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Effect of Tobacco Smoking on Oral Mucosa in Orthodontic Patients - A Cytomorphometric Study

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ABSTRACT:

Objectives: To analyse the cytomorphometrical changes in oral buccal mucosal cells of tobacco smokers undergoing fixed orthodontic treatment. Materials & Methods: Buccal smears were prepared, stained with Papanicolaou's stain and observed at 400x magnification. Digital images were obtained and analysed with 'Image J' software and data entered. One way ANOVA & Post Hoc test were performed to test the difference of various parameters among and between groups. Results: Cell diameter showed a significant reduction (p=0.032) in smokers undergoing orthodontic treatment as compared to normal individuals. Conclusion: Tobacco smokers undergoing fixed orthodontic treatment did not show any significant cellular and nuclear changes other than a reduction in cellular diameter.

Key Words: cytomorphometry, exfoliative cytology, fixed orthodontic treatment, oral buccal mucosal cells.

1. INTRODUCTION:

Oral cancer due to tobacco is one of the leading causes of mortality worldwide.[1] Tobacco is consumed as smoked and chewable forms. The number of tobacco smokers is increasing at an alarming rate and is a cause of concern.[2]

The carcinogenic effects due to tobacco smoking is primarily attributed to the production of nitrosamines which effects the cells of the oral mucosa.[3] Numerous studies published in the literature have described the ill effects of tobacco smoking on the oral mucosal cells.[4,5,6] There is no study in current literature describing the effects of tobacco smoking on the oral buccal mucosal cells undergoing fixed orthodontic treatment. Few available studies have described the effects of the orthodontic appliances on the oral buccal mucosal cells but none studying the effects of tobacco smoking during the course of orthodontic treatment.[7,8,9]

Exfoliative cytology is a simple and inexpensive procedure for studying the epithelial cells.[10] Ever since its application in cervical pathology, it has found application in study of oral mucosal cells.[10,11] It is painless and well accepted by the patients and has found usefulness in a lot of research studies.[4,5,6,7,8,9] This study is thus aimed at evaluating the cytomorphometrical changes on the oral buccal mucosal cells in patients undergoing fixed orthodontic treatment.

2. MATERIALS AND METHODS:

This cross-sectional study involved adult patients undergoing fixed orthodontic treatment at College of Dental Surgery, BP Koirala Institute of Health Sciences, Nepal. The study group consisted of twenty adult individuals undergoing fixed orthodontic treatment for at least six months duration and who had habit of tobacco

smoking. Individuals with habit of alcohol consumption, tobacco chewing, denture wearers, with systemic diseases and potentially malignant & malignant oral disorders were excluded from this study. Comparisons were made with two control groups: Postive control (n=20) including adult orthodontic patients not having habit of tobacco use and Negative control (n-=20) including adult individuals not undergoing orthodontic treatment and who were non-smokers. Ethical approval was obtained from Nepal Health Research Council prior to conducting the study. Sample was collected following the principles of exfoliative cytology from the oral buccal mucosa after taking informed consent.

The sample included oral buccal mucosal scrapings collected with a sterile wooden tongue depressor. Smears were prepared on a clean glass microscopic slide after which the cells were fixed using a spray fixative following which staining was done with PAP stain using the RAPID PAP (Biolabs, India) kit, following the recommendations of the manufacturer. The slides were then assessed under a binocular light microscope (Olympus BX 20, Japan) for adequacy of the smear. Digital images were obtained for 20 non-overlapping cells with clear boundaries per slide at 400x magnification. Digital image of the calibrations of a stage micrometer was also obtained at similar magnification (Figure 1).

Cytomorphometric analysis was done with the Image J software developed by the National Institute of Health, USA (Figure 2). The parameters which were studied included: nuclear diameter (ND), cell diameter (CD), ND:CD ratio, nuclear area (NA), cell area (CA) and NA:CA ratio. The values were entered in Microsoft excel sheet.

Statistical analysis was done using SPSS software version 11.5. One way ANOVA test was done to study the difference of the various parameters among the groups and Post hoc Test (Tukey test) to study the differences between the groups.

3.1 Results:

Based on the results, it was observed that the cell diameter showed a significant difference (p=0.024) among the various study groups. Group-wise comparison revealed that there was a significant reduction (p=0.032) in the cell diameter of orthodontic patients who were tobacco smokers as compared to normal individuals not undergoing any orthodontic treatment who were non-smokers. The results are summarized in tables 1, 2 and 3.

3.2 Discussion:

Based on the above findings no significant differences were observed among the various parameters other than the cell diameter among various study groups. These findings are in agreement with the findings of Ferreira et al and Sharma et al who found insignificant differences in nuclear & cellular parameters in smokers as compared to non-smokers.[12,13] The nuclear diameter showed a reduction in smokers undergoing orthodontic treatment and in smokers not under any going orthodontic treatment as compared to normal individuals. However, these differences were not significant. Most researches that were conducted to study the effects of smoking on the oral mucosa have observed an increase in the nuclear area which is thought to be an adaptive response of the oral mucosal cells to the tobacco smoke.[4,5,14] There was no significant differences between the values of nuclear diameter between the study group and the positive controls. Similarly, cell diameter showed reduction in both the smokers group but significant differences (p=0.032) were observed only between the study group and non-smokers. This finding corresponds to the data available in many studies conducted in relation to tobacco habits. Einstein and Sivapathasundaram observed a decrease in cell diameter in their study involving tobacco users.[4] Similar results were observed by Sharma et al but these findings were not significant.[13] The values of ND:CD ratio did not show any significant differences between any of the study groups.

Comparison of the values of nuclear area and cell area showed reduction in values in orthodontic patients who are smokers and non-orthodontic patients who are smokers. However, these differences were not significant. The NA:CA ratio did not show any statistically significant differences. These findings are in contrast with that of the study conducted by Ferreira et al., Goregen et al and Ramesh et al; who suggested an increase in nuclear area of cells in case of smokers.[12,14,15] However, de Arruda et al and Raighi et al; who studied the effects of orthodontic appliances on the oral mucosa observed a reduction in the nuclear area of cells adjacent to the oral mucosa which correlated to this study.[7,9] Since this study involved orthodontic patients who were smokers, the observed results could be based on the prolonged chronic irritation of the oral buccal mucosal cells rather than the tobacco smoke. Finally, Sharma et al in their study had observed a significant increase in nuclear diameter and nuclear area in oral squamous cell carcinoma patients as compared to normal individuals and tobacco users suggesting that the cytomorphometric changes could be relied upon for studying the cellular alterations in response to various agents and for detecting any malignant alterations.[13]

The varying results of this study in comparison to the previous literature could be attributed to the small sample

size of this study. A larger sample size could have provided more comparable results. Also, the frequency and duration of the tobacco smoking habit was not considered which could have resulted in variable results.

4. CONCLUSION:

The cytomorphometric analysis of oral buccal mucosal cells revealed a reduction of cell diameter of oral buccal mucosal cells in tobacco smokers undergoing fixed orthodontic treatment.

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Table 1: Anova test showing comparison of the mean values of various parameters among the different study groups.

Variable	Group 1 (Mean±SD)	Group 2 (Mean±SD)	Group 3 (Mean±SD)	p Value
ND	8.671 ±0.902	8.515 ±1.367	9.222 ±0.857	0.098
CD	52.228 ±6.567	53.014 ±9.329	58.28 7±5.742	**0.024**
ND:CD	0.171 ±0.015	0.166 ±0.013	0.162 ±0.019	0.185
NA	65.13 2±11.574	64.630 ±20.450	72.610 ±13.330	0.203
CA	2449.97 3±665.772	2399.138 ±851.214	2866.668 ±547.890	0.077
NA:CA	0.031±0.005	0.030±0.004	0.027 ±0.004	0.066

Table 2: Tukey test showing comparison of nuclear diameter, cell diameter and ND:CD ratio between the three study groups.

Groups Compared		Nuclear Diameter		Cell Diameter		ND:CD ratio	
		Mean Diff	**p**	**Mean Diff**	**p**	**Mean Diff**	**p**
Group 1	Group 2	0.155	0.890	-0.785	0.939	0.005	0.523
	Group 3	-0.550	0.241	-6.058	**0.032**	0.009	0.162
Group 2	Group3	-0.706	0.101	-5.272	0.070	0.003	0.728

Table 3: Tukey test showing comparison of nuclear area, cell area and NA:CA ratio between the three study groups.

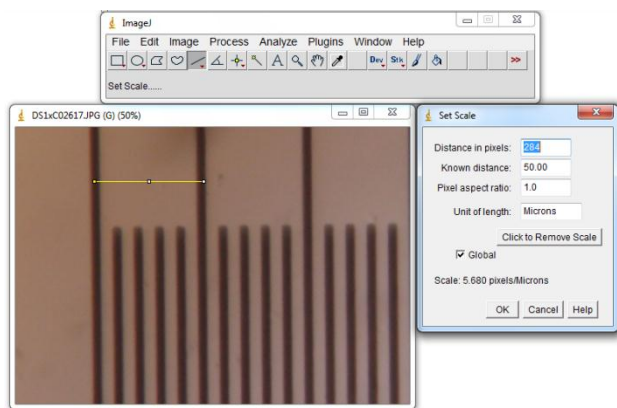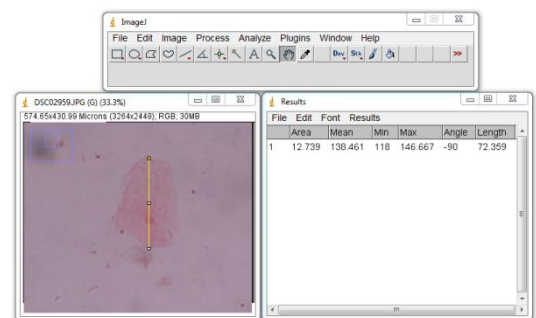| **Groups Compared**		**Nuclear Area**		**Cell Area**		**NA:CA ratio**	
		Mean Diff	p	Mean Diff	p	Mean Diff	p
Group 1	Group 2	0.501	0.994	50.834	0.971	0.001	0.788
	Group 3	-7.477	0.291	-416.71	0.153	0.003	0.061
Group 2	Group3	-7.979	0.247	-467.548	0.096	0.002	0.230

Figure 1: Calibration done using the image of a stage micrometer.

Figure 2: Analysis of cell diameter using Image J.