

# The Effect of C-Reactive Protein and Interleukin 12 in Dental Caries Class V among Diabetic Patients

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## Abstract

**Background:** Dental caries is a multifactorial disease; it is affected by many factors such as sugar consumption, exposure, fluoride, and others. **Objectives:** To investigate the immune status by studying the levels of interleukin 12 (IL-12) and C-reactive protein (CRP) in gingival crevicular fluid (GCF) of individuals with dental caries among diabetic patients compared with diabetic patients with free dental caries and control groups. **Materials and Methods:** The study was designed to include 90 samples of GCF obtained from patients with diabetes and dental caries, patients with diabetes only without dental caries, and healthy subjects attending the Marjan medical city. The study duration was prolonged from November 2021 to March 2022. **Results:** The results showed that there were highly significant differences ( $P \leq 0.05$ ) in the concentration of IL-12 among healthy subjects. Both diabetic patients with and without dental caries were represented by the increase in the concentration of IL-12. However, diabetic patients with dental caries ( $74.86 \pm 37.45$ ) were more than those with free dental caries ( $53.26 \pm 17.75$ ) and even more than the control group. The contrast result showed a decrease in the level of CRP concentration in patients with diabetic and dental caries ( $2.589 \pm 2.262$ ) less than in patients with diabetes only ( $3.783 \pm 2.408$ ) and less than healthy subjects with significant differences ( $P \leq 0.05$ ). **Conclusion:** This study found that there was an elevation of the IL-12 level in patients with significant differences compared with control groups in contrast to a reduction in the concentration level of CRP patients with significant differences compared with the control group.

**Keywords:** C-reactive protein, dental caries, diabetes mellitus, IL-12

## INTRODUCTION

Numerous oral disorders have been reported in the literature as impacting oral health-related quality of life. Dental caries is a complex, chronic, infectious illness that develops due to a mix of environmental and behavioral factors such as the reaction between dental plaque bacteria, diet, and a variety of host variables, including fermentable carbohydrates, saliva flow, and exposure length.<sup>[1]</sup>

Genetic variables such as vulnerable teeth based on the production of dental enamel and immune response to cryogenic bacteria were influenced.<sup>[2,3]</sup> Dental caries is considered a common multiple various disorders, and the cervical form of the disease has been in several clinical situations.<sup>[4]</sup> The chemical characteristics of oral fluid have a crucial function in the beginning, occurring, and development of this illness because there is constant contact between teeth, gingival crevicular fluid (GCF),

and saliva.<sup>[5,6]</sup> Class five or cervical caries is a soft, uneven, progressing carious lesion that arises at an apical tooth exposed cervical amelo-cemental junction cemento-enamel junction known as root surface caries.<sup>[7]</sup>

Diabetic mellitus (DM) is a metabolic disorder with complications throughout the body related to disease, control, and duration. A mechanism directly spurts higher caries occurrence in an individual with DM. In addition, this mechanism is responsible for the decreased flow of saliva caused by DM, which elevates the risk of tooth decay in diabetic patients.<sup>[8]</sup> In addition, there are many

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oral complications linked to diabetic patients.<sup>[9,10]</sup> Such as xerostomia, taste impairment, oral candidiasis, and periodontal disease.<sup>[11]</sup> The tissues and organs with the most capillary capillaries primarily affected by DM problems are the kidney, the retina, and nerves. As a result, secondary to the emergence of microangiopathy are these problems.

The oral tissues display similar modifications in tiny veins.<sup>[12]</sup> However, severe periodontal inflammation can lead to the deterioration of the alveolar bone structure, the loss of tooth support, and the eventual loss of teeth. In periodontitis, an attachment loss leads to gingival recession, leading to root exposure to the oral environment. As a result, these sites become a possible source of root caries.<sup>[13]</sup> Moreover, in persons with type 2 diabetes, periodontitis and gingival erosion may induce increased cervical caries, aggravated by reduced salivary flow and raised gingival crevicular glucose levels. Even in the absence of periodontal disease, root surfaces might be exposed through other ways, such as a brushing force, and so become vulnerable to root cervical caries.<sup>[14,15]</sup>

C-reactive protein (CRP) is the other protein found in GCF and releases to the oral cavity due to inflammation during the acute phase; it is synthesizing by the liver and then transports into the bloodstream. It is thought to be a gold standard for determining inflammation, and it is a non-immunoglobulin protein composed of five subunits identical in structure. One of the essential functions of CRP is its role in innate immunity; it aids in the binding of complements to foreign and injured cells, as well as assisting in the repair of damaged cells and improving phagocytosis.<sup>[16-19]</sup> The amount of CRP is thought to be a potential risk for certain life-threatening disorders, such as diabetes<sup>[20]</sup>; it is suggested that dental caries could be a risk factor for this disorder in the long run. As a result, dental caries can lead to prolonged illness caused by infection with oral bacteria that persist in the tooth if not treated. Moreover, dental caries could have a detrimental influence on the well-being of senior citizens.<sup>[21]</sup>

The aim of this study was to investigate the immune status by studying the levels of IL-12 and CRP in GCF of individuals with dental caries among diabetic patients.

## MATERIALS AND METHODS

### Subjects

Ninety specimens were collected for the present study for individuals aged from 20 to 50 years, and 45 samples from GCF of patients with dental caries and diabetes were collected. 20 GCF samples from healthy subjects as control, and 20 samples from diabetic individuals who were in good oral health and without dental caries; these samples were used to detect the IL-12 levels in GCF of diabetic patients with dental caries by using Enzyme-Linked Immunosorbent Assay (ELISA) assay.

### Sample collection

GCF samples were collected.<sup>[22]</sup> Teeth dried with cotton wool rolls and short air blasts to avoid contamination and mixing with saliva. Following, three paper points with size 30 were inserted 1 mm into the gingival crevice and held in position for half a minute. Blood-stained paper points were excluded. The paper point placed in Eppendorf's tubes after GCF collection contains (300 µL) phosphate buffer saline. GCF was extracted from the paper point by placing the sample at centrifugation at 3000 rpm for 15 min. Following the paper point was removed, and then the GCF sample was stored at -40°C until the analysis.

### Determination of interleukin-12 and C-reactive protein in gingival crevicular fluid

The detection of these markers was done in GCF using the ELISA; according to the manufacturing company Elabscience produced in China, 100 mL of the GCF sample was added to each well of the plate, after that it was incubated for 90 min at an optimal temperature of 37°C. After incubation, GCF was removed from each well, and then 100 mL of a biotinylated detection antibody was also added to each well and incubated for 1 h to 90 min at 37°C. After that, the antibody was discarded from the plate and the plate was washed by a washing buffer (1×) for three times and dried by a towel paper very well. Then, the enzyme horseradish peroxidase (HRP) was added to each well and incubated within a half hour, the enzyme was also discarded by the same way of removing the unconjugated antibody, but washing was used for five times and dried. Then, a 90 mL of tetramethylbenzidine-substrate (the substrate specific to HRP) was added to each well and incubated within a few minutes until appearing of a blue color, and finally, 50 mL of the stop solution was added carefully. Finally, the OD was measured spectrophotometrically by an ELISA reader at 450 nm.

### Statistical analysis

Statistical analysis was carried out using SPSS version 25.0 (SPSS, IBM Company, Chicago, IL, USA). Means and standard deviations were used to present continuous variables. The means of the three groups were compared using an ANOVA test. *P* values of 0.05 or less were regarded as statistically significant.

### Ethical consideration

The study was conducted following the ethical guidelines specified in the Helsinki Declaration. The patient's verbal and written consent was acquired before collecting the sample. Using document number 566 (with the number and date November 1, 2022), a regional ethics committee examined and approved the study protocol, subject information, and consent form to get this approval.

## RESULTS

### Evolution of the gingival crevicular fluid interleukin 12 levels

Figure 1 shows that the mean and standard deviation of the concentration IL-12 in healthy individuals ( $46.76 \pm 12.78$ ) which is less than in the diabetes patient's group with ( $74.286 \pm 37.45$ ) and without ( $53.265 \pm 17.752$ ) dental caries respectively, with a significant differences of 0.001.

### Evolution of the gingival crevicular fluid C-reactive protein levels

Figure 2 shows that the mean and stranded deviation of concentration CRP in healthy individuals ( $5.307 \pm 2.129$ ) which is less in diabetes patients than that without dental caries patients ( $3.783 \pm 2.408$ ) and less in diabetic patients than that with dental caries patients ( $2.589 \pm 2.262$ ) with a significant difference of 0.000.

## DISCUSSION

The findings of this investigation were analyzed in three various categories of people. The first group included relatively healthy and without dental caries without diabetes individuals, and the second group included subjects with diabetes, but without dental caries. The last

group included patients with DM and class five dental caries.

Our study was compatible with several authors<sup>[23,24]</sup> who found an increase in the concentration of IL-12 in GCF in patients more than in control group. This result may be supported by for several reasons such as:

1. One of the causes reveals that oral bacteria cause dental caries to invade the teeth and initiate a process that causes inflammation. These lesions increase the generation of pro-inflammatory cytokines and trigger inflammatory cytokines in the host through of a wide variety of biological events.
2. Also, DM is an autoimmune disease that impairs the immunity of the body to make pathogens have more ability to invade the body and elevate the concentration of IL-12.
3. Maybe also to other infections or diseases interfering with diabetes and dental caries.

Concerning CRP, the study showed a decrease in the concentration of CRP GCF in diabetic patients with and without dental caries compared with a control group. Still, in contrast, the study disagreed with,<sup>[25]</sup> which found an increase in CRP in GSF of diabetic patients compared with healthy subjects. Furthermore, the results were incompatible with Fitzsimmons *et al.*<sup>[26]</sup> So, the decreased level of CRP in our study may be related to lifestyle changes or a reduction in the CRP which can lead to increase aerobic activity such as running, fast walking, cycling, and smoking cessation or perhaps controlling the glucose level in diabetic patients. Also, It may be owing to the type of certain drugs that inhibiting the level of CRP protein inside the mouth. Therefore, the general level of CRP in GCF lower in serum is different from person to person, according to many factors such as those mentioned above.

## CONCLUSION

This study found that there was an elevation of the IL-12 level in patients with significant differences compared with control groups in contrast to a reduction in the concentration level of CRP patients with significant differences compared with the control group.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

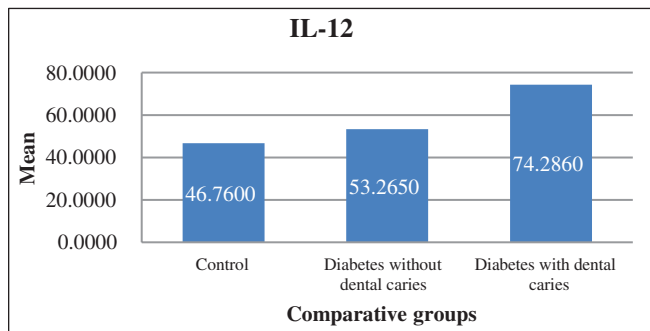


Figure 1: Mean concentration of IL-12 among healthy and patients

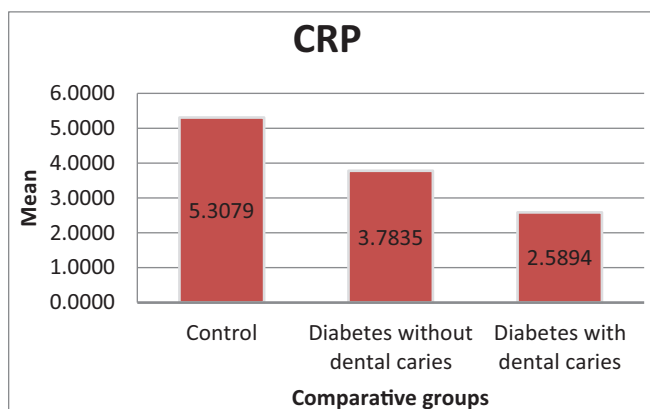


Figure 2: Mean concentration of CRP among healthy and patients

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