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RESEARCH ARTICLE

CYTOMORPHOLOGICAL ALTERATIONS IN ORAL BUCCAL MUCOSAL CELLS IN PATIENTS UNDERGOING FIXED ORTHODONTIC TREATMENT - A CASE CONTROL STUDY

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Abstract

Objective: This was a cross-sectional study undertaken to analyze the effects of 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 (Biolabs, Maharashtra). The slides were analyzed under a compound light microscope and images of cells taken with a digital camera. Cytomorphometric analysis was performed using Image J software.

Results: The nuclear and cellular parameters showed an overall reduction in patients undergoing fixed orthodontic treatment as compared to normal individuals. However, statistically significant reduction could be observed in the values of cell diameter (p=0.039) and cell area (p=0.047) only.

Conclusion: These findings suggest that the oral buccal mucosal cells adjacent to the orthodontic brackets shows features suggestive of atrophy.

Introduction:-

An increasing number of individuals are seeking correction of malocclusion these days. (Sharma, 2009; Singh & Sharma, 2014) This involves use of different form of treatment options ranging from removable to fixed orthodontic appliances.(Chaturvedi & Upadhayay, 2010) Fixed orthodontic treatment is the most commonly adopted procedure; and involves attachment of wires by means of brackets and bands on to the tooth surface; and subsequent application of controlled forces to bring about the tooth movement.(Jian et al, 2013) This is however, a time consuming procedure and extends to an average of around two years.(Fink &Smith, 1992)

Application of brackets and bands onto the tooth has numerous clinical implications, the chief ones being, the deterioration of oral hygiene and decalcification of tooth.(Travess, 2004) Numerous studies have observed these changes within the oral cavity.(Boyd & Baumrind, 2006; Naranjo et al, 2006; Al-Anezi, 2014) However, other undesirable effects occurring during orthodontic treatment have been sparsely mentioned in the current literature. Among these, the effect of metallic appliances on the oral mucosa needs further consideration. The oral mucosa, especially the buccal and labial mucosa are constantly exposed to friction from the orthodontic brackets and wires; frequently resulting in oral ulcerations and keratinization of the oral mucosa (table 1).(Travess, 2004) There are very few studies in the literature indicating the effect of chronic irritation of orthodontic appliances on the cells of oral mucosa.(Pereira et al, 2008; de Arruda et al, 2011; Rafighi et al, 2012; Mei et al, 2013) This study was hence undertaken to evaluate the cytomorphological alterations in oral buccal mucosal cells in patients undergoing fixed orthodontic treatment.

Materials and methods:-

This was a cross sectional study involving patients visiting the College of Dental Surgery, BP Koirala Institute of Health sciences, Nepal; and involved the cytomorphometrical comparison of oral buccal mucosal cells in patients

undergoing fixed orthodontic treatment (treatment group); with individuals not undergoing any treatment (control group). These two groups consisted of 20 healthy individuals each belonging to the age group 18-30; and in whom treatment was initiated atleast 6 months back. The study was conducted after obtaining ethical approval from the Nepal Health Research Council. All individuals meeting the above criteria were invited to participate in the study and informed consent was taken. The exclusion criteria included individuals with tobacco & alcohol drinking habits; with any systemic diseases, inflammation, potentially malignant oral disorders or malignant lesions and presence of any prosthesis or tooth restorations. Orthodontic treatment was undertaken with stainless steel bands & brackets; and Nickel-Titanium (NiTi) & stainless steel wires at different points of time.

Sample collection:-

Oral buccal mucosal cells were obtained by following the principles of exfoliative cytology. Prior to collection of sample the individual was asked to rinse his/her mouth with water to remove any debris; and then scrapings were collected from the oral buccal mucosa using a sterile wooden tongue blade and the scrapings were immediately transferred to a coded clean microscopic glass slide and fixed using a spray fixative. The slides were then stained with Papanicolaou's (PAP) stain using the RAPID PAP$^{(T)}$ kit according to the instructions provided by the manufacturer. The slides were then analyzed for the cell yield and quality of staining, under a compound light microscope (Olympus BX 20, Japan).

Data extraction:-

Data was extracted by performing cytomorphometric analysis on all the samples collected. Slides were observed at 400x magnification and images of 20 individual cells showing clear nuclear & cell boundaries; and that did not overlap with adjacent cells were taken using a digital camera at a fixed zoom of 2x. For calibration, image of the graduated markings of a stage micrometer was also taken with the same zoom settings of the camera as done for each of the smears. The images were then transferred to a computer and analyzed using the public domain Java based software Image J 1.48V; developed by the National Institute of Health (NIH), USA. First calibration was done using the image of the stage micrometer and then 20 cells per slide were analyzed. The cytomorphometrical parameters that were studied included: nuclear diameter (ND), cell diameter (CD), ND:CD ratio, nuclear area (NA), cell area (CA) and NA:CA ratio. The findings were immediately entered into Microsoft excel data sheet.

Stastical analysis:-

Statistical analysis was done using SPSS software version 11.5. Descriptive statistics were performed. Comparative analysis of the various parameters between the two groups were done using independent't' test.

Results:-

The average measurements for nuclear diameter (ND), cell diameter (CD) and ND:CD ratio have been summarized in table 1. Similarly, average values of nuclear area (NA), cellular area and NA:CA ratio for both the groups have been summarized in table 2. Overall there is a general decrease in the various nuclear and cellular parameters in patients undergoing fixed orthodontic treatment as compared to the normal group. However, statistically significant difference was observed in the values of cellular area and cellular diameter between the two groups (Table 1 & 2).

Discussion:-

Cytomorphometric analysis to compare the cells of the oral buccal mucosa between patients undergoing fixed orthodontic treatment and normal individuals revealed that the cells showed an overall reduction in size in orthodontic patients. It was observed that there was a decrease in the nuclear diameter in orthodontic patients; which was not statistically significant. However, a significant ($p=0.039$) decrease in cell diameter was observed in orthodontic patients as compared to normal individuals. The nuclear diameter to cell diameter ratio between normal individuals showed a reduction which was not statistically significant. Direct comparison of these findings cannot be made with other articles available in the literature; since there are only few studies that evaluated the effect of fixed orthodontic treatment on the oral buccal mucosal cells that included analysis of nuclear area, cell area and the nuclear to cell area ratio only.

Comparison of nuclear and cellular areas of patients undergoing fixed orthodontic treatment revealed that there was a reduction in both these parameters as compared to normal individuals. However, statistically significant (0.047) differences were observed only in the values of cellular area. These findings are in agreement with that of de Arruda et. al. and Rafighi et .al.(de Arruda et al, 2011; Rafighi et al, 2012) Contrasting results were obtained by Mei et. al.

who suggested that the cytoplasmic area showed an increase in orthodontic patients. However, this was suggested to be an adaptive response of the oral buccal mucosal cells to orthodontic brackets, since the sample were collected one month after placement of brackets and showed a return to baseline values after removal of brackets. (Mei et al, 2013) Similarly, Pereira et. al. also observed an increase in cell area, two months after placement of orthodontic brackets.(Pereira et al, 2008) Both the above mentioned studies involved experimental application of few brackets in normal individuals for a short period of time. The present study involved collection of samples from actual orthodontic patients, atleast 6 months after initiation of the treatment. The nuclear area to cell area ratio showed a decrease in value which was not found to be statistically significant. These parameters were analyzed only among healthy adult patients requiring orthodontic treatment resulting in a smaller sample size during the study period. A larger sample size would have indicated more representative results.

The above observations suggest that the oral buccal mucosal cells adjacent to orthodontic brackets undergo atrophic changes. According to de Arruda, this is an adaptive phenomenon of the buccal cells to counter the frictional effects of the orthodontic brackets, which if persistent to could lead to cell death. (de Arruda et al, 2011)

Cytomorpological analysis has been performed in a number of studies; involving the changes in oral mucosal cells due to tobacco related habits and in oral squamous cell carcinoma. There are varied results but still these serve as valid indicators of mucosal changes in such conditions (Khandelwal and Solomon, 2010; Hande and Chaudhary, 2010; Goregen et al, 2011 and Acarya et al, 2013). So, more studies involving patients undergoing fixed orthodontic treatment and using a larger sample size is needed in the future to establish a fair indication of cellular changes occurring in these patients.

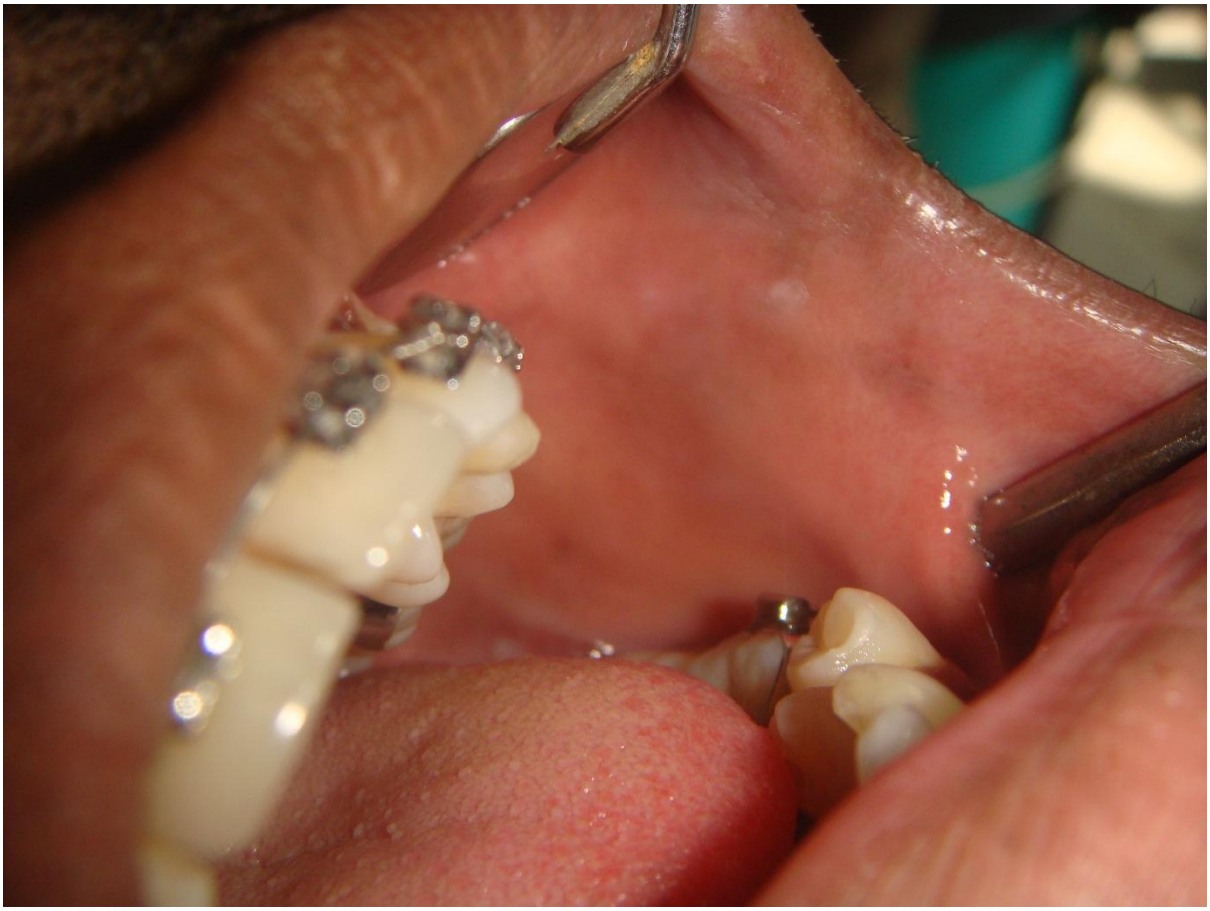

Figure 1: Keratinization of buccal mucosa adjacent to the brackets and arch-wires

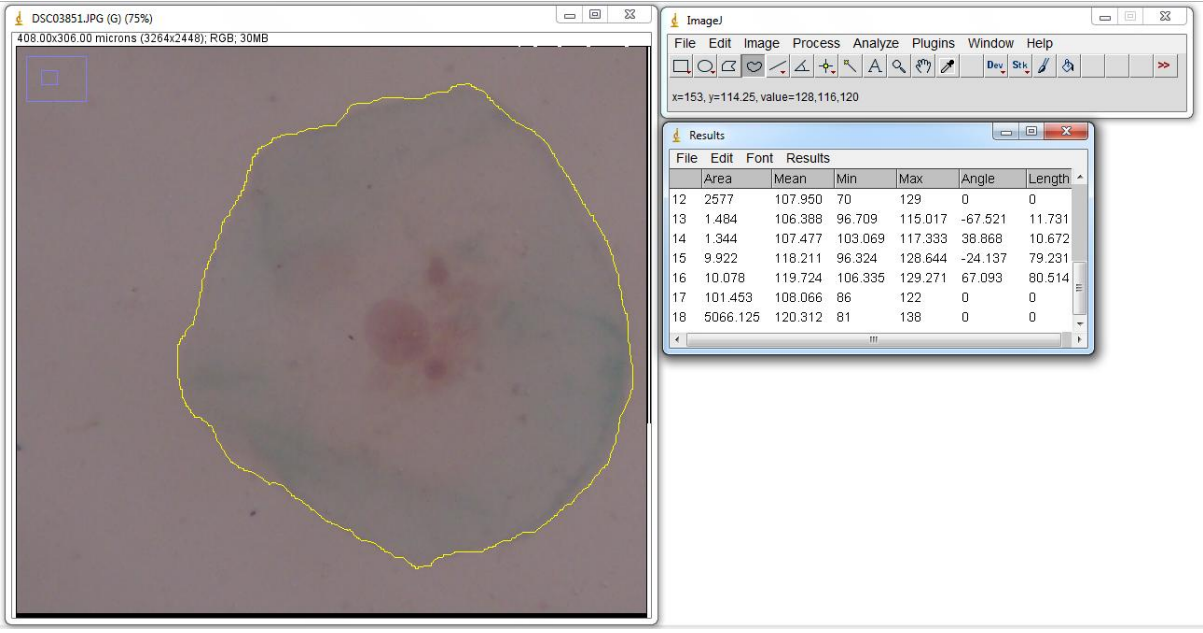

Figure 2: Image J analyzer used for calculation of cell area. Additionally, the cell shows two micronuclei.

Group	n	ND		t	p	CD		t	p	ND:CD		t	p
Normal	20	Mean	9.222006	2.0639	0.059	Mean	58.28715	2.0369	**0.039**	Mean	0.162144	2.0301	0.461
		SD	0.857749			SD	5.742978			SD	0.019113		
Orthodontic Patients	20	Mean	8.515784			Mean	53.01455			Mean	0.16608		
		SD	1.367182			SD	9.329777			SD	0.013869		

Table 1: Average Nuclear Diameter (ND), Cell Diameter (CD) and ND:CD ratio of both the groups.

Group	n	NA		t	p	CA		t	p	NA:CA		t	P
Normal	20	Mean	72.61034	2.0345	0.153	Mean	2866.687	2.0369	**0.047**	Mean	0.027632	2.0243	0.087
		SD	13.3309			SD	547.8941			SD	0.004891		
Orthodontic Patients	20	Mean	64.63092			Mean	2399.138			Mean	0.030232		
		SD	20.4503			SD	851.2142			SD	0.004482		

Table 2: Average Nuclear Area (NA), Cell Area (CA) and NA:CA ratio of both the groups.

Conclusion:-

It can be concluded that the oral buccal mucosal cells show reduction in cellular diameter and area; suggestive of atrophy in response to fixed orthodontic treatment.

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