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**ORIGINAL ARTICLE**

# Evaluation of Salivary Levels of Plasminogen Activator Inhibitor-2 in Patients with Moderate Generalized Chronic Periodontitis: A Case-Control Study

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**Abstract**

**Objective:** To compare salivary levels of PAI-2 in patients with moderate generalized chronic periodontitis before and after treatment and healthy subjects. **Material and Methods:** The present case-control study evaluated patients with generalized moderate chronic periodontitis (the case group) and subjects with healthy gingiva (the control group). The healthy subjects were evaluated once and the cases were evaluated twice (before and after treatment) by collecting their salivary samples. ELISA technique was used to determine PAI-2 salivary levels. Data were analyzed with the use of SPSS 17. The level of significance was set at 5%. **Results:** The mean salivary levels of PAI-2 in the case and control groups were 45.63 ± 8.63 and 22.01 ± 9.77 ng, respectively ($p<0.0001$). In addition, PAI-2 salivary levels in the case group subjects after treatment was 27.43 ± 5.79 ng, which was lower than that before treatment (45.63 ± 8.63 ng) ($p<0.0001$). The mean salivary level of PAI-2 in subjects with periodontitis after treatment (27.43 ± 5.79) was not significantly different from that in healthy subjects (22.01 ± 9.77) ($p>0.05$). **Conclusion:** The salivary levels of PAI-2 in patient with moderate generalized chronic periodontitis were higher than these in healthy subjects. However, the salivary levels of PAI-2 decreased in the case group subjects after treatment, with no significant difference from the healthy subjects.

**Keywords:** Periodontics; Chronic Periodontitis; Plasminogen Activators; Saliva.

**Introduction**

Periodontitis is a progressive inflammatory condition, which is the most common chronic infection in man and has a multifactorial etiology; it is initiated by microbial plaque. However, the extent and severity of periodontitis depends on environmental factors, acquired diseases and genetic predisposition. The condition is manifested in the form of inflammation of tooth-supporting tissues (gingiva, periodontal ligament, bone and cementum) and bone loss [1,2].

Periodontitis is categorized into three major forms: chronic, aggressive and as a manifestation of a systemic condition. Chronic periodontitis is the most common form of the condition and in its generalized form, over 30% of the areas are affected [1,3]. Apart from the local manifestations of periodontal disease such as destruction of tooth-supporting structures, tooth mobility and tooth loss [2], based on observational studies, there is strong evidence about a relationship between periodontitis and an increased risk of cardiovascular diseases and cerebral vasculature, including myocardial infarction and cerebrovascular accident [4], indicating the importance of the diagnosis and proper treatment of periodontal diseases.

Periodontal diseases are usually diagnosed based on clinical parameters such as bone loss as depicted on radiographs and determination of probing depths, clinical attachment levels and bleeding on probing (BOP). Since a diagnosis is reached based on these clinical parameters after significant injuries have been inflicted on periodontal tissues, researchers are trying to find other techniques for the early diagnosis of periodontal diseases and evaluation of the treatment outcomes [3]. Recent studies have shown that tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-2 (PAI-2) have important roles in the destruction and regeneration of periodontal tissues and can be used as markers for the diagnosis of periodontal diseases and evaluation of treatment outcomes [5].

The plasminogen activating system has a role in various processes, including tissue repair and remodeling and local inflammatory reactions. Plasminogen activators (PA) consist of two types, the tissue/blood vessel-type plasminogen activator (t-PA) and the urokinase-type plasminogen activator (u-PA), which convert the proenzyme plasminogen into the broad-spectrum proteinase plasmin. Directly and indirectly, Plasmin assists in degradation and turnover of the extracellular matrix (ECM) by activating latent collagenase. The activities of the PAs are regulated in turn by plasminogen activator inhibitor-1 (PAI-1), predominantly synthesized by endothelial cells, and PAI-2 released by macrophages and epithelial cells [6,7].

PAI-2 is found in very low concentrations in the plasma in healthy subjects; however, under specific conditions such as pregnancy or leukemia its concentration increases in the plasma [8]. In patients with periodontitis, changes occur in the blood clotting system, including an increase in blood clotting and an increase in fibrinolysis inhibitors and an increase in the secretion of PAI-2 in inflamed tissues [9]. A study showed that in biopsy samples from the inflamed gingival tissues of dogs the concentrations of t-PA and PAI-2 were higher than those in healthy tissues [10]. In addition, the concentrations of tissue plasminogen activator and PAI-2 were higher in the gingival crevicular fluid of patients with gingivitis and periodontitis, with higher concentrations in

periodontitis, followed by gingivitis and healthy gingiva, in descending order. The concentration of PAI-2 decreased two weeks after periodontal treatment [5].

Since no studies in Iran have evaluated salivary levels of PAI-2 and a similar study has evaluated PAI-2 concentrations in gingival crevicular fluid or gingival tissues, the present study was designed to determine salivary levels of PAI-2 in patients with moderate generalized chronic periodontitis before and after periodontal treatment as a clinical marker for the diagnosis of periodontal diseases and evaluation of treatment outcomes, because it is easier, safer and less costly to collect salivary samples compared to collecting biopsies or gingival crevicular fluid. The saliva contains local and systemic markers and various enzymes, enzymatic products and immunologic markers that can be used in the diagnosis and management of diseases [11-13].

## Material and Methods

### Study Design

In the present case-control study, the subjects consisted of patients with moderate generalized chronic periodontitis (the case group) and individuals with healthy gingiva (the control group), who referred to the Departments of Oral Medicine and Periodontics, Faculty of Dentistry, Tabriz University of Medical Sciences in 2017.

### Sampling

The sample size was estimated using the results of a previous study [5]. A total of 14 samples were calculated for each group by considering mean PAI-2 levels of 30.49±11.38 and 21.99±9.26 before and after treatment, respectively, and also by considering $\alpha$=0.05 and a study power of 80%. To increase the validity of the study the sample size was increased up to 20% and 17 subjects were included in each group, based on inclusion and exclusion criteria.

### Inclusion and Exclusion Criteria

The inclusion criteria consisted of affliction with moderate generalized chronic periodontitis, with more than 30% of the teeth exhibiting attachment loss in the case group and absence of gingival disease in the control group, an interest to take part in the study, and an age range of 20-55 years.

The exclusion criteria consisted of any systemic condition, including cardiovascular and respiratory diseases, cancer, diabetes mellitus, debilitating diseases such as arthritis that might affect oral health, any surgical and non-surgical periodontal treatment during the previous 6-month period and antibiotic therapy during the previous 3-month period, smoking in the last 3-months, current pregnancy or lactation and postmenopausal women, use of alcohol, use of anti-inflammatory agents, calcium, phenytoin, cyclosporine and antagonists [7].

The subjects with periodontal disease were selected using convenience sampling technique and the control group subjects were selected randomly (simple random sampling) using the website at www.randomizer.org.

The control group consisted of subjects with no clinical inflammation, no clinical attachment loss or radiographic evidence of alveolar bone loss. Salivary samples were collected from the control group subjects only once.

Patient with the involvement of more than 30% of the areas, probing pocket depths of >4 mm, attachment loss of 3-4 mm, positive BOP and radiographic evidence of bone loss were included in the case group as patients with moderate generalized chronic periodontics. These subjects had at least 10 teeth in the oral cavity. Salivary samples were collected from these subjects twice (before and after periodontal treatment).

Patients with periodontitis received non-surgical periodontal treatment consisting of oral hygiene instructions, scaling and root planning with the use of ultrasonic and hand instruments, without any adjunctive therapy. After two weeks, salivary sampling was collected again in the periodontitis group [14]. To prevent biases, all the necessary treatment procedures were carried out by one periodontologist who was blinded to the aims of the study.

The salivary samples were collected according to previously describe [15]. The subjects were asked to refrain from toothbrushing for 12 hours before collecting salivary samples and refrain from eating and drinking for 1.5 hours before collecting the samples. The subjects rinsed their oral cavities with water 15 minutes before collecting the samples. Then they were examined to make sure that there were no debris and food remnants in their oral cavities. Before collecting the salivary samples, the subjects were asked to swallow their saliva. Then each subject was asked to collect his/her saliva into clean and dry polyethylene tubes without chewing anything, from 9 to 11 in the morning in a seated position in a calm manner. A total of 5 mL of salivary sample was collected from each subject. All the samples were immediately stored at -80ºC until immunologic analyses were carried out. An ELISA kit (MyBioSource Inc., San Diego, CA, USA) was used to determine salivary levels of PAI-2 according to manufacturer's instructions.

## Ethical Aspects

The present study was conducted in accordance with the Helsinki Declaration. The protocol of the study was approved by the Ethics Committee of the Tabriz University of Medical Sciences. The aim of the study was explained to all the patients and consent forms were obtained before the study was instituted.

## Data Analysis

Data were analyzed with descriptive statistics (means and standard deviations, frequencies and percentages) using SPSS 17 (IBM Corp., Armonk, NY, USA). Paired t-test was used to compare salivary levels of PAI-2 before and after treatment. In addition, independent t-test was used to compare salivary levels of PAI-2 between the control group subjects and case group subjects before and after treatment. Statistical significance was set at $p<0.05$.

## Results

No statistically significant differences were found in gender (p=0.72) and mean age (p=0.29) of the participants between the groups (Table 1).

**Table 1. Genders and mean ages of the subjects.**

| Group | Gender | | Mean Age |
|---|---|---|---|
| | Male N | Female N | |
| Case | 9 | 8 | 35.64 ± 7.11 |
| Control | 11 | 6 | 32.94 ± 7.64 |

The mean salivary PAI-2 levels before and after treatment in the case group and control groups are presented in Table 2.

**Table 2. The mean salivary PAI-2 levels in the case group before and after treatment and in the control group.**

| Group | Mean ± SD (ng) |
|---|---|
| Control Group | 22.01 ± 9.77 |
| Case Group (Before Treatment) | 45.63 ± 8.63 |
| Case Group (After Treatment) | 27.43 ± 5.79 |

The mean PAI-2 level in the control group was 23 units lower than that in the case group before treatment, which was significant statistically (CI: 17.18 to 30.06; p<0.0001). The mean PAI-2 level in the control group was 5 units lower than that in the case group after treatment, which was not significant statistically (CI: -0.191 to 11.031; p=0.0578).

There was a difference of 18 units in mean PAI-2 levels before and after treatment in the case group, indicating a decrease after treatment; the difference was statistically significant (CI: -23.334 to -13.066; p<0.0001) (Figure 1).

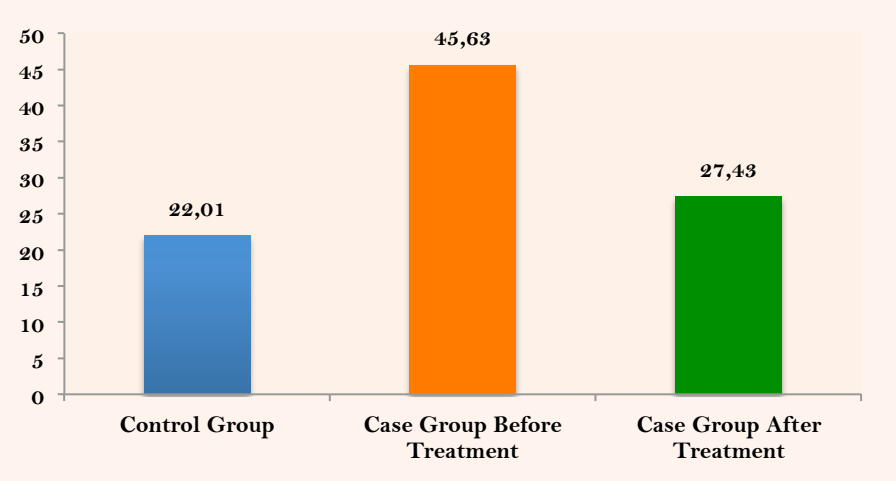


**Figure 1. The mean salivary levels (ng) of PAI-2 in different study groups.**

**Discussion**

Periodontal diseases are chronic inflammatory disorders, resulting from the activation of the host's immuno-inflammatory response to oral pathogens. The condition gives rise to local and systemic increases in proinflammatory cytokines, leading to destruction of tissue [16,17]. Destruction due to periodontal disease, referred to as periodontitis, is associated with proinflammatory cytokines such as IL-1β [18], PA system, including t-PA and PAI-2 [5], and MMPs, such as MMP-3 [19].

The results of the present study showed that the mean salivary level of PAI-2 in patients with moderate generalized chronic periodontitis (45.63 ± 8.63 ng) was approximately twice that in healthy subjects (22.01 ± 9.77 ng), indicating a 23-unit difference. However, after treatment in the group with periodontitis, the salivary levels of PAI-2 decreased, almost reaching the levels in the healthy subjects. In addition, the salivary levels of PAI-2 after treatment in patients with periodontitis decreased up to 50% compared to that before treatment, which was significant. This increase in salivary levels of PAI-2 levels in subjects with periodontitis might be due to the stimulation by inflammatory mediators [20].

It has been shown in relation to inflammatory tissues in patients with periodontitis that the chief cells involved in the secretion of PAI-2 are gingival fibroblasts and macrophages [21]. In a previous study, the concentrations of tissue plasminogen activator and PAI-2 in gingival crevicular fluid increased in patients with gingivitis and periodontitis, with higher levels in periodontitis compared to gingivitis and healthy gingiva, respectively, in descending order. The PAI-2 levels decreased two weeks after periodontal treatment [5]. In biopsy samples from the inflamed gingival tissues of dogs, t-PA and PAI-2 levels were higher than those in healthy tissues [10].

Previous authors evaluated gingival crevicular fluid levels of t-PA and PAI-2 in patients with gingivitis and reported decreases in their levels after non-surgical periodontal treatment [22]. Another researchers evaluated gingival crevicular fluid levels of t-PA and PAI-2 in aggressive periodontitis, chronic periodontitis and periodontally healthy controls. The results showed significant decreases in GCF levels of PAI-2 in patients with chronic periodontitis patients after non-surgical periodontal treatment [14]. Some authors evaluated the gingival crevicular fluid levels of PAI-2 in patients with chronic periodontitis, aggressive periodontitis and healthy controls; that the results showed increases in gingival crevicular fluid levels of PAI-2 in the disease, with no significant differences between the groups. However, positive correlations were found between the gingival crevicular fluid levels of PAI-2 and the probing pocket depths and the clinical attachment levels [7].

In a previous study, the gingival crevicular fluid levels of PAI-2 in patients with periodontal disease with or without type II diabetes mellitus and a group of systemically and periodontally healthy (control) subjects were evaluated. The results showed that the PAI-2 levels in the gingival crevicular fluid were similar in patients with periodontal disease with or without type II diabetes mellitus but PAI-2 levels were higher in diabetic patients with periodontal disease compared to healthy subjects [23].

The results of the studies above are consistent with those of the present study, with all indicating higher levels of PAI-2 levels in patients with periodontitis, which decrease after periodontal treatment. However, it should be pointed out that in contrast to previous studies in the present study the salivary levels of PAI-2 were determined; in previous studies PAI-2 levels were determined in the gingival crevicular fluid or gingival tissues. Since it is easier, inexpensive and less aggressive to collect salivary samples, evaluation of salivary factors is more favorable for both the patients and physicians.

Anyway, the results of the present study showed that PAI-2 has an important role in the destruction and regeneration of periodontal tissues and it can be used as a clinical marker for the diagnosis of periodontal diseases and evaluation of treatment outcomes. However, further clinical studies are necessary with more extensive scopes to reach a better understanding of complex relationship between inflammatory mediators, the PA system and the destructive processes of periodontal diseases. Therefore, it is suggested that in future studies the salivary levels of other proteins involved in inflammatory process such as IL-1β, TNF-α and IL-6 be evaluated in patients with chronic periodontics in comparison with healthy subjects. In addition, it is suggested that PAI-2, PAI-1 and t-PA levels be evaluated at molecular and tissue levels by the relevant techniques and at a wider level for more accurate conclusions. Considering the possible relationship between CVA and cardiovascular diseases and periodontal problems, it appears it is necessary to evaluate PAI-2 levels in patients with a history of MI and CVA.

## Conclusion

Salivary levels of PAI-2 in patients with moderate generalized chronic periodontitis were significantly higher than those in healthy controls. However, the salivary levels of PAI-2 decreased after periodontal treatment and almost reached the levels in healthy subjects.

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