STUDY COMPLEMENT ACTIVITY AND HUMORAL IMMUNE RESPONSE IN TYPE 2 DIABETES MELLITUS.

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ABSTRACT

Diabetes mellitus (DM) is a syndrome of metabolic disease characterized by hyperglycemia, resulting from deficiency of insulin secretion or insulin action or both, and results in abnormal metabolism of carbohydrate, protein, and fat. Because diabetic patients suffer from lingering infections, the aim of this study was to determine the functions of serum complement and its relationship with immunoglobulin profiles in patients with type 2 DM. A total of 57 patients and 30 healthy volunteers were included in this study with age range between 34 - 63 years among patients group and age range between 30 - 61 years among control group. The results of the study showed that the mean duration of disease for diabetic patients was found to be 10.25 year (±5.92). Serum IgG, IgM and IgA were measured by single radial immunodiffusion method, also complement components C3 and C4 were measured by single radial immunodiffusion method. In the type 2 DM patients, the results of immunoglobulin profiles IgG, IgM and IgA were found to be significantly lowered (p<0.05) in comparison with healthy control group whereas the levels of C3 and C4 were higher significantly decreased (p<0.001) in comparison with healthy control group. The results of this study indicate that serum complement and immunoglobulins activities was impaired in type 2 diabetic patients, so as result to decrease of immune response which might be a cause for delayed wound healing and repeated infections.

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INTRODUCTION

Diabetes Mellitus is a syndrome of metabolic disease of heterogeneous etiology, characterized by hyperglycemia which is due to deficiency of insulin effect, and results in abnormal metabolism of carbohydrate, protein and fat (Sperling and Jenson, 2000). There are two main types of diabetes, type I insulin dependent diabetes mellitus (IDDM), which is usually manifested in childhood or adolescence, and characterized by pancreatic β-cell destruction mediated by immune mechanisms, severe insulinopenia and dependence on exogenous insulin to preserve life, whereas type II non insulin dependent diabetes mellitus (NIDDM), is characterized by insulin resistance, with association and often progressive defect in insulin secretion, it occurs after age 40, but it may occur at any age ,other types of diabetes could be latent, gestational, or secondary due to other diseases (Barnett and Braunstein, 2001). On the other hand not all diabetic patients are at equal risk for complications, some variations are apparently related to the differences in the diabetic status of the patient, age at diagnosis, duration of diabetes, presence of organ complication ,HbA1c , complement components and immunoglobulins level may play role (Kuzuya etal., 2002).The immune system of the body consists of two major components which are B-lymphocytes and T-lymphocytes. The B-Lymphocytes are mainly derived from bone marrow cells in higher animals. The B-cells are responsible for the synthesis of circulating, humoral antibody; also known immunoglobulin , also there are five immunoglobulin classes which are designated IgM, IgG, IgA, IgE and IgD (Parslow,2001). An IgM is the first antibody to appear in the circulation after stimulation of B-Lymphocytes. An IgG is the classical gamma globulin, this antibody is major circulating antibody. IgG appear about 24-48 hours after antigenic stimulation and continues antigen antibody interaction begun by IgM . The complex of Bactria with IgG activate complement thereby chemolactically attracting polymorph nuclear
phagocytic cells. An IgA appears selectively in the sero-mucous secretion such as saliva, nasal fluid… etc. It clearly has the job of defending the exposed external surface of the body against attack by micro-organisms, this antibody provides resistance in the respiratory and gastro intestinal tract, possibly by inhibiting the attachment of parasites to the tissue (Roitt et al., 2001). The functions of the complement system include control of inflammatory reactions and chemotaxis, clearance of immune complexes, cellular activation and antimicrobial defense. In children, the deficiency of complement protein C3 has been found to result in overwhelming bacterial infections (Brozy et al., 1988). Because of repeated infection episodes, the functions of serum complement in killing intracellular bacteria could be impaired in the diabetic patients. In addition, the role of humoral immune response has not been extensively studied in diabetic patients. However, a study conducted in Nigeria had shown that humoral immunity provided by complement C3, C4 and IgM is more deranged in Type 2 diabetic patients compared with Type 1 (Akinlade et al., 2004). In view of the scarcity of data, this study was undertaken to evaluate humoral immune response with respect to complement activity and immunoglobulin profiles in patients with Type 2 diabetes mellitus.

MATERIALS AND METHODS

**Subjects**
A Cross sectional study was conducted on the following groups in the period between May 2010 to January 2011.

**Patient Study Group**
Fifty seven patients of diabetes mellitus (type 2) who attending the outpatient clinic in the National Diabetes Center, Al-Mustansiriya University for monthly clinical examination and laboratory investigation which
included certain hematological and biochemical tests. Their age range were between (34-63) years with (33) males and (24) females.

**Healthy Control Group**

Thirty apparently healthy volunteers from both sexes were included in this study as a control group. They were 16 males and 14 females. Samples were collected from those individuals only if they were not receiving any medication and did not had a history of a chronic or acute illness.

**Samples collection**

From each individual included in this study, 5 ml of blood was drawn by vein puncture using disposable syringes. The blood was placed in plastic disposable tubes, it was left to stand at room temperature (20-25°C) to allow it to clot, then the sera was separated by centrifugation for 5 minutes, and divided into aliquots (250 µl) and stored at -20°C till examination. Each aliquot of the serum was used once to avoid thawing and freezing. All sera and reagents were allowed to stand at room temperature before use in the test.

**Immunological markers**

**A- Single Radial Immunodiffusion (SRID)**

The method of Mancini *et al.* (1962) was employed for the measurement of immunoglobulin classes (IgG, IgM, IgA) and complement components (C3 and C4).

**Principle**

Equal volume of reference sera and test sample were added to wells in an agarose gel containing a mono specific antiserum. The sample diffuses radially through this gel and substance being assayed (antigen) form a precipitin ring with the mono specific antiserum. Ring diameters were measured and compared to a reference table.
Procedure

- The endoplate kit and reference sera were allowed to reach room temperature.
- The lid of the kit was removed and wells were inspected for any moisture, if moisture is present, the uncovered plate was allowed to remain at room temperature (approximately 15 minutes) until moisture had evaporated.
- Five μL of patient’s sera, controls and reference sera were dispensed into the appropriate wells.
- The plates were closed tightly and left on the bench at room temperature on a leveled surface.
- After 48 hours for IgG, IgA, C3 and C4 and 72 hours for IgM, the diameter of the precipitin rings were measured by suitable viewing device, and the final results were obtained from the reference table.

Statistical analysis

The usual statistical methods were used in order to assess and analyze our results and included:

Descriptive statistics: including
a. Mean (M).
b. Standard deviation (SD).
c. Statistical tables.

Inferential statistics: Data have been analyzed statistically using SPSS program version 10. Analysis of quantitative data was done using t-test and ANOVA (analysis of variance). Acceptable level of significance was considered to be below 0.05 (Sorlie, 1995).

RESULTS AND DISCUSSION

Patients with diabetes have an increased risk of infections, but information on their immune response is incomplete and contradictory. It has been suggested that chronic low-grade inflammation may be involved in the pathogenesis of
insulin resistance and type 2 diabetes. A total of 87 subjects were divided into two groups type II Diabetes mellitus patients (57) and Healthy control group (30) as is shown in tables (1 and 2). The age of diabetes patients in this study ranged from 34 - 63 years with mean of 49.9 years and age range between 30 - 61 years with mean of 43.07 years among control group, moreover the results of this study showed that the mean duration of disease for diabetic patients was found to be 10.25 year (±5.92). These results coincide with the previous study done in Brazil as Vozarova et al. (2002) who establish that 49 y was the mean age for patients having type 2 diabetes with duration of disease about 11.1 year, also other study was done by Lindsay etal. (2001) registered that the mean age for persons having of diabetes mellitus type 2 was 49.5 y. Both sexes can be affected by type 2 diabetes mellitus it can be seen in table (1) that 33 (57.8%) were males and 24 (42.2%) were females. According to Islam et al. (2000) study, 58 % of the patients with type 2 diabetes mellitus are males, mainly 30-50 years old, moreover, Bagust et al. (2002) reported that diabetes mellitus type 2 affect men 2 to 4 times more than women. Illness due to dysfunction of the endocrine gland can occur in both sexes but many more men than women are likely to develop diabetes problems at some time in their lives.
Table 1. Age and sex distribution of study groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Type 2 diabetes mellitus patients</th>
<th>Healthy control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sex</td>
<td>sex</td>
</tr>
<tr>
<td>Age (year)</td>
<td>Male, Female, Total</td>
<td>Age (year), Male, Female, Total</td>
</tr>
<tr>
<td>30-39</td>
<td>5, 3, 8</td>
<td>8, 5, 13</td>
</tr>
<tr>
<td>40-49</td>
<td>11, 12, 23</td>
<td>3, 6, 9</td>
</tr>
<tr>
<td>50-60+</td>
<td>17, 9, 26</td>
<td>5, 3, 8</td>
</tr>
<tr>
<td>Total</td>
<td>33, 24, 57</td>
<td>16, 14, 30</td>
</tr>
</tbody>
</table>

Complement system serves as an important effectors arm of both innate and acquired immunity. Complement acts in a wide variety of host defenses, inflammatory, homeostatic, and immune reactions. Serial measurements of C3 and C4 are useful in monitoring disease activity or treatment in patients with some forms of disorders (Benjamini et al., 2000). Mean serum complement components (C3 and C4) measured in this study was higher in healthy control group than the patients group, though the difference was statistically highly significant (p=0.001) as shown in tables (2 and 3). Pickup and Mattock (2003) reported a similar result in persons having diabetes type 2 for a period of less than 10 years. Complement promotes the clearance of immune complexes, an important way of eliminating antibody-coated bacteria. If, however, immune complexes cannot be eliminated, complement becomes chronically activated leading to increased consumption of the components (Walport, 2001). This might be the reason for lower level
of complement C3 and C4 that could affect the formation of membrane-attack complex and lower bactericidal activity. Hostetter (1990) pointed that the binding of glucose molecules with the biochemically active sites of the C3 component will prevent the protein molecule from attaching to the bacterial surface, thus interfering with the process of opsonization and increasing susceptibility to infection. Others (Pietruska et al., 1989) showed that reduced complement is due to consumption of those proteins as a result of microangiopathic complications and autoantibodies found especially in type I diabetes. Furthermore, The comparison between patients group and control group according to humoral immunity (immunoglobulins profiles) reviewed that significant difference (P<0.05) in most parameters (IgA, IgM and IgG). The level of IgA, IgM and IgG was lower in patients group than control group, as pointed out in tables (2 and 3). These results are in concurrence with study done by Recasens et al. (2005) who recorded an decreased level of IgA, IgM and IgG among patients group as compared to control group, also a study done by Akinlade et al. (2004) in Nigeria who demonstrated an decreased levels of IgA, IgM and IgG in patients with type 2 diabetes mellitus.

Serum immunoglobulin levels are dependent on a variety of condition such as genetic factor, chronic disease and environmental factors. These include ethnic back ground, age, sex, history of allergies or recurrent infections, and geographic factors (Al-Dabbagh et al., 1988). In addition to, the concentration of the immunoglobulins IgG, IgM, and IgA are reduced by 10-20% in the serum of diabetic patients (Jakelic et al., 1995). A possible explanation of reduced immunoglobulin level is reduced percentage of activated (CD38+) B-cells found in diabetic patients which may contribute to the reduced humoral immune response observed in those patients (Recasens et al., 2005). In conclusion, the altered levels of serum complement and
immunoglobulins might be responsible for depressed immune response in patients with type 2 diabetes.

**Table 2. Descriptive Statistics for Type 2 diabetes mellitus patients and Healthy control group.**

<table>
<thead>
<tr>
<th>parameter</th>
<th>Type 2 diabetes mellitus patients</th>
<th>Healthy control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Std. Dv</td>
<td>Mean</td>
</tr>
<tr>
<td>Age</td>
<td>7.461</td>
<td>49.98 year</td>
</tr>
<tr>
<td>C3</td>
<td>29.369</td>
<td>123.75 mg/dl</td>
</tr>
<tr>
<td>C4</td>
<td>20.227</td>
<td>44.72 Mg/dl</td>
</tr>
<tr>
<td>IgA</td>
<td>63.783</td>
<td>130.05 Mg/dl</td>
</tr>
<tr>
<td>IgM</td>
<td>70.593</td>
<td>91.25 Mg/dl</td>
</tr>
<tr>
<td>IgG</td>
<td>369.79</td>
<td>891.0 Mg/dl</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>5.920</td>
<td>10.25 year</td>
</tr>
</tbody>
</table>


Table 3. **t-test for Type 2 diabetes mellitus patients and Healthy control group.**

<table>
<thead>
<tr>
<th></th>
<th>Healthy control group</th>
<th>t-test</th>
<th>p-value</th>
<th>C.S</th>
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<tr>
<td>Type 2 diabetes patients</td>
<td>Age</td>
<td>3.656</td>
<td>P&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>5.061</td>
<td>P&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>4.122</td>
<td>P&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>2.238</td>
<td>P&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>2.065</td>
<td>P&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>2.277</td>
<td>P&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>Duration of disease</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

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count is associated with a worsening of insulin sensitivity and predicts
دراسة فعالية المتمم والاستجابة المناعية الخلطية في النوع الثاني من مرض السكر.

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الخلاصة

يعتبر مرض السكري متلازمة مرضية أضداد، والتي تتضمن ارتفاع نسبة السكر بالدم نتيجة لنقصان إفراز الأنسولين أو فعالية الأنسولين أو الانتهاك في إفراز السكريات، البروتينات والدهون. بسبب كون مرضى السكري يعانون من إصابات متعددة وطويلة، لذلك فان الدراسة هدفت لدراسة فعالية مكونات المتمم وعلاقتها بمجموعة الكليوبيلات المناعية في المرضى المصابين بالنوع الثاني من مرض السكري. أجريت هذه الدراسة على مجموعه 57 مريض مصاب بالنوع الثاني من مرض السكري و30 متطوع سليم والتي تم استخدامها كمجموعة السيطرة وكان معدل الأعمار بالنسبة لمجموعة المرضى يتراوح من 34 – 63 سنة وأما معدل الأعمار بالنسبة لمجموعة السيطرة فأنه يتراوح من 30-61 سنة. نتائج هذه الدراسة أظهرت بأن متوسط فترة الإصابة بالمرض كانت 10.25 سنة (±5.92).

تم استخدام طريقة الانتشار المناعي الإشعاعي الأحادي لقياس الكليوبيلات المناعية (ج، م، أ) وكذلك قياس مكونات المتمم (س3، س4) باستخدام طريقة الانتشار المناعي الإشعاعي الأحادي. فيما يتعلق بمرضى السكري النوع الثاني فان نتائج الكليوبيلات المناعية (ج، م، أ) وجدت انخفاضاً معنويًا (p<0.05) بالمقارنة مع مجموعة السيطرة السليمة، بينما أظهرت مستويات مكونات المتمم (س3، س4) انخفاضاً معنويًا عالياً (p<0.01) بالمقارنة مع مجموعة السيطرة السليمة، لذلك فان نتائج هذه الدراسة أكدت بأن فعالية المتمم والكليوبيلات المناعية كانت ضعيفة في المرضى المصابين بالنوع الثاني من مرض السكري وبالتالي انخفاض مستوى الاستجابة المناعية والتي من المحتمل أن تسبب في تأخر شفاء الجروح وحدوث الإصابات الأمراضية المتكررة.