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In vivo study of gingival crevicular fluid interleukin 1-beta (IL1-β) and prostaglandin-E2 (PGE-2) levels with pain perception after placement of elastomeric separators with and without low level laser therapy: An in vivo study

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ABSTRACT

Background: Orthodontic tooth movement following application of force features bone remodelling changes in periodontal and dental tissues. These necessary orthodontic tooth movement achieved by numerous orthodontic procedures that evokes pain sensations in patients, such as separator placement, archwire placement and activations.

Objective: The aim of the study is to compare the levels of interleukin 1-beta and prostaglandin E2 in GCF with pain perception after placement of elastomeric separators with and without low level laser therapy.

Materials and Methods: 12 patients scheduled for orthodontic treatment, were screened test and control. Mesial and distal elastomeric separators flanked the maxillary first molar arch. The experimental side received 20s of Ga Al As, diode laser irradiation at 810 nm, 2 J/cm2, 200 mW power output, while the control tooth did not. GCF was collected from the mesiobuccal and mesiopalatal sides of first molars in the maxillary quadrant before, 1hr, 24 hours, and 48 hours after separator installation from both groups to quantify IL1-b and PGE2.

Result: The control and experimental group had IL-1β levels of 18.609 ng/ml (SD = 3.833) and 17.582 ng/ml (SD = 2.425) at the 'Before' time point, with p< 0.001. Significant variations in IL-1β and PGE2 were observed from baseline, with p < 0.001. After 1 hour, IL-1β levels significantly increased to 132.678 ng/ml (SD = 9.628)/ 83.848 ng/ml (SD = 8.833).In the 24-hour interval, IL-1β levels increased dramatically to 185.283 ng/ml (SD = 9.875) and 116.998 ng/ml (SD = 5.680). By 48 hours, IL-1β levels remained high at 157.459 ng/ml (SD = 10.141) and 103.664 ng/ml (SD = 9.662).

Conclusion: Low-level laser therapy has been shown to reduce pain perception and decrease inflammatory mediators IL-1β and PGE2 in GCF patients with elastomeric separators. A positive correlation exists between these biomarkers, pain perception, and laser irradiation across all time intervals.

1. Introduction

The process of orthodontic tooth movement can be described as a sophisticated phenomenon characterized by the adaptive biological reaction to disruptions in the natural balance of the dentofacial structures caused by the application of an external force.[1] The phenomenon of orthodontic tooth movement, which occurs subsequent to the application of force, can be attributed to the remodeling alterations that take place in the periodontal and dental tissues.[2] When exposed to mechanical loads of different amplitude, frequency, and duration, these tissues undergo noticeable macroscopic and microscopic alterations.[3] In this study, we aim to investigate the effects

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of a specific intervention on the cognitive development. The initial phase in the process of banding in ordinary orthodontic therapy is the separation of teeth in order to generate interproximal space. In orthodontic practice, separators are frequently employed to establish interdental gaps, primarily in the molar area. This is done to facilitate precise positioning of orthodontic bands. The posterior teeth, being subjected to greater masticatory forces compared to the front teeth, necessitate the use of separators for optimal placement.[4] Various separation methods have been employed in contemporary orthodontics, encompassing a range of techniques such as brass wire, elastic rings, Kesling separators, C separators, dumbbell-shaped separators, NiTi spring separators, Kansal separators, and others.[5] Nevertheless, the elastic ring separator is commonly employed. The patient's perception of orthodontic treatments may be called into question due to the intense discomfort associated with the insertion of separators.

When a dental band is applied following an improper separation, it can lead to hyalinization of the periodontal ligament, resulting in pain.[6] Despite a multitude of recent advancements, the attainment of comprehensive pain reduction remains elusive. Various strategies have been employed in order to mitigate orthodontic discomfort. These include cognitive behavioral therapy, the utilization of chewing gum or viscoelastic wafers of varying hardness, and pharmacological interventions involving drugs like Ibuprofen or locally applied anesthetic gels.[7] Nonsteroidal anti-inflammatory medicines (NSAIDs) have been found to have the potential to alleviate orthodontic pain. However, it is important to note that their usage may have adverse effects on the process of tooth movement, as they might inhibit bone resorption.[8] Recent research have demonstrated that non-pharmacological approaches, such as LASER therapy, have emerged as a novel technological advancement in the field of dentistry. Low-level laser therapy (LLLT) has demonstrated efficacy in the suppression of inflammation, with no documented instances of significant adverse effects.[9]

Previous studies have demonstrated that irradiation within the wavelength range of 670–830 nm, with doses ranging from 1–20 J/cm2, light intensities between 10–100 mW, and durations spanning from 10 seconds to 2.7 minutes, can effectively mitigate the inflammatory process and alleviate pain.[10] The Visual Analogue Scale (VAS) has been extensively employed in many research studies as a subjective instrument for assessing pain severity.[11] In contrast, employing questionnaires as a means of evaluating patients' discomfort represents an impartial method for measuring pain associated with separators. Prostaglandin E2 (PGE2) and interleukin 1-beta (IL-1β) are present in the gingival crevicular fluid (GCF). The release of biomarkers occurs in the periodontal ligament (PDL) and the gingival crevicular fluid (GCF) shortly after the application of pressure.[12] Mediators, such as prostaglandin E2 and interleukin-1β, play a role in the modulation of the pain pathway. Interleukin-1 beta (IL-1β), a multifunctional cytokine belonging to the interleukin family, has a significant role in regulating bone metabolism and actively participates in the inflammatory processes.[13] According to existing literature, it has been suggested that the earliest indication of inflammation during orthodontic tooth movement is followed by the presence of PGE2.[14] Given the independent associations of IL-1b and PGE2 with pain, it is plausible that the considerable variation in pain experienced by individuals using elastic separators can be attributed to the differing quantities of these substances in the gingival crevicular fluid (GCF).[15] The utilization of the GCF analysis serves as a valuable diagnostic tool for establishing associations between the concentrations of inflammatory biomarkers, including cytokines (such as interleukins, tumor necrosis factor, interferons, growth factors, and colony stimulating factor), prostaglandins, leukotrienes, hydroxyproline, and their notable clinical outcomes.[16]

The process of bone remodeling during orthodontic therapy and separator implantation is closely associated with the generation of inflammatory biomarkers, specifically prostaglandin-E2 and interleukin-1-beta.[17] The previous investigations have not provided a comprehensive understanding of the impact of low-level laser therapy on the pain pathway and its influence on inflammatory biomarkers, including prostaglandin E2 (PGE2) and interleukin-1 beta (IL-1β).[18] Therefore, the objective of this study was to establish a correlation between the inflammatory markers IL-1b and PGE2 and the experience of pain following the implantation of elastomeric separators. Additionally, we sought to examine the levels of these markers in gingival crevicular fluid (GCF) with and without the use of low level laser treatment (LLLT).

2. Materials and methods

2.1. Source of data

This study comprised patients who underwent treatment at the Department of Orthodontics and Dentofacial Orthopaedics, M. R. Ambedkar Dental College and Hospital, located in Bengaluru, Karnataka, India. The inclusion of patients in this study was contingent upon obtaining approval from the ethics committee (EC128). In this investigation, all patients included provided written and informed consent. A cohort of 12 individuals, all of whom were above the age of 15, underwent assessment to examine the impact of low level laser therapy (LLLT) on pain perception subsequent to the insertion of elastomeric separators.

Additionally, the study aimed to compare the levels of two inflammatory markers, namely IL-1b and PGE2, in gingival crevicular fluid (GCF) both with and without the use of LLLT.

2.2. Inclusion and exclusion criteria

In this study, inclusion and exclusion criteria were set to choose suitable participants. Patients over 15 with good general health and no systemic disorders were eligible for extensive orthodontic treatment[19]. Participants also had to have a healthy periodontium with a generalized probing depth of less than 3 mm and no radiographic bone loss. Inclusion required adequate oral cleanliness (gingival index scores below 1), completely erupted first and second molars, and antagonist teeth in the opposing dental arch.However, exclusion criteria excluded certain groups. Patients on NSAIDs or opioids within the month before the study and those on antibiotics during the last 6 months were excluded. Excluded were people with systemic illnesses, oral lesions or pathologies, pain from any orthodontic technique save separator placement, posterior open bite, and interdental gaps, significant caries, or fixed or removable prosthesis. Participants with poor oral hygiene (Gingival index scores > 1) and periodontal disease were excluded from this study. These criteria were carefully designed to choose a homogeneous and suitable research participant group.

The utilization of elastomeric separators (manufactured by American Orthodontics, located in Sheboygan, Wisconsin, USA) is observed on both the mesial and distal sides of the maxillary first molars in every patient. The teeth in the experimental and control groups of these patients were subjected to cleaning using a prophylaxis paste that did not contain fluoride.[20] This cleaning process involved the use of rubber prophylactic cups for a duration of 10 seconds. Following the cleaning, the teeth were rinsed and dried before the separators were placed. In the control group, no irradiation was administered following the implantation of elastomeric separators. In contrast, the experimental group received low level laser treatment (LLLT) using the AMD Lasers LLC device, a product of A DENTSPLY International Company, USA, after the placement of separators.[21] In order to assess the degree of pain following the placement of separators, patients were provided with post-treatment instructions and cautioned against the simultaneous use of analgesic medications.

In this study, a diode laser manufactured by AMD Lasers LLC, DENTSPLY, USA, was utilized for the purpose of delivering low-level laser treatment to the experimental molar subsequent to the implantation of a separator.[22] The diode laser employed in this experiment was composed of Gallium-Aluminum-Arsenide (GaAlAs) and operated at a wavelength of 810-nm. The energy density of the laser was set at 2 J/cm2, while the power output was maintained at 200 mW for a duration of 20 seconds. In the experimental group, a total of 10 doses of laser irradiation (LLLT) were administered, with 5 doses applied on the buccal side and 5 treatments on the palatal side of the experimental teeth, specifically targeting the cervical third of the roots.[23] Gingival crevicular fluid (GCF) was collected at four distinct time intervals from the mesiobuccal and mesiopalatal sides of the first molars in the maxillary quadrant of each patient, belonging to both the experimental group that underwent low level laser therapy (LLLT) and the control group.[24]

The subsequent time intervals were employed for the purpose of gathering the Gingival crevicular fluid (GCF):

1. T0: Prior to the application of elastomeric separators.
2. T1: One hour after the placement of separators, with and without the implementation of low-level laser therapy on the experimental and control sides, respectively.
3. T2: Twenty-four hours after the placement of separators, with and without the utilization of low-level laser therapy.
4. T3: Forty-eight hours after the placement of separators, with and without the implementation of low-level laser therapy on the experimental and control sides, respectively.

The GCF was obtained by employing a calibrated volumetric micro capillary tube (10-100μL, Fisher Scientific, Leicestershire, UK) with graduated markings at 5μL intervals until a standardized volume of 20μL was attained. Capillary tubes manufactured by Eppendorf A G in Hamburg, Germany, with a certain internal diameter, were carefully placed into the entrance of the gingival fissure. Gingival crevicular fluid (GCF) was then gathered into the capillary tubes through the process of capillary action. All samples that were tainted were discarded.

The GCF samples were diluted using 250 μL of sterile phosphate buffered saline (pH 7.4) and afterwards stored at a temperature of -80°C until they were analyzed to assess the levels of IL1-b and PGE2. The samples were sent to the laboratory in a thermos-sealed package containing ice packs in order to conduct an ELISA test using the Parameter Assay Kit from R&D Systems, located in McKinley Minneapolis, USA.

2.3. The assessment of pain perception via the Visual analog scale (VAS)

The researchers measured the pain perception (PP) following the installation of elastomeric separators by utilizing a 10 cm visual analogue scale (VAS) at four different time points: T0, T1, T2, and T3[25]. Following the placement of elastic separators, patients were administered a pain questionnaire for a duration of 7 days. They were requested to indicate their pain levels on a 10 cm Visual Analog Scale (VAS)[26]. Participants were instructed to

indicate the degree of pain experienced in both the control and experimental quadrants at three specific time intervals: 1 hour, 24 hours, and 48 hours following the installation of separators, both with and without the application of low level laser therapy (LLLT).

2.4. Statistical analysis

IBM Corp., Armonk, NY's 2013 SPSS for Windows 22.0 was used to analyze data in this study. To summarize the data, descriptive statistics were used. Frequencies and proportions were used for categorical data, whereas mean and SD characterized continuous values. Data correlations and differences were examined using inferential statistics. At different time intervals, the Mann Whitney Test was used to evaluate GCF levels of IL-1β and PGE2, as well as VAS scores between the two sides. The study used Repeated Measures Analysis of Variance (ANOVA) and Bonferroni's post hoc test to compare GCF IL-1β and PGE2 levels across time points. Friedman's Test and Wilcoxon Signed Rank post hoc test were used to compare mean VAS scores between time intervals on each side. Throughout the analyses, the significance level was set at $P < 0.05$, ensuring that observed differences and correlations were considered statistically significant if the probability of their occurrence due to random chance was less than 5%. These statistical approaches were crucial in deriving meaningful insights from the data and drawing valid conclusions regarding the relationships and disparities observed in the study.

3. Results

Throughout the duration of the trial, it was seen that all 12 individuals consistently upheld proper oral hygiene practices. The Chemiluminescent assay was conducted to evaluate the concentrations of interleukin 1-beta (IL-1b) at four distinct time periods (T0, T1, T2, and T3) in the absence of low level laser therapy (LLLT).The study evaluated mean GCF IL-1β levels (ng/ml) across control and experimental groups at various time intervals using Independent Student t Tests. Prior to intervention, the control side had a mean IL-1β level of 18.609 ng/ml with an SD of 3.833, while the experimental side had 17.582 ng/ml with an SD of 2.425. The mean difference was 1.028 ng/ml, however the p value was 0.44, indicating no statistical significance (Table 1). IL-1β levels significantly rose in both control and experimental groups after 1 hour. The control side had a mean IL-1β level of 132.678 ng/ml (SD = 9.628), while the experimental side had 83.848 ng/ml (SD = 8.833). A substantial difference between the two sides was observed, with a mean difference of 48.829 ng/ml and a p-value of < 0.001. At 24 hours post-intervention, IL-1β levels remained considerably higher. The mean IL-1β level in the control group was 185.283 ng/ml (SD = 9.875), while in the experimental group, it was 116.998ng/ml (SD = 5.680), a difference of 68.285 ng/ml. A substantial difference ($p < 0.001$) was observed. At 48 hours post-intervention, IL-1β levels remained substantially higher than baseline. Results: The control group had a mean IL-1β level of 157.459 ng/ml (SD = 10.141) while the experimental group had a mean of 103.664 ng/ml (SD = 9.662), a difference of 53.795 ng The significant difference between the control and experimental groups at this time point is indicated by the p-value, $p < 0.001$. The intervention led to a considerable increase in GCF IL-1β levels, indicating an inflammatory response in the examined patients.

Gingival crevicular fluid (GCF) Prostaglandin E2 (PGE2) levels (pg/ml) were compared between control and experimental groups at various time intervals using the Independent Student t Test. Before any intervention, the control side had a mean PGE2 level of 49.971 pg/ml with an SD of 4.556 and the experimental side 50.860 with an SD of 6.255. The baseline mean difference was -0.889 pg/ml, and the p-value was 0.69, showing no significant difference (Table 2). Both control and experimental groups had significant PGE2 increases one hour post-intervention. Control PGE2 levels averaged 157.878 pg/ml (SD = 16.222) and experimental levels 111.797 (SD = 12.990). The mean difference was 46.082 pg/ml, with a p-value of $p < 0.001$, indicating a significant difference between the two groups at this time.PGE2 levels increased significantly 24 hours post-intervention. The control side had 255.254 pg/ml (SD = 28.500) and the experimental side 145.958 (SD = 25.459). A substantial difference between the control and experimental groups was observed (mean difference = 109.296 pg/ml, p-value = < 0.001).Also, 48 hours post-intervention, PGE2 levels remained increased. The control side had a mean PGE2 level of 96.019 pg/ml (SD = 5.338) and the experimental side 69.884 (SD = 10.154), a difference of 26.135 pg/ml. The significant difference between the control and experimental groups at this time point is indicated by the p-value, $p < 0.001$. These results show that GCF PGE-2 levels increased significantly after the intervention, indicating a strong inflammatory response in the individuals.

The study compared mean GCF levels of IL-1β (ng/ml) and PGE2 (pg/ml) in the control group at various time intervals using the Repeated Measures of ANOVA Test. At the 'Before' time point, IL-1β levels ranged from 14.44 to 28.81 ng/ml, with a mean of 18.609 ng/ml (SD = 3.833). Meanwhile, PGE2 levels were 49.971 pg/ml (SD = 4.556), ranging from 42.32 to 57.88. Significant variations in IL-1β and PGE2 were observed from baseline, with p-values < 0.001 (Table 3). After one hour, IL-1β levels considerably increased to 132.678 ng/ml (SD = 9.628), ranging from 114.57 to 145.68 ng/ml. PGE2 levels also increased to 157.878 pg/ml (SD = 16.222), ranging from 125.36 to 185.54. Over the 24-hour period, IL-1β levels increased dramatically to 185.283 ng/ml (SD = 9.875), ranging from

Table 1: Differential GCF IL-1β Levels: unveiling significance in elastomeric separator variants. A atatistical exploration using independent student t test. control vs. Experimental: Laser therapy influence examined.

Comparison of mean GCF IL-1β levels (ng/ml) between 2 sides at different time intervals using Independent Student t Test						
Time	**Side**	**N**	**Mean**	**SD**	**Mean Diff**	**p-value**
Before	Control	12	18.609	3.833	1.028	0.44
	Experimental	12	17.582	2.425		
1 hr	Control	12	132.678	9.628	48.829	<0.001*
	Experimental	12	83.848	8.833		
24 hrs	Control	12	185.283	9.875	68.285	<0.001*
	Experimental	12	116.998	5.680		
48 hrs	Control	12	157.459	10.141	53.795	<0.001*
	Experimental	12	103.664	9.662		

Table 2: Differential GCF PGE-2 Levels: Unveiling significance in elastomeric separator variants. A statistical exploration using independent student t Test. Control vs. Experimental: Laser therapy influence examined.

Comparison of mean GCF PGE2 levels (pg/ml) between 2 sides at different time intervalsusing Independent Student t Test						
Time	**Side**	**N**	**Mean**	**SD**	**Mean Diff**	**p-value**
Before	Control	12	49.971	4.556	-0.889	0.69
	Experimental	12	50.860	6.255		
1 hr	Control	12	157.878	16.222	46.082	<0.001*
	Experimental	12	111.797	12.990		
24 hrs	Control	12	255.254	28.500	109.296	<0.001*
	Experimental	12	145.958	25.459		
48 hrs	Control	12	96.019	5.338	26.135	<0.001*
	Experimental	12	103.664	9.662		

165.68 to 198.54 ng/ml. Meanwhile, PGE2 levels rose to 255.254 pg/ml (SD = 28.500), ranging from 210.25 to 298.65. After 48 hours, IL-1β levels remained increased at 157.459 ng/ml (SD = 10.141), ranging from 141.41 to 174.52 ng/ml. However, PGE2 levels declined dramatically to 96.019 pg/ml (SD = 5.338), ranging from 88.47 to 104.35. Results show dynamic changes in IL-1β and PGE2 levels in the control group over time, demonstrating a complicated and time-dependent inflammatory response in gingival tissues.

The study compared mean GCF levels of IL-1β (ng/ml) and PGE2 (pg/ml) at various time intervals in the experimental side using the Repeated Measures of ANOVA Test. At the 'Before' time point, IL-1β levels ranged from 12.69 to 21.85 ng/ml, with a mean of 17.582 ng/ml (SD = 2.425). Meanwhile, PGE2 levels were 50.860 pg/ml (SD = 6.255), ranging from 41.25 to 59.64. Significant variations in IL-1β and PGE2 were observed from baseline, with p-values < 0.001(Table 4). After one hour, IL-1β levels rapidly increased to 83.848 ng/ml (SD = 8.833), ranging from 66.58 to 98.65 ng/ml. PGE2 levels also increased to 111.797 pg/ml (SD = 12.990), ranging from 95.84 to 138.46.IL-1β levels increased considerably over 24 hours, reaching a mean of 116.998 ng/ml (SD = 5.680) and ranged from 104.25 to 128.25 ng/ml. PGE2 levels also rose to 145.958 pg/ml (SD = 25.459), ranging from 117.25 to 201.36.

After 48 hours, IL-1β levels remained increased at a mean of 103.664 ng/ml (SD = 9.662), ranging from 88.65 to 118.25 ng/ml. In contrast, PGE2 levels declined dramatically to a mean of 69.884 pg/ml (SD = 10.154), ranging from 59.68 to 88.65. The results show that IL-1β and PGE2 levels in the experimental group alter over time, indicating a complicated and time-dependent inflammatory response in gingival tissues.

There were substantial variations in Gingival Crevicular Fluid (GCF) Interleukin-1β (IL-1β) levels in the Control (Ct) group throughout time. A significant drop in IL-1β levels was seen between 'Before' and '1 hr', with a mean difference of -114.07 (95% CI: -124.29 to -103.85) and a p-value of <0.001*. IL-1β levels decreased significantly from 'Before' to '24 hrs' and '48 hrs', with mean differences of -166.67 (95% CI: -175.98 to -157.37) and -138.85 (95% CI: -147.39 to -130.31), both with p-values <0.001*(Table 5). Comparing IL-1β levels at '1 hr', '24 hrs', and '48 hrs' in the Control side showed substantial differences. The mean difference between '1 hr' and '24 hrs' was -52.606 (95% CI: -61.894 to -43.317) and '1 hr' and '48 hrs' was -24.782 (95% CI: -36.275 to -13.288), both with p-values <0.001*. In the Control group, IL-1β levels showed a significant difference between '24 hrs' and '48 hrs', with a mean difference of 27.824 (95% CI: 17.674 to 37.975) and a p-value <0.001*.

On the Experimental (Ex) side, significant changes in IL-1β levels were detected over time. IL-1β levels decreased significantly at 'Before', '1 hr', '24 hrs', and '48 hrs', with mean differences of -66.267 (95% CI: -74.848 to

Table 3: Comparative analysis of GCF IL-1β Levels (ng/ml) and PGE2 Levels (pg/ml) at Different time intervals in the control side. Exploring variations through repeated measures ANOVA Test and independent student t Test."

Comparison of mean GCF IL-1β levels (ng/ml) and PGE2 levels (pg/ml) between different time intervals in Control side using Repeated Measures of ANOVA Test						
Time	**N**	**Mean**	**SD**	**Min**	**Max**	**p-value**
Before	12- IL-1β	18.609	3.833	14.44	28.81	
1 hr	12- IL-1β	132.678	9.628	114.57	145.68	<0.001*
24 hrs	12- IL-1β	185.283	9.875	165.68	198.54	
48 hrs	12- IL-1β	157.459	10.141	141.41	174.52	
Before	12- PGE2	49.971	4.556	42.32	57.88	
1 hr	12- PGE2	157.878	16.222	125.36	185.54	<0.001*
24 hrs	12- PGE2	255.254	28.500	210.25	298.65	
48 hrs	12- PGE2	96.019	5.338	88.47	104.35	

Table 4: Variations in GCF IL-1β Levels (ng/ml) and PGE-2 Levels (pg/ml) Over Time: Exploring dynamics in the experimental side through repeated measures ANOVA test

Comparison of mean GCF IL-1β levels (ng/ml) and PGE2 levels (pg/ml) between different time intervals in Experimental side using Repeated Measures of ANOVA Test						
Time	**N**	**Mean**	**SD**	**Min**	**Max**	**p-value**
Before	12- IL-1β	17.582	2.425	12.69	21.85	
1 hr	12- IL-1β	83.848	8.833	66.58	98.65	<0.001*
24 hrs	12- IL-1β	116.998	5.680	104.25	128.25	
48 hrs	12- IL-1β	103.664	9.662	88.65	118.25	
Before	12- PGE2	50.860	6.255	41.25	59.64	
1 hr	12- PGE2	111.797	12.990	95.84	138.46	<0.001*
24 hrs	12- PGE2	145.958	25.459	117.25	201.36	
48 hrs	12- PGE2	69.884	10.154	59.68	88.65	

-57.686), -99.417 (95% CI: -105.31 to -93.525), and -86.082 (95% CI: -96.122 to -76.043), all with p-values <0.001In the experiment, IL-1β levels showed a significant difference between '1 hr' and '24 hrs', with a mean difference of -33.15 (95% CI: -43.557 to -22.743) and a p-value <0.001*. Comparing IL-1β levels between '1 hr' and '48 hrs' revealed a significant difference, with a mean difference of -19.816 (95% CI: -31.759 to -7.872) and a p-value of 0.001*. Finally, comparing '24 hrs' and '48 hrs' on the Experimental side showed a mean difference of 13.334 (95% CI: 4.067 to 22.601) and a p-value of 0.004*.(*p-values < 0.05 are significant.) IL-1β levels fluctuated throughout time in both the Control and Experimental groups, indicating significant variability in inflammatory responses.

Bonferroni's post hoc test was used to compare Control (Ct) and Experimental (Ex) Gingival Crevicular Fluid (GCF) Prostaglandin E2 (PGE2) levels. On the Control side, PGE2 levels significantly decreased from 'Before' to '1 hr', '24 hrs', and '48 hrs', with mean differences of -107.91 (95% CI: -122.87 to -92.947), -205.28 (95% CI: -233.28 to -177.29), and -46.048 (95% CI: -51.996 to -40.101), all with p-values <0.001*.

The Control group showed significant differences in mean differences between '1 hr' and '24 hrs' and '1 hr' and '48 hrs', with p-values <0.001*. Additionally, a significant difference was found between '24 hrs' and '48 hrs' in the Control side, with a mean difference of 159.235 (95% CI: 132.143 to 186.327) and a p-value <0.001* (Table 6). In the experiment, 'Before' showed significant reductions compared to '1 hr' and '24 hrs', with mean differences of -60.937 (95% CI: -76.25 to -45.624) and -95.098 (95% CI: -121.27 to -68.928), both with p-values <0.001*.

However, comparing 'Before' to '48 hrs' showed a significant decrease of -19.024 (95% CI: -29.932 to -8.116) with a p-value of 0.001*. In the Experimental side, '1 hr' and '24 hrs' had a mean difference of -34.162 (95% CI: -62.497 to -5.826) with a p-value of 0.02*. Significant changes were identified between '1 hr' and '48 hrs', with a mean difference of 41.913 (95% CI: 29.43 to 54.395) and a p-value <0.001*. Finally, a significant difference was found between '24 hrs' and '48 hrs' on the Experimental side, with a mean difference of 76.074 (95% CI: 49.393 to 102.756) and a p-value <0.001*. (*p-values < 0.05 are significant.) These findings show that PGE2 levels change over time in both Control and Experimental groups, highlighting inflammatory response alterations.

The Mann-Whitney Test was used to compare mean Visual Analogue Scale (VAS) pain scores between Control and Experimental groups at different time intervals (Table 7). On Day 1, the Control group had a mean VAS score for pain of 7.58 (SD = 1.00), substantially greater than the Experimental group's 4.75 (SD = 1.22). The Mann-Whitney Test showed a significant difference ($p < 0.001$*) in

Table 5: Uncovering significant differences in GCF IL-1β Levels between time intervals in control (Ct) and Experimental (Ex) Sides: A multiple comparison analysis utilizing bonferroni's post hoc test

Multiple comparison of mean diff. in GCF IL-1β levels b/w time intervals in Control(Ct) and Experimental (Ex) side using Bonferroni's post hoc Test					
(I) Time	**(J)Time**	**Mean Diff (I-J)**	**95% CI for the Diff.**		**p-value**
			Lower	**Upper**	
Before-Ct	1 hr	-114.07	-124.29	-103.85	<0.001*
	24 hrs	-166.67	-175.98	-157.37	
	48 hrs	-138.85	-147.39	-130.31	
1 hr-Ct	24 hrs	-52.606	-61.894	-43.317	<0.001*
	48 hrs	-24.782	-36.275	-13.288	
24 hrs-Ct	48 hrs	27.824	17.674	37.975	<0.001*
Before-Ex	1 hr	-66.267	-74.848	-57.686	<0.001*
	24 hrs	-99.417	-105.31	-93.525	
	48 hrs	-86.082	-96.122	-76.043	
1 hr-Ex	24 hrs	-33.15	-43.557	-22.743	<0.001*
	48 hrs	-19.816	-31.759	-7.872	
24 hrs-Ex	48 hrs	13.334	4.067	22.601	0.004*

Table 6: Uncovering significant differences in GCF PGE-2 Levels between time intervals in control (ct) and experimental (Ex) Sides: A multiple comparison analysis utilizing bonferroni's post hoc test

Multiple comparison of mean diff. in GCF PGE2 levels b/w time intervals in Control(Ct) and Experimental (Ex) side using Bonferroni's post hoc Test					
(I) Time	**(J)Time**	**Mean Diff (I-J)**	**95% CI for the Diff.**		**p-value**
			Lower	**Upper**	
Before-Ct	1 hr	-107.91	-122.87	-92.947	<0.001*
	24 hrs	-205.28	-233.28	-177.29	
	48 hrs	-46.048	-51.996	-40.101	
1 hr-Ct	24 hrs	-97.376	-126.52	-68.234	<0.001*
	48 hrs	61.859	47.475	76.243	
24 hrs-Ct	48 hrs	159.235	132.143	186.327	<0.001*
Before-Ex	1 hr	-60.937	-76.25	-45.624	<0.001*
	24 hrs	-95.098	-121.27	-68.928	
	48 hrs	-19.024	-29.932	-8.116	
1 hr-Ex	24 hrs	-34.162	-62.497	-5.826	0.02*
	48 hrs	41.913	29.43	54.395	<0.001*
24 hrs-Ex	48 hrs	76.074	49.393	102.756	<0.001*

Table 7: Comparative analysis of mean VAS scores for pain between control and experimental sides at various time intervals using mann-whitney test

Comparison of mean VAS scores for Pain b/w Control & Experimental side atdifferent time intervals using Mann Whitney Test						
Questions	**Groups**	**N**	**Mean**	**SD**	**Mean Diff**	**p-value**
Day 1	Control	12	7.58	1.00	2.83	<0.001*
	Experimental	12	4.75	1.22		
Day 2	Control	12	5.08	0.90	2.92	<0.001*
	Experimental	12	2.17	0.72		
Day 3	Control	12	2.33	0.78	2.25	<0.001*
	Experimental	12	0.08	0.29		

Table 8: Significant variations in mean vas scores: Exploring differences between time intervals in control and experimental sides utilizing friedman's test, with further insights from wilcoxon signed rank post hoc analysis

Comparison of mean VAS scores b/w diff. time intervals in Control & ExperimentalSides using Friedman's Test followed by Wilcoxon Signed Rank Post hoc Test							
Groups	**Time**	**N**	**Mean**	**SD**	**p-value** [a]	**Sig. Diff**	**p-value** [b]
Control	Day 1	12	7.58	1.00	<0.001*	D1 vs D2	0.002*
	Day 2	12	4.25	2.14		D1 vs D3	0.002*
	Day 3	12	0.00	0.00		D2 vs D3	0.004*
Experimental	Day 1	12	4.75	1.22	<0.001*	D1 vs D2	0.002*
	Day 2	12	1.08	1.68		D1 vs D3	0.002*
	Day 3	12	0.00	0.00		D2 vs D3	0.07

Table 9: Evaluating relationships: Spearman's rank correlation test examining the association between vas scores for pain, GCF IL-1β, and PGE2 levels. statistical significance unveils insightful correlations

Spearman's Rank correlation test to assess the relationship b/w VAS scores for pain,GCF IL-1β & PGE2 levels						
Time	**Variable**	**values**	**Control**		**Experimental**	
			IL-1β	PGE2	IL-1β	PGE2
Day 1	VAS scores	rho	0.22	0.16	0.35	0.68
		p-value	0.49	0.63	0.26	0.02*
Day 2	VAS scores	rho	0.23	0.37	0.63	0.31
		p-value	0.48	0.23	0.03*	0.32

VAS scores between the Control and Experimental groups, with a mean difference of 2.83. The Control group had a mean VAS score of 5.08 (SD = 0.90) on Day 2, while the Experimental group had 2.17 (SD = 0.72).

The Mann-Whitney Test showed a significant difference ($p < 0.001$*) in mean VAS scores between the two groups, with a difference of 2.92 Day 3 showed that the Control group's mean VAS score dropped to 2.33 (SD = 0.78), while the Experimental group's dropped to 0.08 (SD = 0.29). The Mann-Whitney Test revealed a significant difference ($p < 0.001$*) in VAS scores between the Control and Experimental groups, with a mean difference of 2.25. These data show that the Control group had considerably higher pain levels than the Experimental group across all days. The Mann-Whitney Test showed that the experimental intervention reduced participant pain. (*p-values < 0.05 are significant).

Friedman's Test and Wilcoxon Signed Rank Post hoc Test were used to compare mean Visual Analog Scale (VAS) scores between Control and Experimental time periods (Table 8). On the control side: On Day 1, the Control group had a mean VAS score of 7.58 (SD = 1.00), substantially higher than Day 2's 4.25 (SD = 2.14) ($p < 0.001$*). The Wilcoxon Signed Rank Post hoc Test showed a significant difference between Days 1 and 2 ($p = 0.002$*). On Day 3, the mean VAS score was 0.00 (SD = 0.00), much lower than Day 1 and Day 2. The post hoc test showed significant differences between Day 1 and Day 3 ($p = 0.002$*) and Day 2 and Day 3 ($p = 0.004$*). The Experimental Side: The Experimental group had a substantially higher mean VAS score on Day 1 (4.75, SD = 1.22) compared to Day 2 (1.08, SD = 1.68) ($p < 0.001$*). The post hoc analysis showed significant changes between Day 1 and Day 2 ($p = 0.002$*). The mean VAS score dropped to 0.00 (SD = 0.00) on Day 3. Although not statistically significant compared to Day 2 ($p = 0.07$), pain levels decreased significantly. With considerable pain decreases in both the Control and Experimental groups, the intervention was beneficial. (*p-values < 0.05 are significant).

A Spearman's Rank correlation test was used to assess the correlation between VAS pain scores and GCF IL-1β and PGE2 levels on different days in both the Control and Experimental groups.

Day 1:The correlation between VAS scores and IL-1β levels in the Control group was weak (rho = 0.22) and not statistically significant ($p = 0.49$). The correlation between VAS scores and PGE2 levels was weak (rho = 0.16) and not significant ($p = 0.63$)(Table 8). The Experimental group showed a moderate positive correlation between VAS scores and IL-1β levels (rho = 0.35), but not statistically significant ($p = 0.26$). Significantly, VAS scores correlated positively with PGE2 levels (rho = 0.68) with a p-value of 0.02*. On Day 2, the correlation between VAS scores and IL-1β levels in the Control group was minimal (rho = 0.23) and not significant ($p = 0.48$).

VAS scores and PGE2 levels had a moderate positive correlation (rho = 0.37) but not a significant one ($p = 0.23$). The Experimental group showed a significant positive correlation between VAS scores and IL-1β levels (rho = 0.63, p-value = 0.03*). The correlation between VAS scores and PGE2 levels was weak (rho = 0.31) and not significant ($p = 0.32$).Both groups' pain assessments and GCF biomarker levels correlated differently on different days. On Day 1, pain scores and PGE2 levels were significantly

correlated in the Experimental group, suggesting a link between pain perception and prostaglandin E2 levels. (*p-values < 0.05 are significant).

Evaluating Relationships: Spearman's Rank Correlation Test Examining the Association Between VAS Scores for Pain, GCF IL-1β, and PGE2 Levels. Statistical Significance Unveils Insightful Correlations.Table 9

4. Discussion

In this present study, before the separators were placed, the mean GCF IL-1β and PGE2 levels in this study were 18.609 ± 3.833 and 49.971 ± 4.55, respectively. After one hour, the control side showed an increase in mean GCF IL-1β and PGE2 levels, measuring 132.678 ± 9.628 and 157.87 pg/ml. The levels of PGE2 and GCF IL-1β peaked around twenty-four hours, with a mean of 255.254 ± 28.500 and 185.283 ± 9.875, respectively. The biomarkers PGE2 and IL-1 β have increased and have been linked to pain perception at one hour and twenty-four hours, respectively. These results showed that increased levels of biomarkers in GCF considerably increase pain, with the most severe pain occurring about 24 hours after the elastomeric separators were placed. Pain began about an hour after that. The synergistic effect of prolonged mechanical stress induced in the periodontal ligament by elastomeric separators, which is known to induce the expression of cyclooxygenase-2 (COX-2), facilitates the formation of PGE2, and increases the formation of cyclooxygenase, may be the cause of this increased biomarker production.[27] Similarly, in the current study, synergistic up-regulation of PGE2 was observed with elevated IL-1β level following the placement of the separators. Increased nociception at peripheral inflammatory sites is a significant mechanism by which elevated cytokine levels contribute to the development of hyperalgesia. Similarly, IL-1 β, a neuropeptide released by nociceptors in the site of tissue damage that enhances the firing rate of neurons that relay nociceptive information, and pain were found to be significantly correlated by Yamaguchi et al.[28]

This provided oblique evidence for a relationship between pain, IL-1b, and PGE2 levels. The current study's findings are consistent with those of Giannopoulou et al., who found that the degree of pain following separator implantation was linked to IL-1β and PGE2 levels in GCF, which were markedly higher at treatment teeth with elastomeric separators than at control teeth (highest day 1). Subsequently, there was a significant decrease in the levels of PGE2 and IL-1β (ng/ml) at 48 hours (mean of 157.459 ± 10.141 and 96.019 ± 5.338) on the control side. Declining biomarker levels also demonstrated a correlation with pain and the VAS scores, with the latter showing a significant decrease in scores from day 1 (7.58 ± 1.00) to day 3 (0.00). The strong decomposition or absence of active force in elastomeric separators is the cause of this notable decline in biomarker levels; in the absence of force reactivation, PGE2 and IL-1β levels return to baseline within 48 hours. As a result, after the force decay of elastomeric separators, there is a decrease in discomfort because PDL cells experience less mechanical stress.[29] Our current study's findings on biomarker levels and pain perception are consistent with those of Giannopoulou et al., who found that after seven days, pain and PGE2 and IL-1β levels declined from their peak levels but did not return to the baseline. The experimental side experienced a decrease in both IL-1β and PGE2 levels when low level laser treatment (LLLT) was administered.

On average, T1 displayed considerably lower levels of PGE2 and IL-1β than the control side (111.79 pg/ml and 83.848 ± 8.833). The mean PGE2 level was 145.958 ± 25.459 and the mean IL-1β level was 185.283 ± 9.875 at their highest, which occurred around 24 hours. A considerable drop in both PGE2 and IL-1β levels was seen after 48 hours (mean of 69.884 ± 10.154 and 157.459 ± 10.14). These findings unambiguously demonstrated that LLLT dramatically reduces the rise in IL-1β and PGE2 production that elastomeric separators elicit in human PDL cells in response to mechanical therapeutic stress. Pain perception has been linked to both IL-1β and PGE2 levels, as both levels considerably dropped on the experimental side. This was demonstrated by the dramatic drop in the VAS score from Day 1 (4.75-2.22) to Day 3 (0.00). We hypothesize a relationship between laser irradiation, inhibition of IL-1β and PGE2, and pain relief because LLLT has been shown to reduce synthesis of inflammatory mediators, IL-1β and PGE2, which are the most important chemical mediators in acute phase inflammation and are thought to have pain producing activity. The most severe pain experienced was around 24 hours, but the magnitude of pain was much lower than the control side, and finally the pain disappeared around 32 hours, which was earlier than the control side due to LLLT. This suggests that low-level laser therapy (LLLT) is helpful in reducing pain perception because it decreases the synthesis of inflammatory mediators PGE2 and IL1- in stretched human periodontal ligament cells, as well as in neural tissue and more quickly matures and regenerates, especially axonal growth. Additionally, low-level laser therapy accelerates wound healing and reduces pain by modulating inflammatory responses and stimulating oxidative phosphorylation in mitochondria. The aforementioned findings corroborated a study by Mizutani K et al.,[30] which demonstrated that in effective cases, post-irradiation IL-1β and PGE2 levels were lower than pre-irradiation IL-1β and PGE2 levels. The analgesic effects of LLLT were found to be effective, and as a result, the serum IL-1β and PGE2 levels are thought to be a direct indicator of nociceptive pain.

In line with the current study, a different investigation by Shimizu N[31] revealed that interleukin (IL)-1 beta is present in the periodontal ligament (PDL) during tooth movement and is implicated in the pain-inducing process. Al-Ga-After applying a 60 mW low-power diode laser once a day for 3, 6, or 10 min (ranging from 10.8 to 36.0 J) for 1, 3, or 5 days, the results demonstrated a significant decrease in both pain perception and the downregulation of IL-1β production caused by laser irradiation.The current study found that the experimental side, which was exposed to 810 nm laser irradiation (LLLT), effectively reduced the discomfort associated with separator insertion in orthodontics. There was a notable drop in the levels of the biomarkers PGE2 and IL-1β in both the experimental and control groups, as these variables were closely related to how PDL cells responded to mechanical stress from elastomeric separators. Comparatively speaking, the side that was exposed to laser radiation experienced far less discomfort than the control side. The amount of pain perception and laser irradiation, as well as the biomarkers, IL-1β and PGE2, showed a positive correlation. Therefore, low-level laser therapy may be quite beneficial for treating discomfort in conjunction with orthodontic treatment.

5. Conclusion

Orthodontics has made great strides, but patients still associate tooth movement with pain. Fixed orthodontic appliances like bands, brackets, buccal tubes, archwires, separators, and others helps to move teeth[32]. Ordinary orthodontic banding begins with teeth separation to create interproximal space. Separators are used in orthodontics to create enough interdental space, especially in the molars, for correct band positioning. The soreness after orthodontic tooth separation or arch wire implantation deters many from getting treatment. Inflammatory exudate is released when orthodontic pressures damage the periodontal ligament (PDL) matrix[33]. Exudate from periodontal tissues contains serum and locally generated chemicals, including inflammatory mediators. PGE2 and IL-1b are strongly linked to pain. Gingival crevicular fluid contains these chemicals. Gingival crevicular fluid (GCF) can diagnose inflammatory biomarker correlations. Non-pharmacological methods for orthodontic discomfort include LASER therapy. Low-level laser treatment (LLLT) can reduce inflammation, relieve pain, and increase tissue bioactivity[34].

LLLT reduces pain without affecting tooth movement, unlike other painkillers that may slow bone resorption. This study aimed to compare IL-1β and PGE2 concentrations in gingival crevicular fluid after elastomeric separator implantation, with and without low-level laser treatment (LLLT), to examine their potential impact on pain perception. The study found that 810 nm low-level laser therapy (LLLT) reduced pain perception from the first to the third day after orthodontic separation. A drop in IL-1β and PGE2 concentrations in gingival crevicular fluid (GCF) on the experimental side was linked to a reduction in discomfort. These data corroborate LLLT's systemic and local photobiomodulation.

This implies that low-level laser therapy (LLLT) reduces PGE2 and IL1-b, which improves pain perception.IL-1b and PGE2 concentrations peaked at 24 hours in both control and experimental groups. Since the most severe pain occurred at 24 hours, this biomarker elevation may be linked to pain perception. Interestingly, both groups followed this tendency. However, pain severity and magnitude were much lower on the experimental side than the control side. Unlike the control side, where pain subsided around 48 hours, biomarkers dropped and disappeared within 32 hours on the experimental side.A significant positive correlation was found between IL-1β and PGE2 levels, pain perception, and laser irradiation. Thus, low-level laser therapy may reduce orthodontic treatment discomfort.

6. Future Prespective

Further study is needed to examine how mediators interact with other measures of bone remodelling and systemic mediators. Because pain intensity is subjective and varies among patients, it is important to standardize pain measurements among patients. Stereological investigations on laser-irradiated tissues are important to analyze the conflicting effects of lasers used to relieve pain after orthodontic force application. Costly and time-consuming assay techniques are the main drawbacks of using it in clinical practice. Additionally, a larger sample size is needed to determine the specific biomarker levels that correspond with orthodontic treatment pain.

7. Declaration

Ethics approval and consent to participate: The institutional review board of the Department of Orthodontics & Dentofacial Orthopedics, M. R. Ambedkar Dental College and Hospital, Bengaluru India approved the study protocol. All the patients provided informed consent for their data to be used in this study EC Number: EC128/ DHR Reg No: EC/NEW/INST/2020/901 dated 07-October-2020

8. Consent for Publication

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9. Author Contributions

Conceptualization: SS, SVS. Data curation: SS, RSN. Formal analysis: RSN, NT and SS. Methodology: AJ, NT. Writing- original draft: SS, AJ. Writing- review & editing: SS&AJ.

10. Conflicts of Interest

The Authors declare that there are no conflicts of interest.

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