

## Review Article

## Evaluation of Salivary Level of Resistin in Patients with Chronic Periodontitis

Solmaz Pourzare Mehrbani<sup>1</sup>, Farzaneh Pakdel<sup>1</sup>, Masoumeh Faramarzi<sup>2</sup>, Paria Motahari<sup>1\*</sup>, Parisa Khalifeh<sup>1</sup>, Leili Aghebati Maleki<sup>3</sup><sup>1</sup>Department of Oral Medicine, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran<sup>2</sup>Department of Periodontology, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran<sup>3</sup>Immunology research center, Tabriz University of Medical Sciences, Tabriz, Iran**Corresponding Author:** Paria Motahari, E-mail: paria.motahari@yahoo.com

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## ABSTRACT

In periodontology, clinical criteria are usually not sufficient to distinguish the presence of active disease, monitoring response to treatment, or assess disease progression. Studies have shown that resistin is involved in the etiology of periodontitis. The purpose of this paper was to assess the salivary resistin levels in patients with chronic periodontitis (CP). In this case-control study the saliva samples were collected from 45 patients with CP and 45 age- and sex-matched healthy individuals. Salivary resistin was determined by ELISA method. The results were analyzed by Mann-Whitney test. The software used in this study was SPSS 17. P value less than 0.05 was considered significant. In this study, the mean salivary resistin level was 0.769 (+0.396) ng/ml in the patients group and 0.145 (+0.068) ng/ml in the healthy group, with a significant difference ( $P < 0.05$ ). The results of this research showed that the salivary levels of resistin in patients with CP are higher than in healthy individuals and salivary resistin can be considered as a marker in the pathogenesis, early diagnosis and determination of the inflammatory status of CP.

## INTRODUCTION

Periodontitis is a disease that affects dental support structures. Periodontitis can be divided in three main forms: chronic, aggressive and as a manifestation of a systemic disease. Chronic periodontitis (CP) is prevalent form of this disease and generalized form involves more than 30% of dentition (1). Periodontitis is an inflammatory condition in which the infiltration of mononuclear cells into the gingival tissue results in connective tissue and alveolar bone destruction. Although bacteria are causes of periodontitis, development and severity of disease are characterized by the host immune reaction. The exact mechanism of periodontal tissue destruction has not yet been clarified. Immunohistochemical studies have shown that infiltration of mononuclear inflammatory cells below the basal layer of periodontal pockets increase (2). Resistin is a lipid-derived agent and a cysteine-rich peptide encoded in humans by the RETN gene and is secreted by immune and epithelial cells (3-5). Inflammation is the first response to the body's innate immunity to infection, which is caused by stimulation of leukocytes (neutrophils and mast cells). Studies have shown that resistin involves in inflammatory responses by increasing the expression of some pro-inflammatory cytokines such as Interleukin

1, 6, 12 and Tumor necrosis factor alpha (6-8). It has also been proven that resistin increases the expression of chemokine (C-C motif) ligand-2, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1; all of which are on the pathway of Chemotactic Leukocytes to the infection location (9).

In periodontology, clinical criteria are usually not sufficient to distinguish active disease sites, monitor response to treatment, or measure the susceptibility to disease progression. Saliva is an important source of clinical information and is considered a mirror of oral health since it comprises specific markers for the biological characteristics of periodontal diseases (10). Previous studies indicate that serum and gingival crevicular fluid (GCF) resistin levels were associated with periodontal diseases (11). In the study of Devanoorkar et al., it has been shown that serum resistin levels have been increased in patients with diabetic periodontitis (12). A study by Mital et al. suggests that GCF resistin levels increase in patients with CP and rheumatoid arthritis (13).

Several studies have been performed on salivary resistin levels in CP. In some of these studies, no significant relationship was found between salivary resistin levels and periodontal resistin, while in some other studies there was a positive relationship. Because of these contradictory results and since saliva

preparation is non-invasive method; the purpose of this study was to assess the level of resistin in saliva of patients with CP.

## MATERIALS AND METHODS

### Sample Size, Location and Duration of Study

In a case-control study, we compared salivary level of resistin in patients with CP and healthy controls.

In the case group, all patients with moderate or severe generalized CP (N = 45) were selected from the patients of the Oral Medicine and Periodontology Department, Tabriz Faculty of Dentistry from 2019 to 2020. Inclusion criteria were general health, agreement with sample collection for examinations and diagnosis of CP according to the International Workshop for a Classification of Periodontal Diseases and Conditions for CP (14). Exclusion criteria included history of heart disorders, diabetes, malignant diseases, smoking, immunodeficiency and existing pregnancy or lactation. The control group (healthy/non-periodontitis subjects, N = 45) were chosen randomly in the same period and matched for age and sex. All controls had at least 20 teeth, with no history or clinical signs of periodontitis. They were generally healthy, and agreed with examinations. Exclusion criteria are the same as for patients with periodontitis.

The parameters assessed were clinical attachment loss (CAL), probing pocket depth (PPD), bleeding on probing (BOP) and radiographs. CAL and PPD were assessed using a William's probe from six sites on each remaining teeth. At least 30% of sites must have PPD > 5 mm and attachment loss > 3 mm. All patients should have no history of antibiotic treatment and periodontal therapy for at least 3 months prior to contribution in the study.

### Saliva Sampling

Saliva sampling was performed using NAVAZESH method (15). Participants should not eat or drink anything two hours before sampling. 15 minutes before sampling, the volunteers washed their mouths, and then their oral cavity was examined with adequate light and mirrors for assuring of no material in the oral cavity. The patient's saliva samples were collected within 16-20 minutes using sterile disposable plastic container and transferred to the laboratory immediately. The laboratory was then centrifuged and the granular particles were discarded and the supernatant was partitioned into a micro tube and then stored at  $-70^{\circ}\text{C}$ .

### Enzymelinked Immunoassay for Resistin

Salivary resistin level was determined by enzyme-linked immunosorbent assay (ELISA) kit (human Resistin ELISA Kit – AB108896). Prior to measurement, the saliva samples were defrosted and centrifuged at 10,000 rpm for 1 minute.

### Statistical Analysis of Data

In this study the level of salivary HSP70 in both groups will be measured by ELISA method. The results of the study reported using descriptive statistics (mean + standard deviation).

To investigate the normal distribution of the resistin level variable, the Kolmogorov-Smirnov test was used. Mann-Whitney test used to compare salivary resistin in healthy subjects and patients with CP. To examine the existence of a significant difference between the mean ages of the participants in the two groups, independent t-test was used. The software used in this study was SPSS 17. P value less than 0.05 was considered significant.

### Ethical Considerations

Participants in this study were consented and no unnecessary intervention was performed. Therefore, this study had no adverse effects on patients and their therapeutic process. It should be noted that the agreement of the Research Ethics Committee has also been obtained by Code of Ethics (IR.TBZMED.REC.1397.913).

## RESULTS

The results of this the Kolmogorov-Smirnov test showed that the distribution of the variable is not normal; therefore, nonparametric test (Mann-Whitney) was used. The significance level of the test was 0.05.

Table 1 shows the means and standard deviations (SD) of salivary levels of resistin in CP and control groups. This Table also shows the age and gender characteristics of the groups and the results of the Mann-Whitney test. According to Mann-Whitney test, there was a significant difference in salivary resistin level between these groups ( $P < 0.05$ ).

The mean age in the control group was  $36.6 (\pm 9)$  and in the case group was  $40.1 (\pm 10.7)$ . The results of t-test presented that there is no statistically significant difference between the mean age of the participants in the two groups of study ( $P\text{-value} > 0.001$ ).

## DISCUSSION

Currently, Diagnosis of periodontal disease mainly depends on clinical parameters and radiography. These methods are useful in diagnosing the disease or confirming periodontal health, but provide restricted information about patients and put them at risk for future periodontal damage (16). Numerous markers have been used in saliva to diagnose periodontal disease. Ideally, diagnostic markers should be very specific and sensitive (17). Due to the complex nature of periodontal disease, the presence of a sensitive and specific marker seems unlikely. A combination of two or more markers may provide a more accurate assessment of periodontal patients. Interest in saliva as a diagnostic tool is increasing. Both saliva and blood serum contain similar proteins, so saliva is a "mirror of the body" (18).

In the present study, salivary resistin level in healthy individuals is significantly lower than moderate and severe periodontitis. In recent years, a number of studies have highlighted the relationship between resistin and periodontal diseases; Devanoorkar et al., (12) concluded that, probably, resistin plays a role in periodontitis and is one of the agents associated with obesity, periodontitis, and diabetes

**Table 1.** Comparison of salivary HSP70 levels in the study groups with one-way ANOVA analysis. (p<0.05)

Groups	Sample size		Age range	salivary resistin (ng/ml) Mean + SD	P-value
	Male	Female			
Chronic periodontitis	24	21	30-50	0.769 (+0.396)	0.001
Healthy control	24	21	30-50	0.145 (+0.068)	

which is consistent with our study. A number of studies have shown that saliva resistin levels are higher in patients with CP than in healthy individuals, and there is a significant reduction in salivary resistin levels after treatment of periodontitis (19-22). However, Akaram et al., and Rao et al., showed that there was no significant relationship between salivary resistin and clinical parameters of chronic periodontitis (23, 24). This difference appears to be due to differences in sample size, inclusion criteria, different sample populations; different saliva sampling methods or different resistin analysis methods.

The increase in salivary resistin can be explained by the fact that resistin is produced by immune cells in response to lipopolysaccharide of periodontopathic microorganisms and exudes from the GCF into the saliva (25). In periodontal disease resistin binds to human leukocytes and induces cytokine production from immune cells through the nuclear factor- $\kappa$ B way (26). Resistin is also involved in bone metabolism, which increases the levels of resistin along with the differentiation of osteoclasts (27). Limitations of the study include small sample size and changes in salivary compounds under different conditions (28). However, more studies with larger sample sizes are needed to assess the diagnostic value of salivary resistin levels in the early diagnosis of periodontal disease.

## CONCLUSION

Based on the results of the present study, we can emphasize the role of resistin in the pathogenesis, early diagnosis and determine the inflammatory status of CP. Also, periodontitis treatment targets can focus on this protein. However, more extensive studies should be conducted on the diagnostic value of this protein.

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