number of pixels detected into square microns (Figure.1).

Nuclear-cytoplasmic ratio

The nuclear-cytoplasmic ratio was calculated by dividing the nuclear area by the difference between the cellular area and nuclear area (Figure.1).

$$\text{N:C ratio} = \text{N area}/(\text{C area} - \text{N area}).$$

All the measurements were saved in Microsoft excel and subjected for statistical analysis.

ISSN: 0975-8585

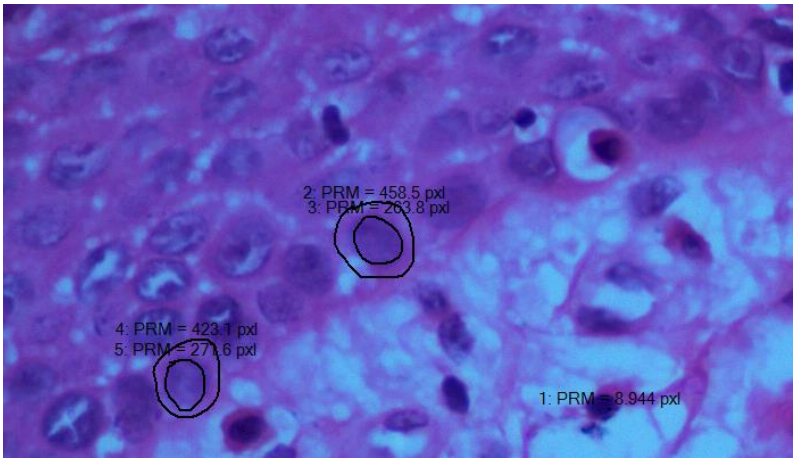

Figure 1: In each field 10 most representative cells were selected and measured

RESULTS

Our study consisted of a total of 45 cases which included 20 cases of OL, 20 cases of OSMF and 20 cases of NOM.

Among the OL patients, 3 were females and 17 were males which accounted for 15% and 85% of the total OL cases included in the study (Table.1).

Among the OSMF patients, 7 were females which accounted for 35% and 13 were males which accounted for 65% of the total OSMF cases included in the study (Table.1).

Among 5 cases of NOM, 1 was female and 4 were males which accounted for 20% and 80% of the cases (Figure.2).

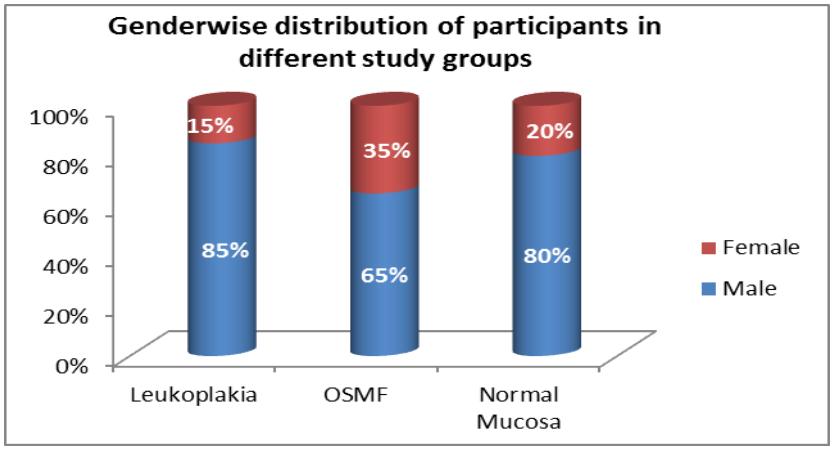

Figure 2

Among the 45 cases included in the study, patient's age ranged from 11 to >60 years. Patient's age range was divided into different groups as: 11-20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years and >60years. In OL cases, patients with age groups of 31-40years and 41-50years predominated accounting for 35% each.Age group of 21-30years was highest in OSMF and NOM cases accounting for 60% and 80% of the total OSMF and NOM cases respectively.

ISSN: 0975-8585

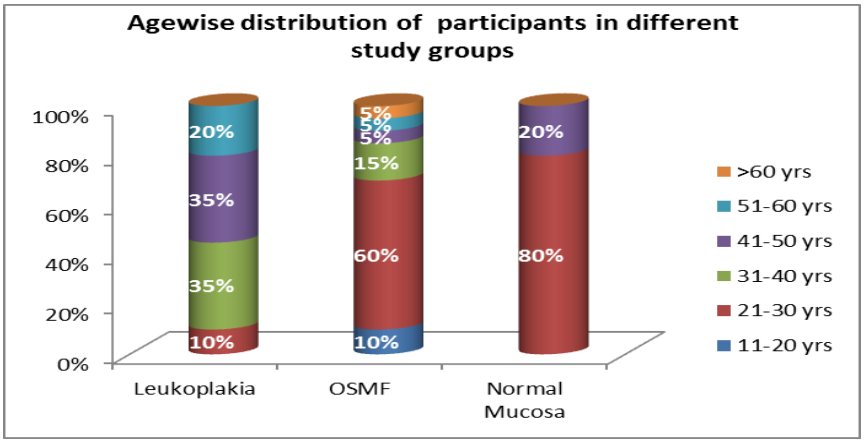

Figure 3

Approximately 4,500 measurements were carried out. The mean values of CA, NA and N:C of the study group and control group are shown in Table.1. The data collected in this study were analyzed statistically by computing descriptive statistics, viz., percentage, mean, standard deviation, standard error of mean, 95% confidence interval for mean.

ANOVA Test followed by Bon Ferroni's Post Hoc Analysis was done to know if significant difference was there between the group and to know the significant difference between the individual groups respectively.The results showed that the values of CA, NA and N:C obtained, were highest in OSMF compared to that of leukoplakia and normal oral mucosa, with the lowest in normal oral mucosa. (Figure.4,5,6). There was a statistically significant difference in CA, NA and N:C of oral leukoplakia, OSMF and normal oral mucosa with P-values of <0.001, <0.013 and <0.019 respectively. The morphometric parameters were statistically significant to differentiate between OL and NOM, and between OSMF and NOM with P-values <0.05. However, the parameters were not statistically significant to differentiate between OL and OSMF.

Paramete	Condition	Mean
CA	Leukoplakia	425.72
	OSMF	451.267
	Normal Mucosa	374.796
NA	Leukoplakia	290.5
	OSMF	296.8
	Normal Mucosa	261.8
NCR	Leukoplakia	1.48
	OSMF	1.53
	Normal Mucosa	1.45

Table 1

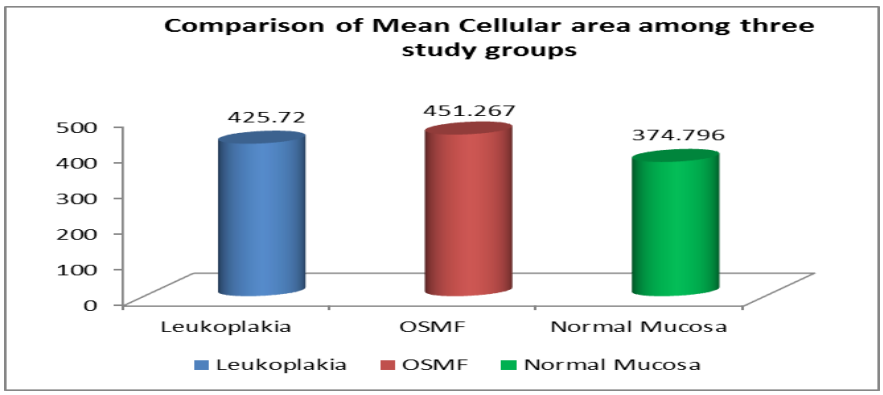

Figure 4

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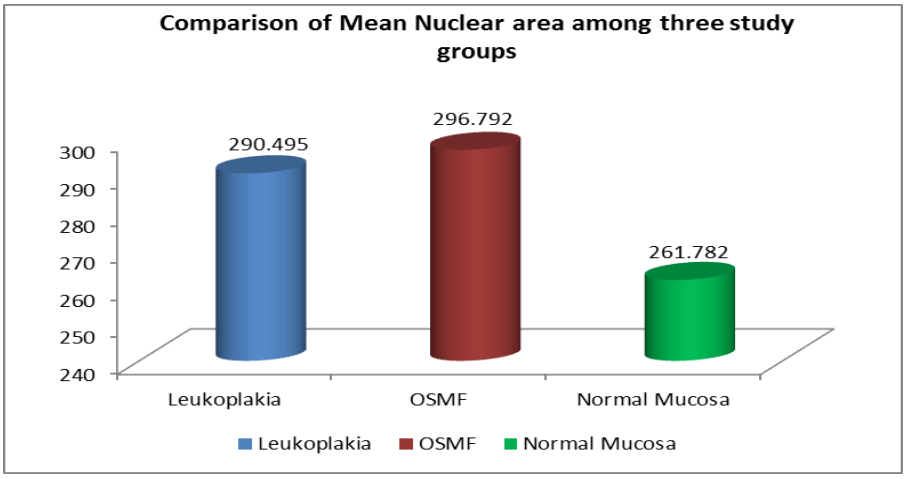

Figure 5

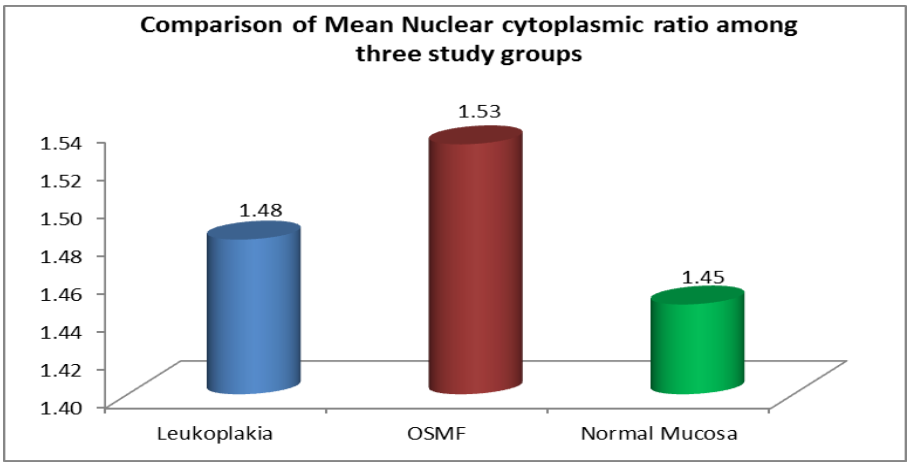

Figure 6

DISCUSSION

The histopathological diagnosis of Oral leukoplakia (OL) and Oral Submucous Fibrosis (OSMF) is based on the individual judgement. There is, therefore, a considerable need to improve the histologic evaluation of epithelial dysplasia.

Of late, interest has turned toward applying sophisticated technique of computer-assisted morphometry to investigate the cellular and nuclear changes in correlation with the histological behavior of the lesions. It is a method of assessing the computerized images of histological stained sections. The results have been more reliable, objective and reproducible.

In the literature, various cytomorphometric studies have been undertaken to evaluate the alterations in quantitative parameters like nuclear area, cellular area and nuclear-cytoplasmic ratio.

Smitha et al. did a study on morphometry of the basal cell layer of oral leukoplakia and oral squamous cell carcinoma using computer-aided image analysis. The mean values of nuclear area and perimeter, cellular area and perimeter and nuclear-cytoplasmic ratio were highest in oral squamous cell carcinoma and lowest in normal oral mucosa. The study suggests that the morphometric parameter, size, was useful to differentiate between normal oral mucosa, potentially malignant leukoplakia and SCC [3].

Shilpa B. Rajesh et al. studied the squames obtained from normal oral mucosa and lesions of oral submucous fibrosis. A significant reduction in the mean of the cell diameter and an increase in nuclear

diameter were observed in oral submucous fibrosis patients compared to that of normal oral. The study suggests that reduction in ND and increase in CD could serve as an early indicator of malignant change [4].

Veda Hedge conducted a cytomorphometric analysis of OL, OSMF, oral lichen planus, OSCC and NOM. Results showed that there was a statistically significant reduction in the mean cytoplasmic and nuclear diameter in all the lesional cases compared to that of normal oral mucosa. The nuclear to cytoplasmic ratio was significant in only OL, OSMF and OSCC. The study demonstrates that cytoplasmic changes alone without any nuclear change could be considered as an early parameter in exfoliated cells especially when there is no histologic evidence of dysplasia [5].

Priya Shirish Joshi and Manasi Sandipak Kaijkar conducted a cytomorphometric analysis of leukoplakia, oral squamous cell carcinoma (OSCC) and normal oral mucosa using Feulgen Stain and Exfoliative Brush Cytology. The results showed that N:C ratio, mean nuclear area and diameter value was highest in OSCC and lowest in normal oral mucosa. The mean cellular area and diameter was highest in normal oral mucosa and lowest in OSCC. There was a significant difference in mean values of the parameters between all the groups except between OSCC and leukoplakia. The study was able to differentiate dysplastic and malignant cells from normal ones using analysis based on nuclear and cellular parameters [6].

Metgud R et al did a cytomorphometric analysis of OSMF and OL using methyl green-pyronin Y, Feulgen staining and exfoliative brush cytology. The results showed a significant reduction in CA and NA in OL and OSMF compared to NOM. The study suggests that a decreased mean cytoplasmic diameter of exfoliated buccal mucosal cells could serve as an early indicator of dysplastic change in lesions that otherwise appear benign [7].

Ramesh et al measured cellular and nuclear diameter of squames obtained from normal oral mucosa, lesions of oral leukoplakia and SCC. The study groups consisted of Group 1: normal buccal mucosa; Group 2: lesions with no epithelial dysplasia; Group 3: lesions with epithelial dysplasia and Group 4: SCC cases. Results showed that CD was highest in normal mucosa followed by dysplastic lesions and lowest in SCCs. By contrast, ND was the lowest in normal mucosa, higher in dysplastic lesions, and highest in SCCs. According to this study, reduced nuclear size and increased cytoplasm size are useful early indicators of malignant transformation [8].

Shabana et al did a morphometric analysis of basal cell layer in four groups of white lesions (traumatic keratosis, lichen planus, leucoplakia, and a "risk group") in addition to two control groups (normal mucosa and squamous cell carcinoma). The results showed a significant increase in the dimensions (area, perimeter, and maximum diameter) of the nuclei from normal mucosa through traumatic keratosis, lichen planus, leucoplakia and the "risk group" to carcinoma. The nuclear-cytoplasmic ration was reduced in risk group compared to control group but was not statistically significant. The study concludes that the size of the basal cell and its nucleus can be of diagnostic value for lesions with a high risk of malignant transformation [9].

CONCLUSION

Our study was able to differentiate OSMF and leukoplakia from normal oral mucosa by applying cytomorphometric analysis based on nuclear and cellular parameters. The significant values obtained in the results suggest that estimation of CA, NA, and N:C ratio by image analysis can be used as an effective aid in the diagnosis of OSMF and leukoplakia.

Based on the results obtained, we would like to state that cytomorphometry is a quantitative structural technique that is becoming increasingly important in the diagnosis of potentially malignant disorders as it allows objective information to be acquired from sections of cells and tissues, and the method possesses the potential to differentiate between the lesions when the diagnosis is ambiguous. The advent of sophisticated computer programs has made the interpretation of findings much more reliable and convenient than earlier by using morphometric techniques that have been advocated as objective and reproducible methods of detecting changes before they are visible by routine light microscopy.

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