قياس مستويات المتممات C4 وC3 والكلوبيولينات المناعية عند المرضى العراقيين المصابين بالسكري المعتمد على الأنسولين

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الخلاصة

المقدمة: يرتبط داء السكري بارتفاع نسبة الوفيات والأمراض التي تصيب الأجهزة الأخرى وتكثر فيه الإصابات بالالتهابات في مختلف أعضاء الجسم. لذلك فانه من المهم دراسة المكونات المناعية المهمة في مصل الدم لمعرفة أنماط تغيرها في هذا المرض اذ إن دراستها مازالت من المواضيع المهمة في الطب التي لم تدرس بشكل كاف و ليست مفهومة تماما. في هذا البحث تمت دراسة تراكيز المتممات المناعية C3وC4 والكلوبيولينات المناعية (IgA, IgM،IgG)   في مصول المرضى العراقيين المصابين بداء السكري المعتمد على الأسولين.

طريقة العمل: اشترك في هذه الدراسة21 شخصا مصابا بداء السكري المعتمد على الأنسولين واخذ 24 شخصا سليما من مرض السكر لغرض المقارنة. قدِّرت المتممات C4 وC3 والكلوبيولينات المناعية IgG, IgA, IgM باستخدام طريقة الانتشار المناعي لدى مرضى السكري المعتمد على الأنسولين ومقارنتها بمجموعة السيطرة.

النتائج: أظهرت النتائج ارتفاعا معنويا (p<0.05) في تركيز المتمم C3 وكذلك في تركيز IgA بينما لا يوجد اختلاف معنوي (p>0.05) في تركيز المتممC4 و تراكيز الكلوبيولينات المناعية IgG وIgM في مصول مرضى السكري مقارنة بمجموعة السيطرة.

الاستنتاج: إن أنماط التغيرات في تراكيز بعض المكونات المناعية لدى مرضى داء السكري من الممكن تفسيرها في ضوء التغيرات المناعية المحتملة بوصفها استجابة التهابية لدى مرضى داء السكري التي تعود أساسا إلى الارتفاع في نسبة السكر في الدم. إن الحاجة ماسة إلى دراسة شاملة لكل المكونات المناعية في مصول مرضى السكري للحصول على تفسير كامل لهذه النتائج.

مفاتيح الكلمات: مرض السكري المعتمد على الأنسولين؛ IDDM، المتممات،C3, C4   الكلوبيولينات المناعية، IgG, IgA, IgM.

Level of Serum Complements and Immunoglobulins in Iraqi Insulin Dependent Diabetes Mellitus

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Abstract

Background: Diabetes mellitus (DM) accompanied with an increase in the death rate and represents a significant public health challenge. It is the cause of other disorders and infection in many body organs. Hence, it is important to study the possible changes in the immunological components in the serum of diabetic patients which are not well understood. In this work, serum C3, C4, IgA, IgG, and IgM were estimated in the patients with insulin dependent diabetes mellitus (IDDM) and compared with healthy persons.

Patients and Methods: Twenty-one insulin dependent diabetic patients in addition to twenty-four healthy persons as control group were participated in this study. Serum C3, C4, IgA, IgG, and IgM were measured by using immunodiffusion plates.

Results: The results showed a significant increase ($p<0.05$) in serum C3 and IgA while there is no significant difference ($p>0.05$) in the concentration of the complement C4 and serum IgG and IgM in IDDM patients as compared with healthy control group.

Conclusion: The changes profile of some serum immunological components in IDDM can be explained in the means of the possible changes in immunity system as an inflammatory response in DM as a consequence of hyperglycemia. Comprehensive immunological study of all immunological changes in the IDDM patients is required for a complete explanation.

Keywords: Diabetes mellitus, IDDM, IgM، C4, C3 ،IgG, IgA, and hyperglycemia.

Introduction

Diabetes mellitus is one of the most common disorders in the world. It affects about 6% of the U.S. population [1], 1.5% of the United Kingdom population [2] and 4.5% of Canadians [3] Prevalence is increased with the decrease of the income category and educational attainment in both genders [4] Although in a study carried out in Bangladish, which is one of the poorest countries, the prevalence of type 2 diabetes in a rural population of Bangladesh was 4.3% [5].

Diabetes mellitus is a reason for many other important disorders such as vascular brain disorders [6], coronary disease [1], microvascular complications [7], Sensor neural hearing loss [8], and glaucoma [9] which were more common in patients with diabetes than in the control nondiabetic patients.

An estimated 29 million (14.4%) persons aged ≥20 years had either diagnosed diabetes, undiagnosed diabetes, or impaired fasting glucose; 29% of diabetes cases were undiagnosed. Persons can reduce their risk for diabetes through weight management and physical activity [10].

In diabetes mellitus, the increase in blood sugar contributes to a weakened defense against bacterial infection [11] and oxidative stress seems primarily due to both increased plasma free radical concentrations and a sharp reduction in antioxidant defense [12] Among the causes of

enhanced free radical production are hyperglycemia and hypoinsulinemia that are the main feature of diabetes [13].

Immunological disturbances were recorded in many other disorders. The circulating complexes that consist of the complements and the immunoglobulins A, G and M found to change in many disorders although it is not specific [14].

The immune system can be divided into two branches; the innate and the adaptive immunity. The innate immunity provides the first line of host defense with macrophages, granulocytes and dendritic cells as central player. The adaptive, in which B and T lymphocytes play a crucial role, is a second line of defense when the innate immunity is not able to clear the infection. This branch has a delayed onset, but it is highly specific and has memory. The memory enables us to mount a more efficient secondary response if we are exposed to the pathogen a second time [15].

Immune–mediated (type 1) diabetes (IMD) is an incurable disease that is increasing in incidence throughout the western world [16]. Several studies have reported a viral etiology associated with IMD [16,17] of which congenital rubella is clinically established. Immune responses against a determinant shared by host cells and a viral protein could cause a tissue-specific immune response by generation of cross-reactive, cytotoxic effectors lymphocytes or antibodies that recognize self-proteins located on the target

β-cells [16]. Macrophages may also be involved in the pathogenesis of IMD early on because inactivation of macrophages results in the near complete prevention of diabetes in NOD mice as well as BB rats[18]. This could be because T cells in a macrophage-depleted environment lose their ability to differentiate into β-cell reactive cytotoxic T cells[18]. The determination of immunoglobulins and complements in any disease points the role of the humoral mechanism on pathogenesis and granuloma formation[19].

Genetic disorders can not be excluded in the explanation of the changes in the complement concentration in IDDM. Genetic deficiencies of complement proteins are clearly associated with high levels of Circulation immune complexes and immunecomplex–mediated C4 and C3 are usually accompanied by systemic lupus erythematosis or lupus-like diseases[20]. Unlike other complement factors, C4 is encoded at two polymorphic gene loci, C4A and C4B, and null or unexpressed alleles are common[21]. An increased prevalence of null alleles of C4 was reported in a variety of diseases as diverse as insulin-dependent diabetes mellitus, rheumatoid arthritis and systemic lupus erythematosis[22]. Individuals who are heterozygous for C4A*Q0 or C4B*Q0 also have an increased risk of developing such autoimmune diseases as well as immune complex–mediated.

The presence of antibodies to glycosylated albumin was found in normal and diabetic subjects, but higher titers were significantly more prevalent in the diabetic patients with vascular complications. These antibodies may represent the result of immune tolerance breakdown or, alternatively, be natural antibodies. Although their function remains to be established, their raised prevalence in Type 1 diabetes may be relevant to diabetic microvascular disease and the possible increase in the antibody titer in patients of the present studied is to be studied in this work[23].

A previous report suggested that natural killer (NK) T cell clones developed from patients with type 1 diabetes secreted IFN(Interferone)- but not IL-4(Interleukine-4)[24], while NK T cell clones from their identical twins and siblings who were discordant for diabetes secreted IL-4 [24], while treatments with rIL-4 or rIL-10(Interleukine receptor) have shown that they would protect them from diabetes[25]. Defective apoptosis of activated T cells were reported in animal models of diabetes[26]. T regulatory (Treg) cells are involved in almost all experimental animal models of autoimmunity, and natural killer (NK) T cells and resting CD4+CD25+ T cells have emerged as important immunoregulatory T cell subsets. Importantly, reconstitution of animal models by populations of Treg cells has shown to prevent the development of autoimmunity[26]. Besides NK T cells, CD4+CD25+ T cells can

prevent the development of autoimmune diseases such as thyroditis[27], gastritis[28], and diabetes[29] when transferred into experimental animal models.

Kukreja1 *et al* (2002) findings suggested that there is an underlying global defect in T cells in IMD leading to immune deficiencies affecting immunoregulation[30]. Others have suggested a global T cell defect in the disease, too[31]. Low T cell IL-2 production was reported in IMD patients that appeared to be related to marked β-cell destruction[32]. Another study found IL-2 and soluble IL-2 receptor secretion defects in both newly diagnosed and long-standing diabetic patients[33].

Hence, it is important to study the possible changes in the immunological components in the serum of diabetic patients which are not well understood. In this work, serum C3, C4, IgA, IgG, and IgM were estimated in the patients with insulin dependent diabetes mellitus (IDDM) and compared with healthy persons.

Materials and Methods

Twenty-one insulin dependent diabetic patients in addition to twenty-four healthy persons as control group were participated in this study. These cases were collected from Al-Hussein hospital at Karbala city. The healthy persons were volunteers coming as relevant to patients and other persons are the staff of the hospital. All patients were examined by a senior doctor, and there are no other disorders recorded. Venous blood samples were collected from patients before taking any medications. Sera were separated and stored at (-20° C) until analysis.

After placing 5 ml of serum on each cavity on plates IgG, IgA and IgM and C3 and C4, levels of both groups were quantitatively studied with immunodiffusion plates (Biomaghreb). Serum samples were incubated on plates for 72 hours at room temperature. At the end of this period the diameter of precipitation was measured and converted to mg/dl units. Normal values of the plates used are as follows:

IgG: (710-1520mg/dl), IgA: (90-310mg/dl), IgM: (40-250mg/dl), *C3*: (84-193mg/dl), *C4*: (20-40mg/dl). The student's t assessed comparisons between groups.

Results and Discussion

The results of serum immunoglobulins in diabetics and healthy individuals are introduced in Figure (1) and Table (1). These results showed an increase ($p < 0.05$) in serum IgA and no significant difference ($p > 0.05$) in the concentration of IgG and IgM in diabetics as compared with healthy group. Figure (2) and Table (1) showed that there is an increase ($p < 0.05$) in serum C3 in diabetics as compared with healthy group. C4 level showed no difference ($p > 0.05$) in both groups.

The result of this research indicates different profiles of changes in immune system components in diabetes mellitus. T cells may be responsible for the increased immunoglobulin response through causing B cell growth factor mediation. Determining the IgG-A-M and the complement fragments in any disorder may prove the presence of immunological reactions. The major function of IgA is to prevent pathogens from adhering to epithelia and penetrating the underlying tissues. Therefore the increase in IgA noticed in the diabetic patients in this work may be due to the increase in the demand to IgA to protect the tissue of diabetics from infection by different types of pathogens. Antibodies that are required for the formation of immune complexes may explain our observation of the increased levels of IgA. These results are in accordance with the result of other researches studies of IgA concentration in diabetics with different complications[33a]. They concluded that the increase in IgA concentration in diabetics indicate that high serum IgA level is a sign of the existence

of IgA nephropathy superimposed on diabetes mellitus. Therefore, the patients of our sample should be monitored for diabetic nephropathy at different intervals.

In a study carried out on Nigerian diabetics[33b], different results were noticed; circulating immune complexes were significantly higher in Type 1 diabetic subjects while there is no significant change in IgA concentration as compared with the controls, whereas C3c, C4 and IgM were significantly increased in Type 2 diabetic subjects compared with the controls. These differences may be due to the sample size, diet habits, age ranges in addition to other factors that affect the results of any research carried out in different areas. Insulin dependent diabetes mellitus (IDDM) is an autoimmune disease associated with the presence of different types of autoantibodies. The presence of these antibodies and the corresponding antigens in the circulation lead to the formation of circulating immune complexes (CIC). CIC are known to persist in the blood for long periods of time. Such CIC following deposition in the small blood vessels have the potential to lead to microangiopathy with debilitating clinical consequences[34].

Patients with type 2 diabetes[35] were reported to have impaired cell-mediated immune responses, explaining the increased incidence of infections in these patients that leads to the increase in different types of antibodies and complements. The metabolic glucose disturbance is the probable explanation[36,37].

The complement proteins are always present in plasma but must be activated by pathogens to exert their effects. Two pathways present for the activation process; the classical and alternate pathways. Both pathways converge on a step where complement C3 is split into two fragments C3a and C3b. Complement helps destroy pathogens in three ways: enhanced inflammation by stimulating mast cells and basophils to secrete inflammatory chemicals, by coating bacteria surface and make bacteria easier to phagocytize (opsonization), and by cytolysis by triggers the insertion of a group of proteins called the membrane attack complex[38]. Hence the increase in serum concentration of C3 in diabetic patients may be due to the increase in the susceptibility of diabetic patients to be infected by different types of pathogens that require an increase in the C3 concentration as noticed in Figure (2) and Table (1).

The results of this work are not in accordance with the results of other research carried out in Poland. In that work, they found that the mean level of IgA was lower and IgM and IgG higher in both types of diabetic patients. But the low level of IgG was observed in 11% patients with insulin dependent diabetes. It was not asignificant difference in the concentrations of complement components proteins in both diabetic groups and control subjects. Insulin dependent diabetic patients with microangiopathic complications had lower level of serum C4, but this difference was not statistically significant[39].

Other important explanations came from the study of serum IgA and IgA-class circulating immune complexes (IgA-CIC) elevation in patients with non-insulin-dependent diabetes mellitus (NIDDM) carried by Eguchi *et al* (1995). They have postulated that the elevations of serum IgA and IgA-CIC were based on subclinical infection of the mucosa and/or deterioration of IgA clearance in patients with   NIDDM[40].

Once the macrophages get activated, they mixed histocompatibility antigens on the surface release interleukin1, which is a T cell activator[41]. These informations that affect immunity may affect the complement and immunoglobulin concentration of diabetic patients that are found in our study. Lately, another gene on chromosome 6 could alter immunological responsiveness in general, such as via the inducible polymorphism of the immunoglobulin constant region (Fc) receptors and the associated cytotoxic T-lymphocyte adhesion ligand (CTLA-4)[42]. In addition, it was postulated that the increased susceptibility of islet cells to the induction of apoptosis by cytotoxic T cells may also be responsible for the facilitated death of islet β-cells[43]. The pathogenesis of IMD has been extensively studied, but the exact mechanism involved in the initiation and progression of β-cell destruction is still unclear[44]. The presentation of β-cell-specific autoantigens by antigen-presenting cells (APC)

[macrophages or dendritic cells (DC)] to CD4+ helper T cells in association with MHC class II molecules is considered to be the first step in the initiation of the disease process[44].

In an interesting work carried previously [45], the researchers noticed that there are deposition of different immunoglobulins and complement C3 in the skin of diabetic patients. The type of deposits found occasionally to be related to the diabetes complications. From the present work, the researchers concluded that humorally mediated immunological processes are active in IDDM. However, the exact role of this activity remains to be defined [46].

The other important factor, but less understood, is the effect of the changes in serum metal ions on the activity of immune system. Many researchers noticed a deficiency in serum zinc in diabetic patients which is an important factor in immune system [46,47] .Zinc is an essential metal for maintaining the integrity of immune system [48]. The Increase in blood sugar can suppress the immune system [49]. The underlying requirement of zinc in maintaining immunocompetence requires further study, but may be a result of its requirement in many enzyme systems, or its ability to stabilize biologic membrane [48]. Several laboratories have found that zinc deficiency depresses antibodies responses possibly owing to a loss of T-helper–cell function [49]. Zinc deficiency affects the biological activity of thymus hormones and has a major effect on cell mediated immunity perhaps as a result [50]. Hence, the decrease in zinc may one possible cause for the attenuation of immunity.

Zinc deficiency in diabetics found in other research and associated with a low concentration of zinc in lymphocytes, granulocytes and platelets and also associated with high total body clearance [51,52].

This work advise to determine the serum immunoglobulin and complement levels with other immune and biochemical parameters in checking the activity and follow up of diabetic patients. These results suggest that testing in a larger trial with greater power is needed.

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Table (1): Serum immunoglobulins and complements C3, C4 in diabetic patients as compared with healthy controls.

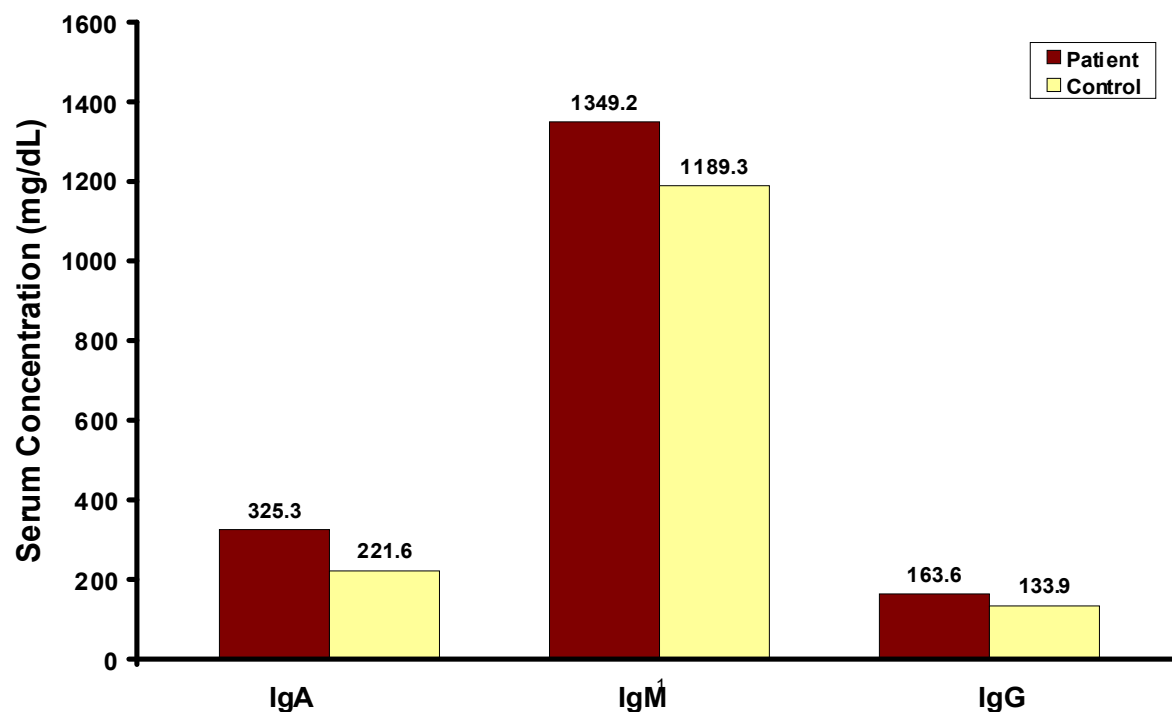| Serum Parameter	Serum Concentration (Mg/dL) (Mean±Standard Deviation)	
	Patients	Control
C3	138.7 ± 41.5	86.4 ±33.7
C4	20.8 ± 10.4	27.5±12.9
IgA	325.3 ± 99.7	221.6±65.2
IgG	1349.2 ± 587.0	1189.3 ±783.4
IgM	163.6 ± 58.2	133.6 ±72.2

Fig. (1) Serum immunoglobulins in diabetic patients as compared with healthy controls.
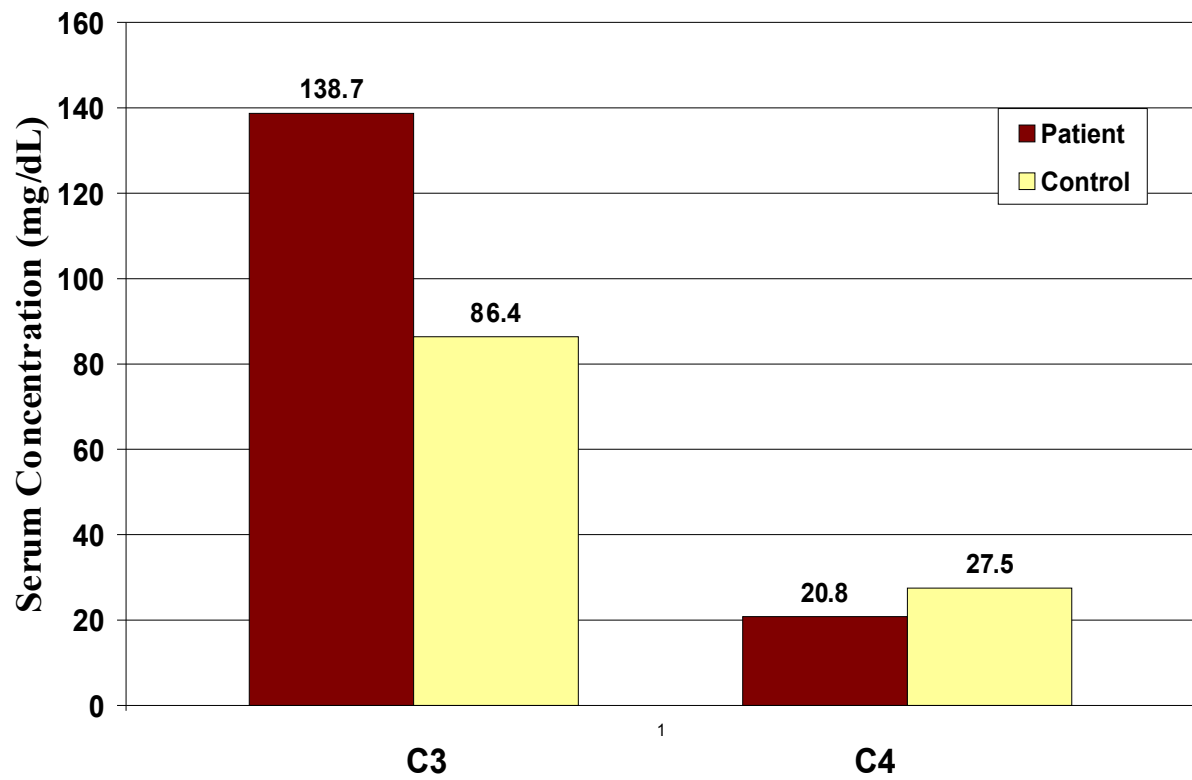
Fig. (2) Serum C3, C4 in diabetic patients as compared with healthy controls.