



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Alkaline phosphatase and acid phosphatase levels in saliva and serum of patients with healthy periodontium, gingivitis, and periodontitis before and after scaling with root planing: A clinico-biochemical study

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ARTICLE INFO

Article history:

Received 13 September 2020

Revised 28 September 2020

Accepted 6 October 2020

Available online 14 October 2020

Keywords:

Alkaline phosphatase

Acid phosphatase

Saliva

Serum

ABSTRACT

Periodontitis is commonly diagnosed based on clinical parameters. However, the analysis of a few unique biomarkers of the disease process present in the saliva and blood can further assist the estimation of the rate of disease progression.

Aim: The present study attempted to correlate the alkaline phosphatase (ALP) and acid phosphatase (ACP) levels in saliva and serum between patients with healthy periodontium, gingivitis, and chronic periodontitis.

Materials and methods: The present study was conducted in 135 subjects between 20 and 55 years of age. The subjects were divided into three groups, namely healthy (Group A), gingivitis (Group B), and chronic periodontitis (Group C). The clinical parameters were recorded using the plaque index (PI), gingival index (GI), and probing depth (PD). Saliva and serum were analyzed for ALP and ACP levels using an auto analyzer. All patients underwent scaling and root planning (SRP) along with oral hygiene instructions. Patients were then recalled after four weeks, and blood and saliva samples were collected to estimate ALP and ACP levels prior to clinical examination.

Results: The clinical parameters exhibited a statistically significant decrease in the PI and GI in both group B and group C after SRP. A significant change in the PD and attachment levels (AL) was observed in the periodontitis group after SRP. The mean salivary & serum ALP levels exhibited a statistically significant decrease in group B & C after SRP. The mean serum ACP levels exhibited a statistically significant decrease in group B & C after SRP. However, the salivary ACP levels decrease after SRP was only statistically significant in group C.

Conclusion: Serum and salivary ALP and ACP levels were markedly decreased in the gingivitis and periodontitis groups after SRP and were positively correlated with the clinical parameters.

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Peer review under responsibility of King Saud University.



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1. Introduction

Periodontal disease is a chronic oral polymicrobial infection characterized by periods of exacerbation and remission. It is also coupled with a number of gram-negative microorganisms, along with these pathogenic bacteria, a susceptible host is also crucial (Koppolu et al., 2013). Traditional methods for periodontal disease diagnosis such as probing depth (PD), attachment level (AL), and

<https://doi.org/10.1016/j.sjbs.2020.10.016>

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gingival recession, diagnose the pathology only after the disease process and the damage associated with it has already occurred.

Estimation of the serum and saliva levels of a few components can predict the disease risk. These organic, inorganic, enzymatic, immunoglobulin, or hormonal components are called as biological markers or biomarkers. Biomarkers have been defined by Hulka and colleagues (Hulka, 1990) as “cellular, biochemical, or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids.” This definition has been modified by Naylor (2003) to include biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.

Host response to periodontal disease includes production of several tissue degradation enzymes released from various inflammatory or bacterial cells, of which bone markers such as alkaline phosphatase (ALP) and acid phosphatase (ACP) play a crucial role as altered levels of these markers are observed in disease (Todorovic et al., 2006).

ALP, a vital marker associated with bone formation, is an enzyme found in many periodontal cells, including osteoblasts, fibroblasts, and neutrophils (Kinane, 1997). ALP is released from polymorphonuclear leukocytes during inflammation, osteoblasts during bone formation, and periodontal ligament fibroblasts during periodontal regeneration. Thus, it is involved in the process of both periodontal inflammation and regeneration. ALP allows bone mineralization by releasing an organic phosphate and hydrolyzing inorganic pyrophosphate (Daltaban et al., 2006).

ACP is usually present in neutrophils and is associated mainly with the osteoclastic activity of bone metabolism. Desquamated epithelial cells, macrophages, and several bacteria such as actinobacillus, capnocytophaga, and veillonella also produce this enzyme. Enzyme histochemistry studies have demonstrated elevated levels of ALP and ACP in chronically inflamed gingiva (Desai et al., 2008; Sanikop et al., 2012).

ALP and ACP biomarkers are associated with the formation, resorption and turn over of the bone. These mediators are frequently associated with systemic bone metabolism and also could be associated local bone metabolism in periodontitis. Thus, the present study attempted to estimate the ALP and ACP levels in the saliva and serum of patients with gingivitis and periodontitis before and after scaling & root planning (SRP), and its correlation with healthy individuals.

2. Materials and methods

The present study was conducted in 135 patients of both sexes between 20 and 55 years attending the outpatient department of Periodontics. Of the 135 patients, 75 were males and 60 were females, with 24 males and 24 females in both gingivitis and periodontitis groups and 24 males & 25 females in the healthy group. The power analysis was used to regulate the minimum sample size essential to distinguish the effect of treatment; a sample size of minimum 40 was set for this study, based on an estimated value of 80% power that is essential to discard the null hypothesis. The study population was divided into groups A, B, and C, with 48 patients each in groups B and C, and 49 patients in group A. Systemically healthy patients with more than 20 teeth and within the age group of 20–55 years, patients with either healthy gingiva or chronic gingivitis based on clinical appearance, and patients with periodontitis associated with a PD of ≥ 4 mm were included in the study. Patients being medicated for any medical conditions, patients on drugs which interfere with blood coagulation, patients with history of bleeding disorders, smokers, pregnant and lactating women, patients who had undergone oral prophylaxis within

6 months of the study, and patients who used analgesics and antibiotics since 3 months were excluded from the study.

The patients were divided into the following groups:

- Systemically healthy patients with healthy gingiva: Healthy group (group A) gingival index (GI) ≤ 0.1
- Chronic gingivitis based on the GI: Gingivitis group (group B)
- Systemically healthy patients with probing depths of ≥ 4 mm: Chronic periodontitis group (group C).

Unstimulated saliva and venous blood from the antecubital fossa were collected in a sterile container before clinical examination and centrifuged. Serum and saliva were separated from the centrifuged samples, and the ALP and ACP were estimated using an auto analyzer. The clinical examination consisted of the plaque index (PI) (Silness and Loe), GI (Loe and Silness), PD, and clinical attachment loss with a thorough medical and personal history. PD was measured with UNC 15 probe. All patients underwent SRP along with oral hygiene instructions.

Patients were then recalled after four weeks, and blood and saliva samples were collected to estimate ALP and ACP levels prior to clinical examination.

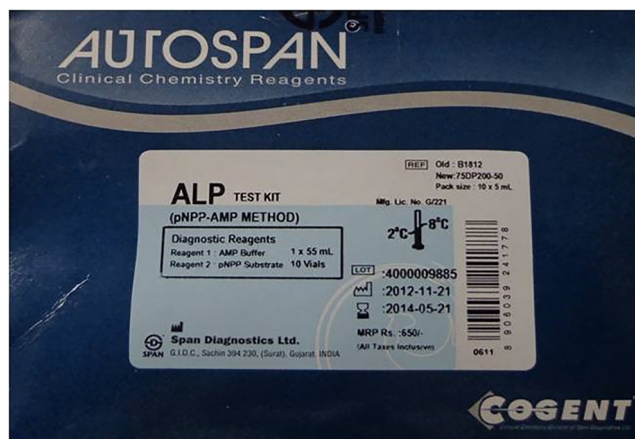


Fig. 1. Test kits used as reagents.



Fig. 2. Auto analyzer used for estimation of serum and salivary ALP and ACP levels.

2.1. Enzyme assay for saliva and serum

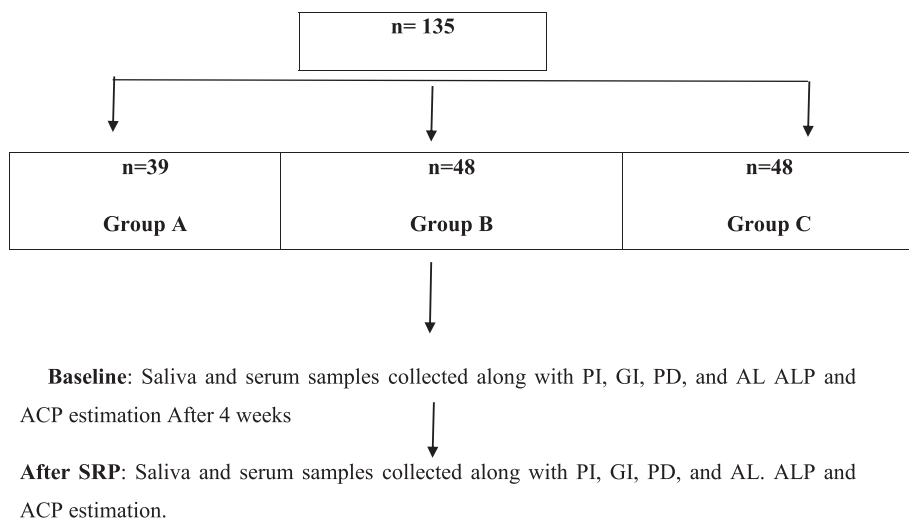
ALP and ACP levels were estimated with an auto analyzer (Nex-Gen, Span, USA) by using Autospan clinical chemistry reagents (Span Diagnostics, India). Saliva and blood were centrifuged at 3000 rpm for 10 min, the supernatant saliva sample and serum were added to 5 µl of the specific ALP reagent and ACP reagent used as substrates, and the values were determined and expressed as units per liter (U/L) (Figs. 1, 2).

2.2. Statistical analysis

Statistical analysis was performed using statistical package for social science, Chicago, IL version 18. The clinical indices and parameters among the gingivitis and periodontitis groups were compared using independent sample *t* test, whereas the serum and salivary ALP and ACP among the three groups were compared using analysis of variance with post hoc Games–Howell test. A *p* value of < 0.05 was considered statistically significant.

2.3. Study design

Study design:



3. Results

For each subject, the mean values of the clinical indices for AL, PD, plaque, and bleeding along with biochemical parameters such

Table 1
Comparison of parameters for Group A (Healthy) with Group B (Gingivitis) and Group C (Periodontitis) patients before and after treatment.

Parameter	Healthy	Gingivitis Mean ± SD		P value	Periodontitis Mean ± SD		P value
		Before SRP	After SRP		Before SRP	After SRP	
PI	0.13 ± 0.11	1.15 ± 0.34	0.67 ± 0.30	0.036*	2.48 ± 0.27	0.98 ± 0.25	0.039*
GI	0.09 ± 0.07	1.72 ± 0.46	0.98 ± 0.15	<0.001#	2.10 ± 0.41	1.26 ± 0.26	<0.001#
PD	2.53 ± 0.34	2.74 ± 0.13	2.56 ± 0.20	0.031*	6.36 ± 1.03	3.55 ± 1.02	0.027*
CAL	–	–	–	–	4.98 ± 1.07	2.70 ± 1.04	0.043*

#P = <0.001 highly significant.

*P = <0.05 statistically significant.

as salivary and serum ALP and ACP were assessed for 90 patients (age range: 20–55 years). Tables 1 and 2, along with Graph 1 present the summary of the statistics for these means across all subjects.

The mean serum and salivary ALP levels were higher in both the group B & C at baseline (prior to SRP). A statistically significant reduction in the mean serum and salivary ALP was observed after SRP in both B & C groups. Similarly, a statistically significant reduction in the mean serum ACP levels was observed after SRP in groups B & C. Whereas the decrease in mean salivary ACP level was not statistically significant in group B. The mean serum and salivary ALP and ACP levels were highest in the group C, followed by the group B and group A at baseline. The mean serum & salivary ALP levels were higher in the group C as compared with the group B before and after SRP. Correspondingly, the mean serum & Salivary ACP levels were higher in the group C when related to the group B at baseline and after SRP consequently. (Tables 3 and 4, Graph 2).

4. Discussion

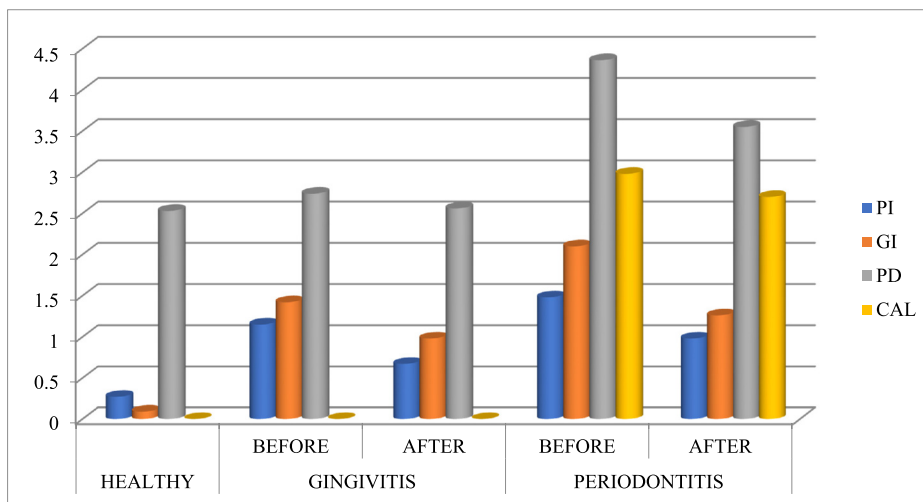
Periodontitis is a major threat to both oral and systemic health. The periodontal destruction process is highly complex and involves

Table 2
Differences between ALP, ACP activity (U/L ± SD) in saliva and serum of healthy before and after periodontal treatment in Gingivitis and Periodontitis patients.

Parameter	Healthy mean ± SD	Gingivitis mean ± SD		P value	Periodontitis mean ± SD		P value
		Before SRP	After SRP		Before SRP	After SRP	
ALP serum	98.18 ± 34.47	109.18 ± 35.62	94.07 ± 22.95	<0.001*	132.54 ± 17.24	102 ± 10.05	0.03 [#]
ALP saliva	43.56 ± 10.87	52.06 ± 30.47	41.03 ± 24.77	<0.001*	123.54 ± 83.90	57.24 ± 36.28	0.04 [#]
ACP serum	4.45 ± 2.73	6.55 ± 4.78	5.04 ± 4.23	0.03 [#]	6.83 ± 3.51	4.74 ± 3.007	0.01 [#]
ACP saliva	32.63 ± 10.01	41.45 ± 16.82	30.49 ± 16.20	0.06	54.43 ± 34.43	42.40 ± 29.95	0.04 [#]

[#]P = <0.05 statistically significant.

*P = <0.001 highly significant.



Graph 1. Comparison of parameters for Group A (Healthy) with Group B (Gingivitis) and Group C (Periodontitis) patients before and after treatment.

Table 3
Comparison of mean values of the variables (before treatment) between different study groups.

	GROUP	Mean ± SD	P Value	Significant groups at 5% level Interval
ALP SERUM	A	98.18 ± 34.47	1.000	A Vs B
	B	109.18 ± 35.62	0.020*	B Vs C
	C	132.54 ± 17.24	0.020*	A Vs C
ALP SALIVA	A	43.56 ± 10.87	0.731	A Vs B
	B	52.06 ± 30.47	0.007*	B Vs C
	C	123.54 ± 83.90	0.003*	A Vs C
ACP SERUM	A	4.45 ± 2.73	0.244	A Vs B
	B	6.55 ± 4.78	0.857	B Vs C
	C	6.83 ± 3.51	0.322	A Vs C
ACP SALIVA	A	32.63 ± 10.01	0.352	A Vs B
	B	41.45 ± 16.82	0.051	B Vs C
	C	54.43 ± 34.43	0.282	A Vs C

*P = <0.05 statistically significant.

various biological mediators. The present study attempts to evaluate the levels of hydrolytic enzymes ALP and ACP as potential biochemical markers for gingivitis and periodontitis.

ALP is a highly significant enzyme in the periodontium as it is a part of the normal turnover of the periodontal ligament, root cementum, and bone homeostasis. ACP is a lysosomal enzyme and has high activity in bone resorbing cells such as osteoclast and macrophages. It catalyzes a variety of hydrolytic enzymes that occur in multiple molecular forms with cell lysosomes from a variety of tissues. ACP could also be bacterial in origin and play a role in pathological pocket formation (Pushparani and Nirmala, 2013).

Periodontal disease may be diagnosed through the evaluation of multiple clinical parameters. However, the salivary analysis can

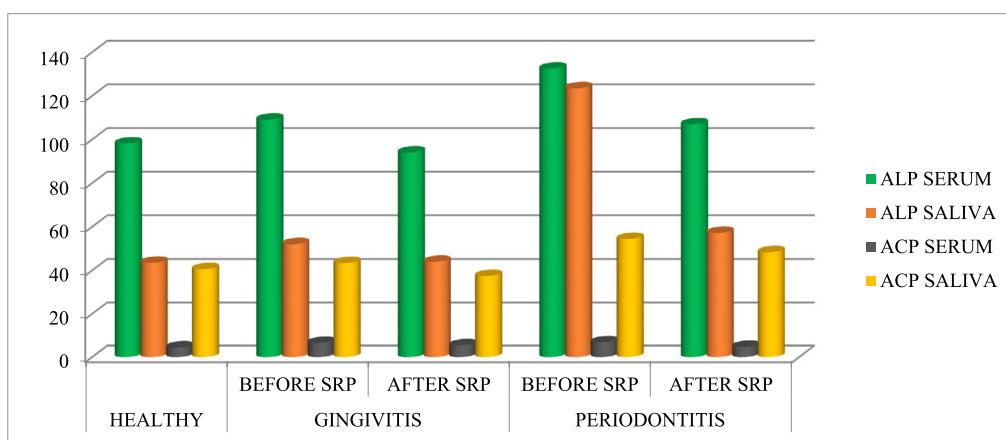
contribute to both the disease diagnosis and prognosis. Numerous salivary markers such as intracellular enzymes [creatine kinase, lactate dehydrogenase, aspartate aminotransferases (ASTs), alanine aminotransferases, gamma glutamyl transferase, ALP, and ACP] have been proposed as diagnostic markers for periodontal disease and appear to be useful for testing the periodontal disease activity and measuring the effectiveness of periodontal therapy (Kaufman and Lamster, 2002).

ALP, ACP, AST, alanine aminotransferases, gamma glutamyl transferase, are indicators of high level of cellular damage of periodontal tissue, and their increased activity in the gingival crevicular fluid (GCF) and saliva is due to their increased release from the damaged cells of the periodontal soft tissues and the reflected

Table 4
Comparison of mean values of the variables (After treatment) between different study groups.

	GROUP	Mean ± SD	P Value	Significant groups at 5% level Interval
ALP SERUM	A	98.18 ± 34.47	0.425	A Vs B
	B	94.07 ± 22.95	0.001	B Vs C
	C	102 ± 10.05	0.010*	A Vs C
ALP SALIVA	A	43.56 ± 10.87	0.970	A Vs B
	B	41.03 ± 24.77	0.292	B Vs C
	C	57.24 ± 36.28	0.275	A Vs C
ACP SERUM	A	4.45 ± 2.73	0.536	A Vs B
	B	5.04 ± 4.23	0.661	B Vs C
	C	4.74 ± 3.007	0.856	A Vs C
ACP SALIVA	A	32.63 ± 10.01	0.127	A Vs B
	B	30.49 ± 16.20	0.039*	B Vs C
	C	42.40 ± 29.95	0.554	A Vs C

*P = <0.05 statistically significant.



Graph 2. Differences between ALP, ACP activity (U/L ± SD) in saliva and serum of healthy before and after periodontal treatment in Gingivitis and Periodontitis patients.

metabolic changes in the inflamed gingiva (Mohammad and Aziz, 2018).

The present study revealed increased serum and salivary ALP and ACP levels with the disease progression. This can be attributed to high levels of cellular damage to the periodontal tissues and increased release of both enzymes from the damaged cells of the periodontal soft tissue into GCF and consequently into saliva.

Serum and salivary ALP and ACP levels decreased after SRP in both the groups exhibiting that decrease in disease progression resulted in decrease in the markers of bone destruction. This finding is concurrent with that of Todorovic et al. (2006) and Mohammad and Aziz (2018). The present study demonstrated a statistically significant difference in the mean serum and salivary ALP levels in both the gingivitis and periodontitis groups before and after SRP.

Although there was a difference in the higher serum and salivary ACP levels in both the groups, it did not exhibit any statistical significance. The reduction in the enzyme levels may be due to the role of scaling in the removal of plaque, which consists mainly of bacteria and may be a source for ALP and ACP in GCF and consequently into saliva. Thus, their release was reduced after treatment.

Increased ALP and ACP levels were positively correlated with the increased PI and GI scores in gingivitis and periodontitis group, and the PD and AL levels in the periodontitis group. After SRP, decrease in the clinical parameters coincided with the decrease in the serum and salivary ALP and ACP levels.

The small sample size and single-center design are major limitation of the present study because the findings cannot be generalized to the entire population. Further multicenter studies

with a larger sample size will strengthen the findings of this study.

5. Conclusion

ALP and ACP can be considered as potential periodontal disease markers as they can differentiate amongst healthy & inflamed sites. Both ALP & ACP are valuable to clinicians as they can quantitatively estimate the inflammatory status of gingival and periodontal tissues. Accordingly, they can be used as an aide to clinical indices of inflammation.

The serum and salivary ALP and ACP levels were markedly decreased in both the groups after SRP and were positively correlated with the clinical parameters. The reduction in the levels of serum and salivary ALP and ACP levels was observed with the reduction in the disease-causing factors. This reduction in the serum and salivary levels of ALP and ACP can be attributed to the tissue repair process after periodontal therapy. The estimation of serum and salivary ALP and ACP levels can be considered as a valuable diagnostic marker for the diagnosis of periodontal disease.

Funding

Self.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The project was supported by Deanship of Graduate Studies and Scientific Research at Dar Al Uloom University, Riyadh, KSA.

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