The comparison of GCF and Salivary level of ALP in smokers, non-smokers with periodontitis and in healthy subjects

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ABSTRACT

Background and Aim: Periodontal destruction occurs in an episodic fashion, with periods of exacerbation and quiescence. It is therefore important to diagnose periodontal diseases in active phase. Various diagnostic markers have been identified in GCF and saliva. The aim of this study was to compare the salivary and GCF levels of alkaline phosphatase (ALP) in patients with periodontitis, smokers with periodontitis and healthy subjects.

Materials and Methods: In this descriptive study 90 subjects aged 30-50 without any apparent systemic disease were chosen and divided to 3 equal groups: smokers with periodontitis, non-smokers with periodontitis and healthy subjects. In healthy subjects, GCF samples were collected from gingival sulcus. In patients with periodontitis, GCF was collected from pockets of 5-7 mm of depth. About 5 ml of un-stimulated whole saliva was collected from all subjects. All GCF and salivary samples were sent to the laboratory for ALP measurement. ANOVA and Tukey’s tests were used for statistical analysis.

Results: The results of this study showed that the mean level of ALP in saliva and GCF was higher in the first and second groups compared to the third group. The mean level of ALP in GCF was higher in the first group compared to the second group. The mean level of ALP in saliva was not statistically different between the first and second group.

Conclusion: It is possible to use both GCF and salivary levels of ALP as diagnostic markers for periodontitis. Higher levels of ALP in GCF samples of smokers with periodontitis could explain the higher rate of alveolar bone destruction in smokers.

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Introduction
Periodontal diseases are among common diseases of oral cavity. Severe periodontitis is manifested as alveolar bone loss around teeth with eventual loosening and loss of dentition. One of the characteristics of these diseases is the occurrence of bone destruction in a periodic fashion. Active periods of the disease are short which are followed by a prolonged phase of passiveness and quiescence. This characteristic along with the multifactorial nature of periodontal diseases creates difficulty in the recognition, classification and treatment of these diseases. If the disease is detected in the short active phase, the treatment will be easier and more effective. Therefore, in the past few decades a number of inflammatory and destructive markers for periodontitis have been presented. Potential resources for collection of inflammatory and destructive markers of periodontal diseases in order of importance include gingival crevicular fluid, saliva, serum and urine. Saliva can be easily collected and includes local and systemic markers for detection of periodontal diseases. Saliva may be collected in stimulated or unstimulated form solely from the parotid, submandibular or sublingual glands or as a mixture from the mentioned glands along with the secretions of minor glands.

Gingival crevicular fluid (GCF) is the most important resource for the analysis of inflammatory and destructive markers of periodontal diseases. These markers are generally enzymes, cytokines and chemical mediators which are significantly increased in the active phase of the disease. The main markers isolated from GCF are chemical mediators of inflammation and edema. Absorbent paper strips (intra gingival and in the margin of gingiva), pre-weighted twisted threads, micropipettes and intra-crevicular washing are among the common methods for collection of GCF. One of the disadvantages of these strips is stimulation of sulcular epithelium and alteration in the volume and flow rate of GCF when they are placed in the gingival sulcus. In the present study paper points were used for collection of GCF which is an easy and cost effective method.

Alkaline phosphatase (ALP) is an important marker of bone loss. In the patients with severe periodontitis the level of ALP in GCF is significantly increased. Azizi et al. and Darba et al. showed that the level of ALP enzyme in unstimulated saliva of periodontitis patients is higher than that of healthy subjects. On the other hand, it has been proved that tobacco smoking is an important risk factor in incidence and severity of advanced periodontal diseases. Though cigarette smoking decreases inflammatory reactions and gingival bleeding. The results of numerous studies show that tobacco smoking is an important risk factor in prevalence and severity of periodontal destruction. The various effects of cigarette smoking on the periodontium are as follows:

Almost 90% of the patients with refractory periodontitis are smokers. Smoking causes undesirable response to the surgical and non-surgical periodontal treatments. The response of smokers to the regenerative treatments is dissatisfactory compared to that of non-smokers and there is a direct correlation between smoking and dental implant failure. On the other hand, the results of some studies indicate the effectiveness of smoking on alteration of the level and activity of biomarkers and chemical mediators in GCF and saliva and a limited number of studies have shown that the level of ALP in the serum is higher in smokers. Considering that the association between ALP level in saliva and periodontitis has been investigated in a limited number of studies and the level of ALP has not been investigated simultaneously in GCF and saliva of smokers, the present study with the aim of comparing the level of ALP in GCF and saliva of smokers and non-smokers with periodontitis and also healthy subjects was conducted.

Materials and Methods
The samples of this descriptive study were chosen from among individuals referring to the departments of diagnosis and periodontology of dental school of Islamic Azad University of Isfahan (khurasgan). The inclusion criteria were as follows:
1- No systemic diseases
2- The smokers should have had a history of smoking 10 cigarettes or more per day for at least a year
3- No medication consumption in the last 6 months
4- Patients with periodontitis should have had at least 20 teeth and pockets with depth of 5 mm or more in five areas.

With the use of calculation formula for this type of study, the minimum sample size was determined to be 84 individuals and eventually 90 individuals in accordance to the inclusion criteria were selected and assigned to three groups of periodontitis and smoking, periodontitis and non-smoking and healthy subjects.

All samples were advised to refrain from eating and drinking for 2 hours prior to the collection of saliva. 15 minutes prior to saliva collection they were asked to rinse their mouth thoroughly with water for 1 minute. After 15 minutes, subjects swallowed their entire saliva. Immediately afterwards, they were asked to evacuate their whole saliva in sterile containers.

GCF was collected from the areas with pocket depth of 5-7 mm from individuals in groups one and two and from intact gingival sulcus of group 3 with paper point number 35. Approximately 5 ml of unstimulated whole saliva was collected from all samples.

Specimens collected from GCF and saliva were sent to the laboratory for measurement of ALP level. Measurement of enzyme level was performed by a specialist with the aid of enzyme activity measurement kit. The measurement of ALP enzyme level in GCF was done with alteration compared to its measurement in serum due to the small sample size collected from GCF and dilution of the mentioned liquid. The samples collected from GCF were diluted in 250 µl of 20 mM MgCl2, 200 mM Tris (pH 9.8 ± 0.1) and 1 mg/ml of p-nitrophenol phosphate buffer and were incubated at 37 ºC for 3 hours. The interaction was then terminated by addition of 5 µl of NaOH and the amount of absorption was determined by spectrophotometry and recorded in the form of enzyme activity unit.

ALP level in saliva was measured with the automatic biochemistry analyzer (Furuno, Japan). Data were analyzed with ANOVA and Tukey’s test.

Results
In the present study with the aim of comparing the ALP level in GCF and saliva of smokers and non-smokers with periodontitis and healthy subjects, the average ALP level in GCF and saliva of smokers and non-smokers with periodontitis was higher than healthy subjects. This difference was statistically significant. (P = 0.001) (Table 1)

The average ALP level in GCF of smokers with periodontitis was higher than that of non-smokers with periodontitis. This difference was statistically significant. (P= 0.023). While the average ALP level in saliva was not statistically different between smokers and non-smokers with periodontitis. (Table 1)

### Table 1 – The average ALP enzyme level divided by non-smokers with periodontitis and smokers with periodontitis based on ANOVA test results

<table>
<thead>
<tr>
<th>Level of ALP</th>
<th>Healthy</th>
<th>Periodontitis non smoker</th>
<th>Periodontitis smoker</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCF</td>
<td>8.36±2.9</td>
<td>16.4±5.4</td>
<td>26.53±5.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Saliva</td>
<td>8.75±3.2</td>
<td>19.2±7.6</td>
<td>18.7±7.5</td>
<td></td>
</tr>
</tbody>
</table>

![Diagram 1 – Mean ALP level in GCF](http://www.jrds.ir)
Discussion

The studies of the past two decades have shown that periodontitis is a multifactorial disease which accrues in active and quiescent phases. The active phase is usually short and destructive while the quiescent phase or passive phase is long. It is clear that the common diagnostic methods such as pocket depth, attachment level and radiographic images although may reveal history of periodontal disease but do not possess a real value in predicting the future progression of the disease. Due to the limitation of diagnostic methods and the active and quiescent fashion of periodontitis, recognition of inflammatory and destructive markers in GCF and saliva has gained attention in the recent decades and in numerous studies these biomarkers have been used for predicting the progression of chronic periodontitis. 24 Therefor, proper and early treatment of periodontal diseases will be possible with the presence of the diagnostic kit in dental unit for detection of the activity of disease with determining the markers in GCF. Alkaline phosphatase is an effective enzyme in the metabolism of bone. The measurement of the level of this enzyme in GCF and saliva as an important marker of alveolar bone loss has been investigated in different studies. 25 Tobacco smoking especially in the form of cigarette smoking increases the risk of alveolar bone loss. On the other hand, inflammation and gingival bleeding are less in smokers compared to non-smokers due to the alteration in small gingival blood vessels. The aim of this study as mentioned before was to compare the salivary and GCF level of ALP in smokers and non-smokers with periodontitis and healthy subjects. The average ALP level in all samples was significantly higher in GCF than saliva. This finding is rational considering that the volume of saliva is many times more than that of GCF. 26 Another result of the present study showed that the ALP level in GCF and saliva of smokers and non-smokers with periodontitis was higher than that of healthy subjects. This finding is in line with the results of most studies. 7, 8 In a study by Azizi et al. salivary level of ALP was compared between periodontitis patients and healthy subjects. The results indicated a significant difference between ALP level in saliva of healthy subjects and periodontitis patients which is in accordance with our results. In another study Totan showed no significant difference in ALP level among groups of periodontitis patients with different severities. But ALP level was statistically different between areas with periodontitis and healthy areas which is relatively in line with the results of our study. 27 The results of our study contradicts that of the study by Yoshie which showed that the ALP level of saliva was the same for healthy subjects and periodontitis patients. The main reason for this difference may be the lack of definition and classification of periodontal diseases in the study by Yoshie with unclear differentiation between gingivitis and periodontitis. 28 Probable association between smoking and ALP level in GCF has not been investigated before. Heikinen et al. assessed the level of salivary biomarkers such as MMP-8 and PMM elastases in smokers with periodontitis and concluded that the level of these biomarkers changes in smokers. 29 The present study although followed numerous studies investigating correlation between increased ALP level and periodontitis but is the only study of this type that simultaneously assessed the ALP level of saliva and GCF in smokers and non-smokers. Another result of this study was that the ALP level in GCF of smokers with periodontitis was higher than that of non-smokers with periodontitis. This matter may be one of the reasons for the higher
probability of bone loss in smokers. Although in this regard few studies are available, but this finding is in line with the results of a study by Saito et al. 21 which showed that the level of ALP in serum is higher in smokers. In the mentioned study the serum level of ALP was measured. The present study can be considered as a fundamental study which will become more applicable with the aid of future complementary studies. The elevated level of ALP enzyme in GCF and saliva can be considered as a marker for periodontitis and alveolar bone loss. The difference between smokers and non-smokers with periodontitis regarding the level of ALP in GCF may be a reason for the higher probably of alveolar bone loss in smokers. Although definite prove of this matter needs more extensive complementary studies. Moreover, it is advised to compare the ALP level between healthy smokers and non-smokers.

Conclusion
It is possible to use both GCF and salivary levels of ALP as diagnostic markers for periodontitis. Higher levels of ALP in GCF samples of smokers with periodontitis could explain the higher rate of alveolar bone destruction in smokers.

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Conflict of interests
Authors report no conflict of interest related to this study.

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