

Evaluation of Resistin Levels in Saliva of Patients with Chronic Periodontitis and Healthy Subjects

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Objective: To evaluate resistin levels in the saliva of patients with chronic periodontitis, and healthy subjects.

Methods: Thirty-four subjects aged between 25 and 50 years were included and divided into healthy group ($n = 19$) and chronic periodontitis group ($n = 15$). The saliva levels of resistin were assessed by enzyme-linked immunosorbent assay. Comparisons of resistin levels between the two groups were made with the Mann-Whitney Test.

Results: The chronic periodontitis group showed significantly higher resistin levels than the control group ($P = 0.001$).

Conclusion: The level of resistin in saliva might help to determine the inflammatory status of periodontal diseases.

Key words: periodontitis, resistin, saliva

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Chronic periodontitis is an inflammatory disease characterised by different pathogenic bacteria and results in the tooth supporting structure's destruction, which may lead to tooth loss¹. It seems that patients with periodontitis predispose to rheumatoid arthritis, cardiovascular disease, cerebrovascular disease and respiratory disease². In inflammation-related disorders, some of the adipokines such as resistin have been identified³. Resistin is a cysteine-rich polypeptide hormone produced in low levels by adipocytes and largely produced by monocytes, macrophages⁴, and bone marrow⁵. It has been proposed that adipokine like resistin plays a key role in inflammation and immune responses⁶. Resistin increase in numerous inflammatory cells is stimulated by periodontal pathogenic components such as LPS⁷.

In addition, resistin has been considered as a pro-inflammatory cytokine⁸. In some studies, there was a positive correlation between serum and gingival crevicular fluid (GCF) levels of resistin and the severity of periodontitis^{9–11}, but in other studies the significant difference of resistin level in healthy and periodontitis patients^{12,13} was not observed. The use of saliva as a diagnostic fluid for determination of systemic diseases is a relatively recent trend because it is a non-invasive and cost-effective method¹⁴.

Due to discrepancies among studies with regard to the association between resistin levels and periodontal disease and since studies have seldom been conducted to assess salivary resistin levels and periodontitis^{15,16}, the purpose of this study was to evaluate the resistin levels in the saliva of patients with chronic periodontitis, and healthy subjects.

Materials and methods

This case-control, cross-sectional study was performed from 2016 to 2017 at the Periodontology Department of Shahid Beheshti University of Medical Sciences, Tehran, Iran. A total of 34 male and female subjects aged between 25 and 50 years participated in this study. All individuals gave their written informed consent and Institutional ethics review committee approval was

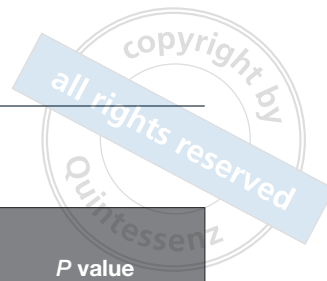
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**Table 1** Salivary resistin levels in the two groups.

Sample	Healthy group		Periodontitis group		P value
	Median (ng/ml)	Mean ± SD	Median (ng/ml)	Mean ± SD	
Resistin	5.1	8.1 ± 7.0	13.0	19.7 ± 15.0	0.001*

*Significant

obtained. The exclusion criteria included: 1) a history of smoking, 2) systemic diseases, 3) pregnancy, 4) a history of periodontal as well as antibiotics or anti-inflammatory drugs treatment within the past 6 months.

Subjects were divided in to two groups, based on the gingival index (GI), probing pocket depth (PPD), and clinical attachment level (CAL). Group I (control group): Nineteen individuals with clinical healthy periodontium, with GI < 1mm, PPD < 3 mm, and no CAL. Group II (test group): Fifteen patients with chronic periodontitis according to American Academy of Periodontology (AAP) criteria 1999, having PPD ≥ 5 mm with CAL 4 mm at least in two teeth.

Sampling

The saliva samples were collected between 9:30 am and 11:30 am. All participants were requested not to eat or drink at least 2 h prior to sampling. They were instructed to sit in a relaxed position and then were asked to allow saliva to pool in the bottom of their mouths and split it into smile tubes every minute for 5 min – known as a non-stimulated spitting method. Samples were then centrifuged at 1100 g for 10 min within 1 h after collection of eliminated debris and cellular matter. The supernatant saliva was immediately stored at -70°C until analysis.

Detection of resistin

The human resistin enzyme-linked immunosorbent assay (ELISA) kit (BioVendor Laboratory Medicine, Brno, Czech Republic) was used to assessing the resistin concentration in the saliva. All procedures were carried out in accordance with the manufacturer's instructions. Absorbance was measured by a microplate reader (Bio-Tek Instruments, Winooski, VT, USA) at a wavelength of 450 nm. The concentration of resistin was estimated using the assay standard criteria.

Data analysis

Statistical analyses were performed using SPSS software (version 21). Comparisons of resistin levels between the two groups were made with the Mann-Whitney Test. The values < 0.05 were considered statistically significant. Data were presented as the mean ± standard deviation (SD).

Results

Median and mean values of the salivary concentration of resistin are shown in Table 1. The levels of resistin in the saliva of patients with chronic periodontitis were significant higher than that in the healthy subjects ($P = 0.001$).

Discussion

The potential role of saliva in the diagnosis of oral and systemic health is evident in researches. Salivary biomarkers could be used to screen periodontal health status or periodontal disease initiation and progression. Therefore, saliva could be a possible alternative approach to classic examination for early disease diagnosis^{17,18}. The results of the present study revealed that mean salivary resistin level in the periodontitis group was significantly higher than in the healthy subject group ($P < 0.01$). In research conducted recently by DA Sabir et al¹⁶ and TA Karam et al¹⁵ there was a significant difference in the level of resistin in the saliva of periodontal disease and healthy subjects, which is in accordance with our findings. Numerous researches have been conducted on GCF and serum resistin levels in periodontitis⁹⁻¹³, whereas little was known about the association between salivary resistin level and periodontal disease. Increased saliva resistin in periodontitis patients may be due to proinflammatory cytokines produced by chronic inflammatory cells in periodontitis. Proinflammatory cytokines including tumour necrosis factor- α (TNF- α), interleukin-6 (IL-6) and IL-1b play an important role in inflammation and immune response and tissue damage in peri-



odontitis^{19,20}. Moreover, it is shown that these cytokines induced resistin expression *in vitro*⁹.

It is suggested that resistin as a pro-inflammatory molecule could be triggering the synthesis and secretion of TNF- α and IL-12²¹. In addition, resistin may contribute to inflammatory procedures produced by bacteria²². Resistin is also expressed during the differentiation of monocytes to macrophages that may indicate its role in inflammatory process^{4,23}. Elevated resistin levels during osteoclast differentiation by increasing levels of ICAM-1 and VCAM-1, suggest a potential role for resistin in bone metabolism^{24,25}.

Moreover, resistin release from macrophages was stimulated by endotoxin lipopolysaccharide (LPS) of the bacteria in an *in vitro* study²¹. Both *Porphyromonas gingivalis* (*Pg*) and *Escherichia coli* (*E. coli*) LPS have been reported to stimulate neutrophils to produce resistin²⁶. Resistin increase in numerous inflammatory cells stimulated by periodontal pathogenic components such as LPS7. Leukotoxin of the *Aggregatibacter actinomycetemcomitans* (*Aa*) have been revealed to induce the extracellular release of human neutrophil-derived resistin, suggesting a contribution between the prevalence of *Aa* in periodontal disease and levels of resistin²⁷.

Evaluation of levels of multiple biomarkers might be more beneficial to determine the inflammatory status of periodontal diseases due to the contribution of a complex network of biomarkers in disease initiation and progression. Furthermore, to evidently clarify the role of resistin in the pathogenesis of periodontal diseases, longitudinal studies should be carried out. A significant difference of resistin in the saliva of patients with chronic periodontitis and healthy subjects suggested that levels of resistin in saliva might help to determine the inflammatory status of periodontal diseases.

Conflicts of interest

The authors reported no conflicts of interest related to this study.

Author contribution

Dr Zeinab Rezaei Esfahrood designed the study and revised the paper; Drs Sahar Vardian Tehrani and Farshid Dehnavi participated in sample collection; Dr Zahra Yadegari performed laboratory testes; Drs Bahareh Shams and Nasim Shams participated in all parts of this study.

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