Mustansiria Dental Journal
MDJ

Vol.21, No.01, 06/2025
P- ISSN: 2618-0944
E- ISSN: 1813-8500

Assessment of Salivary C Reactive Protein level in Type Two Diabetic Patients with Different Stages of Periodontitis

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Received 31/08/2024 ***Accepted in revised form 19/12/2024*** ***Published 30/06/2025***

Abstract

The purpose of this study is to contrast the salivary levels of C-reactive protein between healthy controls and diabetic patients at various stages of periodontitis.

Materials and methods: One hundred sixty men participated in this study; 32 of them were healthy control groups, while the other 128 men with type -2 diabetes were equally categorized into four groups according to stages of periodontitis (stage I, stage II, stage III, and stage IV). Following the collection of unstimulated salivary samples from each participant, (PPD, CAL, and BOP) clinical periodontal parameters were assessed. ELISA technique was used to measure the levels of CRP in saliva samples. **Results:** CRP was higher in diabetic patients with all stages of periodontitis than healthy controls. The level of CRP is increased when periodontitis progresses to higher stages. Stage IV group (515.33±258.51), stage III group (315.52±206.71), stage II group (206.61±124.64), stage I (160.07±97.49), control group (45.63±25.29) in which their salivary levels were the least with significant differences among the groups (p=0.000).

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Conclusion: The inflammatory marker CRP is increased in diabetic patients than in Control, especially when the stage of periodontitis gets worse.

Keywords: CRP, saliva, periodontitis, diabetic, inflammation.

Introduction:

The most prevalent conditions affecting oral health are periodontal disorders, such as gingivitis and periodontitis. By definition, gingivitis is an inflammatory condition brought on by tooth plaque buildup and bacterial dysbiosis in the gingival area. [1] By decreasing the number of bacteria within the biofilm and improving dental hygiene procedures, gingivitis can be prevented. [2] Periodontitis, a more severe form of gingival disease, is characterized by bone loss, gingival recession, tooth movement, and clinical attachment loss. [3] Since periodontitis is irreversible and the deterioration of the periodontium cannot be fully restored, a patient diagnosed with the condition is believed to have it for the rest of their life. [4]

The pancreatic beta cells secrete the peptide hormone insulin, which controls the adsorption and glucose levels. Diabetes mellitus (DM) is an integration of numerous metabolic diseases marked by the body's inability to create enough insulin or use it efficiently. Insulin deficiency or improper insulin use can cause hyperglycemia, which can potentially lead to life-threatening consequences. [5, 6]

Diabetes can impact many organs and can change their structure when chronic hyperglycemia reaches advanced levels. Examples include cardiovascular disease and an increase in the risk of atherosclerotic processes in blood vessels. Moreover, diabetes may increase the risk of infections.[7]

Numerous investigations have shown a reciprocal association between diabetes mellitus and periodontitis.[8] Periodontitis and diabetes mellitus interact reciprocally and affect each other, regardless of associated risk factors.[9]

The primary source of C-reactive protein production is hepatocytes. In the innate immune system, where it acts as an early line of defense against external pathogenic microorganisms, C-reactive protein predominantly performs physiological activities. [10]

Because C-reactive protein is a sensitive inflammatory measure, its relationship to insulin resistance and type 2 diabetes has been investigated. Numerous cross-sectional research has indicated a connection between increased C-reactive protein levels and elements of insulin resistance such as obesity, elevated fasting blood sugar, and reduced insulin sensitivity. These results raised the possibility that those with pre-diabetes or insulin resistance could be identified by elevated C-reactive protein levels .[11]

Giannobile et al.,2009 discovered that the degree of periodontal disease is directly correlated with C-reactive protein levels, with higher levels seen in cases with aggressive periodontitis. [12]

The current study aims to evaluate the C-reactive protein level in type two diabetic patients with periodontitis.

Materials and methods:

Study design

A total of one hundred sixty (160) male participants, ages ranging from 40 to 60 years, were included in this case-control study. One hundred twenty-eight patients with Type-2 diabetes mellitus and periodontitis (were collected from the Diabetes and Endocrinology Center at Marjan Hospital – Babylon. Thirty-two control people were selected from individuals visiting the dental clinic for regular dental check-ups who do not have any diseases.

The Mustansiriyah University College of Dentistry- Institutional Ethics Committee Board granted ethical approval. (approval number and date "REC145" on 01/December/2023).

All participants gave their informed consent after agreeing to take part willingly. In an isolated room in the Diabetes and Endocrinology Center, each participant underwent an oral examination with a Michigan periodontal probe, and a complete medical and dental history was recorded on a consent form.

Patients who did not give their consent, those under 40 years old, those who had undergone periodontal therapy within the previous six months, those who had taken an antibiotic within that same time frame, those with systemic diseases other than diabetes mellitus, female patients, those who smoked or had smoked cigarettes within the previous three years, edentulous patients, and patients with fewer than

twenty teeth were not included in this study.

Saliva collection

Before the examination of clinical periodontal parameters, entire, unstimulated saliva samples from the patients were collected in the morning. Patients have to fast for one hour before the sample collection and only drink water. The patients were told to wait one to two minutes for the water to clear and to thoroughly rinse their mouths to eliminate any debris.[13]

The plain tube had been saturated with saliva that drooled passively over the bottom lip. Samples that contained blood were discarded after collection[13], and 2ml of saliva was collected. The subject's number from the case sheet was written on the label of the tube. The collected samples were centrifugation for 10 minutes at 5000 rpm, and the supernatant was then aspirated into Eppendorf tubes using a micropipette. The samples were then kept in the laboratory freezer at -20°C until the day of analysis[14].

After saliva was collected, a comprehensive periodontal examination was carried out, which included measuring bleeding on probing (BOP) [15], probing pocket depth (PPD), and clinical attachment loss (CAL). All periodontal parameters were measured with a periodontal Michigan O probe. Every tooth, except for the wisdom teeth, had six surfaces inspected. Each subject underwent an identical clinical evaluation conducted by the matching examiner.

The patients in the control group did not have any CAL, PPD ≤3 mm, BOP, or diabetes[2]. Range in control group for "bleeding on probing BOP= 0.99, probing pocket depth PPD= 2.33, and clinical attachment loss CAL"= 2.74

diabetes patients with interproximal CAL at two non-adjacent teeth were classified as part of the periodontitis and diabetes group. CAL≥3 mm buccal or oral, with pocket > 3 mm seen at two teeth. Range in diabetic and periodontitis group for "bleeding on probing BOP= 0.817, probing pocket depth PPD= 2.31, and clinical attachment loss CAL"= 3.462.

Quantitative analysis of Saliva C-reactive protein

Using an ELISA kit from Elk Biotechnology company, the quantities of C-reactive protein in the saliva of the diabetic within periodontitis groups and the control group were analysis by following the assay procedure of the

company in Al-Amin Center for Research and Biotechnology in Najaf.

Statistical analysis

To characterize, analyses, and present the data, Statistical Package for Social Science (SPSS) version 24 (USA, Illinois) was utilized. The two types of statistical analyses are inferential, which includes the one-way ANOVA test and Tukey's test, and descriptive, which includes the mean and standard deviation (SD).

Results

In Table 1 the mean of salivary C-reactive protein was increased from control until stage IV with a significant difference (0.00) (ANOVA test was used), the mean value of C-reactive protein for control was (45.63±25.29), for stage I mean value was (160.07±97.49), for Stage II mean value was (206.61±124.64), for Stage III mean value was (315.52±206.71), for Stage IV mean value was (515.33±258.51).

After several merges comparisons (using Tukey's test) among groups as seen in **Table 2** all results showed significant differences (p=0.000), except group I with group II and group II with group III were no significant difference (p>0.05).

As seen in **Table 3** there was a significant positive correlation between C-reactive protein and each of PPD (r= 0.35, p=0.00) and CAL (r= 0.64, p=0.00) in diabetic patients with periodontitis groups.

Discussion:

In terms of salivary C-reactive protein levels, the results of this investigation showed that all diabetic groups with periodontitis had considerably higher C-reactive protein levels in comparison to controls. These results were consistent with the results of (Dholey et al.,2017)[16], and (Bachtiar et al.,2023)[17] who concluded that C-reactive protein levels were significantly increased in the diabetic patients with periodontitis than in control subjects, implying that the level of C-reactive protein in saliva may help in the detection, good diagnosis, and treatment of diabetic and periodontal diseases. These results may be due to the truth that diabetes mellitus and periodontitis made the inflammatory situation worse. Elevation of C-reactive protein is a component of inflammation's acute phase response (Dholey et al.,2017)[17].

An additional study finding is that there was a significant positive correlation between C-reactive protein

with PPD and CAL in diabetic patients with periodontitis groups. This results in agreement with (Shojaee et al.,2013)[18]; who demonstrated that there is a significant positive correlation between C-reactive protein levels, PPD, and CAL in diabetic patients with periodontitis. Increased periodontal disease severity is correlated with elevated C-reactive protein levels, emphasizing the inflammatory nature of periodontitis and its consequences for systemic health in diabetics.[19]

These effects may result from inflammation or infection activating leukocytes, which in turn causes C-reactive protein (CRP) to be produced and secreted. TNF-alpha, interleukin-6 (IL-6), and IL-1 are inflammatory cytokines that cause the liver to produce C-reactive protein, an acute-phase reactant protein. As a result, both acute and chronic inflammation in the body cause a rise in the level of C-reactive protein in saliva and blood serum. (Ramamoorthy et al.,2012; Gupta et al.,2020).[20,21]

Conclusion

The investigation's outcome demonstrated that the inflammation increased in diabetic patients when the severity of periodontitis increased. The inflammatory marker C-reactive protein is increased in diabetic patients than in healthy controls, and when the stage of periodontitis gets worse.

Conflicts of Interest

According to the writers, they don't ha ve any conflicts of interest.

Acknowledgment:

Sajjad Ali Jreo and Dalia Kudier Abbas would like to express gratitude to Mustansiriyah University in Baghdad, Iraq (www.uomustansiriyah.edu.iq) for their assistance with this study.

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Table 1: Mean , standard deviation and ANOVA test of salivary C- reactive protein for study groups.

	groups	Mean ± SD	F	P value
CRP ((pg/ml))	I	160.07±97.49	37.39	0.000
	II	206.61±124.64		
	III	315.52±206.71		
	IV	515.33±258.51		
	controls	45.63±25.29		

CRP: C- reactive protein; SD: standard deviation ; F: ANOVA

Table 2: Tukey's Honest Significant Difference (HSD) test of salivary C- reactive protein among study groups.

Study groups		Mean difference	P value
group I	group control	-114.4356	0.000
group II	group control	-160.982	0.000
group III	group control	-269.8885	0.000
group IV	group control	-469.6983	0.000
group I	group II	-46.5464	0.7893
group I	group III	-155.4529	0.000
group I	group IV	-355.2628	0.000
group II	group III	-108.9065	0.0667
group II	group IV	-308.7164	0.000
group III	group IV	-199.8099	0.000

Table 3: Correlation coefficient between stages, control group of salivary C reactive protein and periodontal parameters.(Pearson correlation (r)test used)

groups	periodontal parameters	salivary CRP biomarkers	
Stage I,II,III,IV groups	**PPD**	**r**	0.35
		p	0.000
	CAL	**r**	0.64
		p	0.000
	BOP	**r**	-0.10
		p	0.24
control	**PPD**	**r**	-0.003
		P	0.98
	CAL	**r**	0.12
		p	0.49
	BOP	**r**	-0.22
		p	0.2

PPD :probing pocket depth ;CAL: clinical attachment loss; BOP: bleeding on probing