Cytomorphometric Analysis of Obtained Squames Obtained from Normal Oral Mucosa and Lesions of Oral Submucous Fibrosis

Shilpa B Rajesh, Satheesha BH Reddy, TK Ramamurthy, K Srinivas, Shilpa Patil

ABSTRACT

Introduction: The oral cavity is the site of many diseases. Not only dental, even medical professionals encounter a wide spectrum of oral mucosal lesions in their day-to-day clinical practice. An adequate diagnosis is essential as these lesions may vary in nature from simple to life threatening ones.

As nuclear changes are the most important criteria for diagnosing precancerous and cancerous lesions and since no single structural change is diagnostic by itself, a combination of several abnormalities is always necessary. There are several studies in the literature that have used cell diameter (CD) and nuclear diameter (ND) parameters in morphometric analysis of cells.

Application of quantitative techniques to smears obtained from oral premalignant lesions like oral submucous fibrosis could possibly improve the diagnostic value of oral exfoliative cytology. The cytological study of oral mucosal cells is simple, rapid and nonaggressive and relatively painless. It is thus well-accepted by patients and suitable for screening large population, for early analysis of suspect lesions.

Materials and methods: Oral exfoliative smears were collected from 50 clinically confirmed cases of oral submucous fibrosis (OSMF) and 50 control group. All cytological smears were fixed with commercially available spray fixative for 15 minutes and all the smears are stained with Papanicolaou’s (PAP) stain and with computer assistance, CD and ND was assessed.

Results: The results showed decrease in CD in OSMF patients when compare to normal patients and increase in ND in OSMF patients when compare to normal patients.

Interpretation and conclusion: The reduction in CD and increase in ND could be early indications toward a malignant change. Application of quantitative techniques to smears obtained from the buccal mucosa of the study groups could possibly improve the diagnostic value of oral exfoliative cytology.

Keywords: Cytomorphometry, Oral exfoliative cytology, Oral submucous fibrosis, Premalignant condition.


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INTRODUCTION

The oral cavity is the site of many diseases. Not only dental, even medical professionals encounter a wide spectrum of oral mucosal lesions in their day-to-day clinical practice.

An adequate diagnosis is essential as these lesions may vary in nature from simple to life-threatening ones.

Oral submucous fibrosis (OSMF) is one such lesion, which though is easy to diagnose but difficult to manage. The disease was first described by Schwartz in the year 1952 and was named ‘Atrophica idiopathica (tropicum) mucosum oris’. Areca nut, which is believed to be major etiological factor which increases the collagen production and decreases the degradation of collagen on basis of this pathogenesis; now OSMF has been considered as collagen metabolic disorder.1

Early detection of premalignant and cancerous mucosal lesions improves the survival and morbidity of patient’s sufferings. Cytological study of oral mucosal cells is a nonaggressive technique that is well accepted by the patients, and is therefore an alternative option for the early diagnosis of oral mucosal lesions. Recent advances in cytological techniques novel aspects of applications of scraped or exfoliative cytology for detecting these lesions and predicting their progression or recurrence can be detected. In recent decades, we have seen a dramatic switch from histopathological to molecular method for obtaining DNA samples.2

Oral exfoliative cytology is particularly valuable for mass screening purposes. It has been used in the detection of oral squamous cell carcinoma and has been shown to have a sensitivity of 94%, specificity of 100% and an accuracy of 95%. The genotoxic and carcinogenic chemicals released from betel nut and calcium hydroxide content of lime present in the betel quid are thought to be responsible for promotion of reactive oxygen species from areca nut extracts. These reactive oxygen species can in turn cause damage to the DNA.3

Application of quantitative techniques to smears obtained from oral premalignant lesions like OSMF could possibly improve the diagnostic value of oral exfoliative cytology. The cytological study of oral mucosal cells is simple, rapid and nonaggressive and relatively painless. It is thus well-accepted by patients and suitable for screening large population, for early analysis of suspect lesions.4

Objective measurements of quantitative parameters could be more useful than subjective parameters. Because
the quantitative parameters are objective and reproducible, they are important adjuncts in making a cytopathological diagnosis. In 1980s, cytological studies were restricted largely to the subjective or qualitative interpretation. But now, with advance technology, more reliable quantitative techniques like cytomorphometry, histometry and use of computer-assisted image analyzer, will nullify the interobserver variation. This quantization aims at reproducibility, enables direct comparison from person to person. Morphometry can be used selectively on structures or samples, which are difficult to assess accurately, like variation in cell and nuclear size. As nuclear changes are the most important criteria for diagnosing precancerous and cancerous lesions and since no single structural change is diagnostic by itself, a combination of several abnormalities is always necessary. There are several studies in the literature that have used cell diameter (CD) and nuclear diameter (ND) parameters in morphometric analysis of cells.

Intrapatent comparisons have displayed gross variation in cytomorphology and DNA profiles between normal and abnormal tissue in the same patient. The results indicate that in the absence of the defined normal baseline for oral smears it is suggested that quantitative techniques, based on evaluation of parameters, such as nuclear area (NA), cytoplasmic area (CA) and nucleus to cytoplasm area ratio (NA/CA) may increase sensitivity of exfoliative cytology for early diagnosis of oral cancers. Since, these techniques are precise, objective and reproducible it was found that tissues undergoing malignant transformation typically show a reduction in CA before the reduction in NA.

**MATERIALS AND METHODS**

Armamentarium

1. Glass slide (Pearl Brand, India)
2. Wooden spatula
3. Biofix
4. Papanicolaou (PAP) stain
5. Cover slips (Blue star, Mumbai, India) (Fig. 1)

There were fifty patients who were clinically confirmed with oral submucous fibrosis and who have not taken any treatment (Figs 2A to C).

 Fifty volunteers with normal buccal mucosa and they should be free from any oral and systemic diseases and nonsmokers are considered as a control group.

Before the procedure all subjects were asked to rinse their mouth with water. Then following informed consent oral mucosal cells were scrapped from buccal mucosa using moistened wooden spatula with patient’s saliva (Fig. 3).

Then, the cells were immediately smeared on precleaned microscopic slides (Fig. 4). Just prior to drying, the smears were fixed with commercially available fixative (Biofix). Then the slides were processed for cytomorphological assessment using PAP staining procedure.
Then the smears were focused under microscope and viewed under 40× magnification, pictures were taken of 15 individual unfolded cells with clear outline of each patient. The picture were taken using Olympus BX41 (Fig. 5) in a stepwise manner, moving the slide from left upper corner to right and then down in order to avoid measuring the same cells again. And outline of cell and nucleus were drawn to measure the perimeter in millimeters (mm). The evaluations were done by computer assistance using C3-ProgRes software (Fig. 6).

Mean of cell perimeter and nucleus perimeter of 15 cells of each patient were calculated separately (Figs 7 and 8).

**Inclusion Criteria**

1. Clinically confirmed cases of OSMF.
2. Patient who had not taken any treatment for this condition.
3. Individuals without any habits and free from oral and systemic diseases considered for control group.

**Exclusion Criteria**

1. Patient with smoking habit.
2. Patient who has taken prior treatment for oral submucous fibrosis.

**RESULTS**

This study was conducted. Buccal exfoliative smears were collected from 50 OSMF patients and 50 normal patients. Then the smears were focused under microscope and viewed under 40× magnification, pictures were taken of 15 individual unfolded cells of each patient using Olympus BX41. And outline of cell and nucleus were drawn to measure the perimeter in millimeters (mm). The evaluations were done by computer assistance using C3-ProgRes software of each cell. Then the measurements were statistically analyzed using t-test at 5% significance and following results were obtained.

**Descriptive Statistics**

**Observation**

There is no association observed between CD and ND in normal case. But in a disease case, a high positive correlation is noticed which is significant (Table 1).

A high significant reduction was observed in the mean of the CD for disease patients compared to normal patient (Graph 1). But a significant increase was observed in the mean of the ND for disease patients compared to normal patient (Graph 2).
In our study, exfoliative squames of normal oral epithelium cells of basal layers are uniform in appearance. The diameter of each cell is about five times that of leukocytes, each cell is roughly oval in outline. The cytoplasm stains were dark to deep blue with PAP stain. The nucleus is round to ovoid in shape and occupies one-third to one-fourth of the cell.

The exfoliates of normal mucosa reveal different cells from epithelium and color ranging from light orange to orange depending on degree of maturations and appearance of the cells in normal patients which were similar to that of OSMF cells.

In present study, we found that the CD in OSMF was reduced when compared to normal patient. The minimum size of CD in normal patients is about 0.1460 mm and maximum is 0.8099 mm with a mean of 0.2975 mm, but in OSMF patients minimum size was 0.1585 mm, maximum was 0.2107 mm and mean value was 0.1871 mm (Fig. 2). Similar result was found by Frost et al and these changes are mainly due to increased cellular activity and diminished activity of cells to mature. Thereby, reducing the CD when it transforms from normal to malignant cell (Table 2).

However, we have also observed that there is an increase in ND in OSMF patients compare to normal patients. These changes are mainly due to increase in nuclear content required for replication. Similar results were reported by many authors. ND of normal patients, minimum is 0.0293 mm and maximum is 0.484 mm and mean being 0.0337 mm. Whereas in OSMF patient’s minimum is 0.0263 mm, maximum is 0.1312 mm and mean being 0.0492 mm (Fig. 1 and Table 2).

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<th>Table 1: Relation between CD and ND in OSMF patients and normal patients</th>
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<td><strong>Items</strong></td>
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*t-test is used at 5% significance; r: Correlation coefficient lies between –1 and +1; NS: Not significant at 5% significance; HS: Highly significant at 5% significance.
Furthermore, we have also observed p-value relation between CD and ND in normal patients was not significant, whereas p-value relation between CD and ND in OSMF patients were highly significant at 5% significance. So, high positive correlation was noticed in OSMF patients compare to normal patients again which is 5% significance and t-values in CD is – 3.204 in OSMF compare to normal which is highly significant, whereas the p-value of ND is – 2.818 compare to normal, hence which is also highly significant at 5% significance (Table 3).

In summary, this study supports and extends the view that exfoliative cytology in evaluation of CD and ND in exfoliative squames of OSMF can serve as a useful diagnostic aid for early detection of OSMF. Increase in size of ND and decrease in cellular diameter is characteristic features of malignant cells. Evaluation of greater number of cases is essential to establish this parameter which can be used as definitive indicator.

CONCLUSION

The basic defect of any cell alteration begins at the molecular level, and triggers a series of reactions which affect an entire cell system. This is ultimately reflected in the cellular morphology. Cytomorphometric analysis of smears can be carried out regularly to detect alterations in the cellular nuclear dimensions especially OSMF patients.

Cytomorphometric assessment of cells of the oral mucosa will assist in monitoring the status of the mucosa. Further, as the acceptance in reliability of measurable values increases, this method can also aid in motivating individuals to withdraw from adverse effects of betel nut and tobacco.

Exfoliative cytology is painless, noninvasive, less time consuming procedure, which causes little discomfort to the patient. So, exfoliative cytology has been widely used as diagnostic tool. It can be repeated number of times for diagnosis follow-up and research purposes.

We are of the opinion that, analysis of mucosal cells can be used as an effective screening tool for early detection of malignant transformation of OSMF patients.

However, more extensive studies with larger sample size are needed to determine its diagnostic sensitivity, specificity and reliability.

REFERENCES


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