Scholars Journal of Dental Sciences
Abbreviated Key Title: Sch J Dent Sci
ISSN 2394-4951 (Print) | ISSN 2394-496X (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Cytopathological Analysis of Oral Buccal Mucosa of Dental College Students

Dr. Kavita Gupta[1*]

[1]Syamala Reddy Dental College, Bangalore

DOI: 10.36347/sjds.2024.v11i05.002 | **Received:** 09.06.2024 | **Accepted:** 14.07.2024 | **Published:** 18.07.2024

***Corresponding author**: Dr. Kavita Gupta
Syamala Reddy Dental College, Bangalore

Abstract **Original Research Article**

In this study Cytopathological observations were recorded, including inflammation, microbial colonies, micronuclei, keratinisation, overlapping and hemorrhage of cells which are obtained from the buccal mucosa of the dental students to assess the fluctuation of the cellular characteristics among relatively normal subjects. This prospective study included 50 dental students with no detectable oral alterations and are submitted to brush cytology. The smears were fixed with 95% ethyl alcohol and stained with hematoxylin and eosin stain. The stained section were observed. Chi-square tests were applied for non-parametric variables. A p-value < 0.05 was considered statistically significant. The results showed close proximity among subjects in the matter of cytopathological observation and measures; no significant influence of sex was found on cytopathological observation, and vice versa. Oral exfoliative cytology combined with cytopathological analysis can be helpful for the study of normal individuals in various investigations of oral and systemic diseases.

Keywords: Buccal Mucosa, Cytopathological Analysis.

INTRODUCTION

Microscopic examination of individual cell in a smear is called cytology and study of tissue is called histology. Each of these steps plays a vital role in cytological diagnosis. Exfoliation of cells is one of the main mechanisms of cell loss participating in the homeostatic control of cell population size. Cell exfoliation process in normal physiological conditions is closely associated with terminal differentiation and orderly loss of dying cells compensated by permanent cell population renewal. Hence, exfoliative cytology is the analysis of cells collected by normal shedding or artificial mechanical desquamation. For appropriate cytological evaluation proper collection, fixation, staining and evaluation of samples are required. Exfoliative cytology is a non-invasive technique, which allows simple and pain-free collection of intact cells from different layers within the epithelium for microscopic examination [1]. It is less time-consuming procedure with sensitivity of 89% and specificity of 89.5% [2], and is one among the best procedures applied for the initial microscopical examination of various oral lesions [3]. It has proved its high reliability and simplicity since its significance was first noted by Montgomery and Von Haam in 1951 [4]. Under normal circumstances, epithelial cells are maintained firmly in place. But these cells can lose their cohesiveness in certain conditions, resulting in exfoliation of cells [5]. Therefore, it can detect changes in oral cells even in normal individuals and provides a promising option for the early detection of various potentially malignant oral mucosal lesions [6]. Its importance has increased over the past years, synchronized with the emergence of new modalities, including immunocytochemistry, molecular analysis, advanced imaging techniques and cytomorphometric analysis [7-9]. Cell and its morphology reflects the biological behavior of the tissue and their genetic and molecular background. Hence, the slightest defect or alteration at the molecular level would lead to a chain of reactions influencing the entire cell system, subsequently, its cellular morphology [10]. This concept led to the application of cytopathological analysis of cells and these parameters have been shown to provide beneficial results in diagnosing the diseases in which some are systemic, such as anemia [11], diabetes mellitus [12], and hormonal changes [13]. Meanwhile, various other studies have examined the associated cytomorphological changes in potentially malignant and malignant lesions [4-13]. However, little research has been done with evaluating the normal oral mucosa [14, 15], as a baseline for comparison with pathological

smears. This study aims to do a cytopathological evaluation of the buccal mucosa of dental students to assess the fluctuation of the cellular characteristics among subjects. Such data can provide a database of potential cytological features in relatively normal individuals and the impact of having such statistics for comparison in future studies of different conditions and diseases.

MATERIALS AND METHODS

Fifty dental students with no detectable oral alterations and lesions were submitted to brush cytology. The demographic and clinical data for each student were registered in a case sheet. The recorded information includes; sex, times of tooth brushing/mouthwash (if used). Ethical approval was obtained from the Local Ethical and Scientific Committee of the college to conduct this prospective study at the laboratory of department of oral and maxillofacial pathology. Verbal consent was obtained from students to participate in this study. The students were asked to rinse their mouths with water thoroughly. A disposable medium-hard nylon brush was sterilized in 0.2% of chlorhexidine gluconate mouthwash for 24 h. Under adequate illumination, the cytobrush was used with moderate pressure in one direction over the buccal mucosa. Specimens were processed by a conventional method, in which the material from the brush was spread on the middle third of one clean, dried glass slide. The smears were then fixed immediately with 97% ethanol alcohol and stained with hematoxylin and eosin. The slides were evaluated under a light microscope for the following cytopathological findings: cellular overlapping, micronuclei and keratinization. At the same time, the background was also assessed for the presence or absence of any hemorrhage, inflammatory cells, and microbial colonies. The slide reading was performed stepwise, moving the slide from the left upper corner to the right and then down to avoid to avoid any misunderstanding. The frequency, percentage, and Pearson Chi-square test were used for non-parametric variables. A *p*-value < 0.05 was considered statistically significant.

RESULT

This study includes 50 first professional year dental students. Hence, the participants shared the same age of about 18 years old. The sample showed a female predominance of 56% (n = 28). We found that all the participants followed brushing once daily in the morning time as an oral health maintenance routine, with no one is using a mouthwash gargle. Regarding hormonal disturbance, (n=7) 14% of female participants underwent menstruation at the time of sample collection. This study assessed the frequency of cytopathological findings of inflammation and microbial colonies in terms of tooth brushing. In which the majority experienced both inflammation and microbial colonies in their smears, among which the difference was not statistically significant (the reported p-value > 0.05). In terms of cytopathological findings, the entire sample revealed overlapping cells in all of the analyzed smears 100% (n = 50). Simultaneously, the majority established microbial colonies 80.2% (n = 41) and micronuclei 90 % (n = 45). Moreover, 62 % (n = 31) demonstrated signs of keratinization in the cytoplasm of cells, and 66 % (n = 33) showed inflammation; meanwhile, only 10.8 % (n = 10) showed hemorrhage. None of the reported findings were influenced by sex. Meanwhile,only 50% showed inflammatory cells in the background of their smears(Figure A and B).

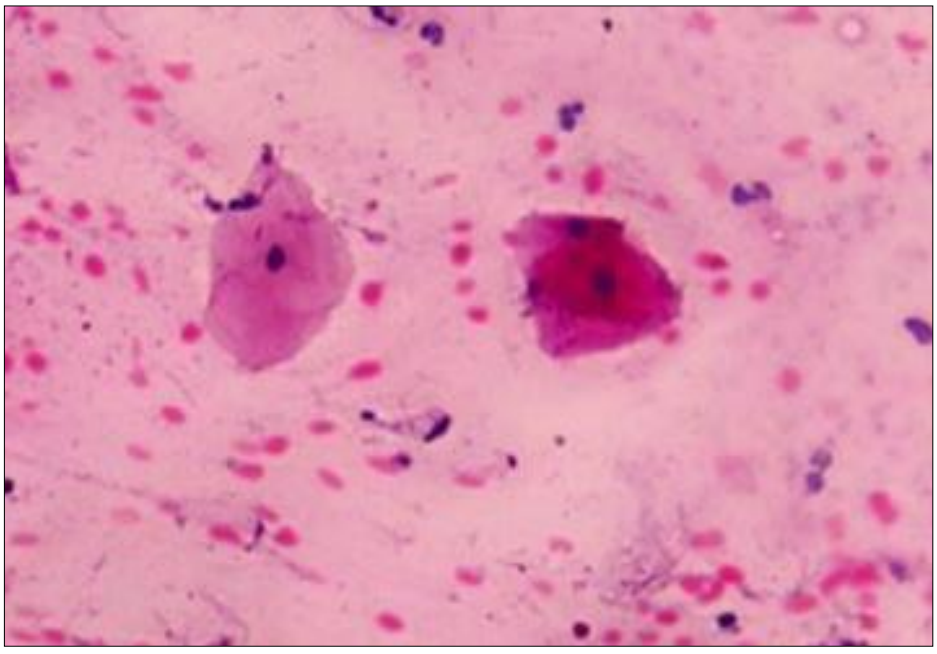

Figure A: Cytological smear (X40), showing Keratinization, Inflammation, Microbial colonies and Hemorrhage

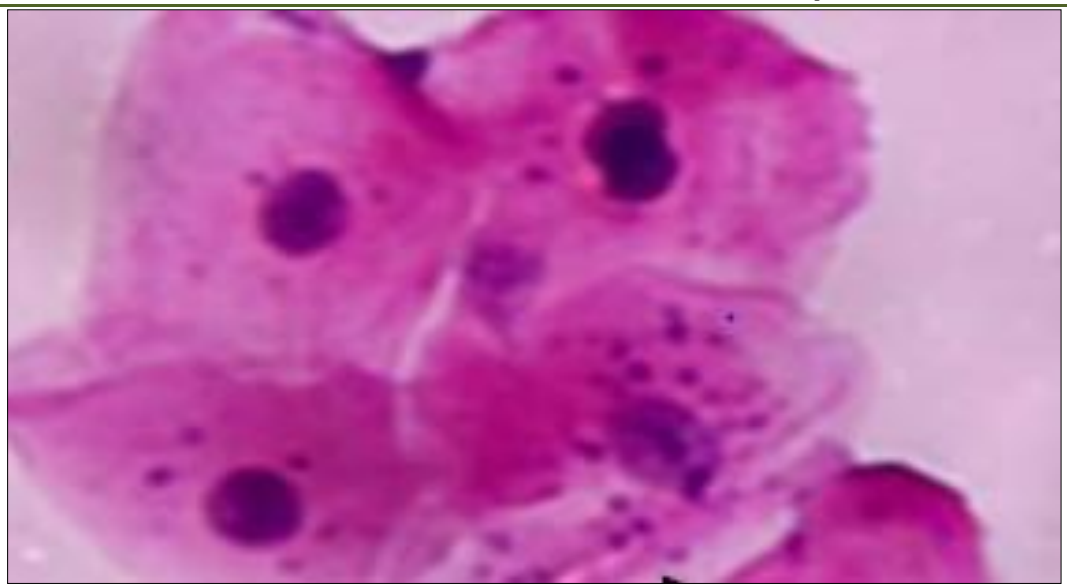

Figure B: Cytological smear (X40), showing cytoplasmic vacuolization, overlapping and Micronuclei

DISCUSSION

Exfoliative cytology and cytological smears is a simple, safe, and practical approach, that is particularly applicable for mass screening due to its high sensitivity and specificity [16].

Furthermore, the application of cytomorphometry offers remarkable development in the diagnosis and prognosis of serious diseases as it can detect cellular alteration, improving diagnosis accuracy and reproducibility [10]. Therefore, exfoliative cytology could be a beneficial adjunct to the clinical evaluation of lesions and influencing factors [17]. This study showed female predominance. However, since the sample was not collected haphazardly or in response to a particular disease or condition; instead, it was based on college admission within a specific age group. Therefore, it would be statistically unjustified to compare our data with other research on the matter of sex and age. Our sample followed the international recommendations for a daily frequency of tooth brushing; this could be attributed to the participant's awareness of the significance of toothbrushing as a preventive measure and an indicator of oral hygiene [18]. Additionally, the procedure was sex-related since females expressed more interest in practicing the globally advised dental hygiene regimen, which agreed with the literature [19-21]. Regarding cytological observations of the obtained smears, 66.7% showed signs of inflammation. This disagreed with Queiroz *et al.*, [15], in which no evidence of inflammation was seen in their sample of normal individuals. Also Ahmed *et al.*, [22], mentioned that exposure to tobacco products could induce inflammatory events in the buccal mucosa, although, in his study, inflammation was noted in both smokers and control groups, with varying degrees of intensity. Additionally, Proia *et al*., [23], linked the presence of inflammation in response to bacterial infection; however, this cannot be supported by our findings. As a result of these contradicted findings, further investigation is required to uncover the underlying causes of inflammation and anticipate their potential consequences. On the other hand, the majority of our sample (80.6%) showed bacterial colonies, which is higher than the findings of Abdelaziz and Osman [24], in their control group. Moreover, this study showed keratinization in 62.4% of samples. This contradicted the findings of other studies [24, 25], since no keratinization was observed in their control groups. Therefore they attributed the keratinization observation to smoking. Meanwhile, our results might be related to vigorous tooth brushing, the type of food intake, and perhaps accidental cheek biting. The sample screening revealed 90.3% of micronuclei expression. As explained by the researches, micronuclei originate from chromosome fragments or whole chromosomes, which stay behind at anaphase during nuclear division, and various genotoxic substances which induce their formation [26]. Since 1983, many studies have used micronuclei detection as a short-term mutagenicity test. Since it is a simpler and much more rapid screening of chromosomal damage in cytological preparations [27], others used it as a reliable indicator for neoplastic progression [28, 29]. Any increase in micronucleus count is a reflection of chromosomal alterations [30]. It has been reported that in normal healthy individuals, exposure to environmental pollutants such as drugs, chemicals, food, and free radical injuries, and lifestyle factors (smoking, alcohol consumption, diet, vitamin deficiencies) are all related factors in producing the higher rates of micronuclei count in buccal mucosa and peripheral blood lymphocytes [31], as shown in our study. Radhika *et al*., in their study have used wooden spatulas to collect buccal smears for exfoliative cytology. In this study we have used cytobrush to collect oral smears. Difference in the collection equipment could improve the nature of the collected cells therefore giving a comprehensive result [32, 33]. Research indicates that female hormones

impact epithelial cell proliferation and development [5]. In general, ovarian hormones have a significant impact on females' life.

These hormones' levels fluctuate throughout puberty, the menstrual cycle, pregnancy, and menopause [34]. It has been discovered that these hormones affect the oral cavity, as the onset of monthly menses evokes oral discomforts, including a burning sensation, bleeding with minor irritation, recurrent mouth ulcers, herpes labialis, and increased tooth mobility [34]. However, studies have not sufficiently proved a direct connection between alterations in the oral epithelium and hormonal changes during menstruation. The measuring of cytomorphometric characteristics in females based on their menstrual cycle was addressed by only three research. Balan *et al*., [35], discovered a significant difference in cellular and nuclear diameters during the different stages of the menstrual cycle of healthy young females.

At the same time, the other two studies [13-36], compared females with menstruation in different age groups. Further research emphasized that cytomorphometric parameters of the oral mucosa are undoubtedly affected by female hormones; hence, they excluded female members from their data to avoid superimposed results [37, 38]. In the meantime, in this study, females with menstruation showed nonsignificant cytopathological variation compared to non-menstruating females. Therefore, it is highly suggested that more studies be conducted on female hormones' influence in different age groups on the oral mucosa to enrich the literature and provide sufficient data for comparison. It can be recommended that future studies in this matter be conducted by physicians of both fields (cytology and gynecology) so that any conclusion would be more reliable.

CONCLUSIONS

In conclusion, cytopathological analysis of the smears can be used in various fields of investigation due to its multiple potential benefits including improved objectivity, enhanced sensitivity, and shorter turnaround times. This study provides a backbone of the record of data of normal individuals to be used as a baseline measurement for comparison with future cytopathological studies. Nonsignificant and minor cytomorphological alterations were related to hormonal alterations in between different sexes. Additional researches with larger samples is required to support and validate these findings. The study sample showed high micronuclei numbers, indicating carcinogen doses from either local or ambient exposure. This was the most intriguing and interesting discovery. Hence, Exfoliated buccal mucosal cells can therefore be utilized as a marker for genotoxicity and as a source of awareness in the general population.

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