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Cytokine levels in gingival crevicular fluid samples of patients wearing clear aligners

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ABSTRACT

Objective: The aim of this study was to assess & compare the changes in cytokine levels in GCF samples of patients wearing clear aligners.
Methods: GCF samples of 10 patients undergoing orthodontic treatment, for correction of lower anterior crowding with clear aligners, were collected on day 0, 1, 3, 7 and 21 using periopaper strips. The upper arch was taken as the control site. The samples collected were further analyzed using commercially available ELISA based kits.
Results: The mean levels of IL-1β at day 0, 1, 3, 7 and 21 were compared between experimental and control sites using unpaired *t*-test and it was found that the levels of IL-1β were significantly elevated on experimental site in comparison to control site.
Conclusion: It was concluded from the study that elevation in levels of IL-1β as a biomarker of orthodontic tooth movement reaches its peak after 24 h of force application. The clear aligners, as an alternative to conventional fixed orthodontic treatment, were found to be efficient in correcting mild to moderate lower arch crowding.

1. Introduction

The orthodontic tooth movement (OTM) is a complex process which is initiated by application of a force allowing tooth to move beyond its limit of physiological tooth movement that happens during functions of the stomatognathic system (like mastication), lifelong mesial eruption and active eruption of tooth in the oral cavity. During orthodontic tooth movement, the components of periodontal ligament, i.e. alveolar bone, gingiva and periodontal ligament and to some amount cementum, all are remodeled. Earlier major implications of tooth movement forces have been studied in bone since teeth must be moved in the bone, however the cellular and biological changes occur in other components of periodontium and latest research has been focused on aspects of molecular events that control this process.

The role of inflammatory and pro-inflammatory mediators and other substances due to tissue injury in orthodontic tooth movement is well known nowadays. These include prostaglandins, leukotrienes, neurotransmitters, cytokines, nitric oxide and various hormones. Orthodontic force application leads to vasodilatation in the capillaries of periodontal ligament (PDL), which in turn causes inflammatory cells to migrate and produce cytokines. This assists in the process of bone remodeling.[1] Cytokines are protein molecules that act as signals between the cells of the immune system, and are produced when the immune cells are activated. These extracellular signaling proteins are found to affect bone metabolism and thereby orthodontic tooth movement. These include Interleukin-1 (IL-1), Interleukin-2 (IL-2), Interleukin-3 (IL-3), Interleukin-6 (IL-6), Interleukin-8 (IL-8), tumour necrosis factor alpha (TNFα), gamma interferon (IFNY) and osteoclast differentiation factor (ODF).[2] The most potent among these is IL-1 which stimulates the osteoclast function and attract leucocytes and other cell mediators to process bone remodeling. IL-1 targets osteoblast cells, thereby controlling osteoclasts that resorb the bone upon activation.

IL-1 predominantly exists in two forms, α and β, of which IL-1β is of main interest since this form is involved in bone metabolism, resorption of bone, and inhibition of bone formation. The most important role in the inflammatory process is played by IL-1β and hence, it is present in abundance in inflamed gingival tissues. Their presence can be noticed 12–24 h after orthodontic force application. It plays a major role in initiating bone resorption and tooth movement.[3]

The rate of tooth movement depends on the rate of bone resorption. The speed of orthodontic tooth movement is related to cytokine release which can be detected in Gingival Crevicular Fluid (GCF). The initial fluid accumulated in a healthy gingival crevice is a transudate of

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Table 1
Descriptive statistics of comparison of IL-1β levels on control and experimental sites.

Group 1 (Aligner group)	Experimental site		Control site				
IL-1β	Mean	SD	Mean	SD	Mean difference	*t*-test value	p-value
Day 0	6.92	0.33	6.61	0.55	0.29	1.856	0.094
Day 1	15.64	0.84	6.19	0.88	8.25	20.564	< 0.001*
Day 3	10.67	1.61	6.04	0.57	5.18	16.083	< 0.001*
Day 7	7.93	1.58	5.81	0.96	2.76	8.157	< 0.001*
Day 21	7.43	1.40	6.19	0.41	2.47	8.806	< 0.001*
Unpaired *t*-test					*** Significant difference**		

interstitial fluids; whereas in inflamed or stimulated conditions, GCF is an exudate that reflects serum concentrations of metabolites.[4,5] The composition of the GCF can be analyzed to study the changes occurring during orthodontic tooth movement. Gingival crevicular fluid (GCF) and the periodontal ligament (PDL) experience molecular changes due to the mechanical stress induced during orthodontic tooth movement.

Among all the modalities available to bring about orthodontic tooth movement, clear aligners are the most aesthetic option available to correct mild to moderate malocclusions especially in adult patients who are more concerned about their appearance. Clear aligners are a series of computer or manually generated, clear, removable orthodontic appliances fabricated from thermoplastic sheets designed to bring about the desired tooth movement. Very few studies have been conducted to assess the actual treatment efficiency of clear aligners and not much has been studied about the biological & molecular changes produced by the clear aligners.

1.1. Aim & objective of the study

The present study aims at evaluating and comparing the changes in the levels of Interleukin-1β in GCF samples of patients being treated with clear aligners for mild to moderate lower arch crowding.

2. Methodology

Source & subjects of the study: A total of 10 patients who had to undergo orthodontic treatment using Kline clear aligners were selected for the study. Pre-treatment records including polyvinylsiloxane impressions and photographs were taken for the study.

Sample size & specifications: The sample size was calculated using the nMaster 2.0 software. The power of the study was taken to be 80% and confidence interval of 95% was taken. A sample size of atleast 10 patients was estimated having mild to moderate lower arch crowding (i.e. 5 mm or less). Adult patients who were compliant and had no periodontal disease were selected. Patients having probing depth more than 3 mm or periodontal bone loss were excluded from the study.

Study Design: All patients were informed about the experimental procedure and the informed consent was obtained. Four weeks before the orthodontic treatment, oral prophylaxis and assessment of the periodontal health was done. Oral hygiene instructions were given. The patients were advised at every appointment to maintain their oral hygiene.

Appliance design: Treatment records including study models, cephalometric radiographs and photographs were made. A well detailed dental impression was taken for the same using polyvinyl-siloxane impression material (Fig. 1) which was sent to the laboratory for assessment and diagnosis. These impressions were scanned into the computer software. The entire treatment was visualized and its details were then forwarded to the laboratory. The laboratory translated the treatment plan into planned tooth movements. On being satisfied with the movements, the laboratory was asked to go ahead with aligner fabrication. Afterwards, a full set of aligners was delivered with a set of instructions concerning the insertion and timing of each aligner (Fig. 2). The aligners were used by the patient and the progress was monitored.

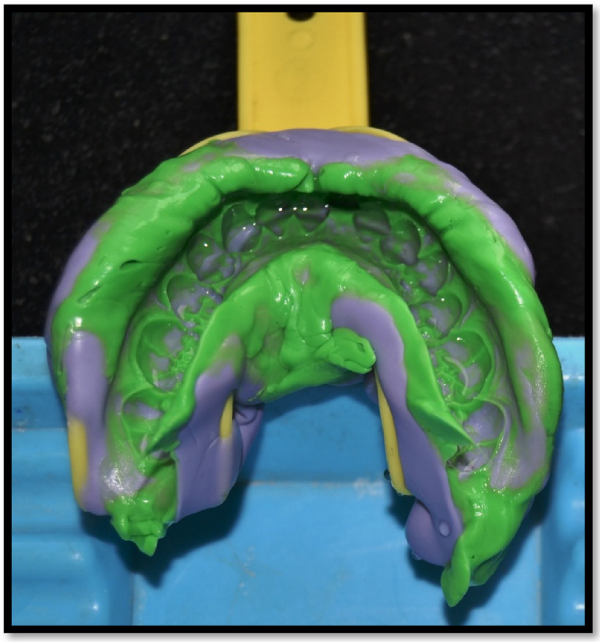

Fig. 1. Polyvinylsiloxane impression.

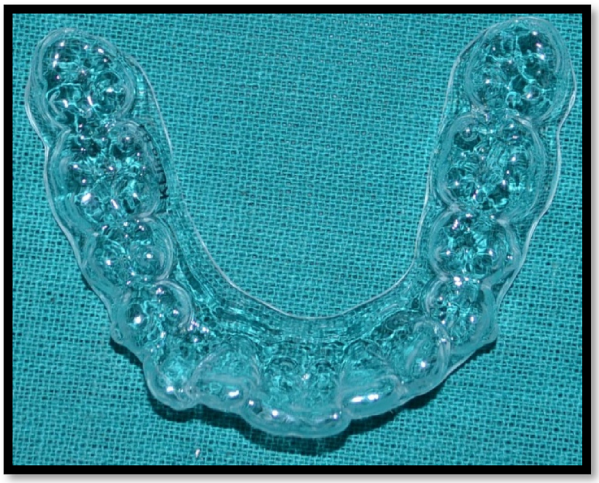

Fig. 2. Lower aligner.

The aligners were changed at an interval of 7 days for the first 4 weeks and after the first four aligners, they were changed at an interval of 2 weeks. Appropriate amount of IPR was done to create space for decrowding.

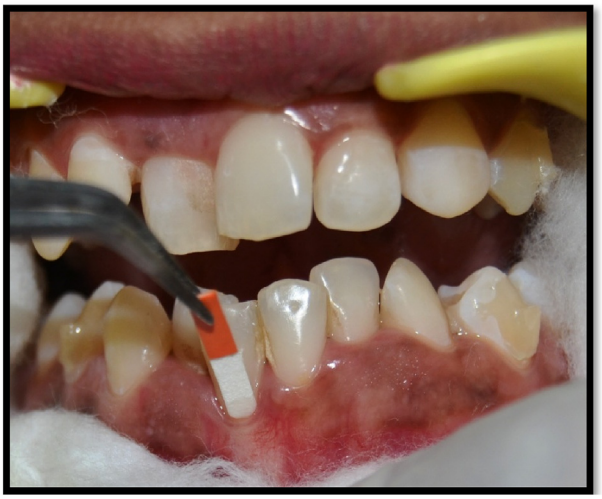

Fig. 3. GCF collection in the lower arch (experimental site).

2.1. Sample collection & assessment

GCF samples were collected before and 1,3,7, and 21 days after initiation of tooth movement from experimental as well as control sites. Before GCF collection, each site was gently dried & isolated from saliva with a cotton roll. GCF was collected by inserting periopaper strip 1 mm in the distolabial sulcus of the incisors (Loe and Holm Pederson technique) for 60 s.

The GCF sample collected from the upper incisor was taken as control since no aligner was given in upper arch in patients. The GCF sample collected from the lower incisor was taken as experimental (Fig. 3) since the aligner was placed only in the lower arch. The GCF periopaper visually contaminated with blood or saliva was discarded.

The GCF samples were then pooled into separate sealed eppendorf tubes containing 100 µl of phosphate buffer solution. The tubes were centrifuged using a centrifuge machine and the supernatant was used for further analysis to determine the levels of IL-1β with commercially available IL-1β enzyme linked immunosorbent assay (ELISA) kit.

3. Results

Data was entered into Microsoft Excel spreadsheet and analyzed by SPSS (21.0 version) and Epi-info version 3.0. Unpaired or Independent *t*-test was used for comparison of mean value between two groups and Paired or Dependent *t*-test was used for comparison of 2 mean values obtained from a same group or a pair of values obtained from the same sample. The p-value less than 0.05 was taken as significant ($p < 0.05$) and Confidence interval of 95% was taken.

In this study, it was found that the levels of IL-1β increased significantly from day 0 to day 1 at experimental sites. The levels remained elevated on day 3, and day 7 compared to day 0 and the control site. The peak was seen on day 1 (See Table 1).

The mean IL-1β at day 0, 1, 3, 7 and 21 was compared between Experimental and Control sites using the unpaired *t*-test. The mean IL-1β at day 1, 3, 7 and 21 was significantly more on Experimental site in comparison to Control site. An overall summary graph compares the changes in the levels of Interleukin 1β in gingival crevicular fluid samples collected from experimental and control sites (Fig. 4).

4. Discussion

During orthodontic tooth movement, an inflammatory process occurs which causes tissue remodeling. One of the first events of this inflammatory process is an increase in vascular permeability. It has been hypothesized that the amount of GCF production and its composition reflects these biological changes.[2]

The present study was aimed at evaluating the composition of GCF for IL-1β levels during orthodontic treatment with clear aligners and to analyze how application of orthodontic forces through the appliance affected the levels of IL-1β over the following 3 weeks after the initial force application. The upper arch was taken as control and no intervention was done. Treatment was carried out in the lower arch and hence the mandibular arch served as the experimental site. Since the patients act as their own controls in the present study design, it proves to be efficient in terms of sample size.

Due to increased concerns for aesthetics and better alternatives for orthodontic braces that are efficient in aligning teeth and at the same time do not compromise the esthetics of the patient, clear aligners have emerged as a good option. Orthodontic treatment with clear aligners consist of a series of removable appliances manufactured with clear polymer and designed to cause tooth movement in small increments from their original position to an ideal position. So far, there has been no study done to assess or evaluate the biological changes occurring during the clear aligner treatment, through the analysis of GCF composition. The present study evaluated the changes occurring in the IL-1β levels in GCF samples of patients who were undergoing clear aligner therapy.

It has been reported that the levels of IL-1β in GCF during orthodontic tooth movement are elevated significantly for the first 7 days and reach a peak at 24 h and then decrease to the baseline levels during a course of 21 days.[6,7] In the present study also, the levels of IL-1β increased significantly from day 0 to day 1 in the experimental group and remained significantly elevated for the next 2 weeks.

Luppanopornlarp et al.[8] and Ren Y et al.[7] reported peak levels of IL-1β that were attained at 24 h which is in concordance with the present study. However, a few studies also report peak levels at 4 h (Grant et al.[9]) and 3 days (Iwasaki et al.[6]). Ren Y et al.[10] systematically reviewed the studies on cytokines that are expressed in gingival crevicular fluid (GCF) during orthodontic tooth movement. Twenty-three out of eighty-five relevant studies were included and the most consistent result was found to be a peak of cytokine levels at 24 h.

Basaran G et al. (2006)[11] conducted a study on 18 patients with fixed orthodontic treatment and analyzed GCF samples collected at baseline, at day 7 and 21 and at 3rd and 6th month of the treatment. They concluded that the leveling and distalization of teeth evoke increases in both IL-1β and TNFα that can be detected in GCF. The elevated levels of IL-1β indicate efficient orthodontic tooth movement since it has been proven that during orthodontic movement there is an increase in the levels of cytokines in the GCF.

Another study by Grant et al.[9] demonstrated that high levels of proinflammatory cytokines and biomarkers of tissue and bone metabolism in GCF are associated with orthodontic force application. It also suggested that orthodontic forces for individual patients can be optimized by GCF biomarker analysis.

However, the present study had limitations regarding the sample size, sex, duration of the study and frequencies of sample collection. Sample collection and analysis for at 4 weeks, 6 weeks and 3 months was the major missing link in the study. Further research may be needed as to study the long term effects of the orthodontic force application through clear aligners on the production of IL-1β. GCF analysis of patients undergoing OTM can be a useful tool for assessing the efficiency of different appliance and techniques of orthodontic tooth movement.

5. Conclusion

After analysing the results of this study, it can be concluded that there was significant elevation in the level of IL-1β after force application in experimental group. The peak levels were attained at 24 h. The clear aligners were found to be efficient in correcting mild to moderate crowding in the lower arch. Clear aligners proved to be an

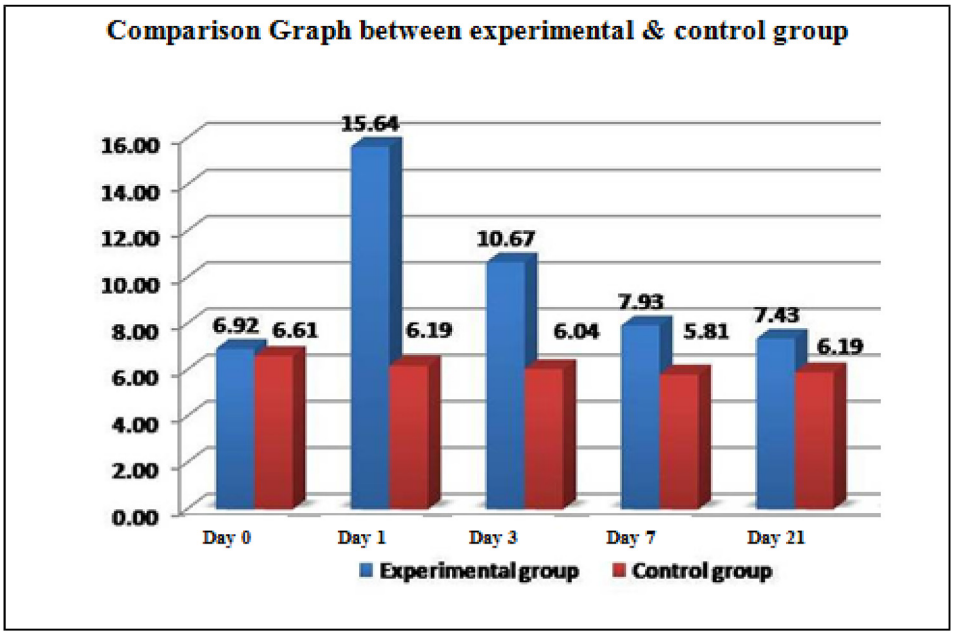

Fig. 4. Summary Graph comparing Experimental & Control Sites.

effective and aesthetic alternatives to braces for treating mild to moderate malocclusions in patients. Also, aligners are removable in nature, and hence oral hygiene maintenance is convenient leading to less issues in patients with special needs.

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