VALUES OF SERUM C-REACTIVE PROTEIN AND SALIVARY IMMUNOGLOBULIN IGA IN DIABETIC PATIENTS: RELATION TO THE SEVERITY OF PERIODONTAL DISEASE

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ABSTRACT

Background: Altered immune response and improper neutrophils chemotaxis, phagocytosis, and adhesion might be the principal causative factor for increased susceptibility to periodontal pathogens and oral complications in diabetic patients. This study aimed to determine the relationship between C-reactive protein and salivary IgA with periodontal disease and the severity of dental caries with the glycemic control state of patients with diabetes mellitus.

Patients and Methods: This study was carried out on 91 subjects, 61 patients with diabetes mellitus, and 30 apparently healthy subjects (as a control group). The patient groups were regularly attended Duhok Diabetes Center, Duhok City, Kurdistan Region (Iraq) for diabetes management. The healthy controls were recruited from the staff and sub staff of Azadi Teaching Hospital. Fasting plasma glucose, HbA1c, and serum high sensitivity C-reactive protein (Hs-CRP) were measured. The whole saliva collection was performed by an unstimulated method for five minutes in a graduated test tube to recognize the salivary secretion rate and then stored at -20 °C for IgA estimation. Periodontal Index was used to determine the periodontal disease status. Each tooth was scored according to the condition of the surrounding tissues.

Results: Significantly higher Hs-CRP (9.2 vs 3.3 µg/ml), fasting plasma glucose (215.4 vs 98.9 mg/dl) and HbA1c (8.5 vs 4.9 %), (P < 0.001 for all parameters, together with lower hemoglobin (13.6 vs 14.5 gm/dl, P=0.03) levels were found in diabetic patients compared to control group, A significantly higher mean salivary IgA level in diabetic patients compared to controls (312.4 vs 177.3 mg/dl, P<0.001), associated with a significant high periodontal index (1.68 vs 0.81, P = 0.003).

Conclusion: Elevated serum Hs-CRP and salivary IgA in patients with diabetes mellitus as inflammatory response sequences raise inflammation potential in the periodontium. Further, the results confirm that periodontal index was associated with poor glycemic control.

Keywords: Hs-CRP, IgA, Periodontal disease, Periodontal index

Epidemiological data support microvascular changes in diabetes mellitus (DM)1. The microvascular changes lead to reduced tissue blood flow, decreased oxygen diffusion, and a better environment for the growth of anaerobic bacteria and susceptibility to infection2. The possible association between oral and systemic health has been highlighted; evidence suggested a bi-directional relationship between systemic and periodontal disease. Oral manifestations in DM include high dental caries, xerostomia, glossitis, oral candidiasis, burning mouth, and gingival and periodontal disease3,4. The altered immune response and improper neutrophils...
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chemotaxis, phagocytosis, and adhesion might be the principal causative factor for increased susceptibility to periodontal pathogens and oral complications. Reduction in the quantity of salivary secretion or changes in its properties is responsible for oral and dental problems. Salivary immunoglobulin A (s-IgA) antibodies could help oral immunity by preventing microbial adherence, neutralizing enzymes, toxins, and viruses. Studies have demonstrated a lower incidence of dental caries as a result of high s-IgA concentration, and low levels have been presented as a risk factor for upper respiratory infection, periodontal disease, and dental caries. On the other hand, numerous studies in the last decade have shown that CRP is an independent predictor of risk for periodontal disease. This work was designed to determine the relationship between C-reactive protein, salivary IgA, and glycemic control with the severity of periodontal disease in diabetic patients.

PATIENTS AND METHODS:
This cross-sectional study was conducted at the Department of Physiology, College of Medicine, University of Duhok and Azadi Teaching Hospital in Duhok city, sixty-one patients with diabetes mellitus (45 with type 2 diabetes and 16 with type 1 diabetes) attended Duhok Diabetes Center, were selected randomly, (35) males and (26) females with ages ranged between 20-55 years. The healthy controls were recruited from the staff and sub-staff of the Azadi Teaching Hospital. Their age and sex-matched with the diabetic group; this group consisted of (19) males and (11) females and with ages ranged between 24-51 years. All participants were informed about the study, and written consents were obtained before the study commenced. The study was approved by the local ethics committee of the Directorate of Health-Duhok. Exclusion criteria are patients with recent acute illness or upper respiratory tract infection in the past one week, history of chronic liver or renal disease, and those taking antibiotics or who underwent periodontal therapy one month before the study, pregnant females, and alcoholics. Data collection: all subjects completed a pre-tested questionnaire, which included anthropometric data and a diabetes record. Body mass index and duration of the diabetes were recorded. History was taken to select healthy subjects (controls) and to exclude those with a known personal or family history of diabetes mellitus or the presence of any of the mentioned abnormalities (exclusion criteria). HbA1c% was estimated using a commercial kit (StanbioGlycohemoglobin Pre-fil procedure no. P350), a qualitative colorimetric determination of glycolhemoglobin in whole blood. Estimation of Serum Hs-CRP level using AccuBind Hs-CRP ELISA kit, product code: 3125-300 (Netherlands), was used for quantitative estimation of serum Hs-CRP. The reaction between various CRP antibodies and native CRP forms a sandwich complex that binds with streptavidin-coated to the well. After equilibrium is attained, the antibody-bound fraction is separated by decantation or aspiration. The enzyme activity presents on the surface of the well quantitated by reaction with a suitable substrate to produce color that is directly proportional to the native antigen concentration. A dose response curve was used to ascertain the concentrations of CRP in the samples. The
quantitative determination of salivary IgA was measured by turbidimetry method using Vital Diagnostics IgA Turbidimetry kit (ACCC 16-010). The reference range provided by the manufacturer (70–400 mg/dl).

**Assessment of the HbA1c, CRP, and IgA**

The Assessment HbA1c (%) at the universal diagnostic decision of the cut-off value of (48 mmol/mol; 6.5%) was considered:

- **Normal**: (< 5.7 %)
- **Prediabetes**: (5.7%–6.4%)
- **Diabetes**: (≥ 6.5%)

And for glycemic control HbA1c (%) in diabetic patients was considered:

- **Good control**: (< 6.5 %)
- **Fair control**: (≥ 6.5% – < 7.5%)
- **Poor control**: (≥ 7.5 %)

The assessment of CRP and IgA was done by using the calculated cut-off value formula:

\[
\text{Cut off value} = \text{mean} + 3 \times \text{SD}
\]

The clinical cut-off value for Hs.CRP = 5.1 (µg/ml) and for salivary IgA = 211.3 mg/dl. The result of higher than 5.1 (µg/ml) for Hs.CRP and 211.3 mg/dl for IgA provided prognostic information as a risk values for periodontal diseases.

**Salivary, Oral and Dental Assessment**

- **Assessment of Salivary Secretion Rate (SSR)** was determined by measuring the amount of unstimulated saliva produced (collected) in a given period of time.

- The Salivary Flow Index (SFI) for unstimulated saliva was used, and subjects were classified into 3 categories:

  - **Normal**: 0.25–0.35 ml/min.
  - **Low**: 0.1–0.24 ml/min.
  - **Very low (Hyposalivation)**: less than 0.1 ml/min.

- **Assessment of Periodontal Index (PI) or Score**: Periodontal Index determines the periodontal disease status. Each tooth is scored according to the condition of the surrounding tissues. On examination, each tooth is assigned a score (from zero to eight) using the following criteria:

  - **0**: Negative. Neither overt inflammation nor loss of function caused by the destruction of supporting tissue is noted.
  - **1**: Mild Gingivitis: overt inflammation in the free gingiva is present, does not circumscribe the tooth.
  - **2**: Gingivitis: Inflammation surrounds the tooth; there is no apparent break in the epithelial attachment.
  - **6**: Gingivitis with pocket formation. The epithelial attachment of the gum to the tooth is broken. There is no interference with normal function. The tooth is not loose or drifting.
  - **8**: Advanced destruction with loss of function. The tooth may be loose or drifting. It may sound dull on percussion and maybe depressible in the socket. The scores for each tooth are added, and the total divided by the number of teeth examined. Scores can be interpreted as follows:

    - **0.0–0.2**: Clinically normal supportive tissues.
    - **0.3–0.9**: Simple gingivitis.
    - **1.0–1.9**: Beginning destructive periodontal disease.
    - **2.0–4.9**: Established destructive periodontal disease.
    - **5.0–8.0**: Terminal periodontal disease.

- **Assessment of Decayed, Missed, and Filled Teeth (DMFT) Index or Score**

  To arrive at a DMFT score for an individual subject's mouth, three values must be determined the number of teeth with carious lesions, the number of extracted teeth, and the number of teeth with fillings.
Participants were divided into three groups according to their DMFT score:

- Group A: DMFT score 0-6
- Group B: DMFT score 7-12
- Group C: DMFT score 13 and more.

**STATISTICAL ANALYSES**

All data were analyzed using the Statistical Package for Social Science (SPSS) version 22.0; independent *t*-test and one-way ANOVA test were used to examine statistical differences between groups.

**RESULTS**

Table 1 shows the baseline characteristics of the studied individuals. No significant differences were found with respect to age and BMI between the diabetic and healthy control groups. Significantly higher fasting plasma glucose, Hs-CRP, and HbA1c levels were observed in diabetic patients compared to healthy controls (P=0.010 for all parameters). Regarding salivary IgA and periodontal index, a significantly higher mean IgA and the periodontal index was found in the diabetic group compared to the healthy control group (P<0.001, P=0.003, respectively). No significant differences were found with respect to salivary secretion rate and DMFT score between the two groups A higher periodontal index (1.86 vs. 1.19) and DMFT score (9.0 vs. 6.6) was observed among patients with type 2 DM compared to patients with type I DM, but the difference was not significant. No significant differences were found with respect to Hs-CRP, salivary secretion rate, and salivary IgA levels between both types of diabetes. No significant differences were found with respect to salivary secretion rate and DMFT score between the two groups. A higher periodontal index (1.86 vs 1.19) and DMFT score (9.0 vs 6.6) was observed among patients with type 2 DM compared to patients with type I DM, but the difference was not significant. No significant differences were found with respect to Hs-CRP, salivary secretion rate, and salivary IgA levels between both types of diabetes.

| Characteristics          | Control Group Mean ± SE, N=30 | DM Patient Group Mean ± SE, N=61 | P-value |
|--------------------------|--------------------------------|---------------------------------|---------|
| Age (years)              | 38.9 ± 1.5                     | 40.4 ± 1.3                      | NS*     |
| Body Mass Index (kg/m2)  | 26.7 ± 0.74                    | 28.5 ± 0.72                     | NS      |
| Serum Hs-C Reactive Protein (µg/ml) | 3.3 ± 0.6 | 9.2 ± 1.2                     | <0.001  |
| Fasting blood sugar (mg/dl) | 98.9 ± 1.4                | 215.4 ± 10.7                    | < 0.001 |
| HbA1c (%)                | 4.9 ± 0.04                     | 8.5 ± 0.3                       | <0.001  |
| Hemoglobin (gm/dl)       | 14.5 ± 0.3                     | 13.6 ± 0.2                      | 0.03    |
| Salivary secretion rate (ml/min.) | 0.34 ± 0.028 | 0.29 ± 0.023 | NS      |
| Salivary IgA (mg/dl)     | 177.3 ± 11.3                   | 312.4 ± 15.5                    | < 0.001 |
| Periodontal index        | 0.81 ± 0.13                    | 1.68 ± 0.2                      | 0.003   |
| DMFT score               | 7.73 ± 0.61                    | 8.36 ± 0.58                     | NS      |

* NS= non-significant
The relationship between Hs-CRP, salivary IgA, and periodontal parameters with glycemic control in people with diabetes and healthy controls are presented in table 2. As shown, diabetic patients with poor glycemic control had significantly higher periodontal index than that in those with good glycemic control ($P=0.003$).

### Table 2: Comparison of Selected Parameters According to Glycemic Control State in Diabetic Patients

| Parameters                          | Glycemic Control State | $P$-value |
|-------------------------------------|------------------------|-----------|
|                                     | Good Control N = 12    | Fair Control N = 10 | Poor control N = 39 | ANOVA |
| Serum Hs-C Reactive Protein (µg/ml) | 7.7 ± 2.6              | 8.7 ± 3.6              | 9.8 ± 1.5              | NS    |
| Salivary secretion rate (ml/min)    | 0.29 ± 0.06            | 0.33 ± 0.05            | 0.27 ± 0.03            | NS    |
| Salivary IgA (mg/dl)                | 313.6 ± 25.9           | 272.8 ± 20.6           | 322.1 ± 22.3           | NS    |
| Periodontal index                   | 1.02 ± 0.21            | 1.1 ± 0.28             | 2.03 ± 0.28            | 0.04  |

Poor control vs Fair control: $P = 0.035$
Poor control vs Good control: $P = 0.003$

DMFT score: 8.83 ± 1.1, 10 ± 1.0, 7.8 ± 0.8, NS

NS: Non-significant

To determine which of the selected or periodontal parameters was significantly associated with periodontal index level in diabetic patients, we performed ANOVA analysis between the periodontal index and Hs-CRP, salivary secretion rate, salivary IgA, and DFMT score. The results are presented in table 3. As shown by ANOVA analysis, salivary secretion rate was significantly higher in the group with low periodontal index (0-0.2) compared with those with periodontal index 2 or more ($P=0.032$).

### Table 3: Comparison of Selected Parameters According to Periodontal Index in Diabetic Patients

| Parameters                          | Groups of Periodontal Index | $P$-value |
|-------------------------------------|-----------------------------|-----------|
|                                     | A (0–0.2) N = 9             | B (0.3–0.9) N = 12 | C (1–1.9) N = 22 | D (≥ 2) N = 18 | ANOVA |
| Serum Hs-CRP (µg/ml)                | 9.8 ± 1.8                   | 8.7 ± 2.3               | 9.2 ± 2.2               | 9.4 ± 2.5               | NS    |
| Salivary secretion rate (ml/min.)   | 0.39 ± 0.06                 | 0.28 ± 0.06             | 0.29 ± 0.03             | 0.22 ± 0.03             | 0.032  |

Group A vs Group D, $P = 0.013$

Salivary IgA (mg/dl) 286.1 ± 22.2, 318.9 ± 26.6, 320.6 ± 28.9, 311 ± 34.3, NS

DMFT score 7.3 ± 1.5, 9.3 ± 1.2, 7.7 ± 0.9, 9.1 ± 1.3, NS

NS: Non-significant

Diabetic patients were divided into three groups according to their DMFT score (A, B, and C). All variables were further analyzed in ANOVA analyses; only salivary IgA level was significantly different between these groups ($P = 0.039$).
as shown in table 4, and it was significantly higher in group C (DMFT score 13 and more) compared to group A (DMFT score 0–6).

**Table 4: Comparison of Selected Parameters According to DMFT Score in Diabetic patients**

| Parameters                          | Group of DMFT Score | P-value   | ANOVA        |
|-------------------------------------|---------------------|-----------|--------------|
|                                     | A (0 – 6)           | B (7 – 12)| C (≥ 13)     |
|                                     | N = 25              | N = 25    | N = 11       |
| Serum Hs-C Reactive Protein (µg/ml)|                     |           |              |
|                                     | 7.6 ± 1.7           | 10.5 ± 2.1| 10.0 ± 3.2   | NS           |
| Salivary secretion rate (ml/min.)   | 0.25 ± 0.03         | 0.29 ± 0.03| 0.39 ± 0.06 | NS           |
| Salivary IgA (mg/dl)                | 274.5 ± 17.8        | 324.7 ± 27.1| 370.5 ± 39.7| 0.039        |
| Group A vs Group C                  |                     |           |              |
|                                     | P = 0.016           |           |              |
| Periodontal index                   | 1.76 ± 0.25         | 1.5 ± 0.3 | 2.15 ± 0.65 | NS           |
| DMFT score                          | 4.3 ± 0.37          | 9.3 ± 0.33| 15.5 ± 0.87 | <0.001       |

NS: Non-significant

**DISCUSSION:**

This study showed significantly higher Hs-CRP and salivary IgA levels in diabetic patients compared with levels in healthy controls. The results confirm a relationship between the periodontal index and glycemic control, as the mean periodontal index in diabetic patients with poor glycemic control was about 2 times higher than that in the good control group, as well as between DMFT and salivary IgA level, as the mean DMFT score was about 3 times in participants with severe dental carries than that with mild dental carries. It has been reported that inflammatory processes are indicated by high Hs-CRP, but data concerning the relation of Hs-CRP levels with the severity of periodontal disease in diabetic patients are scarce. General categories of risk factors associated with the development of periodontitis include genetic, environmental (e.g., tobacco use), and acquired risk factors, e.g., DM and cardiovascular diseases. The elevation in Hs-CRP levels in diabetic patients might be attributed to the enhanced inflammatory process and its complications rather than the severity of the periodontal index. Moreover, CRP is not synthesized locally by the periodontal tissues because CRP-mRNA has not been isolated from periodontal tissue. In patients with DM who are susceptible to periodontitis, the above inflammatory process will eventually extend apically and laterally to involve deeper connective tissues and alveolar bone. The possible explanation is that Hs-CRP and their positive correlation reflect activation of the immune system. In this study, the periodontal index was significantly higher in diabetic patients, with an increase of about two-fold compared with the control group. Many studies have found a higher prevalence of the periodontal disease among diabetic patients than among healthy controls. Large epidemiological studies have shown that diabetes increases the risk of alveolar bone loss approximately three-fold compared with non-diabetic individuals. The mechanisms by which diabetes influences the periodontium are similar to the pathophysiology of classic
microvascular and macrovascular complications. The present study showed a significantly higher periodontal index in people with diabetes with poor glycemic control compared to those with good and fair control; this finding is consistent with previously reported data. Moreover, it has been shown that periodontal disease and DM have a two-way relationship; periodontal disease may be a risk factor for the development of type 2 DM. Additionally, diabetic patients with the periodontal infection have worse glycemic control compared with diabetics subjects without periodontal disease. Salivary Secretion Rate (SSR) in both studied groups was not significantly different, and in both groups, SSR were within normal reference values; the average value for SSR of whole saliva in healthy individuals is about 0.3 ml/min. In this study, subjects spit out the saliva into the test tube once a minute, which may have influenced the flow rate of unstimulated saliva. Panchbhai et al. and Al-Zahawi et al. showed that poorly controlled type II DM has no influence on salivary output; however, Harrison and Bowen concluded that patients with poorly controlled type I DM had the significantly lower flow of the whole saliva compared to well-controlled and healthy subjects. Al-Maroo RH (2010) in line with our finding, he was found that the high concentration of the total proteins level in diabetic patient could be related to the increase in IgA and amylase levels in IDDM, or may be due to the decrease in the flow rate since, total proteins has an inverse relationship with salivary flow rate. Results of the present study revealed a highly significant increase in salivary IgA in diabetic patients. This finding is in agreement with that of Bhuyan et al. Sardari F et al. found that diabetic patients had higher salivary IgA levels compared to non-diabetic individuals. The possible mechanism responsible for increased salivary IgA was sought to the increased severity of periodontal disease in diabetic patients (evidenced by increased periodontal index). In periodontitis, the subgingival plaque accumulation results in an acute inflammatory response with infiltration of lymphocytes and plasma cells and a possible outpouring of immunoglobulins. Data concerning the relation of salivary IgA with DMFT score and dental caries in diabetics, particularly in type II DM, are limited. It has been reported that the bacteria S. mutants is the primary causative agent of dental caries in human beings. The duration of DM has no influence on salivary IgA level, while others found higher salivary IgA in long-duration patients with type I DM and they attributed this increase to decreased SSR due to presence of negative correlation between SSR and IgA level; however, in this study SSR was not changed significantly in diabetic patients; also no correlation was found between SSR and salivary IgA levels.

CONCLUSION

The results confirm that Serum Hs-CRP and salivary IgA levels are significantly elevated in diabetic patients. These findings may have attributed to the activation of the immune system and inflammation with excess production of pro-inflammatory mediators. As expected, higher levels of the periodontal index are associated with poor glycemic control. Therefore, a primary
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پیشگک

نحوخی شکری گلاب‌پزیک یکی از بیماری‌های آپورین زیادی است که در انسان‌ها برجسته است. شکری گلاب‌پزیک باعث افزایش سطح سرم‌پروتئین C-پرتابل (CRP) و افزایش کل است، که این افزایش به وسیلهٔ پروتئین‌های ایمنی در سرماخوردگی می‌باشد. شکری گلاب‌پزیک می‌تواند باعث افزایش سطح قند خون و اختلالات قلبی-عروقی شود.

پیشگک

دندان‌های عیوبی موثر در مصرف بیشتری از مواد غذایی و درک بهبودی باعث می‌شود که در کل مصرف مواد غذایی و درک بهبودی باعث می‌شود که در کل مصرف مواد غذایی و درک بهبودی باعث می‌شود که در کل مصرف مواد غذایی و درک بهبودی باعث می‌شود که در کل مصرف مواد غذا
يرجى ملاحظة أنني قادر على قراءة النص العربي وتحويله إلى نص باللغة العربية بشكل طبيعي.

تم جمع من عينة من دم مرضى السكري وعينات من اللعاب من المشاركين في الدراسة. تم اجراء التحليلات الكيميائية في مختبر تلقائي للعقارب، وقد تم استخدام مكون/statistical utilities للبيانات. 

النتائج والمناقشة

أظهرت النتائج زيادة معنوية في معدل البروتين التفاعلي ج في المصل لدى مرضى السكري مقارنة بالأشخاص (P=0.003، 6.4 ميكروغرام/مل). كذلك وجدت زيادة معنوية لمؤشر امراض السكريrede السكري (P=0.03) في مرضى السكري. 

المجموعة السريرية: نبا، كان متوسط أفزاز اللعاب معنويًا بين مرضى السكري الذين يُعانون من السكري نوع 1 المقابلة (P=0.03). 

وظائف زيادة معنوية في متوسط الكلوبيولين المناعي اللعابي لدى مرضى السكري مقارنة بالأشخاص (P=0.020).
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100ml) بالنتائج. متوسط الكلوبيولين المناعي اللعابي أ كان أعلى معنويًا في مرضى السكري الذين لديهم مؤشر تسوس الأسنان عالي (أعلى من 13 نقطة) مقارنة مع الذين لديهم مؤشر تسوس قليل (0–6) نقطة وتتركز 370.5 مقارنة بـ 274.5 ملم/100 مل (P= 0.016).

الاستنتاج

ازداد متوسط الكلوبيولين المناعي اللعابي أ و مؤشر حول السن لدى مرضى السكري. إن متوسط الكلوبيولين المناعي اللعابي أ وجد أعلى عند مرضى السكري الذين لديهم مؤشر تسوس الأسنان عالي بينما متوسط الكلوبيولين المناعي اللعابي A لم يتأثر بعد السيطرة على مستوى السكر في الدم و مؤشر حول السن لدى مرضى السكري.