Saliva C-reactive protein as a biomarker of metabolic syndrome in diabetic patients

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ABSTRACT

Background: Human C-reactive protein (CRP) has been used in the risk assessment of coronary events. Human saliva mirrors the body’s health and well-being and is noninvasive, easy to collect, and ideal for third-world countries as well as for large patient screening.

Aims: This study aimed to screen the saliva CRP qualitatively in patients with diabetes (Type 1 and 2) taking in considerations, the diagnostic criteria of metabolic syndrome.

Setting and Design: Center for diabetes mellitus, prospective study.

Materials and Methods: A total number of 50 Type 2 diabetes (T2D) patients, 25 Type 1 diabetes (T1D) patients, and 25 healthy subjects were recruited from the center for diabetes mellitus. Each patient was assessed clinically, and the anthropometric measures, glycemic status, and lipid profiles were determined. Stimulated salivary flow rate and saliva CRP were determined.

Statistical Analysis: All calculations analysis was made using Excel 2003 program for Windows.

Results: The results showed that the salivary flow rate in T1D was less than healthy subjects and T2D and CRP was found positive (6 mg/L) in 36% and 56% of patients with T1D and T2D, respectively. Saliva CRP was found to be related to the anthropometric measurement, blood pressure, and glycemic control.

Conclusions: We conclude that saliva CRP may be used as a biomarker for metabolic syndrome and its value is obvious in T2D rather than in T1D.

Key words: C-reactive protein, diabetes mellitus, metabolic syndrome, saliva
MATERIALS AND METHODS

This cross sectional study was conducted according to the guidelines from the Declaration of Helsinki. It was approved by the Local Ethical Review Board (signed agreement, number 2, 2012) obtained from the Institutional Scientific Committee which stated the agreement to do this study as a part of the objectives of research work in the university.

All patients gave written informed consent. The entry criteria included known patients of T1D and T2D (ages 29–62 years), using oral glucose-lowering medication(s) alone and/or with once- or twice-daily insulin, who attended the center for diabetes mellitus for follow-up.

Ascertainment of metabolic syndrome is considered from the laboratory measures, if a person satisfied 3 out of 5 National Cholesterol Education Program (NCEP) criteria for metabolic syndrome (MetS). she/he was deemed to have metabolic syndrome. NCEP criteria include the following:

- Central obesity waist circumference >102 cm (male) or 88 cm (female)
- Fasting blood glucose >110 mg/dl (6.1 mmol/L) or having diabetes
- Systolic blood pressure >130 mm Hg diastolic blood pressure >85 mmHg
- Triglyceride >150 mg/dL (1.69 mmol/L)
- High-density lipoprotein cholesterol <40 mg/dL (1.04 mmol/L in male) <50 mg/dL (1.29 mmol/L in female).

This study did not include patients with a history of hematological, neoplastic, renal, hepatic, or thyroid diseases, or patients receiving treatment with anti-inflammatory drugs. Patients with acute or chronic infections and autoimmune disease were also excluded from the study.

A total number of one hundred patients (32 male and 68 female); 25 patients T1D; 50 patients T2D; and 25 healthy subjects were included in the study. The healthy subjects were recruited from the workers at the center for diabetes mellitus.

Demographic data, medical history, and treatment were collected in the center. Modifiable risk factors, events or complications, and current therapy were recorded. A person who reported smoking on admission was defined as current smoker. Anthropometric measurements including height (m), weight (kg), waist circumference (cm), and hip circumference (cm) were measured. Body mass index (BMI) (kg/m²), waist/hip ratio, and waist/height ratio were calculated. The blood pressure was manually measured (using Mercury Sphygmomanometer, P.M.S. Instrumental Ltd, Berkshire, UK) in the sitting position and the mean of three readings was taken. Peripheral venous blood was drawn immediately after admission into tubes, and then the samples were centrifuged (using Portable Centrifuge, Gallen Kamp, England) at 2500 rpm for 10 min, and the sera were separated for determination of fasting serum glucose (using Glucose Diagnostic Kit, Biocon, Greifswald, Germany); HbA1c (%) (using Labkit reagent, Human Kit Laboratories, Wiesbaden, Germany); and lipid profile using triglycerides diagnostic kit, (Biocon, Greifswald, Germany); and high-density lipoprotein using diagnostic kit (Biocon, Greifswald, Germany) as a routine laboratory test (using Ultraviolet-Visible Spectrophotometer, Cecil instruments, CE 7200 series, Aquarius, France) for patients follow-up.

Very low-density lipoprotein is equal to 1/5 triglycerides level and the low-density lipoprotein is calculated according to the Friedewald equation:

\[ \text{Low-density lipoprotein} = \text{total cholesterol} - (\text{high-density lipoprotein} + \text{very low-density lipoprotein}) \]

Stimulated saliva over 10 min was collected (using 25-mL falcon screw cap containers) from each patient, and the salivary flow rate and qualitative CRP (Fisher Health Care, Berkshire, UK) in the sitting position and the mean of three readings was taken. Peripheral venous blood was drawn immediately after admission into tubes, and then

RESULTS

Table 1 showed the characteristics of the study. The distribution of cases according to the gender showed the ratio of male: female was 2.1:1. T2D patients were characterized to have significant (P < 0.05) positive

Table 1: Characteristics of the study

|                         | Healthy subjects (n=25) | T1D patients (n=25) | T2D patients (n=50) |
|-------------------------|-------------------------|---------------------|---------------------|
| Gender (male:female)    | 7:18                    | 7:18                | 18:32               |
| Age (year)              | 37.12±5.622             | 33.16±3.77          | 50.86±5.066         |
| Smoking                 | 11 (44)                 | 11 (44)             | 40 (80)             |
| Family history of diabetes | 0 (16)                | 16 (32)             | 41 (82)             |
| Duration (year)         | 0                       | 6.04±1.287          | 6.55±1.551          |
| Blood pressure (mmHg)   |                         |                     |                     |
| Systolic                | 106.36±13.74            | 115.12±11.38†       | 138.38±13.67*       |
| Diastolic               | 71.88±5.21              | 73.24±5.847         | 87.28±6.63†         |
| Fasting serum glucose   | 98.64±6.075             | 345.72±51.22        | 188.28±7.301        |
| HbA1c%                  | 5.17±0.451              | 9.28±0.730          | 7.80±0.702‡         |

The results are expressed as means±SD and n (% ) of subjects and patients. * P<0.05, † P<0.02 in comparison with healthy subjects, ‡ P<0.001 in comparison with T1D patients. SD=Standard deviation, T1D=Type 1 diabetes, T2D=Type 2 diabetes, HbA1c= Hemoglobin A1c
family history of diabetes, and lower HbA1c% than corresponding T1D patients. Systolic and diastolic blood pressures were significantly ($P < 0.05$) higher among T2D patients than corresponding T1D patients, whereas systolic blood pressure of T1D was significantly ($P < 0.02$) higher than corresponding healthy subjects [Table 1]. The anthropometric measurements revealed significantly higher values of BMI ($P < 0.001$), waist and hip circumferences ($P < 0.001$) in T2D patients in comparison with T1D patients and [Table 2]. Waist ($P < 0.001$) and hip circumferences ($P < 0.01$) of T1D were significantly lesser than corresponding values of healthy subjects [Table 2]. Significant differences in lipid profile measures were found between T1D or T2D patients and healthy subjects [Table 3]. Fasting serum triglyceride to high-density lipoprotein which represented atherogenic index was significantly ($P < 0.02$) higher in T2D compared to T1D patients. Salivary flow rate was significantly ($P < 0.001$) reduced in diabetic patients compared to healthy subjects. It amounts to 0.217 ± 0.0465 ml/min (T2D patients), 0.313 ± 0.0506 ml/min (T1D patients), and 1.182 ± 0.161, 1.182 ± 0.161 ml/min (healthy subjects). The salivary flow rate of T2D patients was significantly ($P < 0.001$) less than T1D patients. Saliva CRP (≥6 mg/L) was detected in 9 out of 25 T1D (36%) and 28 out of 50 (56%) T2D patients, a difference reached to significant ($P < 0.01$) level. Salivary flow rate tended to be less in diabetic patients with metabolic syndrome and reached a significant level in T2D compared without metabolic syndrome [Table 4]. Further analysis revealed that significant association ($\chi^2 = 13.1, df = 1, P < 0.01$) between negative saliva CRP and metabolic syndrome in T1D patients without evidence of metabolic syndrome. In T2D patients, the saliva CRP pattern tended to be equally distributed in patients with and without evidence of metabolic syndrome [Table 4].

**DISCUSSION**

The results of this study show that saliva CRP is significantly detected in T2D with MetS and its detection is associated with significant hyposalivation. There is growing evidence that saliva can reflect virtually the entire spectrum of normal and disease states. Many studies have shown that saliva is a reliable substance for diagnosing levels of steroids, hormones, drugs, and antibodies. CRP is an independent risk factor for cardiovascular disease. Saliva CRP has exhibited highly significant diagnostic capability (with myoglobin and myeloperoxidase) for acute myocardial infarction, and it was demonstrated with high level in children with poor cardiorespiratory fitness. Significant elevations of 2.0–3.5-fold were observed with CRP in saliva in patients with hypertrophic cardiomyopathy treated with alcohol septal ablation indicated cardiac cell necrosis. There is no doubt that diabetic patients had complaints of hyposalivation, which is demonstrated in this study. Previous studies showed that the salivary pH level was significantly decreased and correlated with several MetS covariates and the number of MetS components. This study adds further information that salivary flow rate is significantly decreased in the presence of MetS covariates in both T1D and T2D. Detection of high saliva CRP level may indicate a low-grade inflammatory process that accompanies T2D and to a lesser extent T1D. Recent study demonstrates significant elevation of saliva uric acid in patients with metabolic syndrome, and it is significantly correlated with covariates of MetS as well as the number of cardiometabolic risk factors present. The results of this study do not agree with DeSantis et al.’s study which demonstrated that MetS was associated with lower rather than higher area under the

### Table 2: Anthropometric measurements

| Anthropometric measures | Healthy subjects (n=25) | T1D patients (n=25) | T2D patients (n=50) |
|-------------------------|------------------------|---------------------|---------------------|
| Weight (kg)             | 77.8±3.01              | 75.36±8.855         | 111.1±8.92*         |
| Height (m)              | 1.67±0.027             | 1.675±0.03          | 1.69±0.046          |
| Hip circumference (cm)  | 104.00±4.102           | 100.96±3.747        | 118.4±5.834*        |
| Body mass index (kg/m²) | 27.76±1.260            | 26.87±1.953         | 38.80±3.528*        |
| Waist/hip ratio         | 0.87±0.016             | 0.87±0.030          | 0.95±0.063          |
| Waist/height ratio      | 0.54±0.029             | 0.526±0.021         | 0.66±0.055          |

The results are expressed as mean±SD. *P<0.01 in comparison with T1D patients, †P<0.01 in comparison with healthy subjects. SD=Standard deviation, T1D=Type 1 diabetes, T2D=Type 2 diabetes

### Table 3: Fasting serum lipid profile

| Serum lipids                | Healthy subjects (n=25) | T1D patients (n=25) | T2D patients (n=50) |
|-----------------------------|------------------------|---------------------|---------------------|
| Triglyceride (mg/dL)        | 144.56±7.21            | 197.04±13.737*      | 209.64±44.934*      |
| Cholesterol (mg/dL)         | 170.72±3.995           | 209.52±15.943*      | 216.3±45.31*        |
| High-density lipoprotein (mg/dL) | 59.52±4.726           | 48.62±2.677†        | 47.36±7.774†        |
| Very low-density lipoprotein (mg/dL) | 28.912±1.44         | 39.408±2.747†       | 41.92±8.986†        |
| Low-density lipoprotein (mg/dL) | 82.288±6.444         | 121.512±17.025†     | 127.02±44.63†       |
| Triglycerides/HDL lipoprotein ratio | 2.441±0.204        | 4.07±0.410            | 4.65±1.626**       |

The results are expressed as mean±SD. †P<0.02 in comparison with T1D patients, †P<0.001 in comparison with healthy subjects. SD=Standard deviation, T1D=Type 1 diabetes, T2D=Type 2 diabetes

### Table 4: Saliva flow rate and C-reactive protein in diabetic patients with evidence of metabolic syndrome according to National Cholesterol Education Program criteria

| MetS (+ve) | MetS (−ve) | MetS (+ve) | MetS (−ve) |
|------------|------------|------------|------------|
| T1D patients (n=25) | T2D patients (n=50) |
| Salivary flow rate (mL/min) | 0.297±0.325±0.203±0.269± | 0.0397±0.0559±0.0397*0.0298 |
| C-reactive protein (≥6 mg/L) | 2°724 ||
curve of saliva CRP level. Further study also demonstrated that salivary lysozyme was significantly associated with non-diabetic MetS, whereas CRP was not significantly associated with MetS. The limitations of this study include small sample size, assessment of periodontal diseases, and determination of other bioinflammatory markers such as interleukins 1β (IL-1 beta). There is no doubt that chronic periodontal diseases and MetS are associated with high saliva CRP and IL-1β. He et al. demonstrated that the increased II-1β level in MetS patients was associated with increasing severity of periodontal disease and increasing component numbers of MetS.

**CONCLUSION**

We conclude that determination of saliva CRP could serve as a component of metabolic syndrome particularly in T2D and also could serve as a noninvasive biomarker in the assessment of hyposalivation and cardiovascular events.

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**Conflicts of interest**

There are no conflicts of interest.

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