



## ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

# Analysis of biochemical markers in the saliva and correlation with clinical parameters in patients with aggressive periodontitis, before and after the therapy

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## SUMMARY

**Introduction/Objective** Aggressive periodontitis (AP) is a progressive disease that damages the periodontal tissues.

The aim of the study was the analysis of intracellular enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) and electrolytes in the saliva of patients with AP and their correlation with clinical parameters before and after the therapy.

**Methods** The study included 30 patients with AP (experimental group) and 35 patients with healthy parodontium (control group). Intracellular enzymes and electrolytes were analyzed in an unstimulated saliva of subjects with AP, before and after the therapy and in saliva of the control group. The analysis of biochemical markers was carried out using kinetic methods with commercial reagents.

**Results** Concentrations of the biochemical markers AST ( $28.18 \pm 25.16$ ), ALT ( $5.48 \pm 5.14$ ), ALP ( $31.13 \pm 37.79$ ), ACP ( $17.53 \pm 14.77$ ), calcium ( $2.80 \pm 1.97$ ), phosphate ( $4.43 \pm 1.92$ ) in the saliva of subjects of the experimental group were statistically significantly higher in relation to the control group ( $p = 0.000$ ;  $p = 0.001$ ). Significant correlation was found between AST values, debris index ( $\rho = -0.444$ ;  $p = 0.026$ ) and calculus index ( $\rho = -0.513$ ;  $p = 0.009$ ), and between the plaque index and ALP level in the saliva after therapy ( $p = 0.020$ ).

**Conclusion** The investigation will contribute to a better understanding and standardization of biomarkers in the saliva that may help in diagnosing the AP and evaluation of the applied therapy.

**Keywords:** aggressive periodontitis; intracellular enzymes; saliva

## INTRODUCTION

Aggressive periodontitis (AP) is a disease followed by rapid and severe destruction of the periodontal tissue. Destruction of the periodontal tissue begins in the area of the first molars and incisors, and with aging it can involve the periodontium of adjacent teeth. It affects the periodontal tissue of at least three permanent teeth, except the first molars and incisors [1].

In the etiology of AP, virulence factors of microorganisms in dental biofilm play a key role including the presence of risk factors and genetic predisposition. In the periodontal lesions, *Aggregatibacter actinomycetemcomitans* is a major causative agent, and its role is in regulating the inflammatory response in the aggressive periodontal inflammatory process [2].

The diagnosis of AP is based on patient's medical history, clinical examination and radiographic analysis [3]. However, all these diagnostic methods tell us about the disease only when there are clear clinical symptoms. Thus, in most cases, the disease has already significantly advanced at the time of the diagnosis.

Over the past two decades, with the progress of technological development, saliva has increasingly been analyzed as a sample of biological material, which might be used to establish diagnosis and to clarify the pathogenesis of oral diseases. It provides evidence and gives useful information on various enzymes and biomolecules, the indicators of pathological processes in periodontal tissue [4, 5]. Literature data show that the concentration of intracellular enzymes responsible for the metabolic processes in the cells has been significantly increased in the saliva of patients suffering from periodontitis compared to healthy subjects [6].

The objective of this study was to analyze the activity of intracellular enzymes of aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP), and calcium and phosphate concentrations in the saliva of patients with AP, before and after the therapy, as well as and correlation of the mentioned biochemical markers with clinical indicators of the condition of periodontal tissues.

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## METHODS

The study included 65 subjects. The experimental group consisted of 30 patients suffering from AP who were coming to the Clinic for Periodontology and Oral Medicine at the Military Medical Academy in Belgrade. The control group consisted of 35 subjects with the clinically healthy periodontium. The study excluded all persons who had a systemic illness, used medicines that could impair the condition of periodontium, consumed alcohol, as well as females during the pregnancy, lactation or menstrual cycle.

Clinical evaluation of the periodontal tissue condition was performed of the following parameters: gingival index (GI), plaque index (PI), probing depth (PD), debris index (DI), calculus index (CI), bleeding index (BI). Measurements were performed using the dental mirror and graduated periodontal probe (Periodont Sex Cp 11).

Patients suffering from AP underwent, a basic / causal therapy, such as: removal of soft deposits with brushes and the Vantal (Galenika) toothpaste, removal of supragingival deposits (supragingival calculus) with the ultrasonic apparatus (Kavo, Sonicflex 2000 N, Biberach an der Riss, Germany), removal of subgingival deposits and treatment of the tooth root with special curettes (Gracey, Kohler, Austria). The free content of the periodontal pocket was removed by washing with chlorhexidine digluconate (12%). After clinically visible signs of inflammation reduced, a surgical intervention was performed (Modified Widman flap surgery). Eight weeks (two months) after basic and surgical therapies were performed, clinical parameters were again measured to determine the condition of periodontal tissues.

### Determination of levels of intracellular enzymes and electrolytes in saliva samples

From the patients (control and experimental groups), during the first visit, samples of mixed unstimulated saliva were taken using special tubes (Salivette; Sarstedt AG & Co. KG, Nümbrecht, Germany). Eight weeks after the basic and surgical therapy, saliva samples were taken only from the experimental group. According to the protocol, saliva was taken in the morning hours at the same time. Centrifugation of samples lasted 10–20 minutes at 3000–5000 rpm and there after they were deposited at -80°C until the beginning of analysis.

The activity of enzymes in the saliva was determined by kinetic methods on the spectrophotometer (Secomam Basic, Ales, France) with commercial reagents (Human, Wiesbaden, Germany). Concentrations of calcium and phosphate in unstimulated saliva were determined by a colorimetric method, commercial reagents (Human, Germany) were used. All analyses were done in the Laboratory for Biochemistry and Hematology at the School of Dental Medicine Belgrade, according to the recommendations of the International Federation for Clinical Chemistry, IFCC.

### Statistical analysis of data

In order to perform necessary statistical testing, IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp, Armonk,

NY, USA) was used. All variables were described by measures of central tendency and measures of variability: mean, median, standard deviation, minimum and maximum values.

To test the difference between subjects in the observed groups, the Mann–Whitney U test was used for the non-parametric data and the t-test for independent samples for the parametric data. The Wilcoxon test was used to compare the values of the observed clinical and biochemical parameters before and after the therapy. The correlation between clinical and biochemical parameters was examined by the Spearman's correlation coefficient.

The factors of difference between the subjects with healthy parodontium and the patients with AP disease were established by logistic regression analysis. Statistical significance was defined as  $p < 0.05$ . The study was approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac (No. 01-4798).

## RESULTS

### Descriptive characteristics of the subjects

The basic descriptive data of the subjects in the control and experimental groups are shown in Table 1.

**Table 1.** Descriptive characteristics of the subjects in the control and experimental groups

Patients' characteristics		Control group	Experimental group	Significance
Number of patients (n)		35	30	
Age (X ± SD (Med., min.–max.))		27.06 ± 3.72 (26; 22–38)	42.30 ± 7.69 (40; 30–57)	$p = 0.000^*$
Sex n (%)	Male	12 (34.3%)	18 (60%)	$p = 0.038^*$
	Female	23 (65.7%)	12 (40%)	
Smoking n (%)	Yes	5 (14.3%)	15 (50%)	$p = 0.002^*$
	No	30 (85.7%)	15 (50%)	

\*statistically significant difference ( $p$ -value < 0.05)

The control group included 35 subjects, with an average age of  $27.06 \pm 3.72$  years. In the experimental group 30 subjects had AP, average age was  $42.30 \pm 7.69$  years, 18 were males (60%) and 12 females (40%).

Intergroup analysis found that male subjects were statistically significantly more represented in the group of patients with AP than in the control group ( $p = 0.038$ ; Table 1). The average number of years statistically significantly differed between the groups, and the subjects in the experimental group were older than those in the control group ( $p = 0.000$ ; Table 1).

### Analysis of clinical parameters of subjects diagnosed with AP before and after the therapy

By analyzing the obtained results, a statistically significant correlation between the mean values of the clinical parameters of patients with AP, before and after the therapy ( $p = 0.000$ ; Table 2) was established.

**Table 2.** Values of clinical parameters in the group of patients with aggressive periodontitis before and after the therapy

Clinical parameters X ± SD (Med., min.–max.)	Aggressive periodontitis		Significance
	Before therapy	After therapy	
GI	1.66 ± 0.65 (2; 0.25–2.5)	0.82 ± 0.56 (0.94; 0–1.75)	ap = 0.000*
PI	1.69 ± 0.71 (2; 0.25–2.71)	0.97 ± 0.51 (1; 0.25–2)	ap = 0.000*
BI	1.47 ± 0.66 (1.5; 0.25–2.14)	0.66 ± 0.39 (0.79; 0–1.2)	ap = 0.000*
PD	5.05 ± 1.08 (5; 3.8–6.95)	4.08 ± 0.98 (4; 2.8–5.9)	ap = 0.000*
DI	1.4 ± 0.61 (1.5; 0.25–2.2)	0.59 ± 0.36 (0.68; 0–1)	ap = 0.000*
CI	0.02 ± 0.71 (0; 0–0.25)	0.1 ± 0.14 (0; 0–0.5)	ap = 0.000*

GI – gingival index; PI – plaque index; BI – bleeding index; PD – probing depth; DI – debris index; CI – calculus index;

\*statistically significant difference;

<sup>a</sup>Whilcoxon test

Two months after the therapy a statistically significant decrease in the value of all observed clinical parameters was noticed, i.e., there was an improvement of periodontal health.

### Correlation of biochemical markers values in the saliva between the subjects of the control group and patients with aggressive periodontitis before and after the therapy

The activity of intracellular enzymes in the unstimulated saliva of the patients with AP was different from the one in the control group (Table 3).

**Table 3.** Values of biochemical markers in the saliva of healthy subjects and patients with aggressive periodontal disease before and after the therapy

Biochemical markers	Aggressive periodontitis		Significance
	Before therapy	After therapy	
AST (U/L)	28.18 ± 25.16 (17.59; 1–98)	26.57 ± 23.1 (16.99; 1.74–96)	ap = 0.845
ALT (U/L)	5.48 ± 5.14 (3.8; 1–23)	5.45 ± 6.75 (4; 1–29)	ap = 0.442
ALP (U/L)	31.13 ± 37.79 (18.36; 7.59–178)	17.61 ± 11.38 (16.98; 3.53–61.0)	ap = 0.100
Ca (mmol/L)	2.80 ± 1.97 (2.075; 0.66–10.31)	2.98 ± 2.67 (2.13; 0.74–15.48)	ap = 0.643
P (mmol/L)	4.43 ± 1.92 (4.31; 1.75–9.46)	3.87 ± 1.42 (3.9; 0.17–7.04)	bp = 0.158
ACP (U/L)	17.53 ± 14.77 (14.58; 2.54–80.97)	15.44 ± 16.08 (11.45; 1.15–87)	ap = 0.309

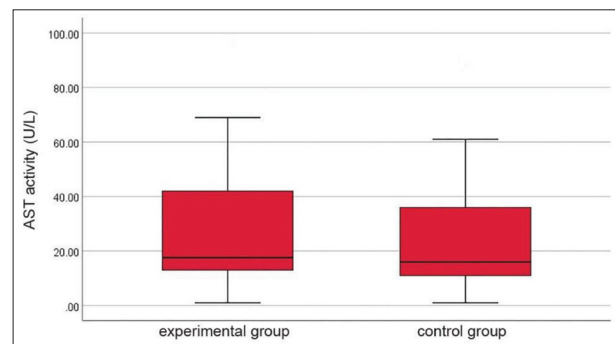
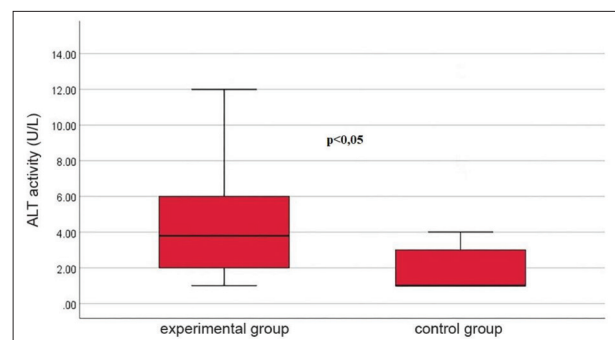
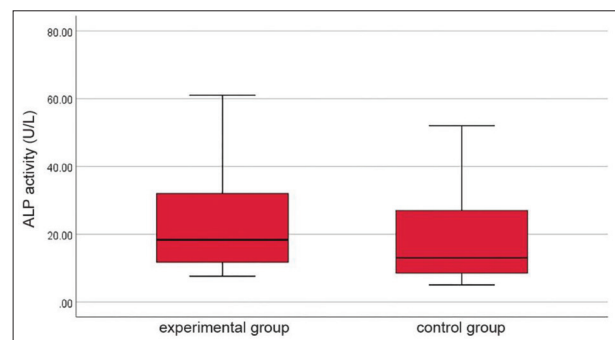
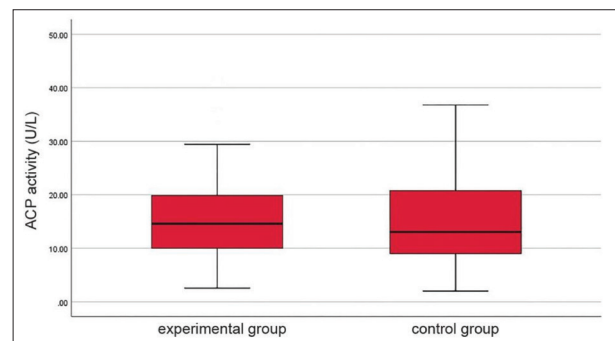
AST – aspartate aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; ACP – acid phosphatase;

\*statistically significant difference;

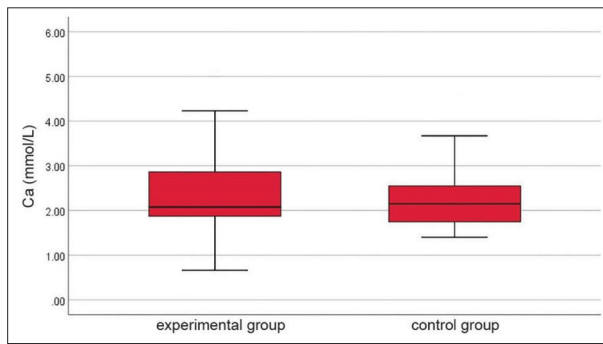
<sup>a</sup>Whilcoxon test;

<sup>b</sup>t-test for bound samples

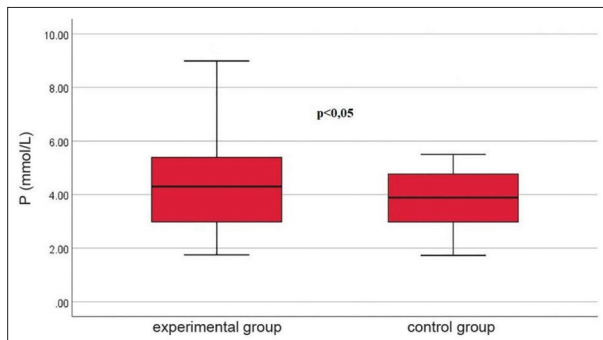
AST was decreased in subjects with AP (28.18 ± 25.16 U/L) compared to the subjects in the control group (29.2 ± 32.67 U/L), but without statistical significance (Figure 1). In contrast to AST, the ALT level in the saliva of subjects in the experimental group (5.48 ± 5.14 U/L) was statistically significantly higher than in the saliva of subjects

**Figure 1.** Value of aspartate aminotransferase (AST) in saliva of control and experimental group before the therapy**Figure 2.** Alanine aminotransferase (ALT) level in the saliva of subjects in the experimental group is statistically significantly higher than in the saliva of subjects with healthy parodontium**Figure 3.** Value of alkaline phosphatase (ALP) in saliva of control and experimental group before the therapy**Figure 4.** Value of acid phosphatase (ACP) in saliva of control and experimental group before the therapy

with healthy parodontium (2.40 ± 2.51 U/L) ( $p = 0.000$ ; Table 3; Figure 2). Mean values of the activity of the enzymes ALP (31.13 ± 37.79 U/L), ACP (17.53 ± 14.77 U/L) in the saliva of subjects in the experimental group were



**Figure 5.** Concentration of calcium in saliva of control and experimental group before the therapy



**Figure 6.** The concentration of phosphate in the experimental group was statistically significantly higher than in the control group

higher compared to the subjects in the control group ( $18.31 \pm 12.39$  U/L,  $15.62 \pm 8.52$  U/L), but without statistically significant difference (Figures 3 and 4). The mean calcium concentration in the saliva of the subjects in the experimental group ( $2.80 \pm 1.97$ ) was higher compared

to the subjects in the control group ( $2.25 \pm 0.69$ ) (Figure 5). Unlike calcium, the mean concentration of phosphate in the experimental group ( $4.43 \pm 1.92$ ) was statistically significantly higher than in the control group ( $3.87 \pm 1.31$ ) ( $p = 0.001$ ; Table 3; Figure 6).

Eight weeks after the basic and surgical therapy, the activity of the enzymes (AST, ALT, ALP, ACP) in the saliva of the subjects with AP was reduced but without statistical significance (Table 3).

**Correlation of clinical parameter values and biochemical markers in patients with aggressive periodontitis before the therapy**

Analyzing the interconnection, no statistical correlation was found between the values of GI, PD, PI, BI and the values of the analyzed markers (AST, ALT, ALP, ACP, Ca, P) in the saliva of subjects with AP, before the therapy (Table 4).

However, the Spearman correlation test showed a statistically significant correlation between the AST values in the saliva and DI values ( $r = -0.444$ ;  $p = 0.026$ ) and CI values ( $r = -0.513$ ;  $p = 0.009$ ). The coefficient of correlation shows that with the increase in the values of CI and DI, there is a decrease in AST values in the saliva of patients with AP before the therapy (Table 4).

**Correlation of the values of clinical parameters and biochemical markers in patients with aggressive periodontitis**

No statistically significant relationship was found between the values of clinical and biochemical parameters, in the saliva of the experimental group after the therapy. Unlike these data, a statistically significant correlation was observed between the PI value and the ALP level in the saliva ( $p = 0.020$ ; Table 5). By the univariate regression analysis, the ALT has been found as statistically significant, therefore the elevated values of this enzyme are always present in patients with AP.

**Table 4.** Correlation of the values of clinical parameters and biochemical markers in the saliva of subjects with aggressive periodontitis before the therapy

Biochemical markers	Gingival index	Plaque index	Bleeding index	Probing depth	Debris index	Calculus index
AST	$\rho = -0.326$	$\rho = -0.236$	$\rho = -0.280$	$\rho = 0.076$	$\rho = -0.444^*$	$\rho = -0.513^*$
ALT	$\rho = 0.023$	$\rho = -0.063$	$\rho = -0.071$	$\rho = 0.147$	$\rho = -0.024$	$\rho = -0.063$
ALP	$\rho = -0.167$	$\rho = -0.035$	$\rho = -0.043$	$\rho = 0.140$	$\rho = -0.112$	$\rho = -0.069$
Ca	$\rho = -0.143$	$\rho = 0.010$	$\rho = -0.069$	$\rho = 0.167$	$\rho = -0.109$	$\rho = -0.080$
P	$\rho = -0.285$	$\rho = -0.029$	$\rho = -0.147$	$\rho = -0.002$	$\rho = -0.248$	$\rho = -0.238$
ACP	$\rho = -0.205$	$\rho = 0.086$	$\rho = -0.094$	$\rho = 0.110$	$\rho = -0.178$	$\rho = -0.156$

ALT – alanine aminotransferase; ALP – alkaline phosphatase; ACP – acid phosphatase;  
 $\rho$  – Spearman's coefficient of correlation; AST – aspartate aminotransferase;  
 \*statistically significant linkage

**Table 5.** Correlation of the values of clinical parameters and biochemical markers in the saliva of patients with aggressive periodontitis after therapy

Biochemical markers	Gingival index	Plaque index	Bleeding index	Probing depth	Debris index	Calculus index
AST	$\rho = -0.010$	$\rho = 0.078$	$\rho = 0.057$	$\rho = 0.309$	$\rho = 0.238$	$\rho = 0.165$
ALT	$\rho = -0.118$	$\rho = 0.089$	$\rho = 0.080$	$\rho = -0.125$	$\rho = 0.097$	$\rho = 0.185$
ALP	$\rho = 0.394$	$\rho = 0.463^*$	$\rho = 0.327$	$\rho = 0.018$	$\rho = -0.180$	$\rho = 0.058$
Ca	$\rho = 0.204$	$\rho = 0.293$	$\rho = 0.043$	$\rho = 0.094$	$\rho = 0.238$	$\rho = 0.123$
P	$\rho = 0.086$	$\rho = -0.062$	$\rho = -0.053$	$\rho = 0.158$	$\rho = -0.259$	$\rho = 0.024$
ACP	$\rho = -0.062$	$\rho = 0.166$	$\rho = -0.027$	$\rho = 0.247$	$\rho = -0.157$	$\rho = 0.196$

$\rho$  – Spearman's coefficient of correlation; AST – aspartate aminotransferase;  
 ALT – alanine aminotransferase; ALP – alkaline phosphatase; ACP – acid phosphatase;  
 \*statistically significant linkage

**DISCUSSION**

In patients with AP, the condition of periodontal tissues before and after the therapy was analyzed, based on the values of clinical parameters and the presence of biochemical markers in the saliva.

During the periodontal infection, various enzymes from stromal, epithelial and inflammatory cells are released into the saliva, gingival fluid and blood [7, 8]. Chambers et al. [9] published the first study that indicated an increase in the AST levels in gingival fluid in dogs during the experimental periodontitis. Since that time, many researchers have found that the AST activity in the

saliva is proportionate to the degree of tissue damage during the chronic periodontitis [10, 11] and gingivitis [12]. Due to the pathological process in periodontal tissues, the integrity of the cells is impaired, the permeability of their membranes is disturbed, and thus the AST is increasingly released from the cytoplasm into the saliva. Also, the AST values in the saliva [13] and gingival fluid [14] were in correlation with the values of the Community Periodontal Index of Treatment Needs. Our study has not proved that there is a correlation between the values of clinical parameters and the AST activity in the saliva.

In patients with chronic periodontitis, three months [11] or one month [12], after the basic therapy, there was a decrease in the AST levels in the saliva. Similar results were obtained in the gingival fluid [14]. Results of this study indicate that the mean value of the AST activity in the saliva of patients with AP was reduced two months after the basic and surgical therapy. Some authors believe that the decrease in AST activity in the saliva after the periodontal therapy, is a consequence of periodontal tissue reparation [8, 15]. In our study, in patients with AP, the mean PD was  $5.05 \pm 1.08$ , but there was a PD decrease ( $4.08 \pm 0.98$ ), after the basic and surgical therapy. This decrease in the PD was followed by a decrease in the AST levels in the saliva, but without statistical significance. After the mechanical therapy, in subjects with the chronic periodontal disease the decrease in AST activity was in correlation with the GI values and depth of the periodontal pocket [11]. The reduced GI value and AST levels indicate that the gingival health has improved [16]. In contrast to these studies, the AST level in the gingival fluid [17] and saliva [18] was not in correlation with the GI and PI values. Similar results have been obtained in our research. However, we have observed a statistical correlation between the increase in the DI and CI values and the decrease in the AST activity. The reason is in that the dental deposits are a predisposing factor which can lead to the development of gingivitis and periodontal disease.

Unlike the AST, the mean value of ALT ( $5.48 \pm 5.14$  U/L) in the saliva of experimental group was statistically significantly higher than in the control group ( $2.40 \pm 2.51$  U/L) ( $p = 0.000$ ). By univariate analysis, it was found that the elevated ALT levels in the saliva are always present in subjects suffering from AP. In addition, this enzyme can be an indicator of the damage degree to the gingival tissue because the GI values were increasing linearly with increasing ALT levels in the saliva [10]. Also, in patients with chronic periodontitis and gingivitis, a correlation was found between the ALT level and the values of the depth of periodontal pocket, the level of clinical attachment and the number of periodontal-pathogenic bacteria in the saliva [15]. Data of this study indicate that there is no statistical correlation between the ALT levels and clinical parameter values. After applying the conventional therapy, in patients with chronic periodontitis, the level of ALT in the saliva [10] was significantly reduced. This is in line with our results. For the stabilization of periodontal tissue in patients with AP, it is necessary to apply the mechanical/surgical and antimicrobial treatment over a longer period [19]. Thus,

the appropriate periodontal therapy for AP significantly changes the condition of the supporting structure of teeth, resulting in the level of ALT in the saliva. The authors consider that intracellular enzymes, especially the ALT, can serve as biochemical markers of acute damage of soft periodontal tissue during AP.

The ALP is an enzyme that catalyses the hydrolysis of the monophosphate ester bond in the alkaline environment. Its increased presence in the saliva reflects changes during the inflammation and destruction of periodontal tissue [20]. In the localized and generalized form of AP, a positive correlation between the pathological changes in periodontal tissues and the ALP concentration in the gingival fluid was demonstrated [21]. In our study, statistically significantly higher ALP values were measured in the group of subjects with AP compared to those with a healthy periodontium. Also, compared to healthy subjects, the level of ALP in the saliva and gingival fluid was increased in patients with chronic periodontitis [22, 23, 24]. The authors believe that, due to the tissue inflammation, there was an accumulation of polymorphonuclear leukocytes (PMN) that release the ALP into oral fluids [24]. Another study demonstrated a greater ALP activity in the gingival fluid in cases of chronic periodontitis, compared to patients with AP [24]. This is probably due to an impaired PMN function in patients with AP. In line with the intensity of the pathological process in periodontal tissue, the release of ALP also correlated. In patients with chronic periodontitis, the GI values and the depth of the periodontal pocket were statistically correlated with the level of ALP in the saliva and gingival fluid [10, 25]. The authors believe that the ALP enzyme can be a predictor of progression of periodontal diseases. In our investigation, no statistical correlation was found between the values of clinical parameters and the ALP concentration in the saliva.

After the basic and surgical therapy, the mean values of the ALP concentrations in the saliva of patients with AP ( $17.61 \pm 11.38$ ) were lower than the mean value of concentration ( $31.13 \pm 37.79$ ) before the therapy. Similar results were obtained in the gingival fluid in subjects with AP and patients with chronic periodontitis, after nonsurgical treatment [24, 26]. The authors believe that the reduction in ALP levels in the saliva can be a useful biomarker to monitor the effectiveness of the applied therapy.

In this study, the ACP as an important marker of remodelling the bone tissue was analyzed in saliva. The mean value of the ACP enzyme activity ( $17.53 \pm 14.77$  U/L) in the saliva samples of AP subjects was higher than the mean value in the control group ( $15.62 \pm 8.52$  U/L). Similar results were obtained in subjects with chronic periodontitis [10]. As periodontal disease is progressing, destructive processes develop in the alveolar bone, and ACP is released as a result of an increased osteoclastic activity [22]. Our results show that the ACP activity in the saliva of patients with AP was reduced, two months after the basic and surgical therapy, but without a statistical correlation. Reduction of the ACP levels in the saliva after the conventional periodontal therapy with a statistically significant correlation was observed in subjects with chronic periodontitis [10, 22].

Changes in the macro and trace element composition of saliva might be indicative for pathological changes in periodontal tissues [27]. Based on literature data, increased concentrations of calcium and phosphate in the saliva are the risk factors for the development of periodontitis [28]. Various studies have shown an increased concentration of calcium in the saliva of patients with chronic periodontitis compared to the group of subjects with healthy periodontium [27]. Also, our results demonstrate an increased calcium concentration in the saliva of subjects with AP, which is in agreement with other studies [27, 29]. Unlike the calcium concentration, a statistically higher phosphate concentration in the saliva of subjects with AP ( $4.43 \pm 1.92$ ) was observed compared to the subjects in the control group ( $3.87 \pm 1.31$ ). It has been proven that with the progression of periodontal disease, the levels of calcium and phosphate in the saliva are correlated with the values of clinical parameters (PI and GI) [29]. This is contrary to our results because the calcium and phosphate levels in the saliva were not in the statistical correlation with clinical parameters of the subjects with AP. Increasing the concentration of electrolytes in the saliva leads to mineralization of the dental plaque (tooth stones),

which makes it difficult to clean, especially in the area of periodontal sulcus.

## CONCLUSION

Based on the obtained results, it can be concluded that the increased level of intracellular enzymes (AST, ALP, ALT, ACP) in the saliva of patients with AP is a consequence of release from damaged cells and / or metabolic changes in periodontal tissues. After the basic and surgical therapy, the values of these enzymes were reduced, and thus they can be used to evaluate the effectiveness of the applied therapy.

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**Conflict of interest:** None declared.

## REFERENCES

- Buziane A, Hamdoun R, Abouqal R, Ennibi O. Global prevalence of aggressive periodontitis. A systematic review and meta-analysis. *J Clin Periodontol.* 2020;47(4):406–28.
- Lee HA, Park MH, Song Y, Na HS, Chung J. Role of Aggregatibacter actinomycetemcomitans-induced autophagy in inflammatory response. *J Periodontol.* 2020;91(12):1682–93.
- Albandar JM. Aggressive periodontitis: case definition and diagnostic criteria. *Periodontol 2000.* 2014;65(1):13–26.
- Martina E, Campanati A, Diotallevi F, Offidani A. Saliva and Oral Diseases. *J Clin Med.* 2020;9(466):1–15.
- Melguizo-Rodríguez L, Costela-Ruiz VJ, Manzano-Moreno FJ, Ruiz C, Illescas-Montes R. Salivary Biomarkers and Their Application in the Diagnosis and Monitoring of the Most Common Oral Pathologies. *Int J Mol Sci.* 2020;21(14):5173.
- Upadhyay M, Bhardwaj P, Sonamben M, Agarwal N, Bhardwaj S, Hundal K. Salivary Enzymes Potential Markers for Periodontal Disease. *Int J Med Res Prof.* 2019;5(5):134–6.
- Kamalabadi YM, Sedigh SS, Fariabi F. A comparison of blood levels with saliva levels of liver enzymes (ALP, ALT, AST) in patients with chronic periodontitis. *Med Sci.* 2020;24(103):1208–16.
- Kishore PK, Kachwala MS, Swetha B. Salivary Enzymes as Diagnostic Markers for Detection of Periodontal Disease. *Inter J Curr Adv Res.* 2019;8(5):18714–6.
- Chambers DA, Crawford JM, Mukherjee S, Cohen RL. Aspartate aminotransferase increase in crevicular fluid during experimental periodontitis in beagle dogs. *J Periodontol.* 1984;55(9):526–30.
- Todorovic T, Dozic I, Barrero MV, Ljuskovic B, Pejovic J, Marjanovic M, et al. Salivary enzymes and periodontal disease. *Med Oral Patol Oral Cir Bucal.* 2006;11(2):115–9.
- Jassim DS. Effects of Non-Surgical Periodontal Therapy on Salivary Aspartate Aminotransferase Levels in Chronic Periodontitis Patients. *Res J Pharm Biol Chem Sci.* 2017;8(2):2058–63.
- Kudva P, Saini N, Kudva H, Saini V. To estimate salivary aspartate aminotransferase levels in chronic gingivitis and chronic periodontitis patients prior to and following non-surgical periodontal therapy: A clinico-biochemical study. *J Indian Soc Periodontol.* 2014;18(1):53–8.
- Deepika V, Vishnu Priya V, Bedre A, Harsha L. Salivary AST, ALP and CK Levels in Patients with Periodontitis. *J Pharm Sci Res.* 2015;7(6):341–3.
- Sheth TS, Verma JS. Analysis of aspartate aminotransferase in gingival crevicular fluid: A study with initial therapy. *J Indian Soc Periodontol.* 2011;15(3):235–9.
- Dabra S, China K, Kaushik A. Salivary enzymes as diagnostic markers for detection of gingival/periodontal disease and their correlation with the severity of the disease. *J Indian Soc Periodontol.* 2012;16(3):358–64.
- Kamma JJ, Nakou M, Persson RG. Association of early onset periodontitis microbiota with aspartate aminotransferase activity in gingival crevicular fluid. *J Clin Periodontol.* 2001;28(12):1096–105.
- Nakayama Y, Takei-Obi M, Toyoshima-Matsumura I, Tsutamorri M, Kato A, Okano C, et al. Clinical usability of aspartate aminotransferase to evaluate the prognosis of periodontal regeneration therapies: prospective, longitudinal study. *Odontology.* 2018;106(3):306–15.
- Silva EB, Salvador SLS, Fogo JC, Marcantonio RAC. Use of aspartate aminotransferase in diagnosing periodontal disease: a comparative study of clinical and microbiological parameters. *J Oral Sci* 2003;45(1):33–8.
- Nomura Y, Shimada Y, Hanada N, Numabe Y, Kamoi K, Sato T, et al. Salivary biomarkers for predicting the progression of chronic periodontitis. *Arch Oral Biol.* 2012;57(4):413–20.
- Shetty SR, Fuoad Al-Bayati SAA, Said Hamed M. Salivary alkaline phosphatase and oral health: A review. *Ital J Dent Med.* 2017;2(2):55–8.
- Castro CE, Koss MA, Lopez ME. Intracytoplasmic enzymes in gingival crevicular fluid of patients with aggressive periodontitis. *J Periodont Res.* 2011;46(5):522–7.
- Dabra S, Singh P. Evaluating the levels of salivary alkaline and acid phosphatase activities as biochemical markers for periodontal disease: A case series. *Dent Res J (Isfahan).* 2012;9(1):41–5.
- Di Lenardo D, Silva FRPD, de Carvalho LF, Carvalho JDS, Alves EHP, VasconcelosDFP. Evaluation of Biochemical Parameters Present in the Saliva of Patients with Chronic Periodontitis: Results from a Meta-Analysis. *Genet Test Mol Biomarkers.* 2019;23(4):255–63.
- Singh N, Chandel S, Singh H, Agrawal A, Savitha AN. Effect of scaling and root planing on the activity of ALP in GCF and serum of patients with gingivitis, chronic and aggressive periodontitis: A comparative study. *J Oral Biol Craniofac Res.* 2017;7(2):123–6.
- Kunjappu JJ, Mathew VB, Hegde S, Kashyap R, Hosadurga R. Assessment of the alkaline phosphatase level in gingival crevicular fluid, as a biomarker to evaluate the effect of scaling and root planing on chronic periodontitis: An in vivo study. *J Oral Maxillofac Pathol.* 2012; 16(1):54–7.

26. Koss MA, Castro EC, Guarnieri SM, Hermosilla D. Determination of Salivary Alkaline Phosphatase and  $\beta$  Glucuronidase in Treated Periodontal Disease Patients. *EC Dental Sci.* 2019;18:1225–31.
27. Inonu E, Hakki SS, Kayis SA, Nielsen FH. The Association Between Some Macro and Trace Elements in Saliva and Periodontal Status. *Biol Trace Elem Res.* 2020;197(1):35–42.
28. Patel RM, Varma S, Suragimath G, Zope S. Estimation and Comparison of Salivary Calcium, Phosphorous, Alkaline Phosphatase and pH Levels in Periodontal Health and Disease: A Cross-sectional Biochemical Study. *J Clin Diagn Res.* 2016;10(7):58–61.
29. Gupta VV, Chitkara N, Gupta HV, Singh A, Gambhir RS, Kaur H. Comparison of salivary calcium level and pH in patients with aggressive periodontitis and healthy individuals: a clinico-biochemical study. *Oral Health Dent Manag.* 2016;15(4):122–6.

## Анализа биохемијских маркера у пљувачки и корелација са клиничким параметрима код оболелих од агресивне пародонтопатије, пре и после терапије

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### САЖЕТАК

**Увод/Циљ** Агресивна пародонтопатија (АП) је прогресивно обољење које доводи до оштећења пародонталних ткива. Циљ истраживања био је анализа интраћелијских ензима аспартат-аминотрансферазе, аланин-аминотрансферазе, алкалне фосфатазе, киселе фосфатазе и електролита (калцијума, фосфата) у пљувачки испитаника са АП и њихова корелација са клиничким параметрима, пре и после терапије.

**Методe рада** У студију је укључено 30 пацијената са АП (експериментална група) и 35 испитаника са здравим пародонцијумом (контролна група). Интраћелијски ензими (аспартат-аминотрансферазе, аланин-аминотрансферазе, алкалне фосфатазе, киселе фосфатазе) и електролити (калцијум, фосфати) анализирани су у нестимулисаој пљувачки испитаника са АП, пре и после терапије (базичне и хируршке) и у узорцима пљувачке испитаника контролне групе. Анализа биохемијских маркера је вршена кинетичким методама, помоћу комерцијалних реагенаса.

**Резултати** Концентрације биохемијских маркера аспартат-аминотрансферазе ( $28,18 \pm 25,16$ ), аланин-аминотрансфе-

разе ( $5,48 \pm 5,14$ ), алкалне фосфатазе ( $31,13 \pm 37,79$ ), киселе фосфатазе ( $17,53 \pm 14,77$ ), калцијума ( $2,80 \pm 1,97$ ), фосфата ( $4,43 \pm 1,92$ ) у пљувачки испитаника експерименталне групе биле су статистички значајно веће у односу на контролну групу ( $p = 0,000$ ;  $p = 0,001$ ). Вредности клиничких параметара су биле статистички значајно ниже након спроведене пародонталне терапије ( $p = 0,000$ ). Спирмановим тестом уочена је статистичка корелација између вредности аспартат-аминотрансферазе и вредности индекса меких наслага ( $\rho = -0,444$ ;  $p = 0,026$ ) и индекса чврстих наслага ( $\rho = -0,513$ ;  $p = 0,009$ ). Након терапије је постојала корелација између вредности плак индекса и нивоа алкалне фосфатазе у пљувачки ( $p = 0,020$ ).

**Закључак** Спроведено истраживање допринеће бољем разумевању и стандардизацији биомаркера у пљувачки који би помогли у дијагностиковању и праћењу спроведене терапије код оболелих од АП.

**Кључне речи:** агресивна пародонтопатија; интраћелијски ензими; пљувачка