Salivary PGE2 as a Potential Biochemical Marker during Orthodontic Treatment Associated with Periodontal Disease

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PGE2 and IL1â are key mediators involved in periodontal disease, strong stimulators of bone resorption, produced by the cells of the human periodontal ligament in response to mechanical stress. Saliva has an impressive number of components with a protective role, but it also contains markers used in identifying oral inflammation processes. This is why studying the levels of PGE2-type markers in saliva can be a very valuable diagnostic instrument. The results of the post-treatment values of PGE2 have shown a significant decrease after both alternatives of treatment. To conclude, the values of PGE2 decreased more in the case of combined treatment. When associated with orthodontic treatment, periodontal therapy determined a significant statistical decrease of PGE2 values, indicating in an obvious manner the decrease of inflammation and the improvement of the periodontal status.

Keywords: PGE2, periodontal disease, combined treatment, biomarker

PGE2 and IL1β are key mediators involved in periodontal disease, strong stimulators of bone resorption, produced by the cells of the human periodontal ligament in response to mechanical stress. PGE2 not only mediates inflammatory responses such as the increase of vascular permeability and dilation, but can also work as strong stimulators of bone resorption and formation. This dynamic mechanism can be influenced by the concentration of PGE2.

Saito et al. have shown that the cells of the periodontal ligament respond to mechanical stress (in vivo and in vitro) by increasing their production of PGE2. Therefore, after the application of mechanical forces, the cells of the periodontal ligament produce such quantities of mediators that they are diffused into the gingival crevicular fluid (GCF) and afterwards into the saliva. Although it is known that the severity of the periodontal disease is influenced by the age of the host, the role of the ageing phenomenon in orthodontic tooth movements has not been very clearly identified. The production of PGE2 in the cells of the human periodontal ligament is exacerbated by mechanical stress. Nevertheless, the role of the changes related to ageing in the susceptibility of the periodontal ligament as a response to mechanical stress remains unclear.

Saliva has an impressive number of components with a protective role, but it also contains markers used in identifying oral inflammation processes. Previous research has shown that saliva can be a viable diagnostic fluid, with genuine practical applicability.

As far as saliva is concerned, its composition, as well as that of other oral fluids, reflects the liquid and tissue levels of immunological, tumoral and therapeutic molecules, as well ad the presence of oral and systemic disease markers. We can thus state that saliva faithfully expresses the totality of systemic modifications.

Experimental part

The aim of this study was to evaluate the differences between the saliva levels of inflammation biomarkers of E2-type prostaglandins in patients wearing orthodontic appliances who had a stabilised pre-existing periodontal pathology (chronic periodontitis localised in minimum 3 teeth) by comparison to patients with the same pathology who did not receive orthodontic treatment.

The 60 patients included in the study were divided into three groups, as follows: **group A**, the control group, represented by 16 patients without periodontal disease, without clinical gingival modifications; **group B**, 22 patients with periodontal disease (chronic periodontitis localised in minimum 3 teeth), who have received periodontal treatment; **group C**, 22 patients with periodontal disease (chronic periodontitis localized in minimum 3 teeth) who have received both orthodontic and periodontal treatment.

Saliva was collected in two instances from the patients included in groups B and C, as follows:

- for **group B,** an initial collection, with a second one six months after the end of the periodontal treatment
- for **group C,** an initial collection, with a second one six months after periodontal stabilisation and instauration of orthodontic treatment

Both for the patients in group B and those in group C, the periodontal treatment was the same and consisted in supragingival and subgingival scaling and root planing.

Saliva, serum and gingival fluid (GCF) have been investigated as research micromedia in the activity of the periodontal disease. Of these, saliva has a number of advantages compared to techniques that use blood as a research:

- easy to collect
- easy to manipulate
- easy to analyse
- easy to store and transport
- lack of discomfort and anxiety typical of parenteral collection

The level of PGE2 in saliva can increase during orthodontic tooth movements, and its quantification provides a non-invasive in vivo model for investigating the dynamics of mediator production [1,2].

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In order to investigate age-related modifications in the orthodontic ligament cells capacity to biosynthesise PGE2, we have examined in vivo the effects of mechanical tension on the expression of PGE2 by the periodontal ligament cells into the saliva during the first month of orthodontic treatment.

The proposed study has used for laboratory determinations sandwich-type ELISA tests, due to their high sensitivity and specificity.

Results and discussions

The results obtained from this research are shown in the tables below.

The values of PEG2 show significant differences between the analysed lots ($F=365.53$, $p<<0.01$).

The study of PGE_2 values before treatment shows significantly higher values in the case of patients whose periodontium was affected ($F=678.58$, $p<<0.01$, 95%CI), as well as significantly higher values in the case of patients receiving associated periodontal treatment ($p=0.0027$).

The results of the post-treatment evaluation of PGE2 values have shown a significant decrease; however, although before treatment the values of PGE2 in the case of patients with combined treatment were higher after treatment, they did not show significant differences ($p=0.903$) by comparison with the values corresponding to patients who received only periodontal treatment. In conclusion, the values of PGE decreased more in the case of combined treatment.

Table 1
EVALUATION OF PGE2 FOR THE PATIENTS IN LOT A, THE CONTROL GROUP (PATIENTS WITHOUT PERIODONTAL DISEASE AND WITHOUT MALOCCLUSIONS)

Control patients	Value of PGE_2 in saliva (ng/ml)	Sex	Malocclusion	Patient's age
1	52.24	F	Angle Cls I	21
2	48.58	M	Angle Cls I	26
3	47.92	F	Angle Cls I	28
4	57.46	M	Angle Cls I	28
5	69.67	F	Angle Cls I	22
6	54.13	M	Angle Cls I	34
7	63.51	F	Angle Cls I	36
8	49.17	F	Angle Cls I	26
9	56.53	F	Angle Cls I	29
10	67.39	M	Angle Cls I	27
11	58.61	M	Angle Cls I	31
12	43.65	F	Angle Cls I	23
13	53.34	F	Angle Cls I	37
14	65.46	M	Angle Cls I	38
15	56.73	F	Angle Cls I	32
16	54.23	M	Angle Cls I	30

Table 2
EVALUATION OF PGE2 FOR THE PATIENTS IN LOT B (PATIENTS WITH PERIODONTAL DISEASE), WHO RECEIVED PERIODONTAL TREATMENT BEFORE THE ORTHODONTIC TREATMENT AND 6 MONTHS AFTER IT

Patients with chronic periodontal disease	Value of PGE_2 in patient's saliva before periodontal treatment (ng/ml)	Value of PGE_2 in patient's saliva at re-evaluation, 6 months after periodontal treatment (ng/ml)	Sex	Malocclusion	Patient's age
1	351.23	185.34	F	Angle Cls I	22
2	376.34	213.28	M	Angle Cls III	34
3	325.12	195.67	F	Angle Cls II/2	30
4	357.73	175.34	M	Angle Cls II/2	31
5	403.23	194.74	F	Angle Cls I with crowding	26
6	333.67	187.23	M	Angle Cls II/2	28
7	312.48	164.85	F	Angle Cls I with crowding	26
8	289.45	175.84	F	Cls II/1	32
9	348.57	275.12	M	Cls II/2	31
10	325.64	169.37	F	Cls II/2	25
11	305.63	179.54	M	Angle Cls III	23
12	412.63	243.65	M	Angle Cls I with crowding	27
13	342.46	173.28	F	Angle Cls II/1	33
14	334.65	181.17	M	Angle Cls III	29
15	324.56	165.39	F	Angle Cls III	36
16	345.75	216.28	M	Angle Cls I	32
17	326.48	227.59	F	Angle Cls I	31
18	338.64	206.47	M	Angle Cls III	34
19	434.75	249.51	F	Angle Cls I with crowding	36
20	338.59	178.52	M	Angle Cls III	38
21	294.17	167.19	F	Angle Cls II/1	30
22	306.78	170.06	F	Angle Cls I	29

Table 3

EVALUATION OF PGE2 FOR THE PATIENTS IN LOT C (PATIENTS WITH PERIODONTAL DISEASE), WHO RECEIVED PERIODONTAL TREATMENT ASSOCIATED WITH ORTHODONTIC TREATMENT, BEFORE TREATMENT AND 6 MONTHS AFTER THE PERIODONTAL STABILISATION AND BEGINNING OF ORTHODONTIC TREATMENT

Patients with cu periodontal disease about to receive orthodontic treatment	Value of PGE_2 in the saliva of patients before periodontal treatment and orthodontic treatment (ng/ml)	Value of PGE_2 in patient's saliva at re-evaluation 6 months after periodontal stabilisation and beginning of orthodontic treatment (ng/ml)	Sex	Malocclusion	Patient's age
1	314,26	234,72	F	Angle Cls I with crowding	25
2	308.69	179.54	M	Angle Cls I with crowding	23
3	338.15	185.27	M	Angle Cls II/2	29
4	326.41	194.28	F	Angle Cls II/1	31
5	317.53	165.89	F	Angle Cls II/2	34
6	278.63	156.34	M	Angle Cls I with crowding	29
7	288.71	183.19	F	Angle Cls II/1	30
8	312.93	208.54	M	Angle Cls III	32
9	320.43	205.14	F	Angle Cls II/2	31
10	296.38	182.64	M	Angle Cls III	32
11	306.82	196.28	F	Angle Cls II/2	34
12	324.75	214.31	M	Angle Cls I with crowding	33
13	294.28	190.65	F	Angle Cls II/2	38
14	305.18	206.87	M	Angle Cls II/1	26
15	316.74	186.56	M	Angle Cls III	34
16	334.19	224.71	F	Angle Cls III	37
17	358.62	235.10	F	Angle Cls II/1	32
18	333.15	216.31	M	Angle Cls II/2	31
19	295.74	175.48	M	Angle Cls III	28
20	318.48	171.83	F	Angle Cls II/2	38
21	336.86	195.18	M	Angle Cls III	36
22	335.29	206.15	F	Angle Cls II/1	31

Table 4

STATISTICAL INDICATORS OF PGE_2 IN PATIENTS WITH PERIODONTAL DISEASE (MINIMUM 3 TEETH AFFECTED) WHO RECEIVED PERIODONTAL TREATMENT IN ASSOCIATION (YES/NO) WITH ORTHODONTIC TREATMENT

		Mean PGE_2 [ng/ml]	Mean -95%	Mean +95%	Std.dev.	Std.err.	Min	Max	Q25	Median	Q75
periodontal treatment	before	342.21	325.83	358.58	36.93	7.87	289.45	434.75	324.56	336.62	351.23
	after	195.25	181.68	208.81	30.59	6.52	164.85	275.12	173.28	183.26	213.28
periodontal treatment + orth. t.	before	316.46	308.02	324.91	19.05	4.06	278.63	358.62	305.18	317.13	333.15
	after	196.14	186.76	205.51	21.15	4.51	156.34	235.10	182.64	194.73	208.54
control	before	56.16	52.23	60.10	7.39	1.85	43.65	69.67	50.70	55.38	61.06

Table 5

TEST FOR COMPARING THE VALUES OF PGE_2 IN PATIENTS WITH PERIODONTAL DISEASE

	F *(95% confidence interval)*	p
Kuskal-Wallis Test	365.5330	0.00

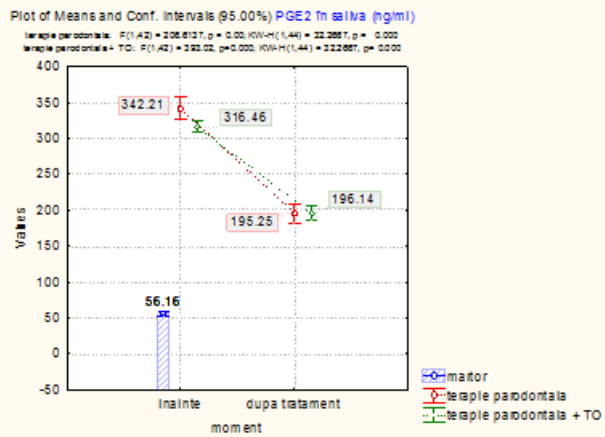

Fig.1. Statistical indicators of PGE_2 in patients with periodontal disease

Fig.2. Statistical indicators of PGE_2 in patients with periodontal disease before treatment

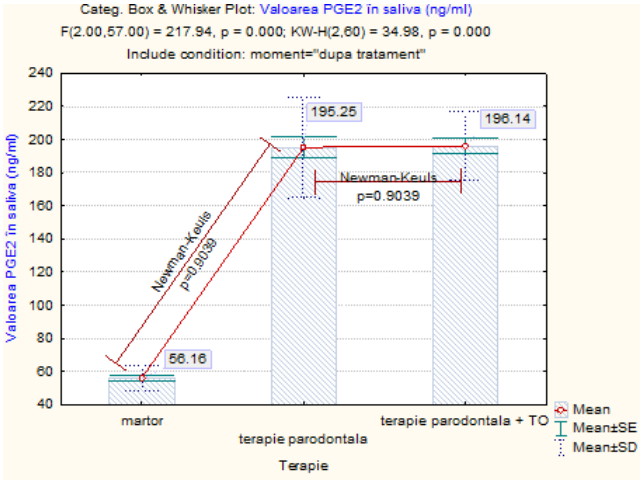

Fig.3. Statistical indicators of PGE_2 in patients with periodontal disease, after treatment

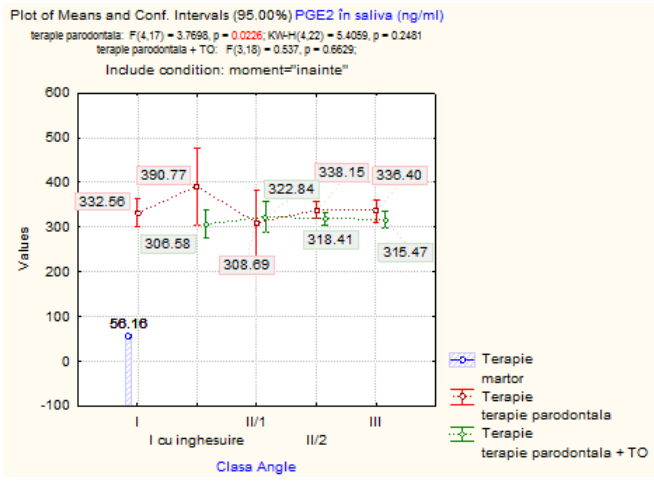

Fig.4. Statistical indicators of PGE_2 in patients with periodontal disease before treatment, in relation to the Angle class

Table 6

STATISTICAL INDICATORS OF PGE2 [ng/mL] IN PATIENTS WITH PERIODONTAL DISEASE (MINIMUM 3 TEETH AFFECTED) WHO RECEIVED PERIODONTAL TREATMENT IN ASSOCIATION (YES/NO) WITH ORTHODONTIC TREATMENT, IN RELATION TO THE ANGLE CLASS

Before treatment	Angle	Mean PGE_2 [ng/ml]	Mean -95%	Mean +95%	Std.Dev.	Std. Err.	Min	Max	Q25	Median	Q75
periodontal treatment	1	332.56	300.42	364.70	20.20	10.10	306.78	351.23	316.63	336.12	348.49
	2	390.77	305.10	476.45	53.84	26.92	312.48	434.75	357.86	407.93	423.69
	3	308.69	235.81	381.57	29.34	16.94	289.45	342.46	289.45	294.17	342.46
	4	338.15	320.17	356.12	14.48	6.47	325.12	357.73	325.64	333.67	348.57
	5	336.40	312.04	360.77	23.22	9.48	305.63	376.34	324.56	336.62	338.64
periodontal treatment + orth. treat.	1										
	2	306.58	275.09	338.07	19.79	9.89	278.63	324.75	293.66	311.48	319.51
	3	322.84	289.27	356.42	27.04	12.09	288.71	358.62	305.18	326.41	335.29
	4	318.41	304.64	332.17	14.88	5.62	294.28	338.15	306.82	318.48	333.15
	5	315.47	296.88	334.07	17.72	7.23	295.74	336.86	296.38	314.84	334.19
control	1	56.16	52.23	60.10	7.39	1.85	43.65	69.67	50.70	55.38	61.06

	F *(95% confidence interval)*	p
Kuskal-Wallis Test	216.7271	0.00

Table 7

THE TEST FOR COMPARING THE VALUES OF PGE2 [ng/mL] IN PATIENTS WITH PERIODONTAL DISEASE

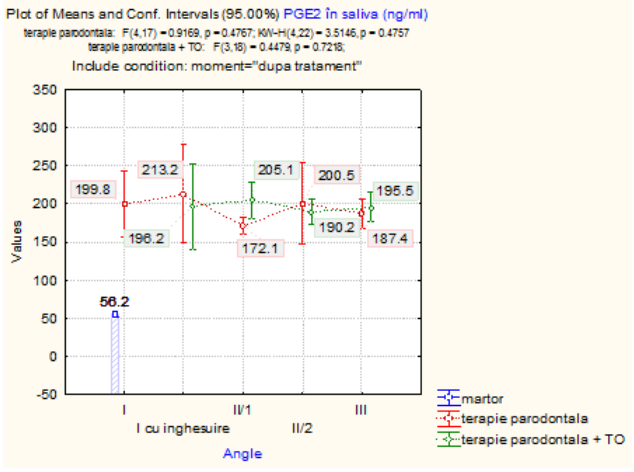

Fig.5. Statistical indicators of PGE_2 in patients with periodontal disease, after treatment, in relation to the Angle class

Evaluation of PGE_2 in relation to the Angle class

After treatment, the values of PGE_2 decreased significantly, but the values remained significantly higher compared to the control lot. Depending on the Angle class, the values of PGE_2 no longer show significant differences either in the patients who received combined treatment (F=0.44, p=0.7218) or in the patients who only received periodontal treatment (F=0.91, p=0.476).

Post-treatment values in relation to the Angle class, do not show significant differences in the case of the two patient groups.

Table 8

STATISTICAL INDICATORS OF PGE2 [ng/mL] IN PATIENTS WITH PERIODONTAL DISEASE (MINIMUM 3 TEETH AFFECTED) WHO RECEIVED PERIODONTAL TREATMENT IN ASSOCIATION (YES/NO) WITH ORTHODONTIC TREATMENT, IN RELATION TO THE ANGLE CLASS

After treatment	Angle	Mean PGE_2[ng/ml]	Mean -95%	Mean +95%	Std.dev.	Std.err.	Min	Max	Q25	Median	Q75
periodontal treatment	1	199.8	157.3	242.3	26.7	13.3	170.1	227.6	177.7	200.8	221.9
	2	213.2	148.7	277.7	40.5	20.3	164.8	249.5	179.8	219.2	246.6
	3	172.1	161.1	183.1	4.4	2.6	167.2	175.8	167.2	173.3	175.8
	4	200.5	147.2	253.8	42.9	19.2	169.4	275.1	175.3	187.2	195.7
	5	187.4	168.1	206.7	18.4	7.5	165.4	213.3	178.5	180.4	206.5
periodontal treatment + orth. treatment	1										
	2	196.2	140.5	251.9	35.0	17.5	156.3	234.7	167.9	196.9	224.5
	3	205.1	181.1	229.2	19.4	8.7	183.2	235.1	194.3	206.2	206.9
	4	190.2	173.8	206.6	17.8	6.7	165.9	216.3	171.8	190.7	205.1
	5	195.5	176.3	214.7	18.3	7.5	175.5	224.7	182.6	190.9	208.5
control	1	56.2	52.2	60.1	7.4	1.8	43.6	69.7	50.7	55.4	61.1

	F (95% confidence interval)	p
Kuskal-Wallis Test	50.21459	0.000000

Table 9

THE TEST FOR COMPARING THE VALUES OF PGE2 [ng/mL] IN PATIENTS WITH PERIODONTAL DISEASE

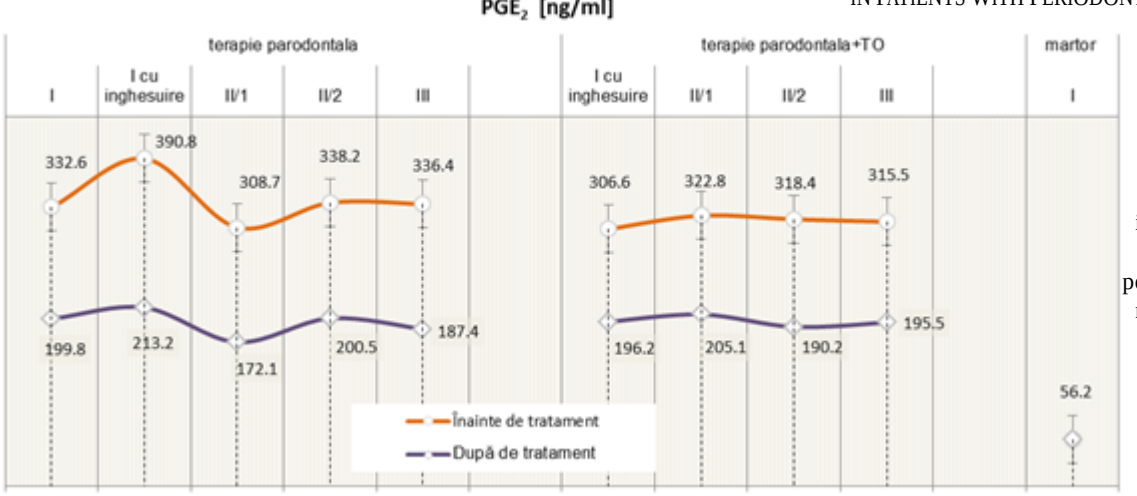

Fig.6. Statistical indicators of PGE_2 in patients with periodontal disease, in relation to the Angle class

Salivary inflammation markers have three important roles: to help identify patients with periodontal risk before tissue destruction occurs, to help evaluate disease activity and evolution, and, last but not least, to guide the development of new treatment protocols that are predictable and effective [3].

Numerous studies exist, carried out using mainly crevicular fluid and venous blood, but researching cytokines in saliva still provides ample perspectives that can be explored.

Javaid et al. underline the fact that the early detection of disease is vital not only in order to reduce the severity of the problem and to reduce complications, but also in order to significantly increase the success rate of treatment. Saliva has been studied intensely in recent decades due to the fact that it represents an easily achieved and non-invasive exploration, as well as due to the abundance of biomarkers, material and proteins that can be determined in it [4].

Kaufman showed that numerous biomarkers in saliva can be associated to the periodontal disease. Of these we can mention inflammation mediators, enzymes, keratinized epithelial cells, immunoglobulins, salivary ions and hormones. [5].

Conclusions

In association with orthodontic treatment, periodontal therapy determined the statistically significant decrease of PGE2 values, indicating in an obvious manner the decrease of inflammation and the improvement of the periodontal status.

This study could not identify a statistically significant correlation between salivary levels of PGE2 after combined treatment and the type of malocclusion.

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