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Original Article

# Interleukin 35 levels in saliva of type 2 diabetic patients with moderate chronic periodontitis

**Anis Moslemi Petrodi[1], Shima Nafarzade[2], Amrollah Mostafazadeh[3], Amir Kiakojori[4]✉, Mohammad Ali Bayani[5], Hemmat Gholinia[6], Monireh Golpour[7]**

1. Dental Student, Student Research Committee, Babol University of Medical Sciences, Babol, IR Iran. **ORCID** (0000-0001-7981-678X)
2. Assistant Professor, Dental Materials Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IRIran.
3. Associate Professor, Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IRIran.
4. Assistant Professor, Oral Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IRIran.
5. Assistant Professor, Clinical Research Development Unit of Rouhani Hospital, Babol University of Medical Sciences, Babol, IRIran.
6. MSc in Statistics, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.
7. PhD Student ,Cellular and Molecular Biology Research Center,Student Research Committee, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari,IR Iran.

✉**Corresponding Author:** Amir Kiakojori, Department of Periodontics, Faculty of Dentistry, Babol University of Medical Sciences, Babol, IRIran.

**Email:** amirkiakojori@gmail.com **Tel:** +989112107490 **ORCID** (0000-0001-7058-1363)

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## Abstract

**Introduction:** Periodontitis is a common disease in patients with diabetes. There is a significant relationship between hyperglycemic degree and severity of periodontitis, but the base of mechanism of this relationship has not been fully defined. Considering the important role of cytokines in periodontal pathogenesis and considering that there has been no study on the comparison of interleukin 35 (IL-35) in these diseases, the aim of this study was to determine the level of this salivary cytokine in patients with type 2 diabetes mellitus with generalized moderate chronic periodontitis.

**Material & Methods:** Totally, 88 subjects (44 female, 44 males) with a mean age of 42.5±10.5 years old participated in this case control study. The subjects were divided into four groups and each group included 22 subjects: Group 1: generalized moderate chronic periodontitis patients with type 2 diabetes, Group 2: generalized moderate chronic periodontitis patients without diabetes, Group 3: diabetic patients with normal periodontium, Group 4: healthy periodontium and non-diabetic group (control) Then saliva were collected and centrifuged, the amount of IL-35 was determined with commercial ELISA kit. Data were analyzed . ANOVA and Tukey post-hoc tests were used to compare the groups.

**Results:** The Mean±SD of IL-35 was significantly higher in the control group (22.59±8.36, $p<0.05$) than other groups (Group1: 13.12±5.62, Group2: 14.27±8.55, Group3: 15.12±8.13). Mean comparison of IL-35 in other groups had no significant difference ($P>0.05$).

**Conclusion:** The salivary IL-35 level is decreased in both periodontitis and type 2 diabetes. However, diabetes mellitus does not exacerbate this decline in patients with periodontitis.

**Keywords:** Diabetes mellitus, Chronic periodontitis, Saliva

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# بررسی سطح اینترلوکین ۳۵ در بزاق بیماران دیابتی نوع ۲ مبتلا به پریودنتیت مزمن متوسط

انیس مسلمی پطرودی[۱]، شیما نفرزاده[۲]، امراله مصطفی‌زاده[۳]، امیر کیاکجوری[۴*]، محمدعلی بیانی[۵]، همت قلی نیا[۶]، منیره گلپور[۷]

۱. دانشجوی دندانپزشکی، کمیته تحقیقات دانشجویی، دانشگاه علوم پزشکی بابل ، ایران.
۲. استادیار، مرکز تحقیقات مواد دندانی، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل،ایران.
۳. دانشیار، مرکز تحقیقات بیولوژی سلولی و مولکولی، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.
۴. استادیار، مرکز تحقیقات سلامت و بهداشت دهان، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.
۵. استادیار، واحد توسعه تحقیقات بیمارستان آیت اله روحانی، دانشگاه علوم پزشکی بابل، بابل، ایران.
۶. کارشناس ارشد آمار، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.
۷. دانشجوی دکتری،،مرکز تحقیقات بیولوژی سلولی و مولکولی،کمیته تحقیقات دانشجویی، دانشکده پزشکی، دانشگاه علوم پزشکی مازندران، ساری،ایران.
*نویسنده مسئول: امیر کیاکجوری، گروه پریودنتیکس ، دانشکده دندانپزشکی، دانشگاه علوم پزشکی بابل، بابل ، ایران.
پست الکترونیکی:amirkiakojori@gmail.com تلفن: ۰۹۸۹۱۱۲۱۰۷۴۹۰+

## چکیده

**مقدمه:** پریودنتیت بیماری شایع در مبتلایان به دیابت است. رابطه معنی‌داری بین درجه هایپرگلایسمی و شدت پریودنتیت وجود دارد. اما مکانیسم پایه این ارتباط کاملاً مشخص نشده است. با توجه به نقش مهم سایتوکاین‌ها در پاتوژنز پریودنتیت و با توجه به اینکه تاکنون مطالعه‌ای در مورد مقایسه اینترلوکین ۳۵ در این بیماری‌ها انجام نشده است هدف از انجام مطالعه حاضر، بررسی سطح این سایتوکاین در بزاق بیماران دیابتی نوع ۲ مبتلا به پریودنتیت مزمن متوسط جنرالیزه است.

**مواد و روش ها:** در مجموع ۸۸ نفر (۴۴ زن، ۴۴ مرد) با میانگین سنی ۱۰/۵±۴۲/۵ سال در این مطالعه موردی شاهدی شرکت داشته‌اند. افراد به ۴ گروه تقسیم شدند و هر گروه شامل ۲۲ نفر بودند. گروه ۱: بیماران واجد پریودنتیت مزمن متوسط جنرالیزه دارای دیابت نوع ۲،گروه ۲: بیماران واجد پریودنتیت مزمن متوسط جنرالیزه فاقد دیابت ،گروه ۳: بیماران دیابتی با پریودنشیوم نرمال وگروه ۴: افراد فاقد دیابت با پریودنشیوم نرمال (گروه کنترل)، سپس نمونه‌های بزاق، جمع‌آوری و پس از سانتریفیوژ کردن، میزان اینترلوکین ۳۵ با کیت تجاری بر اساس ELISA تعیین شد و داده‌ها تحلیل شدند. تست اماری آنوا و تست تعقیبی توکی برای مقایسه گروه ها مورد استفاده قرار گرفتند.

**یافته ها:** میانگین±انحراف معیار اینترلوکین ۳۵ در گروه کنترل (۸/۳۶±۲۲/۵۹ و $p<0.05$) از سه گروه دیگر بیشتر بود.(گروه یک: ۵/۶۲±۱۳/۱۲، گروه دو: ۸/۵۵±۱۴/۲۷ ، گروه سه: ۸/۱۳±۱۵/۱۲ ). مقایسه میانگین اینترلوکین ۳۵ در گروه‌های دیگر تفاوت معناداری را نشان نداد ($p>0.05$).

**نتیجه گیری:** سطح IL-35 بزاق در پریودنتیت و دیابت نوع ۲ کاهش می یابد .با این حال ، دیابت ملیتوس این کاهش را در بیماران مبتلا به پریودنتیت تشدید نمی کند.

**واژگان کلیدی:** دیابت ملیتوس، پریودنتیت مزمن، بزاق

## Introduction

Periodontitis is a periodontal inflammatory disease associated with irreversible loss of connective tissue and supporting alveolar bone. [1] This disease is caused by complex interactions between the periodontium associated pathogens and the host immune cells. The cells in the site of inflammation are responsible for the production of cytokines that are involved in the pathogenesis of the periodontal disease. [2] Chronic periodontitis is the most common form of periodontitis, which is more common in adults but also occurs in children and adolescents. [3, 4] Clinical diagnosis of chronic periodontitis is based on the criteria such as the presence of chronic inflammation in the gingival margin, the presence of periodontal pocket and attachment loss. Attachment loss can be seen in the form of a true periodontal pocket and/or gingival recession. [5] On the other hand, diabetes mellitus is an important disease related to the periodontium. Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. A decrease in insulin production, insulin function impairment, or a combination of both, results

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in impaired glucose transfer and increased blood glucose levels as well as secretion of glucose in the urine. [6] According to available epidemiological data, the potential for periodontitis in people with diabetes is three times that of healthy people. [7] There is a clear relationship between diabetes and the severity of periodontitis. The mechanism of the relationship between these two diseases is not well defined, but it can be affected by the activity of the immune system and its related molecules, particularly cytokines. [8] A variety of possible mechanisms such as oxidative stress and inflammatory immune responses are still under investigation. People with diabetes have higher levels of malondialdehyde and lower levels of glutathione, indicating oxidative stress in hyperglycemia. [9] Both type 1 and type 2 diabetes are associated with an increased level of systemic inflammatory markers. Accordingly, serum levels of IL-6 and CRP (C Reactive Protein) are high in periodontitis. [8] Therefore, the likeness of both diseases is their inflammatory nature. Inflammatory diseases are exacerbated by inflammatory cytokines such as IL-6, TNF-α, IFN-γ, IL-12, and reduced by anti-inflammatory cytokines such as IL-10 and IL-35. The balance between these cytokines determines the mechanism of inflammatory diseases.

IL-35 is a new member of the IL-12 family. [10] IL-12 is one of the cytokines involved in inflammatory reactions in many pathological and physiological processes. [11] It has been shown in some studies that IL-35 is involved in moderating immunity in a severe stage of the disease. [10] Besides, the anti-inflammatory role of IL-35 has been already recognized in research [12], while other members of the IL-12 family have illustrated the role of immunosuppressive. [13]

Recent studies have indicated that IL-35 is an anti-inflammatory cytokine that suppresses the immune response through the proliferation of Treg and suppression of Th17 cell growth. This suggests a possible role for IL-35 in chronic inflammation such as periodontitis. However, little is known about the exact mechanism. [14] Considering the issues mentioned above, the vital role of inflammatory cytokines in the pathogenesis of periodontitis and diabetes in addition to the higher prevalence of periodontitis in diabetic patients, and since there has not been a study on the comparison of IL-35 in these diseases, this study evaluated the level of IL-35 in saliva of type 2 diabetic patients with generalized moderate chronic periodontitis. To the aim of the current study was to answer these questions whether, firstly, reducing this cytokine can advance the equilibrium in both diseases to inflammation, and secondly, diabetes may exacerbate this possible decline to justify the relationship between diabetes and periodontitis.

## Materials & Methods

This experimental study was approved by the Ethical Committee of Babol University of Medical Sciences (Ethical number: mubabol.rec.1396.4427). The statistical population of this study included 88 samples divided into four groups: a) 22 patients with generalized moderate chronic periodontitis with type 2 diabetes, b) 22 patients with generalized moderate chronic periodontitis without type 2 diabetes, c) 22 patient with diabetes and normal periodontium, and d) 22 healthy subjects with normal periodontium as a control group. Diabetic patients were also matched for the duration of the disease.

The study population was selected from patients referred to the Periodontology Department of Babol Dental School and the Department of the Endocrinology of Ayatollah Rohani Hospital in Babol from February to April 2017. Simple sampling was carried out, and the sample size was calculated as 22. This is a case-control study, and 22 patients were sampled in each.

These patients have been selected based on the clinical diagnosis of periodontitis in persons with at least 20 teeth and diabetic disease in their medical history. In addition, the exclusion criteria were pregnancy and lactation, patients receiving topical or systemic antibiotics in the last six months as well as having dental and periodontal abscesses. [10] In terms of clinical diagnosis, the criteria for the presence of generalized moderate chronic periodontitis in patients was having pocket depth between 5 and 7 millimeters, presence of bleeding on probing and clinical attachment loss of 3 to 4 millimeters. [15]

After confirming the diagnosis, saliva samples were collected as follows: samples were collected between 9 to 12 A.M, and people were asked to refrain from eating, drinking, chewing gum and smoking for at least two hours before sampling. At first, the participants swallowed the saliva, then bend the head to the front and poured all of the saliva for 5 minutes in a special 50 milliliters dry tube for centrifugation.

All specimens were centrifuge(Sigma-Aldrich, Munich, Germany) at 4°C for 20 minutes at a speed of

6000 rpm to separate cell debris and then were kept at -80°C for evaluating with Elisa commercial kit (Crystal day,Shanghai,China)with the ability to measure the IL-35 in saliva at a sensitivity of picogram per milliliter.[16]

The collected data were analyzed using SPSS 18. Descriptive statistics were reported as mean ± SD. ANOVA test was used for comparing the groups, and P<0.05 was considered significant level. Tukey post hoc test was used to compare the two groups.

## Results

Generally, 10 males and 12 females with the mean age of 26.09± 3.15 years in control group, 10 females and 12 males with the mean age of 47.1±4.51 years in periodontitis group, 11 males and 11 females with the mean age of 48.8±6.51 years in the periodontitis+ diabetes group and 11 males and 11 females with the mean age of 47.7±4.27 years in diabetes group participated in this study. The results of comparing the average of IL-35 concentration in studied groups are illustrated in figure1.

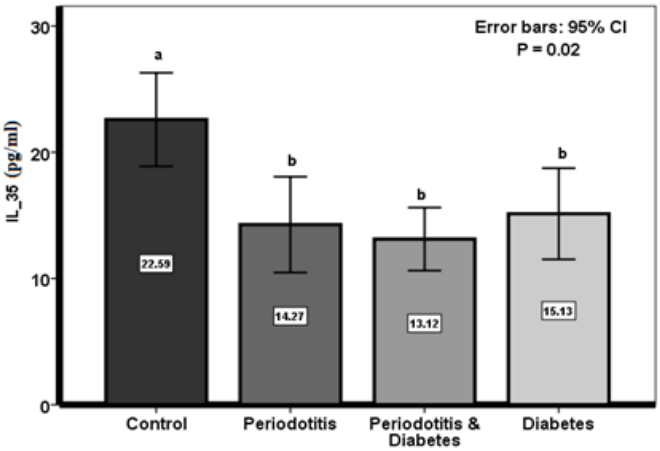


**Fig.1. Comparison of Interleukin-35 average in the studied groups**

According to the results shown in figure1, comparing the IL-35 concentration in pg/ml using one-way ANOVA and Tukey's post-hoc test with 95% confidence interval demonstrated that the difference in concentration between the control and other groups was statistically significant (p<0.001). The difference between groups was not significant sexually (P=0.94), and the level of IL-35 had no significant difference between males and females. The difference between groups in terms of age was significant (P<0.001), while the mean age in the control group was significantly lower than in other groups.

Three-way analysis of variance (3-way ANOVA) illustrated that after controlling the effects of age and sex, there was a significant difference between the IL-35 median (F=3.34, df=3, p=0.02). For comparing the means of IL-35 between four groups pairwisely, the Tukey's post-hoc test was used. The results of this test showed that the mean of IL-35 in the control group was significantly higher than the other three groups, for example, p=0.005 relative to the periodontitis group, p=0.001 relative to periodontitis and diabetes and p=0.01 relative to diabetic group. In other cases, this difference was not significant.

## Discussion

The present study evaluated the salivary levels of interleukin 35 among type 2 diabetic patients suffering from generalized moderate chronic periodontitis. The lowest levels of salivary IL-35 were observed among the patients having both type 2 diabetes and periodontitis. They were followed by patients with periodontitis and then diabetes, respectively. Levels of IL-35 in the saliva of healthy controls were significantly higher than in other groups.

Mitani et al. conducted a study on the levels of IL-35, IL-17, and IL-27 in the gingival crevicular fluid (GCF) and gingival tissues of patients with periodontitis. They suggested that IL-35 and IL-17 have higher levels among the samples taken from patients having periodontitis than healthy subjects. [13] Kalburgi et al. studied the gingival expression of IL-35 mRNA in patients with periodontitis. They showed that IL-35 mRNA was higher in patients with chronic periodontitis than in patients having aggressive periodontitis. The expression level of IL-35 mRNA was the lowest in healthy controls. [11] Maboudi et al. indicated that they found no significant alteration in the serum levels of IL-35 and IL-23 among patients with type 2 diabetes and chronic periodontitis as well as healthy subjects. [17]

These differences may best be explained due to the difference in the sampling sites. In the ongoing study, we used salivary samples, while in the previously mentioned studies, they took samples from gingival crevicular fluid, gingival tissues and serum of the patients.

Köseoğlu et al. performed a study on the levels of IL-35 in GCF, plasma, and saliva of patients suffering

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from periodontitis. The results implied that IL-35 was higher in the GCF of patients with periodontitis. However, the levels of IL-35 in plasma samples had no significant differences. Similar to our findings, they stated that salivary levels of IL-35 were higher in healthy controls than patients with periodontitis. They explained that the salivary concentration of IL-35 is lower in the patients as a result of breakdown due to the salivary proteases. [10]

It should be taken into consideration that the expression level of IL-35 can be affected by other risk factors. According to previous studies, other environmental and systemic factors may affect periodontal status; for instance, new researches have suggested that pro-inflammatory cytokines tend to reach higher levels in patients suffering from periodontal disorders accompanying smoking. [18, 19] The same thing occurs among immunodeficient patients whose systemic condition is associated with more severe and frequent refractory chronic periodontitis. [20] It is crucial to have it in mind that we have studied patients with generalized moderate chronic periodontitis and used salivary samples. Sampling from other sites or other types of periodontal diseases may express different results.

Previously, levels of interleukin 35 were not studied in the saliva of diabetic patients suffering from chronic periodontitis. Due to the limited fund of the project, and interleukin kit price, only saliva samples were used for further analyses.

## Conclusion

In this study, the lowest level of interleukin 35 was observed in the saliva of the diabetic + periodontitis group, and the periodontitis group and the diabetic group were assigned to the next. All groups showed significantly lower levels of interleukin-35 than the control group. Moreover, in other cases, no significant difference was observed. Patients with periodontitis had a reduced level of IL-35 in their saliva. This decrease was not related to type 2 diabetes.

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**Conflict of Interest:** The authors declare no conflict of interest.

## Authors' Contributions

The study was designed by Amir Kiakojori and Shima Nafarzade. The study data were collected by Anis Moslemi and Mohammad Ali Bayani. Analysis and interpretation of data, drafting of the manuscript and critical revision of the manuscript for important intellectual content were performed by Hemmat Gholinia,Monireh Golpour and Amrollah Mostafazadeh. Study supervision was conducted by Amir Kiakojori and Amrollah Mostafazadeh.

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