

EFFECT OF NON-SURGICAL PERIODONTAL TREATMENT ON LIPOXIN A₄ LEVELS IN PERIODONTITIS PATIENTS

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Abstract

The aim of this study was to determine the lipoxin A₄ (LXA₄) in gingival crevicular fluid (GCF) and saliva in chronic periodontitis (CP) patients before and after non-surgical periodontal treatment (NSPT). A total of twenty subjects, ten patients with CP and ten healthy individuals were included in this study. Clinical measurements, GCF and saliva samples were obtained at the beginning and one month after NSPT. GCF and salivary LXA₄ were investigated by ELISA. Salivary LXA₄ were significantly higher in CP than in healthy subjects ($p < 0.05$), but no significant difference was observed after NSPT ($p > 0.05$). GCF LXA₄ were lower in CP and decreased after NSPT, but these differences were not significant ($p > 0.05$). However, GCF LXA₄ in post-treatment CP group were significantly lower than in healthy subjects ($p < 0.05$). Negative correlation was found between probing depth and GCF LXA₄ in CP group ($r = -0.717$; $p < 0.05$). Within the limitations of this study, LXA₄ might have a potential role in both the pathogenesis of periodontitis and the healing process.

Key words: gingival crevicular fluid, lipoxins, periodontitis, periodontal treatment, saliva

Introduction. Periodontitis is an inflammatory disease that develops as a result of the immune-inflammatory response of the host against periodontopathogenic bacteria in dental biofilm. This can lead to tooth loss by affecting the

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supporting tissues of the tooth [1]. With inflammation, proinflammatory lipid mediators cause acute inflammation; conversely, endogenous anti-inflammatory lipid mediators are produced to balance systemic and local pro-inflammatory events. This occurs by “lipid mediator class switching” from pro-inflammatory lipid mediators to the biosynthesis of pro-resolution lipid mediators such as lipoxins (LXs) [2]. In case of a failure in the resolution process that provides homeostasis, the inflammation process cannot be controlled.

According to recent research, the resolution mechanism in the pathogenesis of periodontitis is impaired [3]. This situation has led others to conduct studies addressing the role of pro-resolution lipid mediators in particular lipoxins in periodontal inflammation. Recently, the role of LXA₄ in the pathogenesis of periodontitis has been discussed [4]. LXs are pro-resolution lipid mediators produced by the lipoxygenase enzymatic pathway of AA metabolites, rapidly synthesized in response to stimuli and acting locally. They also have a short half-life and are rapidly inactivated [5]. LXs, particularly LXA₄, are known to have strong counter-regulative signals for pro-inflammatory mediators, thereby inhibiting leukocyte-dependent inflammation. LXA₄ plays a role in the anti-inflammatory process by reducing the responses of polymorphonuclear leucocytes (PMNs), chemotaxis and transmigration through the endothelium and by supporting apoptosis of PMNs [6].

The authors of several studies have reported that LXA₄ is correlated with many chronic diseases, such as periodontitis, and its metabolically stable analogues reduce this inflammation. In the various clinical trials involving LXA₄, only healthy individuals and those with periodontal disease were compared [7,8]. However, as found in a review of the literature, there has been no research evaluating the changes in GCF and salivary LXA₄ levels after NSPT. The aim of this study was to (1) determine the levels of LXA₄ in GCF and saliva in patients with chronic periodontitis, (2) evaluate the effect of NSPT on LXA₄ levels.

Materials and methods. Study design and patient population. A total of twenty individuals participated in this research. Informed consent was obtained from all participants included in the study. All procedures performed in the study involving human participants were in accordance with the ethical standards of the Ordu University research committee (No 2017/117). Two groups were involved in this study: chronic periodontitis (CP; $n = 10$ (4 female, 6 male), mean age 42.33 ± 11.28 yr.) and periodontal healthy individuals (control; $n = 10$ (6 female, 4 male), mean age 38.22 ± 14.49 yr.).

Exclusion criteria were: 1) history of systemic disease, 2) regular use of any drugs which can effect the immune system or inflammatory response in the last 6 months, 3) periodontal treatment received for the last 6 months, 4) smoking, 5) history of radiotherapy or chemotherapy, 6) ongoing orthodontic treatment, 7) aggressive periodontitis, 8) pregnancy or lactation.

All individuals had minimum 20 natural teeth, except for third molars. For the patients with CP, the inclusion criteria were: 1) presence at minimum two

non-adjacent regions per quadrant with probing depth (PD) ≥ 5 mm and clinical attachment level (CAL) ≥ 5 mm with gingival inflammation, 2) $> 30\%$ of the teeth with alveolar bone loss, as detected on clinical and radiographical examinations. The periodontally healthy control group had no sign of gingival inflammation, no PD > 3 mm and no attachment or alveolar bone loss.

Clinical examination and periodontal treatment. Periodontal status of each individual was examined with full mouth plaque index (PI) [9], gingival index (GI) [10], probing depth (PD), clinical attachment levels (CAL) and radiographical evaluation. All clinical parameters were measured with a Williams periodontal probe by a single examiner who was blinded to the whole study (ET).

NSPT including oral hygiene education and scaling and root planing (SRP) was performed in patients with chronic periodontitis in different sessions for each quadrant under local anesthesia. Patients were recalled one month after the completion of NSPT. In periodontal healthy group only oral hygiene instructions were given.

For all individuals, clinical measurements, saliva and GCF samples were obtained at the baseline and one month after the NSPT in CP group. All periodontal treatments were completed by the same researcher (ET). Patients who could not clinically detect periodontal healing during these periods were excluded from the study.

Gingival crevicular fluid and saliva sampling. GCF samples were collected one day after completing the clinical measurements to prevent contamination with blood. Before the GCF sampling, cotton rolls were placed to isolate the teeth and then the area was air-dried to prevent saliva contamination. GCF samples were obtained by using the standardized filter paper strips. Paper strips were placed into the periodontal pocket till mild resistance was felt; these were left there for 30 seconds. Strips contaminated with blood or saliva were discarded. The GCF sample volume was determined with a pre-calibrated Periotron 8010. The two strips for each tooth were placed into an Eppendorf tube and stored at -40°C until the analysis.

Unstimulated saliva samples were collected from individuals with fasting for 1–2 h in the morning one day after the clinical measurements. Each individual was instructed to rinse with distilled water then saliva samples were collected. The saliva samples were stored at -40°C until analysis. The saliva samples were thawed on ice, centrifuged 5000 rpm for 15 min at 4°C , and on the same day immediately used in the analysis.

Measurements of LXA₄ in GCF and saliva. The absorbed fluid was eluted from the pooled paper strips by placing them in 400 μL phosphate buffered saline [PBS-T = (0.05%)] and by shaking the tubes on an orbital shaker (240 rpm) for 15 min. Then the tubes were centrifuged at 13000 rpm for 5 min at $+4^{\circ}\text{C}$. GCF LXA₄ levels were quantitated by the enzyme-linked immunosorbent assay (ELISA) using commercial kits according to the manufacturer's instructions. The

colorimetric reading was performed with a microplate reader at a wavelength of 450 nm. Mediators concentrations were quantitated using the standard curve. GCF results for these mediators were expressed as total amounts (ng) at two samples per sampling time (30 s).

Statistical analysis. Statistical analyses were carried out using the statistical package software (SPSS v.17.0, IBM, Chicago, IL, USA). The normality of the data was determined by Shapiro–Wilk normality test. The equality of variance for all samples was checked by Levene’s test. The significance of the difference between the groups (CP and C group) in terms of mean values was evaluated by Student’s *t*-test, while non-normally distributed parameters were analyzed by Mann–Whitney U test. In CP group (at baseline and 1st month), dependent *t*-test was used for normal distributed parameters and Wilcoxon test was used for non-normally distributed parameters. The relationships between the parameters in each group were evaluated by using Spearman’s rank correlation test. Statistically significant differences were considered at $p < 0.05$ for all analyses.

Results. Demographic and clinical findings. The study involved twenty subjects. The sex and age distribution of the individuals were found similar between the groups ($p > 0.05$).

Clinical measurements and GCF volumes in the study groups are shown in Table 1. All clinical parameters were significantly higher in the CP group than in the controls ($p < 0.001$) and were decreased after the NSPT ($p < 0.05$). GCF volumes were significantly higher in CP group than in the healthy subjects ($p < 0.001$), and a significant reduction was observed after the NSPT ($p < 0.05$). Despite this reduction, GCF volume after the NSPT was statistically higher in the patients with CP than in the healthy subjects ($p < 0.05$).

T a b l e 1

Comparison of clinical findings of the study groups [median (min-max)]

	Control	CP		<i>p</i> value C-BT	<i>p</i> value BT-AT	<i>p</i> value C-AT
		Before treatment	After treatment			
Full mouth						
PI	0.27 (0.10-0.40)	1.76 (1.64-2.26)	0.36 (0.22-1.15)	< 0.001 *	0.008 *	0.013 *
GI	0.15 (0.06-0.22)	1.52 (1.37-1.82)	0.26 (0.14-0.84)	< 0.001 *	0.008 *	0.036 *
PD (mm)	1.27 (1.13-1.39)	2.88 (2.53-4.42)	2.16 (1.66-3.08)	< 0.001 *	0.008 *	< 0.001 *
CAL (mm)	1.27 (1.13-1.39)	4.57 (3.58-7.23)	3.55 (3.10-6.73)	< 0.001 *	0.008 *	< 0.001 *
GCF volume (μ l)	0.02 (0.01-0.11)	1.02 (0.37-1.25)	0.09 (0.01-0.20)	< 0.001 *	0.008 *	0.032 *

*The significant differences level of $p < 0.05$ are shown in bold face.

C-BT: Comparisons between the CP group before treatment and control group.

BT-AT: Comparison between before and after treatment in CP group.

C-AT: Comparisons between the CP group after treatment and control group.

T a b l e 2

Total amounts of GCF and salivary LXA₄ in study groups (mean ± SD)

	Control	CP		<i>p</i> value C-BT	<i>p</i> value BT-AT	<i>p</i> value C-AT
		Before treatment	After treatment			
GCF LXA ₄ (ng/30 s)	1.22 ± 0.52	0.98 ± 0.29	0.72 ± 0.16	0.387	0.051	0.008*
Salivary LXA ₄ (ng/ml)	36.58 ± 12.48	59.06 ± 23.11	53.24 ± 19.99	0.025*	0.523	0.054

*The significant differences level of $p < 0.05$ are shown in bold face.

C-BT: Comparisons between the CP group before treatment and control group.

BT-AT: Comparison between before and after treatment in CP group.

C-AT: Comparisons between the CP group after treatment and control group.

Biochemical findings. The total amount of GCF and salivary LXA₄ levels in study groups are shown in Table 2. Salivary LXA₄ levels were significantly higher in CP group than in healthy subjects ($p < 0.05$) but no significant difference was observed after NSPT ($p > 0.05$). Contrariwise, GCF LXA₄ levels were found to be lower in the patients with CP than in the healthy subjects and decreased after NSPT but the difference was not statistically significant ($p > 0.05$). However, post-treatment GCF LXA₄ levels were found to be significantly lower in the patients with CP than in the healthy subjects ($p < 0.05$). Negative correlation was found between PD and GCF LXA₄ in CP group ($r = -0.717$, $p < 0.05$).

Discussion. Recently, the possible role of LXA₄ in periodontitis has been investigated in a few clinical studies [7,9,11]. However, there is no known study whose authors have investigated LXA₄ levels in periodontitis before and after NSPT. Therefore, in the present study, GCF and the salivary LXA₄ levels were evaluated in the patients with CP before and after NSPT.

ELABDEEN et al. [12] found that salivary and serum LXA₄ levels were higher in individuals with aggressive periodontitis. However, the difference in serum levels was statistically significant, whereas the difference in saliva levels was not. DOĞAN et al. [13] found higher serum LXA₄ levels in CP patients. In our study, salivary LXA₄ levels were significantly higher in the patients with CP. Serum is also a saliva-like bodily fluid as a diagnostic tool; therefore the results of our study may be consistent with those. LXA₄ was detected in neutrophils obtained from peripheral blood samples taken from individuals with localized aggressive periodontitis, but this was not detected in healthy individuals. These results can explain the higher salivary LXA₄ levels in CP patients in our study. Contrary to these results, FREDMAN et al. [3] examined LXA₄ in whole blood samples of individuals with localized, aggressive periodontitis and found they were lower than those of healthy individuals. This can be due to the difference in the method of blood examination for LXA₄. In contrast to our study, TOBON-ARROYAVE et al. [14] reported that salivary LXA₄ levels were lower in individuals with periodon-

titis than in healthy subjects. Additionally, we could not find a significant change in the CP group after treatment. These inconsistent results can be caused by the complex structure and content of saliva being affected by many factors [15]. For this reason, saliva samples were evaluated together with GCF samples in which periodontal status was reflected in a more region-specific manner.

In our study, although the difference was not statistically significant, the GCF LXA₄ levels was lower in the patients with CP compared to healthy subjects. Elabdeen et al. [14] reported that GCF LXA₄ was significantly lower in patients with aggressive periodontitis than in the healthy group. In two different studies by TARANNUM and FAIZUDDIN [8,11] it was found that LXA₄ levels of individuals with CP were significantly lower than those of healthy subjects. In our study, although it is not statistically significant, LXA₄ levels were lower in the CP group compared to those of healthy individuals. LUTFIOGLU et al. [7] could not detect LXA₄ in GCF in healthy subjects. They reported that although GCF LXA₄ levels were lower in non-smokers with generalized, aggressive periodontitis compared to individuals with CP, no significant difference was observed. Also, GCF LXA₄ levels were significantly lower in individuals with periodontitis than in gingivitis subjects [7]. These results can be interpreted that the LXA₄ in GCF can decrease as the severity of periodontal disease increases.

Despite the fact that GCF LXA₄ were found to be lower in patients with CP, salivary LXA₄ levels were found to be higher compared to the healthy group in our study. These findings in saliva are similar to those of several studies in which LXA₄ was examined in serum [12,13]. However, GCF values have become more important in the pathogenesis of periodontal disease, especially because they reflect the region-specific situation. In the CP group, although it was not statistically significant, GCF LXA₄ levels were lower than in those of the healthy group. There was a negative correlation between the PD and GCF LXA₄ levels, which shows that there is a defect in the resolution process during periodontal disease. This supports the finding that LXA₄ plays a role in the pathogenesis of periodontal disease [4,8,11].

In our study, the GCF LXA₄ levels were not statistically significant between the pre-treatment CP group and the healthy group, but it was found to be significantly lower after treatment. In addition, the GCF LXA₄ levels in the CP group were decreased after NSPT but this decrease was not statistically significant. In summary, although it is not statistically significant, LXA₄ levels decreased after treatment in individuals with periodontitis; its levels also became significantly lower than those in the healthy group. To our knowledge, this is the first study in which the effect of NSPT on LXA₄ levels was evaluated. Even though it is known to be pro-resolution mediator, a decrease in levels of this mediator after NSPT may be due to its short half-life [5]. In addition, the fact that sampling was repeated for a single period of time after treatment makes it difficult to clearly interpret the mechanisms by which this mediator is involved during recovery. Fur-

thermore, since changes at one month after treatment were evaluated, it should be considered that histological recovery can continue even if clinical recovery is observed.

In summary, the findings of this research indicate that LXA₄ plays an important role in periodontal inflammation, and the role of LXA₄ in resolution process provides a potential contribution to the healing mechanism. However, the research has a few limitations and therefore the findings should be carefully interpreted. Lipoxins play an important role in resolving periodontal inflammation, but the role of other pro-resolution lipid mediators in this process cannot be ignored. In this study, only one mediator with pro-resolution properties was examined. Examination of LXA₄ with omega-3 fatty acid derivatives known to have pro-resolution properties may be useful in understanding the effects of these mediators on periodontal inflammation and the healing process. Withal, the sample size small for conclusive inferences. These results should be supported by studies in which a higher number of patients and larger groups were included (e.g. different stage of periodontal disease, systemic compromised patients).

In conclusion, it is more reliable to infer on the GCF LXA₄ results due to the inconsistent saliva levels that could be affected by many factors. The fact that GCF LXA₄ levels are different in periodontitis compared to healthy individuals and change their level after NSPT may suggest that LXA₄ might play a potential role in both the pathogenesis of periodontitis and the healing process. Also, the negative correlation between PD and GCF LXA₄ can indicate that LXA₄ plays a role in the development and severity of periodontitis. Future studies with a greater number of patients and a longer period of follow-up are required to fully elucidate the potential role of LXA₄ in these processes.

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