Original Article

Cytomorphometric Analysis on the Effects of Components of Orthodontic Appliances on the Epithelial Cells of the Buccal Mucosa

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Abstract

Aim: The aim of the present study was to evaluate the effect of fixed orthodontic appliances on the epithelial cells of buccal mucosa in patients undergoing orthodontic treatment.

Materials and Methods: The study group included 30 healthy patients who were advised orthodontic treatment. Applying sterile cement spatula, scrapping of exfoliated buccal epithelial cells was performed from the middle part of the inner cheeks before starting the orthodontic treatment and at 1st week, 2nd week, 1 month, and 45 days after the treatment, followed by smearing and staining with Papanicolaou stain. Light microscope was used to score micronuclei, and independent two-tailed *t*-test was used for statistical analysis.

Results: Micronuclei were assessed during the various stages of treatment and were recorded accordingly. At 1 week, there was increase in micronuclei number compared to before starting the treatment (day 0) and at 15th day; 30th day showed decrease in number compared to 1 week but increase compared to day 0. The results of day 45 were almost equal to day 0 with a significant *P* value ($P < 0.001$).

Conclusion: Fixed orthodontic appliances induce increased micronuclei frequency, especially in the first weeks of treatment, however, these genotoxic effects tend to approach baseline levels in later period.

Keywords: *Cytomorphometry, genotoxicity, micronuclei*

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Introduction

Orthodontists in clinical practice are using the acid-etch bonding technique successfully and reliably for fixed appliances. When compared to previously used adhesives, orthodontic light-cured adhesive materials have more advantages such as ease of use and increased time for bracket placement.[1] Biocompatibility remains a concern for orthodontists despite great improvements in the development of photo-activated resin composites because for long periods of time these bonding agents are left in close proximity with oral tissue.[2] Orthodontic appliances causes alterations in the composition of microbial flora of oral cavity such as raised *Streptococcus mutans* colonization and increase in *Lactobacillus* spp, which are closely associated with dental caries,[3] and the presence of microbial plaque increasing periodontal diseases[4] and ulcerations between the bracket and mucosa.[5] Treating a patient with fixed orthodontic appliances also results in the release of metallic ions such as nickel, chromium, and cobalt into the oral environment, which is of significant clinical concern.[6] These metallic ions have genotoxic effects because of either direct interaction by causing oxidative DNA damage or indirect interaction by interfering with DNA replication, hence, necessitating that these patients be periodically evaluated for genetic damage.[7] The genotoxicity of cells can be evaluated by buccal micronuclei cytosome assay in the oral exfoliated cell in the form of micronuclei, karyolysis, karyorrhexis, pycknosis, binucleated cell, broken egg, and condensed

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chromatin.[8] Micronuclei, usually regarded as small nuclei, is a membrane-bound chromatin mass containing abnormal genetic material formed during the metaphase/anaphase of cell cycle either from a whole lagging chromosome or an acentric chromosome fragment detaching from it which do not integrate in the daughter nuclei as a repercussion of toxic exposure of cells to radiation or chemical agents.[9,10] A study by Faccioni *et al*. and Hafez *et al*. revealed that, in patients undergoing fixed orthodontic treatment, the appliances induced DNA breakage in buccal tissues. Subsequently, Angelieri *et al*., in contrast, reported that orthodontic therapy did not generate DNA damage and it was not able to enhance cytotoxicity.[11]

The present study aimed to evaluate the effects of fixed orthodontic appliances on the epithelial cells of the buccal mucosa in the form of morphometric and morphological alterations to examine cells for alterations in the cytologic criteria for malignancy at three time points.

Materials and Methods

Participants and sample

This prospective study included 30 healthy individuals who were referred for various orthodontic treatments and were evaluated for 45 days/3 months. Patient's age group ranged 18–25 years (15 male and 15 female patients). Patients with no tooth decay, no fillings, and good oral hygiene were included. Exclusion criteria included patients who neither consumed alcohol nor smoked, as well as patients with loss of more than four teeth, frequent aphthous stomatitis, and skin reactions. In addition, no patient was allowed to use oral antiseptic solution at the time of the study. Prior to the commencement of the study, patient consent was taken and approval from the institutional review board was attained.

Swab collection

Swabs were collected using a sterile cement spatula. They were collected from the buccal mucosa by scrapping from the middle part of the inner cheeks after rinsing the mouth several times with distilled water to remove exfoliated dead cells on day 0 (before the placement of brackets), day 7, day 30, and day 45. The collected aggregates are smeared on to the slide and fixed immediately to retain the cytoplasmic and nuclear details followed by staining with PAP. The stained cells were examined for micronuclei in a zigzag manner starting from one end of the slide to the other end. For a cell to be micronuclei it should measure approximately one-third the size of the nucleus [Figure 1].

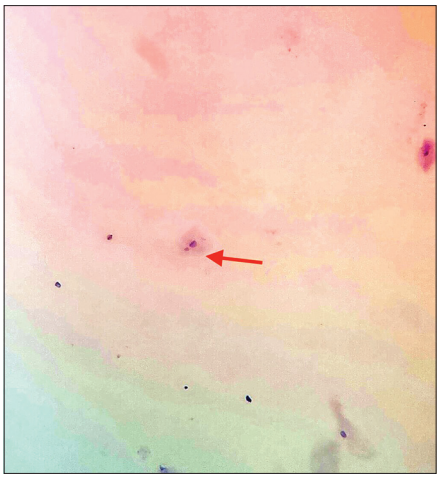

Figure 1: Photomicrograph showing Micronuclei stained with PAP stain

Statistical analysis

Descriptive measurements of this study included mean, standard deviation, and minimum and maximum values of degenerative nuclear abnormalities. The level of significance was fixed at $P < 0.05$. The data was computationally tested using Statistical package for Social Sciences (SPSS) version 11.5 (IBM SPSS Statistics) software at 95% confidence of interval of difference.

Results

Analysis was done using independent student *t*-test for estimating mean micronuclei. The mean micronuclei before placement, day 0, was 1.2 ± 0.2, 3.4 ± 0.2 on day 7; 2.4 ± 0.2 on day 15; 2.1 ± 0.2 on day 30; and 2 ± 0.2 on day 45. The frequency of Micronuclei on day 7, 15, 30 and 45 were significantly increased when compared to day 0. ($P < 0.001$). The application of orthodontic appliances increased during the first few weeks of treatment and later reached to the baseline over a period of time [Table 1].

Discussion

The appliances used for fixed orthodontic therapy are in the form of brackets, bands, and arch wires. These are manufactured to be highly corrosion resistance using stainless steel, nickel-titanium, or nickel-cobalt alloys. However, there may be some localized corrosion caused by certain oral conditions resulting in the release of metal ions. In a study by Kim and Johnson, stainless steel and nickel-titanium wires were shown to be susceptible for pitting and localized corrosion.[12] In addition to these metallic ions, the composites used for bonding the bracket also releases a variety of monomers that will have a toxic effects on adjacent oral tissue.[13] Localized corrosion can be due to plaque, acids produced by the microorganisms, and physicochemical events such as inflated levels of oxygen in saliva leading to DNA

Table 1: The mean and standard deviation values of Micronuclei

Day	*N*	Minimum	Maximum	Mean	Std. deviation
0	30	0.00	2.00	1.2±0.2	0.25
7	30	3.00	4.00	3.4±0.2	0.37
30	30	2.00	3.00	2.4±0.2	0.24
45	30	1.00	2.00	2.1±0.2	0.11

fragmentation in the buccal mucosa.[14] The residual monomers are ingested or absorbed into the digestive system through the saliva from mucosa of the mouth or pharynx.[15] The present study aimed to evaluate the long-term effects of metallic ions and residual monomers on buccal mucosa as they are reported to have harmful and perhaps synergistic effects.[16]

Extraction assay is one of the most commonly used method to study the mechanism of intraoral cytotoxicity caused by orthodontic bonding composities.[1] Micronucleus test (MNT) is an alternative and simple method for the sensitive detection of chromosomal aberrations. The exfoliated oral mucosal cells of individuals consuming tobacco products and alcohol have been examined using micronuclei assay formation.[17] The use of tobacco in fixed orthodontic appliances patients did not show any significant cellular and nuclear changes other than a reduction in the cellular diameter according to the study performed by Marla *et al*.[18] Our study did not include smokers and alcoholics.

The investigation of signs of genotoxicity or chromosomal abnormalities in mammalian cells is an intensive, time consuming process requiring well experienced personnel.[19] However, the cell culture tests are simple, accurate, reliable, and rapid, and can detect the effect not only on isolated cells derived from animal or human tissue but also in culture plates grown for *in-vitro* testing. Studies using animal models are being done to test the biocompatibility of orthodontic adhesives.

Various dental materials have been reported to have genotoxic and cytotoxic capabilities, including aberrations in chromosomal integrity, cell-cycle progression, DNA replication, and repair in normal cultured human lymphocytes, even though investigators have acknowledged that their results could not be directly applied to absolute world clinical framework.[6] Therefore, we evaluated the genotoxic and cytotoxic effects of orthodontic treatment on human oral mucosa cells in such a setting.

Evaluation of increase in the number of micronuclei within the adhesive groups by the MNT showed that the fixed orthodontic treatment had no genotoxic effects. For experimental standardization among all the adhesive groups examined, the same brand of metallic products, including brackets, tubes, arch wires, and ligature wires were applied in the fixed orthodontic treatments during the time period evaluated. According to Ozturk *et al*., band cementation had moderate genotoxic and cytotoxic effects, and hence, we applied bonded tubes to the first and second molars.[20]

While orthodontic fixed attachments bonded with different types of adhesives showed no genotoxic effects during the 2-month periods.

Gamze in a study found a significantly lowered mast cell count and suggested that nanosilver-coated orthodontic brackets have better tissue compatibility than standard orthodontic brackets.[21] In the oral cavity, mitotic activity occurs in the basal layer of the epithelium, which is exfoliated into the oral cavity within 14 days. Hence, any genetic damage caused by continuous uptake of toxic ions released from the metal alloys or due to trauma in the mucosal cells can be reflected in the exfoliated cells as micronuclei, binucleated cell, broken egg, karyolysis, karyorrhexis, and pycknosis.[22]

DNA fragmentation in the buccal mucosal cells is caused by the release of cobalt and nickel ions from metallic brackets and arch wires due to corrosion. Acid produced by microorganisms, plaque, hard calcified deposits, and certain physicochemical events, such as varying chloride combinations and elevated levels of oxygen in saliva, are corrosion inducing agents for orthodontic appliances. Studies have showed that metallic ions were released during the first 4–5 months of orthodontic treatments and afterward moved into systemic transportation. Although the concentration of ions present in saliva or blood samples was significantly less than average, dietary consumption and did not reach toxic levels.[23]

The buccal mucosal cells used for sampling are in close contact with the appliances and may experience damage due to trauma or continuous uptake of toxic ions released from metal alloys.[24] Moreover, these cells have limited potential to repair DNA, hence, they are more suitable to reveal genome instability.[25] The micronucleus is a small extranuclear DNA formed when chromosome fragment or acentric chromosomes lag behind and fails to be included in the main nuclei of daughter cells during anaphase of cell cycle.[26] Micronuclei formation occurs in the progenitor cells of the epithelium during mitosis and is reflected in the exfoliated epithelial cells as chromosomal damage.[27] In our study, MN were analyzed based on certain criteria such as the staining intensity similar to that of the nucleus and that size should be less than one-third of the diameter of the associated nucleus.

Biomarker to monitor genetic damage is the buccal micronuclei assay, which was proposed by Stich *et al*. is becoming famous. The method was initially constrained to the measurement of micronuclei frequency to evaluate the genotoxic influence of inhalation and local exposure to hazardous environmental agents, malnutrition, lifestyle, and inherited genetic defects in DNA repair.[28] It is a minimally invasive method of cell collection involving examination of cells to determine the prevalence of cells with micronuclei and other nuclear abnormalities.[29]

In this study, we used PAP for staining as it is faster, easier to process, and contains a fixative that causes of bacterial lysis and enables better visualization of micronuclei through the clear cytoplasm.[20] Orthodontic appliances lack clastogenic and/or aneugenic effects on exposed buccal cells according to a study reported by Angelieri *et al*., as there was no significant differences in the micronucleus frequencies before, during, and after orthodontic therapy.[30] Baraba *et al*. reported that *in-vitro* alterations induced by dental materials on human leukocytes are reversible, likewise, the changes produced by fixed orthodontic treatment in a minimum 1-year follow-up period did not cause apparent cytotoxic or mutagenic effects in oral mucosa cells.[31] In our study, the mean micronuclei increased during the first few weeks of the treatment, thereby reaching the baseline subsequently. This indicates that the brackets induced localized genotoxic effects, however, these changes are reversible and do not indicate any malignancy.

The effect of tobacco smoking and alcohol consumption on micronuclei formation is controversial. In a study, majority of participants consumed alcohol and tobacco, which hampered researchers in explaining the effects of individual variables.[32] No genotoxic effects with alcohol consumption were detected in a few studies. In contrast, synergistic effect between alcohol and nicotine was detected by Schweikl *et al*. Therefore, we preferred to select nonsmoking and non-drinking individuals for this study.

Research on genotoxicity and cytotoxicity in patients undergoing orthodontic therapy can provide valuable information concerning the carcinogenicity of orthodontic adhesives.

Conclusion

The results of the current study suggest that fixed orthodontic appliances induced cellular alterations due to the release of certain materials; however, these changes cannot be considered malignant because they are reversible. Furthermore, local genotoxic effects were seen during the 45 days of orthodontic treatment, which declined later. This study highlights several deleterious health-related effects of orthodontic treatment, which necessitate further investigation on a large sample to confirm and expand these findings.

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Conflicts of interest

There are no conflicts of interest.

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