



IMMUNOLOGICAL STUDY OF CYTOKINE AND IMMUNOGLOBULIN A IN PATIENTS WITH DIABETES 4 MELLITUS, IRAQ

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ABSTRACT

Diabetes Mellitus type 2 is a heterogeneous group of disorders characterized by tissue-wide insulin resistance and varies widely. Type 2 diabetes is by far the most common, affecting 90 to 95% of the diabetes population. Methods, in this study included a 50 Healthy controls, and total of (100) diabetic type(2) patients as diagnosed by Diabetologist with an age range (25-65 years) who attended to hospitals in Babylon (AL Qasem and Marjan), during the period from January 2015 until June 2015. Result, From the results it was shown of Immunological parameters (IgA, IL4, and IL6) for DM patients were study. In this study the levels of IgA, IL4, and IL6 for DM patients were decreased. In light of the results documented in this study, one can conclude the following; The levels of Immunological parameters of IgA, IL4, and IL6 for DM patients were decreased.

Keywords: Heterogeneous group, Immunological parameters, Autoimmune destruction.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action or both [1]. The World Health Organization recognizes two main forms of diabetes mellitus type I and type II. Type I is usually due to autoimmune destruction of the pancreatic beta cells (β -cells) which produce insulin. Type II is a heterogeneous group of disorders characterized by tissue-wide insulin resistance and varies widely [2]. Type 2 diabetes is by far the most common, affecting 90 to 95% of the U.S. diabetes population.

Changes in human behavior and lifestyle over the last century had resulted in a dramatic increase in the incidence of diabetes worldwide [3]. It is interesting that the proportion of diabetes is higher in women than in men. Common symptoms of diabetes are lethargy from marked hyperglycemia, polyuria, polydipsia, weight loss, blurred vision and susceptibility to certain infections.

Chronic hyperglycemia causes long-term damage, dysfunction and failures of various cells, tissues and organs. Diabetic subjects probably have a higher risk of many infections. In addition, immunosuppression which occurs in those patients, because of increased sugar levels in the blood stream and as a result of dysfunction of the immune system make diabetic patients more incident for microbial infections [4]. Good metabolic control is a major factor in limiting the development and spread of infections and, most importantly, the development of diabetic complications which predispose to infections. Several factors could predispose diabetic patients to infections. These factors include: genetic susceptibility to infection; altered cellular and humoral immune defense mechanisms; local factors include poor blood supply and nerve damage, and alterations in metabolism associated with diabetes [5].

Type of Diabetes Mellitus (D.M)

There is several classification systems established for diabetes mellitus by the WHO Expert Committee on Diabetes (1980, 1985). The current WHO classification system has been established in co-operation with the National Diabetes Data Group (USA). It is mainly based on the pathogenesis responsible for hyperglycemia unlike the earlier classification which were based on the age of the individual (Table 1).

Symptoms and Risk Factors of Diabetes Mellitus

Type 2 diabetes is characterized by elevated fasting blood glucose levels secondary to insufficient insulin action [6]. Other signs and symptoms of diabetes onset may include weight loss, fatigue, frequent urination, blurred vision, increased thirst or hunger, and slow-healing wounds or sores. Patients may even be asymptomatic. Some individuals can develop type 2 diabetes as much as 9 to 12 years before diagnosis. To complicate matters, there can be contrary risk factors for the various forms of the disease. For example, autoimmune diabetes (type 1 and latent autoimmune diabetes of adulthood, LADA) is more common in white people, but metabolic diabetes (type II and gestational diabetes) is more common in people of other races and ethnicities. Type 1 is usually diagnosed in children, but advancing age is a risk factor for type 2 and gestational diabetes. Insulin resistance, prediabetes and metabolic syndrome are strong risk factors for type 2 diabetes. Other diabetic risk factors and causes include [7].

Genetics and family history: Certain genes are known to cause maturity-onset diabetes of the young (MODY) and Wolfram syndrome. Genes also contribute to other forms of diabetes, including types 1 and 2. History of gestational diabetes or delivery of an infant weighing >4.5 kilo. Other disease: Hypertension, hyperlipidemia, Polycystic ovarian syndrome and metabolic syndrome, asthma and sleep apnea have been linked to type 2 diabetes. Impaired fasting glucose tolerance or impaired fasting glucose.

Obesity: Overweight and obesity are leading factors in type 2 diabetes and gestational diabetes. Excess fat, especially around the abdomen (central obesity), promotes insulin resistance and metabolic syndrome. Family medical history is also influential to varying degrees: For example, a person whose parents both have type 1 diabetes has a 10 to 25% chance of developing that disease, according to the American Diabetes Association, and someone whose parents both have type 2 diabetes has a 50% chance of developing that disease.

Race: Black, Hispanic, Native American, some Asian Americans, and native Hawaiian and other Pacific Islanders. Medical treatments: In addition to hormonal therapies, medications including diuretics, beta blockers (another class of antihypertensives), immunosuppressives, antiretrovirals (AIDS/HIV drugs) antipsychotics, lithium, and some antidepressants, anticonvulsants and

chemotherapy drugs have been linked to an increased risk of secondary diabetes. Other environmental factors: Some researchers theorize that free radicals may contribute to the development of type 1 and possibly other forms of diabetes.

Viruses: Some people are diagnosed with type 1 diabetes after a viral infection. Viruses thought to be related to type 1 diabetes.

Smoking: Cigarette smoking is a risk factor for type 2 diabetes and possibly other forms of diabetes.

Alcohol: Excessive use of alcohol is a risk factor for diabetes. For example, it can cause pancreatitis. However, some research has found that light drinking may decrease the risk of becoming diabetic.

Hormones: These chemical messengers can contribute to diabetes in various ways. For example, stress hormones such as cortisol have been linked to fluctuating glucose levels in type 2 diabetes, and stress hormones in women during pregnancy have been linked to risk of type 1 diabetes in the child.

Level of physical activity: Lack of regular exercise is blamed for much of the twin global epidemics of obesity and diabetes.

Diet: The effect of diet in the development of diabetes is controversial.

Other chemicals: In addition to these pharmaceuticals, some studies have linked PCBs, other pollutants and certain pesticides including the defoliant Agent Orange and dioxin (its active ingredient) to insulin resistance and type 2 diabetes. Sedentary lifestyle. Age older than 40 years.

Complications of D.M

There are many complications occurs in diabetic patients include diabetic ketoacidosis (DKA), hypoglycemia which is the most frequent complications in type I diabetes [8], microvascular complications, macro-vascular complications, foot infection and the most important complications that include immunodeficiency and bacterial infections.

Diagnosis

The diagnostic criteria for determining diabetes have recently been changed in order to increase the sensitivity of the test. Currently, diabetes is diagnosed by a fasting glucose of 126 mg/dl or a random glucose of 200 mg/dl [9].

Impaired fasting glucose (IFG) is defined as a blood sugar of 100-125 mg/dl (5.6-6.9 mmol/l). Impaired glucose tolerance (IGT) is defined as an abnormal 2-hour postprandial blood sugar of 144-199 mg/dl. IFG and IGT comprise the category now known as 'prediabetes' [9].

These relatively 'new' criteria differ from the previous diagnostic criteria established in 1985 by the World Health Organization (WHO) [10].

The WHO criterion recommended diagnosis of diabetes with a single random blood sugar greater than 200 mg/dl (11.1 mmol/l) and the use of a 75 g oral glucose tolerance test (OGTT) to diagnose those in "the uncertain range" (blood sugars 140-199 mg/dl). It is important to note that children with diabetes usually present with acute signs and symptoms, including coma or loss of consciousness, critical glucose levels, ketonemia, and marked glucosuria and ketonuria [11].

Immune system

The study of the defense mechanisms of the body against foreign substances including microorganisms that enter into the body constitutes the subject immunology. The study of infectious disease and the body's response to the infections led to the development of immunology. Immunity is due to the existence of a complex defense system in the human body [12].

Immunoglobulins

Immunoglobulins (Ig) are glycoprotein molecules that are produced by plasma cells (terminally differentiated B cells) in response to an immunogen exposure and which function as antibodies; the terms antibody and immunoglobulin are often used interchangeably. Although, the term antibody refers to the specific activity of immunoglobulin. All these antibodies are immunoglobulins, but each one has its own specific activity. An immunoglobulin is a large Y-shaped protein that recognizes a unique part of the foreign target, termed an antigen. Each tip of the "Y" of an antibody contains a paratope (a structure analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision. The production of antibodies is the main function of the humoral immune response (both acquired and adaptive) [13].

Immunoglobulin A (IgA) is the 2nd most common serum Ig. It forms 10-15% of total serum immunoglobulin. When it exits as a dimer, a J chain is associated with it. IgA is found in secretions is also has another protein associated with it called the secretory piece. Unlike the remainder of the IgA which is made in the plasma cell, the secretory piece is made in epithelial cells and is added to the IgA as it passes into the secretions. The secretory piece helps IgA to be transported across mucosa and also protects it from degradation in the secretions. IgA is the major class of Ig in secretions - tears, saliva, colostrums, mucus. Since it is found in secretions secretory IgA (SIgA) is important in local (mucosal) immunity, it is responsible for defense against local infections on mucosal surfaces and acts as a protective coating for the mucous surfaces against microbial adherence or initial colonization. IgA can also neutralize toxin activity on mucosal surfaces and it not

fix complement, unless aggregated. Also it can binding to some cells - PMN's and some lymphocytes. Secretory IgA is also transferred in milk, via the colostrums, from a nursing mother to an infant. This provides passive immunity to many pathogens, especially those that enter by way of the GI tract [13].

Cytokines

Many critical interactions among cells of the immune system are controlled by soluble mediators, which are diverse group of intercellular signaling peptides and glycoproteins with MW (6000-60000)D and concentration 10⁻⁹ - 10⁻¹⁵ M.

These mediators have a generic name "cytokines", those produce by lymphocytes are also called lymphokines, those produce by monocytes/macrophages are also called monokines. Interleukins (IL) are cytokines that regulate the interaction among lymphocytes. Chemokines are a family of cytokines that play an important role in the attraction of inflammatory cells, and also regulate the adhesion, degranulation, development of immune cells, and the genesis of lymphoid organs.

Cytokines represent "the hormones of immune system". They act by binding to specific surface receptors on target cells. Unlike endocrine hormones, they are not produced by specialized glands and secreted into the circulation, but rather are produced locally by a variety of cells and tissues. Also the IL-4 source TH2 cells, mast cells and main functions IgE expression, MHC II expression, eosinophil growth, while IL-6 source TH2 cells, APCs main functions: induce release of acute phase proteins [12].

The interleukin 4 (IL4) is a cytokine that induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. Upon activation by IL-4, Th2 cells subsequently produce additional IL-4 in a positive feedback loop. The cell that initially produces IL-4, thus inducing Th0 differentiation, has not been identified, but recent studies suggest that basophils may be the effector cell. It is closely related and has functions similar to Interleukin [13].

IL-6 is a proinflammatory cytokine produced by many different cell types, including immune cells, skeletal muscles and adipose tissue.

In opposition to IL-6 is a predominantly circulating molecule with relevant systemic effects on peripheral tissues such as skeletal muscle, liver and endothelium. IL-6 is secreted from activated macrophages and lymphocytes, but in non-acute inflammatory conditions, its major source may be adipose tissue. TNF α is a strong inducer of IL-6-release from adipocytes. IL-6 levels in serum correlate positively with body mass index (BMI) and hyperinsulinemia, as well as with insulin sensitivity [14].

Contracting (not resting) skeletal muscle fibers release IL-6 in large amounts into the circulation, and

more so when intramuscular glycogen stores are low [15]. Exercise leads to a gradual increase in IL-6 expression in human adipose tissue, possibly increasing lipolysis by an autocrine signaling mechanism.

IL-6 seems to play a role in general lipid metabolism. In humans, the action of IL-6 is associated with increased plasma free fatty acids (FFAs). These associations seem important because elevated IL-6 levels and C-reactive protein, which is a surrogate marker for IL-6 activity. Raised levels of IL-6 are seen in obesity [16]. Visceral adipose tissue released two to three times more IL-6 than subcutaneous adipose taken from severely obese, non-diabetic patients.

In fact, IL-6 concentration decreases in parallel with both weight loss and improved insulin sensitivity in patients undergoing bariatric surgery [17]. A human polymorphism in the IL-6 gene, which causes a decrease in circulating IL-6 levels, is associated with increased insulin sensitivity.

Serum IL-6 levels have also been found to be associated with insulin resistance and diabetes. In nondiabetic older populations [18] and healthy, middle-aged, white populations, higher serum IL-6 levels correlated with increased insulin resistance. In individuals with impaired glucose tolerance, type 2 diabetes, or the metabolic syndrome, serum IL-6 levels were also found to be higher compared with those with normal glucose tolerance or those who did not meet the criteria for the metabolic syndrome [19] recently reported that elevated IL-6 levels are also associated with an increased risk of clinical diabetes in a large prospective study of postmenopausal women who participated in the WHI (Women's Health Initiative) in the United States.

Immunodeficiency

One of the most complications of D.M is the immunodeficiency which result in greatly increased susceptibility to infections. Immunodeficiency is defect in the development and function of immune system result in an increased susceptibility to infections of certain infectious diseases. The ability of the immune system to respond to pathogens is diminished in age extremes with immune responses beginning to decline at around 50 years of age [20]. Immunodeficiency degree affected by the causes and their affect on the immune system like cancer disease which characterized with neutropenia with defect in humoral response characterized by decreasing levels of immunoglobulins in serum of these patients. In developed countries obesity, alcoholism and illegal drug abuse are common causes of poor immune functions. In diabetic patients, hyperglycemia impairs the immune system and also leads to the accelerated creation and accumulation of advanced glycation end-products (AGEs) that interact with monocytes and macrophages promoting the expression of more pro-inflammatory phenotypes. Both old age and D.M are known to reduce immunity and

increase the risk of infections indicating the importance of special attention to these high risk group.

In humans [21] studied the expression profile of IL-6 and IL-4 in blood of D.M and found that both to be significantly reduced as compared with normal controls. An alteration in the immune responses to the pathogenic agents occurs in immunocompromized patients express a great degree of decrease in opsonization as a result of decrease the levels of specific antibodies, in addition to alteration of surface receptors of fragment constant (Fc) and third complement component (C3) that found on the surface of the phagocytes and this alternate the phagocytosis process and killing of microorganisms.

Regarding diabetic patients, succinate produced by both aerobic and anaerobic bacteria inhibits the chemotaxis of phagocytosis making the probability of infection five times greater than in non-diabetic patients [22]. It was pointed that glycation induced alteration of T-cell immune competence. This provides a biochemical basis for the well recognized association between poorly controlled diabetes and bacterial infections. The hyperglycemic patients had been reported to highly susceptible to microbial infections, in addition binding of glucose to the biochemical active sites of complement components (C3) that inhibits the attachment of this protein to the microbial surface and there by impairs opsonization and phagocytosis functions.

Many proinflammatory cytokines play a central role in inflammatory reaction and were shown to increase the risk of T2DM [23]. These pro-inflammatory cytokines can enhance insulin resistance directly in adipocytes, muscle and hepatic cells, leading to systemic disruption of insulin sensitivity and impaired glucose homeostasis.

It has been reported that normal individuals with detectable levels of IL-1 and elevated levels of IL-6 had an independently increased risk to develop T2DM, whereas those with increased concentrations of IL-6 but undetectable levels of IL-1 had no significantly increased risk [24]. Another study showed that levels of IL-6, TNF-, and TNF-receptor were elevated in insulin-treated, but not in sulfonylurea-treated patients. Moreover, levels of serum glucose, pro-inflammatory cytokines (IL-6, IL-12, and TNF), endothelial dysfunction markers (vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and nitric oxide), and lipid abnormality were highest in T2DM with cardiovascular complications. Recently, it has been shown that T2DM patients had a significantly higher CD14 (+) CD16 (+) fluorescence intensity, TLR4 expression, and serum IL-6 and C-reactive protein (CRP) levels, compared to normal controls [25].

MATERIALS AND METHODS

Methods

Patients

This study included a total of (100) diabetic type (2) patients as diagnosed by clinical physicians with an age range (25-65 years) who attended (AL Qasem and Marjan) hospital in Babylon province during the period from January 2015 until June 2015. The patients complete the questionnaire (Index-1). Diabetic patients were no malignancy, no asthma, no heart failure. In addition, the patients who were admitted in the hospital.

Healthy (controls)

A total of (50) volunteers no diabetes (no prolonged illness and not taking antibiotics) collected as control and the same age which took the patients.

Samples collection

Blood samples

Blood samples were collected from each patient and control (Three milliliters of venous blood) and withdrawn by a disposable syringe under aseptic technique. The three milliliter were out in a sterilized pollen tube and allowed to clot, then serum was separated

by centrifugation at (300) gm for (15) minutes. The serum was stored by freezing until used for immunological tests.

Immunological materials

ELISA Test for estimation of human IL-4 concentrations and IL-6 concentrations :

The kit components detailed in the table (2-2):

1. Wash Buffer:

Twenty ml of Concentrated Wash Buffer of the kit was diluted into (500) ml of Wash Buffer with deionized or distilled water.

2. Standard:

The standard (stock tube) of the kit was Centrifuged at (10,000) g for (1) minute, and reconstituted (1.0) ml of Reference Standard & Sample diluent, and it has been left stand for (10) minutes and turned it upside down for several times, also it has been mixed thoroughly with a pipette. This prepared standard considered as a stock solution (500ng/ml). After this serial dilutions prepared from stock solution as the following:

Standard	1	2	3	4	5	6	7	8
Concentration mg/ml	500	250	125	62.5	31.63	15.63	7.81	0

3. Biotinylated Detection Ab:

Concentrated biotinylated Ab of the kit was centrifuged, and then diluted as (1:100) by of biotinylated Ab diluent of kit. The required amount was (10) ml for (100µl/well) of ELISA plate.

4. HPR Conjugate:

A concentrated HPR conjugate of kit has been diluted as (1:100) by HPR conjugate diluents . The required amount was (10) ml.

B) Assay procedure:

1. All reagents and samples have been brought to room temperature.
2. The samples were centrifuged again after thawing before pipetting.
3. All the reagents were mixed thoroughly by gently swirling before pipetting.
4. (100)µl of each standard and sample was added to appropriate wells.
5. After fist adding, the plate was covered by providing sealer in the kit, and incubate for (90) min at (37)°C.
6. Remove the liquid of each well, and added (100) µl of the prepared Biotinylated Ab to each well. Incubated for (60) min at (37)°C.
7. Washed manually by adding (350) µl of prepared washing buffer into each well, repeated (3) times. The plate was then invited and patted against thick clean absorbent paper.

8. (100) µl of prepared HPR Conjugate was added to each well, covered with a sealer and incubated for (30) min at (37)°C.

9. Washed (5) times with washing buffer as that of step (7).

10. Ninety µl of substrate solution in kit has been added to each well and incubated for (15) minutes at (37)°C avoiding light.

11. Stop solution was added (50) µl/well, the color turned to yellow immediately.

12. The Absorbance was read at (450) nm in microplatereader.

Estimation of IgA Level by Single Radial Immunodiffusion (SRID) test :

Principle: The procedure consists in an immune precipitation in agarose between an antigen and its homologous antibody. It is performed by incorporating one of the two immune reactants (usually antibody) uniformly throughout a layer of agarose gel, and then introducing the other reactants (usually antigen) into wells duly punched in the gel. Antigen diffuses radially out of the well into the surrounding gel-antibody mixture, and a visible ring of precipitation forms. Ring diameters are measured by viewing device (ocular). Unknown concentration is determined from the tables supplemented with each type of plate [26].

Procedure

A- Endo plates and the serum of (patients and controls) were removed from the refrigerator. Reagent were equilibrated to room temperature.

B- Plate was removed from ziplock bag. After lid removed, the wells were inspected for moisture if moisture was present, uncovered plates were allowed to remain at room temperature until moisture evaporated. Sera of patients were thoroughly shaken by inversion pipette (5) μ L of the serum into the appropriate well, and putting wet cotton in the plate center.

C- Plates were incubated at room temperature for 48 hrs. To IgA.

D- End point of diffusion was indicated by a sharp precipitation ring.

E- Diameter of ring was measured with (0.1)mm precision by suitable device and then compared to the standard diameter to conclude the concentrations of serum immunoglobulin (26).

Statistical Analysis

The mean, standard deviation(SD), and analysis of variance (ANOVA) test were calculated [27] for statistical analysis of the results of this study.

RESULTS AND DISCUSSION

Immunological Parameters

Immunoglobulin A levels (Ig A) levels

The mean values of Ig A in D.M type II and control were (482.4 , 533.5 mg/dL) respectively as in table (3-1). The Ig A levels were significantly ($p \leq 0.05$) in DM in comparison to control. These results are in concurrence with study done by [28] who recorded an decreased level of IgA among patients group as compared to control group, also a study done by [29] in Nigeria whodemonstrated an decreased levels of IgA in patients with type 2 diabetes mellitus .

The comparison between patients group and control group according to humoral immunity (immunoglobulins profiles) reviewed that significant difference in most parameters (IgA).The level of IgA was lower in patients group than control group. These results are in concurrence with study done by [28] who recorded an decreased level of IgA , among patients group as compared to control group, also a study done by [29] in Nigeria who demonstrated an decreased levels of IgA in patients with T2DM.

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 [30]. In addition to, the concentration of the immunoglobulins IgA are reduced by 10-20% in the serum of diabetic patients [31]. 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 [28]. In conclusion, the altered levels of serum complement and immunoglobulins might be responsible for depressed immune response in patients with T2DM.

Interleukin 6 (IL-6) levels

The mean values of IL-6 in D.M type II and control were (240.4 , 377.8 pg/ml) respectively as in table (3-2). The results were decreased no significantly ($p > 0.05$) in D.M in comparison to control.The current results are in relative consistence with previous studies [32] who mentioned the decreased production of IL-6 in human T-cells in diabetic patients.

And do not agree with previous studies [33] and [34] have mentioned that IL-6 was elevated in type 2 diabetes.

Interleukin 4 (IL-4) levels

The mean values of IL-4 in D.M type II and control were (564.66 , 756.6 pg/ml) respectively as in table (3-3). The IL-4 levels were decreased significantly ($p \leq 0.05$) in D.M in comparison to control. These results agreed with [35] who mentioned that there was significant reduction in the levels of IL-4 among diabetic patients as compared with controls. And [36], also mentioned the decreased production of IL-4 in human T-cells in diabetic patients. In addition, the levels of IL-4 in blood of D.M patients showed a reduced values [21].

Our data showed decreased levels of IL-6 in T2DM compared to healthy controls. Decreased levels of inflammatory cytokines in our study were in disagreement with previous findings. A recent study by [37] found that subjects with T2DM had increased levels of IL-6while no association was found with IL-1 [37]. Moreover, it was reported that, high levels of inflammatory cytokines appear in early stage of T2DM and capable of predicting the development of type 2 diabetes through diminishing insulin sensitivity [38]. This discrepancy can be attributed to the (I) duration of the diseases; the majority of patients included in our study have a long disease duration (greater than 5 years), (II) small sample size, and (III) the differences in age and sex of the studied groups; the age of the normal controls was lower than that of T2DM patients. These factors may have played essential roles in the cytokine production among these two study groups.

Concerning, the level of IL-4 concentration in diabetic patients was (564.66 pg/ml),and in non- diabetic was (756.6 pg/mlpg/ml) and these results indicate to the levels of IL-4 were decreased significantly ($p \leq 0.05$) in diabetic patients when compared with non diabetic. IL-4 has a differentiation activity leading to the production of immunoglobulin in addition to act as growth factor of B-cells. It is produced by Th2 type of CD⁺T-lymphocytes, following activation by antigen binding to the T-cell receptor and also produce by activated mast cells and basophiles [39]. IL-4 down regulates the production of IFN- γ by Th1 CD4T-lymphocytes, on the B-cells, IL-4

has a growth factor activity mediated via the production of soluble CD23. On monocytes, IL-4 induces an increased number of major histocompatibility complex (MHC) class II antigens. By its pleiotropic activity, IL-4

is a key cytokine in the cytokine network that shows anti-inflammatory properties and is probably involved in mechanisms of allergy by production of IgE [40].

Table 1. Classification of Diabetes Mellitus.

<p>1-Type 1 Diabetes (β cell destruction often leading to absolute insulin deficiency)</p> <ul style="list-style-type: none"> • Immune-mediated (1A DM) • Idiopathic (1BDM) <p>2-Type 2 Diabetes</p> <ul style="list-style-type: none"> • Insulin resistance + relative insulin deficiency • Insulin secretory defect + insulin resistance <p>3-Other specific types of diabetes</p> <ul style="list-style-type: none"> • Genetic defects of (β cell function characterized by mutations in: • Genetic defects in insulin action: • Disease of the exocrine pancreas • Endocrine disorders • Drug induced • Infections • Uncommon forms of immune-mediated diabetes • (Other genetic syndromes sometimes associated with diabetes <p>4-Gestational diabetes Mellitus(GDM).</p>
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Table 2. Mean values of IgA concentration in diabetic patients and control

Testing group	Mean mg/dL
Diabetes mellitus type II	482.4
Controls	533.5

Table 3. Mean values of IL-6 concentration in diabetic patients and control

Testing group	Mean pg/ml
Diabetes mellitus type II	240.4
Controls	377.8

Table 4. Mean values of IL-4 concentration in diabetic patients and control

Testing group	Mean pg/ml
Diabetes mellitus type II	564.66
Controls	756.6

CONCLUSIONS

In light of the results documented in this study, one can conclude. The levels of Immunological Parameters of C3, IgA, IL4, and IL6 for DM patients were decreased, whereas the level of C4 was increased.

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