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GENOTOXICITY AND CYTOTOXICITY CAUSED BY CLEAR ALIGNERS-AN IN-VIVO STUDY

Orthodontics

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ABSTRACT

Aim: To evaluate the possible cytotoxic damage to the oral mucosal cells in healthy patients undergoing orthodontic treatment with clear aligners.
Materials & Methods: 20 patients who required orthodontic treatment were chosen. The first set of aligners were fabricated for each patient using a PETG material. Patients were instructed to wear the aligners for 22 hours a day for 15 days. Buccal mucosal cells were sampled at two time intervals T0- pre treatment and T1- after 15 days and were immediately smeared onto a clean glass slide. The smears were immediately fixed in isopropyl alcohol, following which the slides were hydrated with distilled water, stained with the Papanicolaou method and subjected to cytomorphometric analysis. The two samples obtained at T0 and T1 for each patient were compared to check for an increase in number of micronucleated cells. Student Paired t Test was used to compare the mean micronuclei count between pre and post treatment.
Results: The mean Micro Nuclei count in the post treatment period was significantly higher (4.50 ± 1.96) as compared to pretreatment period (3.10 ± 1.73) with a mean difference of -1.40 at P=0.007.
Conclusion: Within the experimental limits of this study the PETG material used for fabrication of aligners resulted in a change in the nature of buccal mucosal cells with an increase in the number of micronuclei which is an indicator for cytotoxicity. The PETG material may have a cytotoxic effect on the cells of the oral mucosa.

KEYWORDS

Cytotoxicity, Clear aligners, Micronuclei assay.

INTRODUCTION

An increasing demand for esthetic orthodontic treatment in the recent years has led to a paradigm shift towards the development of clear aligner therapy[1]. Aligners are orthodontic appliances that are worn for a duration of 22 hours everyday, they adapt over the teeth and the marginal gingiva. New aligners are given at regular intervals until treatment is finished. Absolute treatment times range from 6 months to 2 years[2]. The plastic materials may be influenced by changes because of the oral environment which may lead to leaching out of products that are potentially harmful to the oral cells.

It is perceived that unpolymerized monomers can drain out of polymeric materials and conceivably cause harmful impacts on biologic frameworks. Potential ranges of cytotoxic effects include an immune reaction to material exposure, cell cycle disturbance, cell apoptosis, and induction of mutagenesis or carcinogenesis[2,3,4]. It is therefore necessary to investigate the cytotoxic potential of the clear aligner materials.

A genotoxicity risk evaluation in the buccal epithelial cells can be performed with some well-established endpoints such as the micronucleus (MN) assay[5]. Micronuclei are extranuclear cytoplasmic bodies. The damaged chromosomes, in the form of acentric chromatids or chromosome fragments, lag behind in anaphase when centric elements move toward the spindle poles. After telophase, the undamaged chromosomes and the centric fragments give rise to regular daughter nuclei. The lagging elements are included in the daughter nuclei cells too, but a considerable portion is transformed into 1 nucleus or several secondary nuclei that appear in the cytoplasm of the daughter cells as a small nuclear particle, termed an MN.[6]

Several authors have employed the the MN assay technique in a few clinical conditions as a noninvasive approach for biomonitoring: eg, as a DNA source to examine oxidative stress furthermore, the relationship of micronuclei with cancer, and in Alzheimer's disease. It has likewise been utilized to assess the impacts of genotoxic specialists like tobacco smoke, liquor, pesticides, and formaldehyde.[7-13]

Genotoxicity can be a mutagenic or a carcinogenic process. Yet, there is an inadequacy of literature about the association of these conditions with clear aligners. Hence this study was conducted to evaluate the possible genotoxic damage to the oral mucosal cells in healthy patients undergoing orthodontic treatment with clear aligners.

MATERIALS AND METHOD

20 patients reporting to The Department of Orthodontics and Dentofacial Orthopaedics of Rajarajeswari Dental College & Hospital, who required orthodontic treatment were chosen for this study. The sample size has been estimated using the GPower software v. 3.1.9.2

Considering the effect size to be measured (dz) at 67% for Two-tailed Hypothesis and 95% Confidence Interval, power of the study at 80% and the margin of the error at 5%, the total sample size needed is 20.

Patients were selected based on the following inclusion criteria: Patients with class I malocclusion with spacing, patients aged between 15-30 years. Patients presenting with systemic conditions, periodontal problems, decayed or restored teeth or prosthesis, previous history of orthodontic treatment and mutagenic hazards unconnected with occupation (eg, smoking, drinking, drug consumption, and illness related to any genetic damage, use of alcohol-based mouthwashes, any lesions on the buccal mucosa) were excluded from the study.

After obtaining written consent from the patients, a detailed case history was recorded, following which alginate impressions of the mandibular arches were made for each patient.

The first set of passive clear aligners were then fabricated with PETG material using a thermoform vaccum machine and were delivered to the patients. Patients will be instructed to wear the aligners for 22 hours everyday for a period of 15 days and also to restrain from the usage of any mouthwashes during the course of the study.

Sampling of oral mucosal cells was done as follows. Samples were collected at two time intervals, once before the appliance delivery(T0) and the second time after a period of 15 days(T1). The oral mucosal

cells were collected from each subject by gentle scraping of the inside part of the lips and buccal mucosa with a metal spatula in a sweeping motion, after rinsing the mouth several times with tepid distilled water, to remove exfoliated dead cells.

The sample obtained was immediately smeared onto the center of a clean glass slide. The smears were immediately fixed in absolute alcohol (isopropyl alcohol, 70%) following which the slides were hydrated with distilled water and stained with the Papanicolaou (PAP) method according to the standard protocol.and subjected to cytomorphometric analysis.[14]

The oral smears were observed at 40-times magnification under a light microscope by the same operator. 500 cells were examined from each subject to determine the presence of micronuclei. In the PAP method, the nuclear material appears blue, and the cytoplasm appears pink. Cells that are not smeared, clumped, or overlapped, and those that contained intact nuclei were included. Cells undergoing degenerative processes such as karyorrhexis, karyolysis, fragmentation of the nucleus, broken egg, and pyknosis were excluded. The micronuclei were identified according to the standard protocol[15] (fig 1&2).

The two samples obtained at different time intervals T0 and T1 for each patient were compared to check for an increase in number of micronucleated cells and evaluate any changes in the buccal mucosa cells.

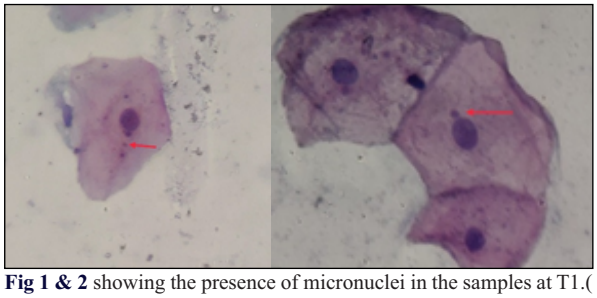


Fig 1 & 2 showing the presence of micronuclei in the samples at T1.(PAP stain 40 times magnification)

METHOD OF STATISTICALANALYSIS:

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analysis.

Descriptive analysis includes expression of Micronuclei count in terms of Mean & SD for each time interval.

Student Paired t Test was used to compare the mean micronuclei count between pre and post treatment periods using Clear aligners.

The level of significance was set at P<0.05.

RESULTS

The MN assay results and the level of cytotoxicity at each time point for the clear aligner evaluated are shown in Table 1.

Student Paired t Test results demonstrated that the mean Micro Nuclei count in the post treatment period was significantly higher (4.50 ± 1.96) as compared to pretreatment period (3.10 ± 1.73) with a mean difference of -1.40 (95% CI -2.31 to -3.50) at P=0.007.

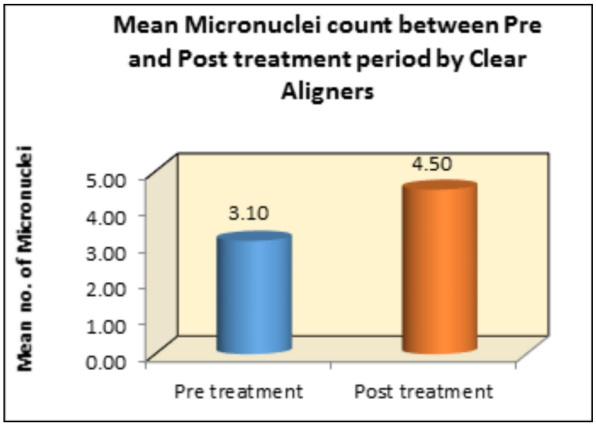
The findings conclude that the material exhibited a slight cytotoxic effect after 15 days.

Table 1:

Comparison of mean Micronuclei count between Pre and Post treatment period by Clear Aligners using Student Paired t Test							
Time	N	Mean	SD	Mean Diff	95% CI for the Diff		P-Value
					Lower	Upper	
Pre treatment	20	3.10	1.73	-1.40	-2.31	-3.50	0.007*
Post treatment	20	4.50	1.96				

Inferential statistics indicating the mean micro nucleated cell count in pre and post treatment samples:

* P , .007 indicates statistically significant differences between pre and post treatment samples.



DISCUSSION

Invisible orthodontic treatment promoted by computed-aided design and computer- aided manufacturing has become a popular orthodontic approach since invisalign was introduced by align technology in 1988. With increase in demand the emergence of various brands of clear aligners have begun in recent times.

Thermoplastics which are used for fabricating clear aligners are a type of polymer material. Polyethylene terepthalate glycol,polyurethane and polycarbonates are the most commomly used thermoplastics for manufacturing these clear aligners. [16]

The plastic materials might be affected by changes due to the oral environment and then release molecules that could be dangerous for oral cells[2,3,4]. Previous studies have reported the leaching of products from these thermoplastic materials, which are potentially hazardous to the biological framework of an individual.[4]

Leaching from thermoplastic sheets generally results in the release of monomers such as bisphenol A (BPA)[17]. The implications of BPA related from dental biomaterials were first reported in a study that assessed dental sealants[18]. BPA is known to cause skin allergies, adverse effects on the reproductive systems of animals, cell death via necrosis, and high hemolytic activity[19]. Previous studies have examined treatment outcomes and patient satisfaction associated with the Invisalign system, there is a paucity of studies that have examined systemic adverse events associated with it. Only a few studies have examined the cytotoxic effects of the Invisalign system; these 2 studies were invitro investigations, and their effects cannot be extrapolated to humans.[4]

This study sought to bridge the literature gap and evaluate the potential cytotoxic effect of aligners made of PETG material on the oral mucosal cells in vivo. The results of the study were in par with the results obtained from Premraj et al[4] and Stefano Martinaa et al[20] which concluded a positive cytotoxic effect of clear aligner materials. The current study showed a positive correlation between the usage of clear aligners and alteration in buccal mucosal cells and an increase in the micronuclei count, suggesting a cytotoxic effect of these materials.

All patients selected to participate for the study were chosen based on inclusion criteria that would result in a homogenous sample without any prevailing conditions or habits that may have an altering effect on the study. However certain factors such as stress, hormonal imbalance and fluctuation which are proven to alter the nature of buccal mucosal cells were not taken into account and may have had an impact on the results obtained[21,22] . The investigation was done by a single operator thereby preventing any bias. The exposure time interval was chosen because patients usually change aligners after 15 days.

The limited sample size owing to the scarcity of patients and economical factors was another draw back of the study. Within the limitations of this study it was noted that the materials used in fabrication of clear aligners have a possible cytotoxic effect on the buccal mucosal cells, however further studies on a larger population with a longer follow up period must be done before any final conclusions can be made on the biocompatibility of these materials.

CONCLUSION

- In conclusion, within the experimental limits of this study the PETG material used for fabrication of clear aligners resulted in a

change in the nature of buccal mucosal cells.

- The material causes an increase in the number of micronuclei in buccal mucosal cells which are an indicator for cytotoxicity.
- The PETG material may have a cytotoxic effect on the cells of the oral mucosa and therefore it is necessary to conduct extensive research on the biocompatibility before its widespread application for the fabrication of appliances.

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