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Genotoxic Analysis of Oral Buccal Mucosal Cells in Patients Undergoing Fixed Orthodontic Treatment - A Case Control Study

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ABSTRACT:

Objective: A cross-sectional study was undertaken to analyse the genotoxic effects occurring during fixed orthodontic treatment on the cells of oral buccal mucosa. Materials & Methods: The study included two groups (n=20 each), one including healthy adult individuals undergoing fixed orthodontic treatment and the other served as control. Oral buccal mucosal cells were collected as per the principles of exfoliative cytology and stained using a Rapid PAP kit. Micronuclei count was done using a compound light microscope in 500 non overlapping cells in a step ladder fashion. Results: Statistical analysis revealed a significant increase in the number of cells showing micronuclei ($p<0.001$) and the total number of micronuclei ($p<0.001$) in patients undergoing fixed orthodontic treatment. Conclusion: Based on these findings it can be suggested that fixed orthodontic treatment causes genotoxic effects on the oral buccal mucosal cells.

KEYWORDS: exfoliative cytology, fixed orthodontic treatment, micronuclei, oral buccal mucosal cells.

INTRODUCTION:

The micronuclei (MN) has been a subject of numerous studies recently.[1,2,3] A micronuclei has been described as a microscopically visible; round to oval cytoplasmic chromatin mass next to the nucleus (figure 1).[4] The micronuclei is thought to arise from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division.[5] The micronuclei can be easily assessed in erythrocytes, lymphocytes and exfoliated epithelial cells including the oral buccal mucosal cells and can be used as a biomarker for genetic toxicology in vivo. This can be achieved by performing the micronuclei assay (MN assay).[6]

The oral buccal mucosal cell is an ideal candidate for performing the micronuclei assay since it acts as the first barrier for establishing contact with different carcinogenic agents and metabolizing it into reactive products. Hence, any early genotoxic events induced by the carcinogenic agents can be assessed satisfactorily in these cells.[4,6] The Micronuclei assay has been used to assess the genotoxic effects of tobacco products, alcohol and many other potentially carcinogenic agents.[7,8] Studies have also been performed in cases of potentially malignant oral disorders and oral squamous cell carcinoma[9,10]

A large number of people undergo fixed orthodontic treatment for correction of malocclusion. This includes the bonding of brackets on to the tooth and use of different types of wires for bringing about the tooth movement.[11] The treatment duration varies from person to person and may last from two to three years.[12] The oral cavity along with saliva acts in a dynamic fashion and may cause degradation of metallic components resulting in leeching out of metallic ions into the saliva.[13] Also any un-polymerized resins from the bonding agents may incorporate into the saliva and directly act upon the oral

mucosa.[14] There are few studies in the literature which analyses the genotoxic effects on the oral buccal mucosal cells occurring during orthodontic treatment.[13,14,15,16,17] This study was hence designed to evaluate the micronuclei assay among patients undergoing fixed orthodontic treatment and compare it with normal individuals.

MATERIALS AND METHODS:

A cross-sectional study was designed which involved adult patients between the age group 18-30 years visiting the department of Orthodontics, BP Koirala Institute of Health Sciences, Nepal. 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 of patients (n=20) who have had started the treatment for at least 6 months after taking informed consent. Individuals with habits related to tobacco and alcohol, suffering from any systemic diseases, potentially malignant oral disorders, oral squamous cell carcinoma or using any prosthesis were excluded from the study. Also, smears were obtained from normal individuals (n=20) which constituted the comparative group.

Scraping were collected from the oral buccal mucosa by using a sterile wooden tongue depressor and smears prepared. The smears were fixed with a spray fixative and stained with 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. Micronuclei count was done by analysing the slide at 400x magnification in a step ladder pattern. A total of 500 cells were analysed per slide according to the study conducted by Kamath et. al.[1] The number of cells showing micronuclei and also the total number of micronuclei per 500 cells were noted and the data entered in Microsoft Excel sheet, 2013. A note of the gender of the participants was also done.

Statistical analysis was done using SPSS software version 11.5. Descriptive statistics revealed that the data did not show a normal distribution. Hence, Mann Whitney U test was done to compare the number of cells showing micronuclei and the total micronuclei count between the two groups.

RESULTS:

The average number of cells showing micronuclei per 500 cells and the total number of micronuclei per 500 cells both showed a statistically significant increase in patients undergoing fixed orthodontic treatment as compared to normal individuals (table 1 & 2). The gender distribution in both the groups is shown as a bar diagram in figure 2. There was no statistically significant difference (p=0.320) in the number of cells showing micronuclei and the total number of micronuclei in relation to gender differences between both the groups.

DISCUSSION:

The micronuclear count was performed in two groups viz. patients undergoing fixed orthodontic treatment and compared with normal individuals. There was no significant difference in the micronuclear counts between males and females. A statistically significant ($p<0.001$) increase was observed among the orthodontic patients suggestive of genotoxic damage occurring in the oral buccal mucosal cells. Similar results were observed in studies conducted by Natarajan et al and Ozturk et al who observed increased frequency of micronuclei in orthodontic patients.[14,18] According to Natarajan et al the increased micronuclear count was attributed to the genotoxic effects of nickel and chromium ions which leeched from the orthodontic appliances. They observed an increased concentration of these ions in the oral mucosal cells but could not correlate it statistically.[18] Ozturk et al on the other hand suggested that the genotoxic effects on the mucosal cells could be due to adhesive cements which were not completely polymerized. They used different types of adhesive cements in their study and observed an increase in micronuclear frequency in all the groups.[14]

In comparison to the current study, contrasting results were observed in two other studies. According to Heravi et al, there was a decrease in the micronuclear frequency nine months after application of orthodontic brackets. However, this difference was statistically insignificant.[15] Toy et al. on the other hand suggested that there was no alteration in the micronuclear frequency which was measured at different times after beginning the orthodontic treatment. However, they observed an increase in other cytomorphological alterations within the buccal cells of orthodontic patients suggestive of some form on genotoxic effects.[17]

This study also measured the number of cells showing micronuclei which was not measured in other studies involving orthodontic patients. A statistically significant ($p<0.001$) increased number of cells were found to be showing the presence of micronuclei within the cytoplasm in patients undergoing fixed orthodontic treatment as compared to normal individuals. The micronuclei frequency has been used as a reliable indicator of genotoxic effects on the cells of oral mucosa as evident in

a number of studies involving the effects of tobacco and alcohol; and also in various potentially malignant oral disorders & oral malignancies.[2,3,19,20] This study is indicative of the potential genotoxic effects on the oral buccal mucosal cells occurring during the course of orthodontic treatment. However, the sample size utilized in this study was small and so a larger sample would be more indicative of these deleterious effects.

CONCLUSION:

It can be concluded that an increased number of oral buccal mucosal cells show micronuclei in its cytoplasm during fixed orthodontic treatment. Also, an increased micronuclei count among the orthodontic patients is indicative of the genotoxic effects on the buccal mucosal cells.

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DECLARATIONS:

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Conflict of interest: Nil

Ethical Approval: Obtained from Nepal Health Research Council. Informed consent obtained from the participants prior to beginning the study.

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Tabels & Figures

Table 1: Comparison of Mean number of cells showing micronuclei (per 500 cells) along with the standard deviation between normal individuals and orthodontic patients.

Sl. No.	GROUP	MEAN NO. OF CELLS SHOWING MICRONUCLEI PER 500 CELLS/ SD	Z value	p
1	Normal	19.95 +/- 7.409	- 4.993	<0.001
2	Orthodontic patients	43.50 +/- 15.726		

Table 2: Comparison of Mean number of micronuclei (per 500 cells) along with the standard deviation between normal individuals and orthodontic patients.

Sl. No.	GROUP	MEAN NO. MICRONUCLEI PER 500 CELLS/ SD	Z value	p
1	Normal	25.55 +/- 13.609	- 4.465	<0.001
2	Orthodontic patients	61.35 +/- 29.770		

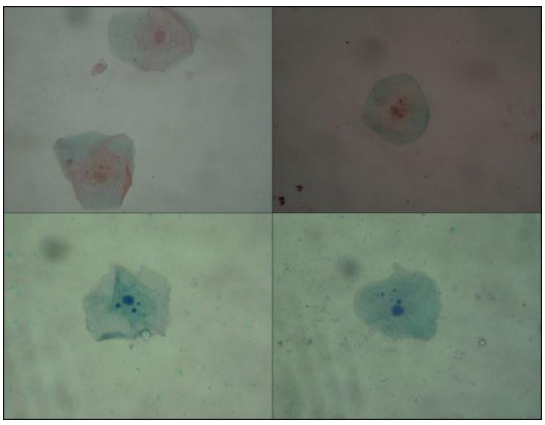

Figure 1: Cells showing micronuclei within the cytoplasm (PAP stain, 400x magnification)

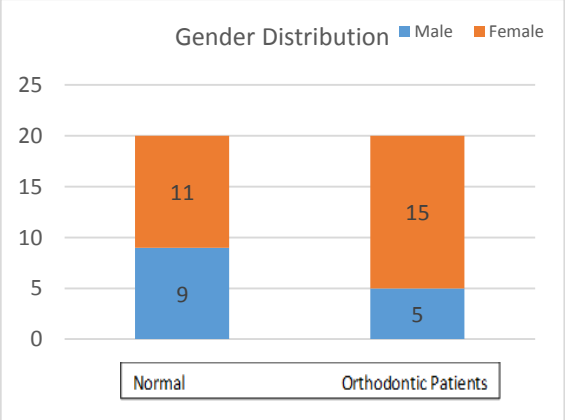

Figure 2: Gender distribution of the participants among the two study groups.