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Assessment of salivary interleukin-1β (IL-1β), prostaglandin E_2 (PGE_2) levels and pain intensity in children and adults during initial orthodontic treatment

Amrit S. Maan and Anand K. Patil

Abstract:

OBJECTIVES: To investigate pain intensity, interleukin-1β and prostaglandin E_2 values in saliva during initial orthodontic treatment among varying age groups and their correlation between these mediators.

MATERIALS AND METHODS: Twenty healthy patients distributed equally in age and gender groups were chosen. Unstimulated saliva was collected before the placement of orthodontic fixed appliance (T_0), 1 hour after the placement of the appliance with 0.014" nickel titanium archwire (T_1), 1 month after the first visit (T_2), and 1 hour after the placement of 0.016" nickel titanium archwire (T_3). The saliva samples were then analyzed for prostaglandin E_2 and interleukin-1β using enzyme-linked immunosorbent assay. Pain intensity was measured using a numerical rating scale.

RESULTS: Prostaglandin E_2 and interleukin-1β levels had increased at T_1 followed by a drop at T_2 and a subsequent increase at T_3. The prostaglandin E_2 and interleukin-1β levels were higher in adults than children. There was an insignificant correlation between the interleukin-1β and prostaglandin E_2 changes in all the patients. No significant differences were seen in pain scores between adults and children. Insignificant correlation was seen between pain scores and prostaglandin E_2 and interleukin-1β.

CONCLUSION: Prostaglandin E_2 and interleukin-1β can be detected in saliva and are increased in during the initial orthodontic treatment but are higher in adults than children. Pain intensity was not significantly different between adults and children.

Keywords:

Adults, children, initial orthodontic treatment interleukin-1β, pain, prostaglandin E2, saliva

Objectives

Orthodontic tooth movement following application of force features remodeling changes in the periodontal and dental tissues. The release of metabolites and molecules produces cellular responses surrounding the teeth which creates an environment that is suitable for tissue resorption and deposition.[1] Biomarkers are substances that are measured and assessed as a marker of normal biological process, pharmacological responses, or pathological processes to a therapeutic intervention.[2] An excellent biomarker is one that has the capability of describing the biological condition with regard to periodontal tissue variations and connections with orthodontic tooth movement phases, be specific and sensitive to changes.[3]

Saliva is clinically informative for prognosis, clinical or laboratory diagnosis and

Department of Orthodontics and Dentofacial Orthopaedics, SDM College of Dental Sciences and Hospital, Dharwad, Karnataka, India

Address for correspondence:
Dr. Amrit S. Maan, Department of Orthodontics and Dentofacial Orthopaedics, SDM College of Dental Sciences and Hospital, Manjushree Nagar, Sattur, Dharwad - 580 009, Karnataka, India.
E-mail: amrit1192@gmail.com

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assessment of patients with oral and systemic diseases. Most biomarkers that are present in blood, urine and gingival crevicular fluid (GCF) are also present in the saliva. Saliva collection is non-invasive as compared to drawing of blood.[4]

Saliva has shown to be able to detect biomarkers such as prostaglandin E_2 (PGE_2) and interleukin-1β (IL-1β).[5,6] IL-1β, a pleiotropic cytokine of the interleukin group, has a role in bone metabolism, suppressing bone formation, inciting bone resorption, and takes part in inflammatory process.[7] It is said to be the earliest marker of bone resorption during orthodontic tooth movement followed by PGE_2.[8,9] PGE_2, a derivative of the arachidonic acid cascade, increases bone resorption by stimulation of osteoclast formation, chemotactic properties, and vascular permeability by vasodilation.[7]

A deterrent to orthodontic treatment is the experience of orthodontic pain.[10] Pain is one of the dislikes during treatment and among the fears prior to the orthodontic treatment initiation.[11] It has been shown that treatment procedures such as separator placement, orthopedic force application, archwire placement, and debonding produce pain in orthodontic patients.[10]

Thus, this research was conducted to identify and estimate of PGE_2 and IL-1β levels in saliva during initial orthodontic treatment among children and adults. This study also aimed to correlate the PGE_2 and IL-1β values for the different age groups during initial orthodontic treatment. Apart from that, a comparison of pain intensity between different age groups and the correlation between the intensity of pain and PGE_2 and IL-1β levels are to be investigated.

Materials and Methods

This was prospective research on 20 healthy patients requiring routine visits for orthodontic treatment in the Department of Orthodontics and Dentofacial Orthopaedics. Twenty patients were divided into a juvenile group aged 12 to 18 years and an adult group with ages above 18 years. Twenty patients were distributed equally in sex with 10 males and 10 females chosen in the study. The sample size was estimated using a power analysis. With an alpha error of 5% and power of 80%, a sample size of 10 for each gender group was adequate for detect the concentrations of IL-1β and PGE2. The Institutional Review Board provided ethical clearance for the study and the patients' consent were taken prior to conducting the study. The inclusion criteria for the patients were (a) healthy patients (both genders) in the group of 12 to 18 years and group of 18 years and above; (b) requiring fixed orthodontic treatment regardless of the type of malocclusion; (c) good oral hygiene; (d) without any systemic diseases; and (e) without any periodontal diseases. Patients with (a) poor oral hygiene; (b) systemic diseases such as hormonal imbalances and bone diseases; (c) periodontal diseases; (d) xerostomia; (e) history of medication during treatment; (f) tobacco related habits such as smoking, tobacco chewing, etc.; and (g) oral pre-malignant lesions were excluded. As for the ethical approval from the SDM Institutional Ethics Committee, the committee had approved the research and was allotted with an ethical clearance number IRB. No. 2016/P/ORTH/37 on 4/11/2016.

All patients were treated with MBT prescription pre-adjusted edgewise brackets (3M Gemini brackets; 3M Unitek Corporation, Monrovia, Calif) with 0.022-inch slots. A passive drool method to obtain unstimulated whole saliva was taken at 4 time periods giving at total of 80 samples from 20 patients for each biomarker. The saliva was collected in a 45 ml sterile plastic tube. The saliva was collected at time intervals of (a) T_0 – Prior to fixed orthodontic appliance placement; (b) T_1 – 1 hour after the placement of the appliance with 0.014-inch nickel-titanium archwire (Ortho Organizers Inc., United States of America); (c) T_2 – 1 month after the first visit; and (d) T_3 – 1 hour after the placement of 0.016-inch nickel-titanium archwire (Ortho Organizers Inc., United States of America).

Collected saliva was transferred into 2ml Eppendorf tubes and stored in a deep freezer at –79°C. The saliva samples were then assessed for the IL-1β and PGE_2 levels using enzyme-linked immunosorbent assay (ELISA). Commercially available IL-1β ELISA kit (Krishgen Biosystems, India) and PGE_2 ELISA kit (KinesisDx, United States of America) were used in this study and the IL-1β and PGE_2 concentrations (pg/mL) were calculated using a spectrophotometric microplate reader (Lisa Plus, India). The pain intensity at time intervals T_1, T_2, and T_3 were assessed using a numerical rating scale which ranges from 0 to 10. The patients were instructed to use the numerical rating scale reflect the intensity of pain felt.

The sample size was estimated using a power analysis. Data analysis was carried out using the software, Statistical Package for Social Sciences (SPSS) version 20.0. The mean and standard deviations of the concentrations of IL-1β and PGE_2 of each group were calculated. Two-way Analysis of Variance (ANOVA) was used to compare the IL-1β and PGE_2 and the time intervals among the gender and age groups. Tukey's multiple post-hoc procedures were done following the two-way ANOVA for pairwise comparisons. The percentage of changes of the IL-1β and PGE_2 levels in each group at different time intervals were also calculated. A comparison of IL-1β and PGE_2 values at different time points between children

and adults of the same gender groups were carried out using paired t-tests. Pearson correlation coefficient was used to study the correlation between IL-1β and PGE_2 values among each group. Mann-Whitney U test was carried out to compare pain scores at different time points between adults and children. The correlations between the pain and the levels of the biomarkers, IL-1β and PGE_2, at different time points were assessed using Spearman's rank correlation coefficient. The significance level was set at $P < 0.05$.

Results

The mean and standard deviations of PGE_2 and IL-1β results of each group are shown in Tables 1 and 2 respectively. There were significant differences between gender groups and age groups with respect to PGE_2 and IL-1β results respectively at different time points. In the PGE_2 levels, significant differences between the male children and male adults and between male adults and female adults were seen. In the IL-1β levels, significant differences between male adults and female adults and between female children and female adults were seen. Significant differences between male children and male adults were seen at T_1, T_2, and T_3. The changes in the percentage of the PGE_2 and IL-1β levels between the time intervals are shown in Tables 1 and 2, respectively.

Tables 3 and 4 showed significant differences of PGE_2 and IL-1β results between the children and adults of the same gender at each time point respectively. Tables 5 and 6 showed insignificant correlation between the changes of PGE_2 results from T_0 to T_3 with regard to the changes in IL-1β results from T_0 to T_3 in children and adults.

Table 7 showed no significant differences between children and adults in terms of the pain scores at different time intervals. Table 8 showed insignificant correlation between the PGE_2 and IL-1β levels respectively to the pain scores.

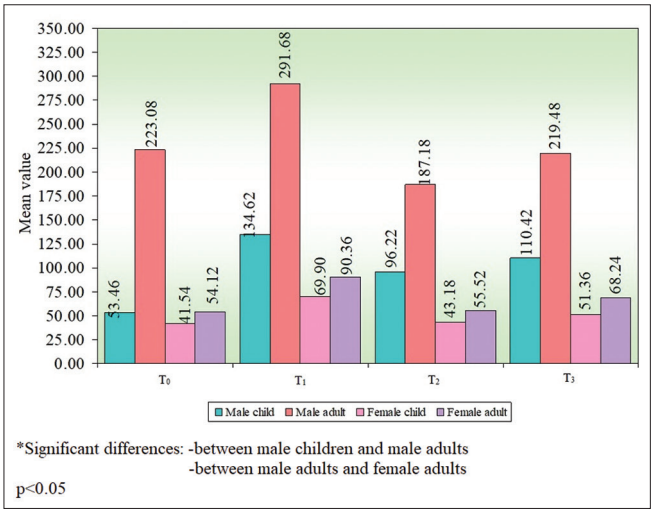

Figure 1: Comparison of gender groups with respect to PGE_2 values at different time points

Table 1: Comparison of gender and age groups with respect to PGE_2 results at different time points by two-way ANOVA, pairwise comparisons by Tukey's multiple post-hoc procedures and changes in percentage of PGE_2 levels at different time intervals

Comparison of Gender and Age Groups with Respect to PGE_2 Results at Different Time Points by Two-Way ANOVA								
Interactions	***n***	**T_0**		**T_1**		**T_2**		**T_3**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Male child	5	53.46	15.87	134.62	26.06	96.22	12.70	110.42	10.83
Male adult	5	223.08	131.05	291.68	146.24	187.18	97.54	219.48	113.30
Female child	5	41.54	9.85	69.90	13.17	43.18	5.73	51.36	5.16
Female adult	5	54.12	10.55	90.36	11.15	55.52	14.72	68.24	14.55
Between genders	*F*	9.2769		15.8248		17.1867		16.7624	
	P	0.0077*		0.0011*		0.0008*		0.0008*	
Between age groups	*F*	9.4128		7.0459		5.3760		6.0115	
	P	0.0074*		0.0173*		0.0340*		0.0261*	
Pairwise Comparisons by Tukey's Multiple Post-Hoc Procedures									
Male child vs Male adult		*P*=0.0048*		*P*=0.0203*		*P*=0.0476*		*P*=0.0381*	
Male child vs Female child		*P*=0.9918		*P*=0.5356		*P*=0.3638		*P*=0.3928	
Male adult vs Female adult		*P*=0.0050*		*P*=0.0032*		*P*=0.0037*		*P*=0.0038*	
Female child vs Female adult		*P*=0.9904		*P*=0.9720		*P*=0.9790		*P*=0.9657	
Changes in Percentage of PGE_2 Levels at Different Time Intervals									
		T_0 - T_1		**T_1 - T_2**		**T_2 - T_3**			
Male Child		151.81%		-28.52%		14.76%			
Female Child		68.27%		-38.23%		18.94%			
Male Adult		25.14%		-35.83%		17.26%			
Female Adult		66.96%		-38.56%		22.91%			

*P<0.05

Table 2: Comparison of gender and age groups with respect to IL-1β results at different time points by two-way ANOVA, pairwise comparisons by Tukey's multiple post-hoc procedures and changes in percentage of IL-1β levels at different time intervals

Comparison of Gender and Age Groups with Respect to IL-1β Results at Different Time Points by Two-Way ANOVA								
Interactions	***n***	**T_0**		**T_1**		**T_2**		**T_3**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Male child	5	4.06	2.21	11.26	3.13	5.14	0.88	7.10	1.00
Male adult	5	4.55	0.94	18.18	3.35	7.98	1.20	10.44	1.29
Female child	5	3.62	1.03	12.14	2.86	6.82	1.64	8.92	1.44
Female adult	5	11.18	1.05	24.76	2.73	13.30	0.89	17.92	1.34
Between genders	*F*	24.1418		7.5849		43.1566		66.5103	
	P	0.0002*		0.0141*		0.0001*		0.0001*	
Between age groups	*F*	40.8361		52.0378		76.5038		117.0990	
	P	0.0001*		0.0001*		0.0001*		0.0001*	
Pairwise Comparisons by Tukey's Multiple Post-Hoc Procedures									
Male child vs Male adult		*P*=0.9458		*P*=0.0114*		*P*=0.0083*		*P*=0.0040*	
Male child vs Female child		*P*=0.9588		*P*=0.9668		*P*=0.1575		*P*=0.1503	
Male adult vs Female adult		*P*=0.0002*		*P*=0.0162*		*P*=0.0002*		*P*=0.0002*	
Female child vs Female adult		*P*=0.0002*		*P*=0.0002*		*P*=0.0002*		*P*=0.0002*	
Changes in Percentage of IL-1β Levels at Different Time Intervals									
		T_0 - T_1		**T_1 - T_2**		**T_2 - T_3**			
Male Child		177.34%		-54.35%		38.13%			
Female Child		235.36%		-43.82%		30.79%			
Male Adult		299.56%		-56.11%		30.83%			
Female Adult		121.47%		-46.28%		34.74%			

*P<0.05

Table 3: Comparison of T_0, T_1, T_2, and T_3 time points with PGE_2 results in children and adults by paired *t*-test

Comparison of T_0, T_1, T_2, and T_3 Time Points with PGE_2 Results in Male Children and Male Adults by Paired *t*-test								
	Time points	**Mean**	**Std.Dv.**	**Mean Diff.**	**SD Diff.**	**% of change**	**Paired *t***	***P***
Male child	T_0	53.46	15.87					
	T_1	134.62	26.06	-81.16	13.79	-151.81	-13.1596	0.0002*
	T_1	134.62	26.06					
	T_2	96.22	12.70	38.40	22.68	28.52	3.7855	0.0193*
	T_2	96.22	12.70					
	T_3	110.42	10.83	-14.20	2.97	-14.76	-10.6976	0.0004*
Male adult	T_0	223.08	131.05					
	T_1	291.68	146.24	-68.60	40.37	-30.75	-3.7993	0.0191*
	T_1	291.68	146.24					
	T_2	187.18	97.54	104.50	59.23	35.83	3.9452	0.0169*
	T_2	187.18	97.54					
	T_3	219.48	113.30	-32.30	16.05	-17.26	-4.5008	0.0108*
Comparison of T_0, T_1, T_2, and T_3 Time Points with PGE_2 Results in Female Children and Female Adults by Paired *t*-test								
	Time points	**Mean**	**Std.Dv.**	**Mean Diff.**	**SD Diff.**	**% of change**	**Paired *t***	***P***
Female child	T_0	41.54	9.85					
	T_1	69.90	13.17	-28.36	13.56	-68.27	-4.6776	0.0095*
	T_1	69.90	13.17					
	T_2	43.18	5.73	26.72	13.17	38.23	4.5356	0.0105*
	T_2	43.18	5.73					
	T_3	51.36	5.16	-8.18	1.81	-18.94	-10.0811	0.0005*
Female adult	T_0	54.12	10.55					
	T_1	90.36	11.15	-36.24	11.85	-66.96	-6.8358	0.0024*
	T_1	90.36	11.15					
	T_2	55.52	14.72	34.84	16.04	38.56	4.8573	0.0083*
	T_2	55.52	14.72					
	T_3	68.24	14.55	-12.72	4.29	-22.91	-6.6286	0.0027*

*P<0.05

Table 4: Comparison of T_0, T_1, T_2, and T_3 time points with IL-1β results in children and adults by paired *t*-test

Comparison of T_0, T_1, T_2, and T_3 Time Points with IL-1β Results in Male Children and Male Adults by Paired *t*-test								
	Time points	Mean	Std.Dv.	Mean Diff.	SD Diff.	% of change	Paired *t*	*P*
Male child	T_0	4.06	2.21					
	T_1	11.26	3.13	-7.20	2.25	-177.20	-7.1466	0.0020*
	T_1	11.26	3.13					
	T_2	5.14	0.88	6.12	2.47	54.35	5.5467	0.0052*
	T_2	5.14	0.88					
	T_3	7.10	1.00	-1.96	0.22	-38.13	-20.0042	0.0001*
Male adult	T_0	4.55	0.94					
	T_1	18.18	3.35	-13.63	3.35	-299.56	-9.0994	0.0008*
	T_1	18.18	3.35					
	T_2	7.98	1.20	10.20	3.24	56.11	7.0487	0.0021*
	T_2	7.98	1.20					
	T_3	10.44	1.29	-2.46	0.43	-30.83	-12.8586	0.0002*
Comparison of T_0, T_1, T_2, and T_3 Time Points with IL-1β Results in Female Children and Female Adults by Paired *t*-test								
	Time points	Mean	Std.Dv.	Mean Diff.	SD Diff.	% of change	Paired *t*	*P*
Female child	T_0	3.62	1.03					
	T_1	12.14	2.86	-8.52	2.43	-235.36	-7.8386	0.0014*
	T_1	12.14	2.86					
	T_2	6.82	1.64	5.32	1.37	43.82	8.6945	0.0010*
	T_2	6.82	1.64					
	T_3	8.92	1.44	-2.10	0.27	-30.79	-17.1464	0.0001*
Female adult	T_0	11.18	1.05					
	T_1	24.76	2.73	-13.58	2.61	-121.47	-11.6473	0.0003*
	T_1	24.76	2.73					
	T_2	13.30	0.89	11.46	2.98	46.28	8.5858	0.0010*
	T_2	13.30	0.89					
	T_3	17.92	1.34	-4.62	0.95	-34.74	-10.8473	0.0004*

*$P<0.05$

Table 5: Correlation between changes in PGE_2 results from T_0 to T_3 with changes in IL-1β results from T_0 to T_3 in children by Pearson correlation coefficient

Correlation Between Changes in PGE_2 Results from T_0 to T_3 with Changes in IL-1β Results from T_0 to T_3 in Children by Pearson Correlation Coefficient							
Changes in PGE_2 results	Summary	Changes in IL-1β results					
		T_0-T_1 (Male)	T_1-T_2 (Male)	T_2-T_3 (Male)	T_0-T_1 (Female)	T_1-T_2 (Female)	T_2-T_3 (Female)
T_0-T_1 (Male)	*r*	0.0135					
	P	0.9830					
T_1-T_2 (Male)	*r*		-0.4761				
	P		0.4180				
T_2-T_3 (Male)	*r*			-0.1576			
	P			0.8000			
T_0-T_1 (Female)	*r*				0.5142		
	P				0.3750		
T_1-T_2 (Female)	*r*					0.5355	
	P					0.3520	
T_2-T_3 (Female)	*r*						-0.2113
	P						0.7330

*$P<0.05$

Figures 1 and 2 shows the mean levels of PGE_2 and IL-1β respectively from T_0 to T_3. PGE_2 and IL-1β increased in T_1 levels from baseline, followed by a drop at and a slight increase seen at T_3. Figure 3 shows the pain scores between the adults and children in which the pain recorded was greater in children than adults at all time points.

Discussion

PGE_2 which is an inflammatory mediator that causes vasodilation and induces the stimulation of osteoclast formation leading to the resorption of bone.[3,7] Shetty *et al*.[12] identified that certain drugs such as ibuprofen

Table 6: Correlation between changes in PGE_2 results from T_0 to T_3 with changes in IL-$_1$β results from T_0 to T_3 in adults by Pearson correlation coefficient

Correlation Between Changes in PGE_2 Results from T_0 to T_3 with Changes in IL-1β Results from T_0 to T_3 in Adults by Pearson Correlation Coefficient							
Changes in PGE_2 results	Summary	Changes in IL-1β results					
		T_0-T_1 (Male)	T_1-T_2 (Male)	T_2-T_3 (Male)	T_0-T_1 (Female)	T_1-T_2 (Female)	T_2-T_3 (Female)
T_0-T_1 (Male)	*r*	0.2740					
	P	0.6560					
T_1-T_2 (Male)	*r*		-0.0969				
	P		0.8770				
T_2-T_3 (Male)	*r*			-0.3278			
	P			0.5900			
T_0-T_1 (Female)	*r*				-0.1121		
	P				0.8580		
T_1-T_2 (Female)	*r*					0.6819	
	P					0.2050	
T_2-T_3 (Female)	*r*						0.6667
	P						0.2190

P<0.05

Table 7: Comparison of children and adults groups with respect to pain scores at different time points by Mann-Whitney U test

Time points	Children group			Adults group			*Z*	*P*
	Mean	SD	Mean rank	Mean	SD	Mean rank		
T_1	6.80	1.03	12.15	5.80	1.40	8.85	-1.2473	0.2123
T_2	2.90	0.74	12.60	2.30	0.82	8.40	-1.5875	0.1124
T_3	4.40	0.84	12.35	3.80	1.03	8.65	-1.3985	0.1620

P<0.05

which is a commonly used drug for pain relief can significantly inhibit the production of PGE_2 during the initial tooth movement. The use of such medications may affect the outcome of the PGE_2 levels in our study. Therefore, our study implemented that medications were not allowed throughout the study as an exclusion criterion.

A study by Kanzaki *et al*.[13] investigated the mechanical stress effects on the osteoclastogenesis in PDL cells' activity. It was found that the PGE_2 levels had increased after force application. Compressive forces and exogenous application of PGE_2 had also increased the RANKL mRNA expression. The stressed PDL cells may induce osteoclastogenesis by increasing the RANKL expression via synthesis of PGE_2 during tooth movement.

IL-1β, a pleiotropic cytokine is said to be the earliest marker of bone resorption during tooth movement in orthodontic treatment.[7,8] Uematsu *et al*.[14] studied IL-1β levels following force application on canines and found IL-1β levels had increased after 24 hours and is associated with resorption of bone during movement of teeth. They concluded that IL-1β regulated bone remodeling processes.

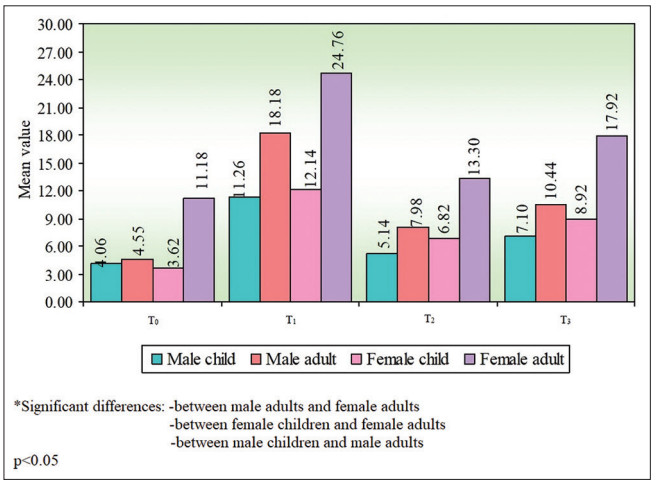

Figure 2: Comparison of gender groups with respect to IL-1β values at different time points

Luppanapornlap *et al*.[15] investigated the force magnitudes of orthodontic treatment on the levels of IL-1β secretion, the pain intensity and amount of tooth movement in canine retraction. IL-1β was found to be increased when forces were applied and correlated with the intensity of pain. The levels were higher when greater force was applied but effective tooth movement could be achieved with lighter forces with less pain. Leethanakul *et al*.[16] studied the effects of vibratory stimulus application on the secretion of IL-1β during distalization of canines. The IL-1β levels were greater at pressure sites than areas of tension. Vibratory stimulus and force application had increased the IL-1β which lead to greater bone resorption and tooth movement.

In this study, significant differences in the levels of PGE_2 and IL-1β in all groups at T_0-T_1, T_1-T_2, and T_2-T_3 were seen. An increase of PGE_2 and IL-1β at T_1 may be caused

Table 8: Correlations between pain scores and PGE_2 and IL-1β levels at different time points by Spearman's rank correlation coefficient

Correlation between PGE_2 Levels at Different Time Points by Spearman's Rank Correlation Coefficient						
PGE_2 levels	Pain Scores					
	T_1		T_2		T_3	
	Spearman *R*	*P*	Spearman *R*	*P*	Spearman *R*	*P*
T_1	-0.5048	0.1367				
T_2			-0.1711	0.6365		
T_3					-0.4917	0.1489
Correlation between IL-1β Levels at Different Time Points by Spearman's Rank Correlation Coefficient						
IL-1β levels	Pain Scores					
	T_1		T_2		T_3	
	Spearman *R*	*P*	Spearman *R*	*P*	Spearman *R*	*P*
T_1	-0.1115	0.7592				
T_2			-0.0790	0.8284		
T_3					0.0197	0.9570

P<0.05

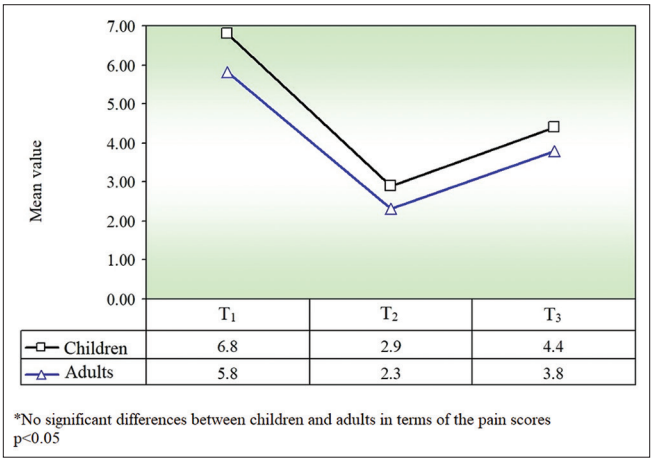

Figure 3: Comparison of children and adults groups with respect to pain scores at different time points

by the acute inflammatory process that occurs during initial orthodontic treatment. This rise of IL-1β concurs with Grieve *et al*.[17] and Kapoor *et al*.[18] in which they found IL-1β to be increased at 1 and 24 hours, whereas the levels of PGE_2 had increased later at 24 and 48 hours.

It was followed by drop in both PGE_2 and IL-1β levels at T_2. This could be associated with a force decay, lack of active force and alignment of the teeth to some degree compared to the first visit. Lee *et al*.[19] and Chibebe *et al*.[20] saw decreases of PGE_2 and IL-1β in their studies after the 24-hour mark. At T_3 in this study, there was an increase in PGE_2 which could be attributed with a larger force applied since an archwire with a greater dimension.

The PGE_2 in adults were higher than children of the same gender. This was contradicted by the studies of Chibebe *et al*.[20] where they found that juveniles displayed greater levels of PGE_2 as their inflammatory systems are more active leading to a faster response to changes in the local environment. However, a study by Ohzeki *et al*.[21] supports that older individuals produce greater amounts of PGE_2 whereby their study showed that the aged periodontal ligament fibroblasts were larger in size than the younger cells and produced greater amounts of PGE_2 when forces were applied.

IL-1β levels were greatest in female adults and with female children being slightly greater than the male children group. This disagrees with the study by Vujačić *et al*.[22] where they found the IL-1β levels to be greater in children as there is increased metabolic activity of the PDL in younger individuals and increased activity of periodontal cells. Giannopoulou *et al*.[23] on the other hand found that the young adults undergoing fixed orthodontic treatment had higher levels of IL-1β than adolescents and children which may be associated with age-related changes, puberty, and hormones. Individual variations such as IL-1β gene polymorphism affects the amount of secretion of IL-1β when same force levels were applied as shown by a study by Iwasaki *et al*.[24] All the studies related to PGE_2 and IL-1β were done in GCF and to our knowledge, no salivary research was done in relation to these biomarkers.

Another aspect to be looked into is the effect of psychological stress during orthodontic treatment. Mirzakouchaki *et al*.[25] showed that cortisol levels increased when rats were stressed leading to a reduction in monocyte numbers. The osteoclasts numbers in turn decrease as monocytes are their progenitors. Cortisol produced in stressful conditions could have also played a role in influencing the values of PGE_2 and IL-1β in this study.

The pain intensity was found to be higher in the children group than the adults but was insignificantly different. Brown *et al*.[26] found that adolescents had higher

levels of pain which may be attributed by the stage of psychological development and lower psychological well-being levels. Similarly, the children group in this study had higher pain levels. In our study, we found no significant correlation between the pain scores and both PGE_2 and IL-1β levels at the time points, T_1, T_2, and T_3. Gameiro *et al*.[27] found no significant correlation between the pain experience with the IL-1β or PGE_2 levels in GCF. Despite these mediators playing a role in investigating cellular responses to mechanical stresses, they could not be identified as the only factor involved describing the process of pain.[28,29]

One of the limitations of this study is that individual variations at a genetic level in each patient may influence the readings of the mediators studied. The severity of the malocclusion could also affect PGE_2 and IL-1β levels that were produced. A larger sample size could have increased the accuracy of the results and reduce the margin of error. Further research should be conducted on how variations in each individual can affect these mediators and the complex interplay within the periodontium during tooth movement as well as the pain intensity.

Conclusion

1. The IL-1β and PGE_2 concentrations in the adults were higher than that of the children
2. In all the groups, PGE_2 and IL-1β levels increased significantly on application of the first archwire at T_1 followed with a decrease in the levels at T_2 and a subsequent rise in these levels on placement of an archwire of larger dimension at T_3
3. Saliva is a non-invasive diagnostic aid to study the variation of the concentrations of PGE_2 and IL-1β during orthodontic treatment
4. The correlation between the variations of IL-1β and the variations of PGE_2 was not significant in all the groups
5. Pain intensity was not significant between children than adults. Insignificant correlation between the pain intensity and the biomarkers, IL-1β and PGE_2 was noted.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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