

Certain Immunological Marker (Mannose Binding Lectin and Interleukin 1-Alpha) in Diabetic Patients in Wasit province

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Abstract

Diabetes mellitus, or diabetes, is a group of disorders described by elevation of blood glucose that come from significance defects in the production and/or usage of insulin ability of patient's body. Diabetes and altered conditions are considered a collection of metabolic syndromes. This study was conducted on 82 samples divided into two groups: Diabetic patients (50) and (35) healthy control. This study included white blood cell level as well as neutrophils ratio. Besides that, HbA1C was estimated for all samples. Biochemical serological test includes random blood sugar test and C - reactive protein. Immunological test including (MBL, IL 1-alpha) was done using ELISA technique. In the current study, Diabetes Patients show significant increase in both family history of DM and BMI. Levels of HbA1C show increase beside RBS who shows also increase in DM patients. White blood cells show highly significant increase, while neutrophils show moderately significant increase in DM patients. Immunological parameter including (IL1-alpha and MBL) shows a significant increase in DM patient as compared with healthy control. We conclude in this study, that the level of MBL and IL1-a increased, as well as increases in WBC count and neutrophils in DM patient combined with the elevation of HbA1C level and RBS as well as BMI value in Wasit diabetic patients, this might be the explanation of the progression in diabetes patients complications.

Keywords: diabetes, mannose/mannan binding lectin, Interleukin 1-alpha, BMI.

1. Introduction

Diabetes mellitus, or basically diabetes, is a group of disorders described by the high blood glucose which come from a consequence of defects in the production and/or usage of insulin ability of patient's body. Diabetes and altered conditions are considered as a collection of metabolic syndrome (1)). Diabetes mellitus has been increased predominant over the latest decades, where the expectation of DM occurrence is increased to 592 million by 2035 (2). Type 1 and type 2 of DM are generally characterized by chronic inflammation. Both diseases implicate in pancreatic islet inflammation, whereas systemic low-grade inflammation is a feature of fatness and type 2 diabetes. Long-term initiation of the innate immune system harms insulin secretion process and action; besides that, inflammation also participate in macro-vascular and micro-vascular complications of DM (3). The complement system can be referred to as a group of plasma proteins called complement proteins, which are created mainly in the liver, and acted in a group to help destroy pathogens (4). On other hand, the complement system implicated in causing and severity of various disorders when this

system disturbed or various related factor as well as in excessive trigger; Complement has the ability to attack host cells causing tissue damage by inflammatory disorders (5). Mannose binding lectin is a soluble pattern-recognition molecule, which can trigger the lectin pathway after tying to the oligosaccharides pattern on the surface of pathogen (6).

2. Materials and Methods

A total 50 specimens of blood samples have been collected from patients with diabetes hospitalized at Al-Karama teaching hospital as well as Al Zahraa teaching hospital in the governorate of Wasit/Iraq, from September 2019 to February 2020. The patients' ages were ranged between 22 and 82 years old. Thirty-two specimens were collected as a healthy control group; those specimens are without a history of DM or other chronic disease or any immune disease. Five milliliter of the venous blood were acquired through vein puncture from each one of the subjected (controls and cases) included in this work, the blood has been almost equally divided into (2.5ml) EDTA tubes, also (3ml) in the Gel tube (the samples of blood have been collected into EDTA

tubes and Gel tubes were prepared and then labeled). (2.5 ml) blood sample obtained from each one was clot at for a period of 30mins at 25 Celsius, after that, the centrifugation has been conducted for a period of 10 minutes, at (4000) rpm for the purpose of separating the serum. The serum was transferred by micropipette into 2 or 3 Eppendorf tubes, used to determine the following Immunological and biochemical markers (MBL, IL 1-alpha, Glucose). The sera were stored at -70 °C until the assay was done. Blood in EDTA tubes has been utilized for performing complete blood counting including WBC and Neutrophils, with the use of (Cell-DYN Ruby Hematology Abbott / USA) as well as in measuring HbA1C level. Biochemical analysis of Glucose level, by Reflotron (Roche company/Germany). HbA1C Determination by The PocketChem™ Alc (Arkray company/Japan). Immunological analyses MBL and

IL-1 α has been done through ELISA technique by kits manufactured by Shanghai (China).

3. Results and Discussion

A total 82 clinical specimens were collected in the current study, which include control samples (32), and DM patient sample (50). The Diabetes mellitus patients showed a significant ($P \leq 0.0001$) increase in BMI in comparison with controls (29.9 ± 3.83 vs. 4.56 ± 0.38 kg/m²), also there is a significant ($P \leq 0.003$) increase in patient with family history of DM in comparison with those haven't any family history with this disease, the control group with positive family history of DM should be followed up because they more likely to have DM in future due to their family history with this disease, as in table (1).

Table 1: General parameters in healthy controls and diabetes mellitus patient.

Gender, family history and BMI.		Cases (n=50)	Controls (n=32)	Sig.*
Family history of DM	Yes	32 (64%)	13 (40.6%)	0.003
	No	18 (36%)	19 (60.4%)	
BMI*** (mean) kg/m ²		$29.9 \pm 3.83^{**}$	4.56 ± 0.38	0.0001

* Chi-square for nominal variables and Independent T-test for numerical variables, **Mann-Whitney U Test, ***Body mass index

Concerning family history, our study was agreed with study in Sweden to Iraqi immigrant by (7) who shows that there is high prevalence of prediabetes and diabetes (95%) in patient of first-degree relatives with diabetes and the prevalence of DM in Iraqi immigrant in Sweden. As well as agreed with study in Korea by (8) who confirm the importance of family history of diabetes in evaluating diabetes. In addition, about BMI our data was agreed with study in USA by (9).

The biochemical parameter including random blood sugar (RBS) in DM patient and healthy control

respectively is (245.0 vs. 86.0), it shows highly significant increase in DM patient, the glycosylated hemoglobin A1C (8.35 vs. 4.5) showed a highly significant ($P \leq 0.0001$) increases in diabetic patients as compared with controls. While the hematological parameter including White blood cells (WBC) in DM patient and healthy control respectively is (9.39 vs. 6.64), also showed a highly significant ($P \leq 0.001$) increase in diabetic patient as compared with control. Besides that, neutrophils level in DM patient and control respectively (59.36 vs. 54.85) showed a moderately significant ($P \leq 0.53$) increase in DM patient compared with healthy group as in table (2).

Table 2: Biochemical and Hematological parameters in diabetic patients and healthy control.

Glucose value, WBC and neutrophil		Cases (n=50)	Controls (n=32)	Sig.*
Random blood sugar (mean) mg/dl \pm SD		245 ± 115.56	86 ± 11.05	0.0001
HbA1C % \pm SD		8.35 ± 2.12	4.5 ± 0.38	0.0001
WBC $\times 10^9/l \pm$ SD		9.39 ± 2.98	6.64 ± 1.27	0.0001
Neutrophil %		59.36 ± 11.36	54.85 ± 7.74	0.053

A high level of HbA1c (7 – 8 %) was observed in more than half of Diabetic cases, while 30.0% of them had a very high level of HbA1c ($\geq 9\%$). Patients with diabetes tend to have leukocytosis with high Blood sugar (RBS). An elevated leukocyte count accompanied by other markers, chronic inflammation that can be shown by this factor could be related to pathogenesis and the progression of these diabetes-related complications. This result agreed with study in USA by (10), also with a study done in other four countries by (11) all of them confirm the significant relation of HbA1C in DM patient as compared with healthy individual.

The result in table 2 showed a moderately significant ($P \leq 0.053$) increase in Neutrophil count in DM patient compared with healthy control. Our study agreed with study in Turkey by (12) who divided

diabetic patients into two groups according to their HbA1C values being lower or higher than 7 and reported that increased Neutrophils was associated with increased HbA1c. Similarly, agreed with study in China by (13) who has been suggested that a high level of neutrophils is a good prognostic marker of early-stage diabetic nephropathy. This study reveals the presence of a low-grade systemic infection process in patients with both types of diabetes compared to the healthy population.

The level of serum MBL demonstrated a significant ($P \leq 0.036$) increase with stactical mean rank in DM patient compared with healthy controls (45.91 vs. 34.61) (table 3). Serum level of IL1-alpha also show significant ($P \leq 0.005$) increase with stactical mean rank in DM patient in comprition with healthy control (47.39 vs. 32.3) (table 3).

Table 3: Mean and mean rank differences for Mannose-binding lectin (MBL) and IL1-alpha (cases and controls).

Indicators ▼	Cases (n=50)	Controls (n=32)	Sig.
MBL ng/ml, mean ± SD (mean rank) *	235 ± 320 (45.91)	46 ± 37 (34.61)	0.036
IL1-alpha pg/ml, mean ± SD (mean rank)	5.1 ± 6.6 (47.39)	1.09 ± 0.6 (32.30)	0.005

* Mann-Whitney U test. **Independent T-test.

The current study agreed with (14) which conclude that diabetes patients have greater levels of MBL than their siblings do. In addition, the high MBL levels was related with the great levels of glucose related to pro-inflammatory cytokines. Beside that (15) suggest that the high levels of MBL, might affect in the excessive constituent triggering and leading adaptive immune response. Study by (16) also conclude from his result on DM patients that the high expression of MBL may be associated with a significantly increased risk of vascular complications in diabetes. Thus, MBL detection in diabetes is an effective and viable method to expect vascular complications of DM patients. Our study also agreed with (17) which conclude that the higher MBL levels, resulting an increase in complement activation, which could support the pathognomonic for early stages of DM.

High level of MBL may indicate the progression of DM patient complication, so that MBL level should inhibited to reduce any serious complication (18). This elevation in MBL that lead to activate lectin pathway came from the presence of mannose subunit. Mannose found in some microbe's cell wall as a short mannose oligosaccharides, plants and fruits such as oranges, apple flesh, peaches, coffee ground, Cranberries, mango and Egg albumin (19).

As in table (3) Interleukin1-alpha (pro-inflammatory marker), shows a significant increase by at least double in compression with healthy control. This result agreed with (20) who see that IL-1 α is high in DM patient compared with healthy persons and conclude that the correlation was observed in the pro-inflammatory cytokine (IL-1 α) is important in the pathogenesis of DM. a study by (21) also noticed that the levels of IL-1 is higher s in DM as compared to controls. Another study by (22) who conclude that the high circulating levels of IL-1 α were highly significant and may be exploited as potential markers for development of T1DM in diabetic patients compared to healthy controls, but their levels are not related to disease duration and body mass index.

In conclusion, this study reveals that the level of MBL and IL1-a increased, as well as increases in WBC count and neutrophils in DM patient combined with the elevation of HbA1C level and RBS as well as BMI value in Iraqi diabetic patients, this might be the explanation of the progression in diabetes patients' complications.

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