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 redial immunodiffusion method , also complement components C3 and C4 were measured by single redial 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.

Received for publication Jan. 9 , 2011 .

Accepted for publication March. 23 , 2011 .

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 *etal.,* 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 *etal.,*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 *etal.,*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.*

Type 2 diabetes mellitus patients				Healthy control group			
parameter	sex			Parameter	sex		
Age (year)	Male	Female	Total	Age (year)	Male	Female	Total
(30-39)	5	3	8	(30-39)	8	5	13
(40-49)	11	12	23	(40-49)	3	6	9
(50-60+)	17	9	26	(50-60+)	5	3	8
Total	33	24	57	Total	16	14	30

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 *etal.,*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.*

Type 2 diabetes mellitus patients				Healthy control group		
parameter	No.	Mean	Std. Dv	No.	Mean	Std.Dv
Age	57	49.98 year	7.461	30	43.07 Year	6.736
C3	57	123.75 mg /dl	29.369	30	152.83 Mg/dl	13.847
C4	57	44.72 Mg/dl	20.227	30	56.77 Mg/dl	8.033
IgA	57	130.05 Mg/dl	63.783	30	178.73 Mg/dl	56.356
IgM	57	91.25 Mg/dl	70.593	30	135.80 Mg/dl	60.652
IgG	57	891.0 Mg/dl	369.79	30	1084.93 mg/dl	260.425
Duration of disease	57	10.25 year	5.920	30	_____	_____

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

Type 2 diabetes patients	Healthy control group	t-test	p-value	C.S
Age	Age	3.656	P<0.001	HS
C3	C3	5.061	P<0.001	HS
C4	C4	4.122	P<0.001	HS
IgA	IgA	2.238	P<0.05	S
IgM	IgM	2.065	P<0.05	S
IgG	IgG	2.277	P<0.05	S
Duration of disease	Duration of disease	----	----	------

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دراسة فعالية المتمم والاستجابة المناعية الخلطية في النوع الثاني من مرض السكر .

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الخلاصة

يعتبر مرض السكري متلازمة مرضية أيضية والتي تتصف بارتفاع نسبة السكر بالدم نتيجة لنقـصان إفراز الأنسولين أو فعالية الأنسولين أو الاثنان معا والتي ينتج عنها خلـل فـي ايـض الـسكريات , البروتينات والدهون.بسبب كون مرضى السكري يعانون من إصابات متعددة و طويلة ، لـذلك فـأن الدراسة هدفت لدراسة فعالية مكونات المتمم وعلاقتها بمجموعة الكلوبيلينات المناعية فـي المرضـى المصابين بالنوع الثاني من مرض السكري. أجريت هذه الدراسة على مامجموعه 57 مريض مصاب بالنوع الثاني من مرض السكري و30 متطوع سليم والتي تم استخدامها كمجموعة سيطرة وكان معدل الأعمار بالنسبة لمجموعة المرضى يتراوح من 34 – 63 سنة وأما معدل الأعمار بالنسبة لمجموعـة السيطرة فانه يتراوح من 30-61 سنة . نتائج هذه الدراسة أظهرت بان متوسط فترة الإصابة بالمرض كانت 10.25 سنة (± 5.92) .

تم استخدام طريقة الانتشار المناعي الإشعاعي الأحادي لقياس الغلوبيلينات المناعية (ج ، م ، أ) وكذلك قياس مكونات المتمم(س3وس4) باستخدام طريقة الانتشار المناعي الإشعاعي الأحادي . فيما يتعلـق بمرضى السكري النوع الثاني فان نتائج الغلوبيلينات المناعية (ج ، م ، أ) وجدت انخفاضـا معنويـا ($p < 0.05$) بالمقارنة مع مجموعة السيطرة السليمة وبينما أظهـرت مـستويات مكونـات المـتمم (س3وس4) انخفاضا معنويا عاليا ($p < 0.01$) بالمقارنة مع مجموعة السيطرة السليمة ، لذلك فـأن نتائج هذه الدراسة أكدت بان فعالية المتمم و الكلوبيلينات المناعية كانت ضعيفة في المرضى المصابين بالنوع الثاني من مرض السكري وبالتالي انخفاض مستوى الاستجابة المناعية والتي من المحتمـل أن تسبب في تأخر شفاء الجروح وحدوث الإصابات الأمراضية المتكررة.