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Assessment of salivary interleukin-1 β (IL-1 β), prostaglandin E $_2$ (PGE $_2$) levels and pain intensity in children and adults during initial orthodontic treatment

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Abstract:

OBJECTIVES: To investigate pain intensity, interleukin-1 β 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-1 β using enzyme-linked immunosorbent assay. Pain intensity was measured using a numerical rating scale.

RESULTS: Prostaglandin E $_2$ and interleukin-1 β 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-1 β levels were higher in adults than children. There was an insignificant correlation between the interleukin-1 β 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-1 β .

CONCLUSION: Prostaglandin E $_2$ and interleukin-1 β 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-1 β , pain, prostaglandin E $_2$, 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

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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 E₂ (PGE₂) and interleukin-1 β (IL-1 β).^[5,6] IL-1 β , 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 PGE₂.^[8,9] PGE₂, 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 PGE₂ and IL-1 β levels in saliva during initial orthodontic treatment among children and adults. This study also aimed to correlate the PGE₂ and IL-1 β 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 PGE₂ and IL-1 β 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-1 β 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) T₀ – Prior to fixed orthodontic appliance placement; (b) T₁ – 1 hour after the placement of the appliance with 0.014-inch nickel-titanium archwire (Ortho Organizers Inc., United States of America); (c) T₂ – 1 month after the first visit; and (d) T₃ – 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-1 β and PGE₂ levels using enzyme-linked immunosorbent assay (ELISA). Commercially available IL-1 β ELISA kit (Krishgen Biosystems, India) and PGE₂ ELISA kit (KinesisDx, United States of America) were used in this study and the IL-1 β and PGE₂ concentrations (pg/mL) were calculated using a spectrophotometric microplate reader (Lisa Plus, India). The pain intensity at time intervals T₁, T₂, and T₃ 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-1 β and PGE₂ of each group were calculated. Two-way Analysis of Variance (ANOVA) was used to compare the IL-1 β and PGE₂ 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-1 β and PGE₂ levels in each group at different time intervals were also calculated. A comparison of IL-1 β and PGE₂ 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-1 β and PGE₂ 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-1 β and PGE₂, 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 PGE₂ and IL-1 β 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 PGE₂ and IL-1 β results respectively at different time points. In the PGE₂ levels, significant differences between the male children and male adults and between male adults and female adults were seen. In the IL-1 β 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 T₁, T₂, and T₃. The changes in the percentage of the PGE₂ and IL-1 β levels between the time intervals are shown in Tables 1 and 2, respectively.

Tables 3 and 4 showed significant differences of PGE₂ and IL-1 β 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 PGE₂ results from T₀ to T₃ with regard to the changes in IL-1 β results from T₀ to T₃ 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 PGE₂ and IL-1 β levels respectively to the pain scores.

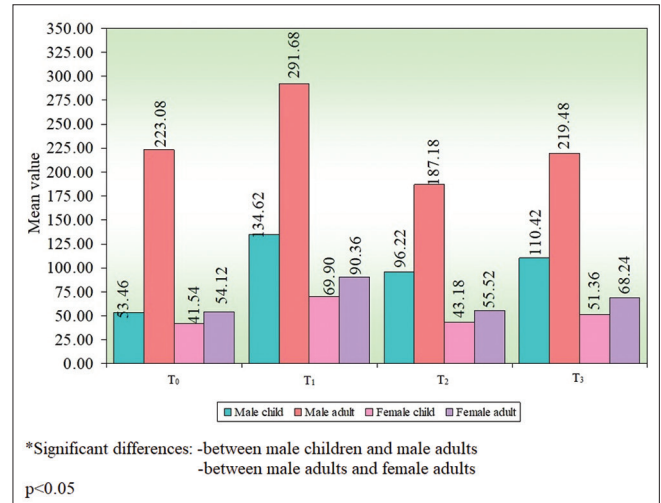


Figure 1: Comparison of gender groups with respect to PGE₂ values at different time points

Table 1: Comparison of gender and age groups with respect to PGE₂ results at different time points by two-way ANOVA, pairwise comparisons by Tukey's multiple post-hoc procedures and changes in percentage of PGE₂ 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-1 β results at different time points by two-way ANOVA, pairwise comparisons by Tukey's multiple post-hoc procedures and changes in percentage of IL-1 β levels at different time intervals

Comparison of Gender and Age Groups with Respect to IL-1 β 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-1 β 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 T₀, T₁, T₂, and T₃ time points with PGE₂ 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 T₀, T₁, T₂, and T₃ time points with IL-1 β results in children and adults by paired t-test

Comparison of T ₀ , T ₁ , T ₂ , and T ₃ Time Points with IL-1 β 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					
Male adult	T ₃	7.10	1.00	-1.96	0.22	-38.13	-20.0042	0.0001*
	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-1 β 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					
Female adult	T ₃	8.92	1.44	-2.10	0.27	-30.79	-17.1464	0.0001*
	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 PGE₂ results from T₀ to T₃ with changes in IL-1 β results from T₀ to T₃ in children by Pearson correlation coefficient

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 Children by Pearson Correlation Coefficient							
Changes in PGE ₂ results	Summary	Changes in IL-1 β 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 PGE₂ and IL-1 β respectively from T₀ to T₃. PGE₂ and IL-1 β increased in T₁ levels from baseline, followed by a drop at and a slight increase seen at T₃. 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

PGE₂ 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 PGE₂ results from T₀ to T₃ with changes in IL-1 β results from T₀ to T₃ in adults by Pearson correlation coefficient

Changes in PGE ₂ results		Summary	Changes in IL-1 β 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 ₀ -T ₁ (Male)	<i>r</i>		0.2740				
	<i>P</i>		0.6560				
T ₁ -T ₂ (Male)	<i>r</i>			-0.0969			
	<i>P</i>			0.8770			
T ₂ -T ₃ (Male)	<i>r</i>				-0.3278		
	<i>P</i>				0.5900		
T ₀ -T ₁ (Female)	<i>r</i>					-0.1121	
	<i>P</i>					0.8580	
T ₁ -T ₂ (Female)	<i>r</i>						0.6819
	<i>P</i>						0.2050
T ₂ -T ₃ (Female)	<i>r</i>						
	<i>P</i>						0.6667
							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 PGE₂ during the initial tooth movement. The use of such medications may affect the outcome of the PGE₂ 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 PGE₂ levels had increased after force application. Compressive forces and exogenous application of PGE₂ had also increased the RANKL mRNA expression. The stressed PDL cells may induce osteoclastogenesis by increasing the RANKL expression via synthesis of PGE₂ during tooth movement.

IL-1 β , 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-1 β levels following force application on canines and found IL-1 β levels had increased after 24 hours and is associated with resorption of bone during movement of teeth. They concluded that IL-1 β regulated bone remodeling processes.

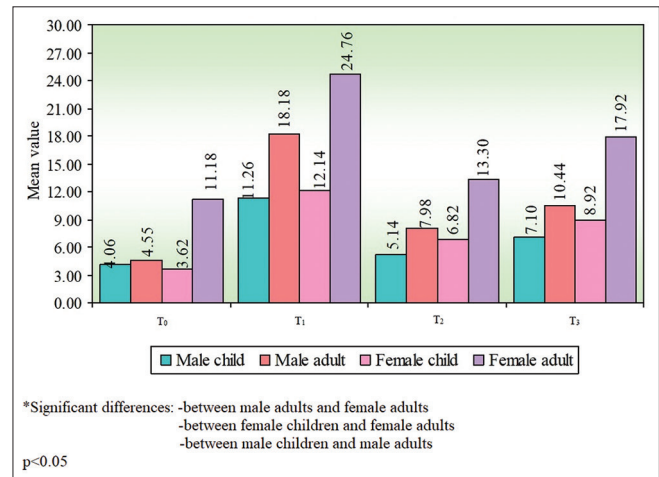


Figure 2: Comparison of gender groups with respect to IL-1 β values at different time points

Luppanapornlap *et al.*^[15] investigated the force magnitudes of orthodontic treatment on the levels of IL-1 β secretion, the pain intensity and amount of tooth movement in canine retraction. IL-1 β 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-1 β during distalization of canines. The IL-1 β levels were greater at pressure sites than areas of tension. Vibratory stimulus and force application had increased the IL-1 β which lead to greater bone resorption and tooth movement.

In this study, significant differences in the levels of PGE₂ and IL-1 β in all groups at T₀-T₁, T₁-T₂, and T₂-T₃ were seen. An increase of PGE₂ and IL-1 β at T₁ may be caused

Table 8: Correlations between pain scores and PGE₂ and IL-1 β 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-1 β Levels at Different Time Points by Spearman's Rank Correlation Coefficient						
IL-1 β 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

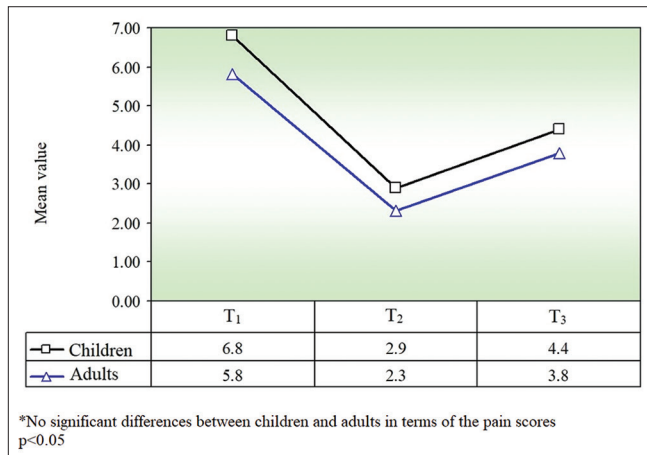


Figure 3: Comparison of children and adults groups with respect to pain scores at different time points

by the acute inflammatory process that occurs during initial orthodontic treatment. This rise of IL-1 β concurs with Grieve *et al.*^[17] and Kapoor *et al.*^[18] in which they found IL-1 β to be increased at 1 and 24 hours, whereas the levels of PGE₂ had increased later at 24 and 48 hours.

It was followed by drop in both PGE₂ and IL-1 β levels at T₂. 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 PGE₂ and IL-1 β in their studies after the 24-hour mark. At T₃ in this study, there was an increase in PGE₂ which could be attributed with a larger force applied since an archwire with a greater dimension.

The PGE₂ 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 PGE₂ 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 PGE₂ whereby their study showed that the aged periodontal ligament fibroblasts were larger in size than the younger cells and produced greater amounts of PGE₂ when forces were applied.

IL-1 β 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-1 β 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-1 β than adolescents and children which may be associated with age-related changes, puberty, and hormones. Individual variations such as IL-1 β gene polymorphism affects the amount of secretion of IL-1 β when same force levels were applied as shown by a study by Iwasaki *et al.*^[24] All the studies related to PGE₂ and IL-1 β 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 PGE₂ and IL-1 β 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 PGE₂ and IL-1 β levels at the time points, T₁, T₂, and T₃. Gameiro *et al.*^[27] found no significant correlation between the pain experience with the IL-1 β or PGE₂ 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 PGE₂ and IL-1 β 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-1 β and PGE₂ concentrations in the adults were higher than that of the children
2. In all the groups, PGE₂ and IL-1 β levels increased significantly on application of the first archwire at T₁ followed with a decrease in the levels at T₂ and a subsequent rise in these levels on placement of an archwire of larger dimension at T₃
3. Saliva is a non-invasive diagnostic aid to study the variation of the concentrations of PGE₂ and IL-1 β during orthodontic treatment
4. The correlation between the variations of IL-1 β and the variations of PGE₂ 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-1 β and PGE₂ 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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