

Investigation on Phagocytosis Index & Humoral Immunity in Patients of Type 1 Diabetes in Thi-Qar Province

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Abstract:

The current study was conducted at the Center for Diabetes and Endocrinology of the Health Directorate in Thi- Qar province, during the period from October 2013 to May 2014. The study aimed and evaluate immune status of diabetes type I patients by measuring the levels of immunoglobulins (IgM, IgG, IgA) by single radial immune diffusion (SRID) and the study included test phagocytic cells on phagocytosis (coefficient of phagocytosis) and measurement hematological parameters (red blood cells count , hemoglobin, packed cell volume and count the total and differential white blood cells) . The study included a total of 100 patients with type I diabetes (51 males) and (49 females) and there aged between 1-40 years. Levels were measured in serum immunoglobulins for 20 a sample of patients with type I diabetes, also examined the levels of immunoglobulins in the serum of 10 samples from healthy people in the control group. The results of the statistical analysis showed high significant increase ($P \leq 0.001$) in the immunoglobulins in serum IgM,, IgG IgA in patients with type I diabetes compared with healthy control group . Decrease coefficient phagocytosis was significantly ($P \leq 0.001$) in all patients with diabetes mellitus type I compared a group of control, either with respect to the hematological parameters have explained the results of the current study, the lack of significant differences ($P \leq 0.05$) in the rates of the values of the RBCs and Hb and PCV in all patients diabetes type I, compared with the control group, also increased the rate of counting the total of WBCs and count differential cell lymphocytes, eosinophil , basophil, and monocyte ($P \leq 0.001$) in patients with diabetes mellitus type I compared with healthy people in the control group .

Key words : T1DM , IgG, IgM, IgA, Phagocytosis, lymphocytes, WBCs count , PCV

Introduction:

Diabetes mellitus type 1 (also known as type 1 diabetes or T1DM ,formerly insulin dependent diabetes mellitus (IDDM) , or juvenile diabetes ^[1,2] . T1DM is a chronic systemic metabolic disorders characterized by increased levels of glucose in the blood (hyperglycemia) and abnormalities in the metabolism of protein ^[3] , that results from the autoimmune destruction of insulin-

producing pancreatic beta cells ^[4,5] , by autoreactive T-lymphocyte ^[6] , and that leading deficiency of insulin ^[7] , and this disease was once thought to be mediated exclusively by CD4_ T cells and is now recognized as one in which autoreactive CD8_ T cells play a fundamental pathogenic role ^[8] . The etiology of T1D is complex and involves both genetic and environmental factors which play important roles ^[9,10,11] . A permissive

genetic background is required for the development of the islet autoimmune process generating antibodies (Ab) against insulin auto antibodies (IAA), glutamic acid decarboxylase isoform 65 (GADA65-Ab), and protein tyrosine phosphatase (IA2)^[12,13].

The incidence of T1DM is reported to be increasing by 3-5% per year, and the number of people with diabetes is estimated to reach 380 million by 2025^[14]. The incidence rate of childhood T1DM is increasing dramatically in many countries over the past 20 years^[15]. The classical symptoms of type 1 diabetes include polyuria (frequent urination), polydipsia (increased thirst), Xerostomia (dry mouth), polyphagia (increased hunger), fatigue, and weight loss^[16,17].

The diabetic complications are divided into microvascular (nephropathy, retinopathy, and neuropathy) and macrovascular (cardiovascular) disease. Once considered a part of type 1 diabetes itself, the observation that similar complications also arise in patients with secondary diabetes led to the view that complications are caused by the hyperglycaemic milieu. The blood glucose values that define diabetes itself are set by the risk of diabetic microvascular complications^[18,19].

Immunoglobulins (Ig) :

The immune system generates billions of different antibody molecules by mature B cells which are capable of secreting antibodies and expressing B cell receptors on their cell surfaces^[20,21]. Ig antibodies are large proteins composed of four polypeptide chains (two identical heavy chains and two identical light

chains) joined together by disulphide bonds. Each Ig recognizes a specific antigen unique to its target and is used by the immune system to locate and destroy invading microorganisms^[22]. Serum immunoglobulin levels provide key information on the humoral immune status^[23,24].

Immunoglobulin M (IgM) :

It is the first isotype to be generated during a primary immune response and it predominates in immune response to most antigens. Its pentameric structure is a highly effective activator of complement. IgM is the first immunoglobulin class to be synthesized by the neonate. IgM is larger than IgG with a molecular mass of approximately 950 kDa that makes up about 8% of the antibody in the serum^[25,26].

Immunoglobulin G (IgG) :

Immunoglobulin G is a major effector molecule of the humoral immune response in man, accounts for about 80% of the total immunoglobulins in plasma of healthy individuals. The IgG (150 kD) is composed of two light chains and two heavy chains (g). The four polypeptide chains are covalently held together by disulfide bonds. It's the major antibody in the blood. Human IgG consists of four subclasses (isotypes), which are numbered in order of their serum concentrations (IgG1, IgG2, IgG3, and IgG4). IgG express predominant activity during a secondary antibody response. IgG antibodies have a relatively high affinity and persist in the circulation for a long time^[26,27].

Immunoglobulin A (IgA) :

The human body produces more IgA daily than any other antibody isotype. IgA has an approximate molecular mass of 160 kDa and, after IgG, is the second most prevalent antibody in the bloodstream. It constitutes about 13% of the antibody in human serum, but it is the predominant class of antibody in extravascular secretions. IgA is the main effector of the mucosal immune system and provides an important first line of defense against most pathogens that invade the body at a mucosal surface^[28]. IgA is found in most external secretions that bathe mucosal surfaces, for example, saliva, tears, mucus and breast milk, and is principally dimeric (i.e. composed of two monomeric IgA subunits and J chain) The total area of these mucosal surfaces is vast and they are vulnerable to exposure to pathogens^[29].

Phagocytosis :

The process of phagocytosis involves the internalization of large particles ($\geq 0.5\mu\text{m}$), phagocytosis is limited to specific Phagocytic cells such as monocytes, macrophage and neutrophils. These cells are vital to both the innate and adaptive immune systems. The innate functions of these molecules (specifically, the internalization and digestion of pathogens bound to receptor on the cell surface) represent the first line of defense against invading microorganisms. In the adaptive response, B cell produce antigen-specific antibodies lead to the opsonization of the pathogen^[27].

Materials & methods :

study design :

This study was performed on (100) Iraqi patients with T1DM patients, who

attended the consultant clinic for Type 1 diabetes mellitus in endocrine and diabetic center in Al-Nasiriya city in the period from beginning October 2013 to end May 2014. This study included too (30) person apparently healthy individuals as a control group, who have no history or clinical evidence of T1DM or any other chronic disease, and no obvious abnormalities.

Blood Samples Collection:

Blood samples were collected by venipuncture from 100 patients and 30 controls (five milliliters of venous blood) were drawn by disposable syringe under aseptic technique. were placed in a sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at -10 C° . These sera (20 T1DM patients and 10 controls) were used for estimating the concentration of Immunoglobulin's (Ig G, Ig M, Ig A).

Methods :

Determination of serum levels of IgA, IgM, and IgG, by single radial immune diffusion (SRID) plate

A-Principle

Kit of (Immunoglobulins IgA, IgG, and IgM) provided by CUSABIO company. The total serum level of immunoglobulins (IgM, IgG, IgA) was determined by means of Single Radial Immune diffusion Assay. It is a single radial immune diffusion test, which was developed by Mancini *et al.*,^[30] for quantitative determination of proteins in the serum. Test sample is added to a well in an agarose gel containing a monospecific antiserum. The sample

diffuses radially through the gel and the substance being assayed forms a precipitation ring with the monospecific antiserum. Ring diameter is measured and the concentration is determined from the reference standard curve.

B-Procedure

Before starting the assay, the plates were opened and left for 5 minutes at room temperature (18-25 °C), and then 5 ml of serum was dispensed into well in the plate. The plate was incubated in flat position at room temperature to 48 hours for (IgG, IgA) and to 72 hours for (IgM). The ring diameter was measured by an ocular and the concentration was obtained the reference curve.

Hank s Balanced salt solution (HBSS)

Prepare this solution according to [31] that contains calcium ions Ca ++ and Mg Mg ++ dissolving substances in 1000 ml of distilled water and adjust the pH to (PH 7.2) and divided to the volumes are equal and then sterilized By autoclave then save in temperature (4 °C) for use when.

Killed Yeast Suspension

was prepared for the purpose of studying the process of Phagocytosis and as the following step :-

- 1- Dry bread *Saccharomyces cervisia* yeast was used .
- 2- 10 grams of the killed yeast was suspended in 150 ml of normal saline .
- 3- The suspension was placed in a boiling water bath for an hour and then it lifted until get cold then filtered via a dual-layer sterile gauze.
- 4- The stuck suspension was divided into many test tubes (5 mL) , stored in (-

20 C °) until used , and The use melted when stuck in a water bath (37 m °) and washing twice before use by using a normal, saline [32] .

Wright' s stain :

The testing equipment (kit) was used for Wright 's stain which consist of fixative solution and Eosine Stain and solution of methylene blue which is produced by syrbio company form republic of Arabia Syria .

Phagocytosis Procedure :

The procedure carried out according Met-Calf *et al.*, [33] as follow: 0.025 ml of the collected blood was put in plane tube , then added for it 0.05 ml from Killed yeast suspension which prepared by soluble 10 grams of *Saccharomyces ceversiae* yeast made in Turkish pakamaya company in 150 milliliters of normal saline and put suspension in water bath for 60 minutes , then this suspension was filtered after it's cooling .

0.025 ml of HBSS were added to the mixture and incubated at 37 C ° for 30 minutes . One drop of the mixture was placed on a slid and smeared, then left to dry , fixed by methyle alcohol (99%) for min and stained for 20 min with Wright stain . then , examined under oil immersion .

No. of phagocytic cells

Phagocytosis index = $\frac{\text{No. of phagocytic cells}}{\text{Total number of cells}} \times 100$

Total number of cells

Hematological assay :

The hematological tests that included (differential WBCs counts) were done by using Genux Auto Hematology Analyzer

in which the results read and printed automatically .

Statistical analysis : The analysis of data were expressed as mean \pm SD. The comparisons between each T1DM patients group with matched healthy control were performed with T-test by using computerized Minitab 14 program. $P < 0.01$ was considered to be the least limit of significance, the statistical analysis were done by using Pentium-4 computer through the (SPSS program) Statistical Package For Social Sciences (version-20).

Results:

RBCs , Hb ,PCV count :

Did not show the results of the current study in table (1) , no significant differences ($p \leq 0.05$) in blood pictures of patients and healthy control group. The red blood cells (RBCs) count , hemoglobin

concentration (Hb) and packed cell volume (PCV) percentage in T1DM compared with the healthy control group, where the RBCs mean were $(4.92) \times 10^6$ cell/ml for patients and $(4.83) \times 10^6$ cell/ml for healthy control group respectively , while the hemoglobin concentration were (13.58) g /dl for patients and (13.07) g /dl for healthy control group respectively . The PCV percentage was $(40.04)\%$ for patients and $(40.86)\%$ for healthy control group respectively .

Phagocytic index :

The results of the current study showed high significant difference ($p \leq 0.001$) in the rate of phagocytosis, as it decreased the rate of phagocytosis in Type I diabetes patients to (30.67) compared to the healthy controls group, and that the rate of phagocytosis (46.98) , as the table (1)

Table(1) : Shows some hematological parameters of T1DM patients and healthy control

Parameter	Subject	No.	Mean \pm SD	T-value	df	P-value
RBC	Pateints	100	4.92 \pm 3.89	0.81	128	0.271
	Control	30	4.83 \pm 3.49			
Hb (g/dl)	Pateints	100	13.58 \pm 1.50	5.52	128	0.082
	Control	30	13.07 \pm 0.94			
PCV (%)	Pateints	100	40.04 \pm 3.73	1.101	128	0.273
	Control	30	40.84 \pm 2.34			
Phagocytosis	Patients	100	30.67 \pm 14.20	0.99	128	0.001
	Control	30	46.98 \pm 10.89			

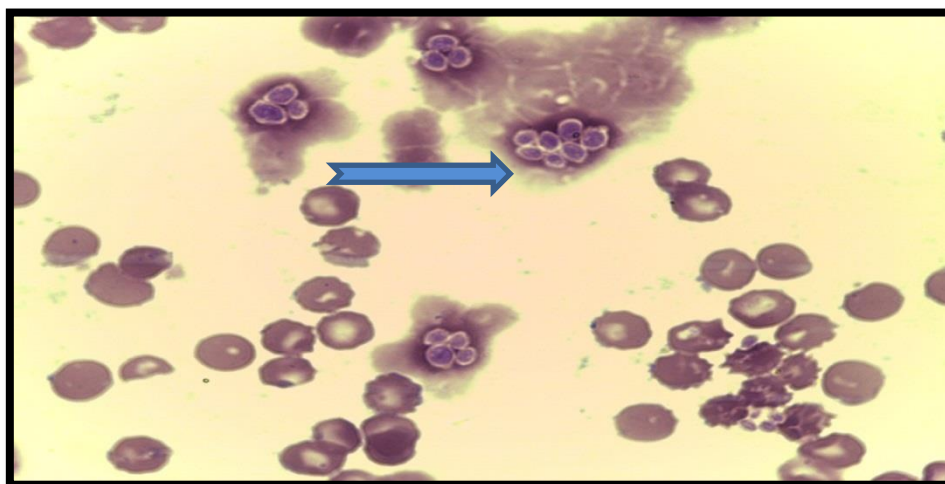


Figure (1): Phagocytosis of yeast cell by Phagocytic cell 1000 x.

WBCs , Lymphocyte , MID , Neutrophil count :

The results of the current study showed high significant difference ($p \leq 0.001$) between the two groups of -patients compared with healthy controls that has been shown in Table (2) .The WBCs count, lymphocytes , MID ,and neutrophil

in T1DM compared with the healthy control group, WBCs count there means were (7.75) for patients and (5.80) for healthy controls group respectively ,while lymphocytes means were (16.68) for patients and (26.30) for healthy control group respectively , the monocytes , eosinophils , and basophils (MID) means were (7.85) for patients and (6.40) for

healthy control group respectively ,eventually the neutrophils there were have high significant difference too , and

the means were(5.623) for patients , and (3.710) for healthy control group .

Table(2) :Hematological parameters in T1DM patients and healthy control group.

Parameter	Subject	No.	Mean ± SD	T-value	df	P-value
WBC Total count	Pateints	100	7.75 ± 2.03	1.82	128	0.001
	Control	30	5.80 ± 1.82			
No. of MID	Pateints	100	7.85 ± 1.73	0.88	128	0.001
	Control	30	6.40 ± 2.17			
No. of lymphocytes	Pateints	100	16.68 ± 4.06	35.90	128	0.001
	Control	30	26.30 ± 7.77			
No of Neutrophils	Pateints	100	5.623 ± 1.118	8.73	128	0.001
	Control	30	3.710 ± 0.784			

Discussion:

The results of the current study showed a lack of significant difference ($P > 0.05$) in each of the concentration of hemoglobin between the two groups of patients (13.58 ± 1.50) and healthy control group (13.07 ± 0.94), and the percentage of the R.BC in patients (4.92 ± 3.89) and healthy control (4.83 ± 3.49), the PCV value (40.04 ± 3.73) for patients and (40.84 ± 2.34) for healthy control ,These findings agree with a studies of [34,35,36] . and attributed the lack of significant differences to the high level of sugar in the blood does not affect the affectiveness of red blood cells and it ability, as well as R.B.C membrane not effect and nor old, and this is reflected in the level of Hb ,RBC and PCV, there has been no change in rates [35] . either phagocytosis which is

the first line of defense against injury and carried out by Neutrophil and Macrophage and Dendritic cells [37] , which should migrate to the places of injuries or infections in order to do its job and be the migration process in response to some of the attraction of chemical [38] . and phagocytosis and one of Non-specific immune mechanism and motivated by the entry of foreign bodies [39] .

The current results showed the significant difference($P < 0.001$) as the percentage of phagocytosis in patients with diabetes mellitus type I (30.67 ± 14.20) compared to a healthy control (46.98 ± 10.89) and these findings are agree with the results of a studies [40,41,42,43] , where the lack of insulin led to use fatty acids as an alternative to glucose and this may cause the formation of large amounts of ketone bodies that cause the occurrence of

ketoacidosis ^[44] , and which is accompanied by much of the metabolic changes such as loss of water and some ions and this causes stop metabolism of glucose is the main source of energy ^[45] . The high level of sugar leads to the flow of calcium ions Ca^{++} to neutrophils and the continued flow inhibits the oxidation process in mitochondria and thus lower production (ATP) which causes low activity of enzymes responsible for the entry and exit of calcium ions to the cells, and this weakens the activity process of phagocytosis, so given diabetic patients drugs depressor Sugar where lead to a reduction of the level of calcium ions and increase the production of the (ATP) and improve the process of phagocytosis ^[46] , and also found that there is a functional failure occurs in the organelles of energy and this directly affects the process of phagocytosis [47], and that the high level of sugar inhibits the enzyme Glucose 6-phosphate dehydrogenase, which reduces the production of ultra-oxygen ^[48] , and that the damage to the Fc receptor for IgG on the surface of neutrophil cells cause low phagocytosis ^[49] .

Showed the results of the current study, high significant differences ($p \leq 0.001$) in total count of white blood cells from the patient group (7.75 ± 2.03) and the control group (5.80 ± 1.82), and these findings are degree with the results studies of each ^[43,50,51] . The reason for increasing white blood cells in patients with Type I diabetes is due to the increased number of granular W.B.Cs (neutrophil, acidophil, basophil (MID)) compared to the healthy control group. The reason for the increase is the rise in the number of neutrophils and this study are degree with ^[52,53] . And that

this increase is occurring as a result of the inhibition of neutrophil cells migration to the site of infection or inflammation ^[54,55,56] . And inhibit antigen pancreatic cell migration neutrophil in diabetes type I and then accumulate in the bloodstream and increase their numbers ^[57] , and may be the chemical factor inhibitory formation proteins plasma compete attraction chemical derived from complement receptor cells of neutrophil, and this competitive inhibition is inhibits the chemical response of neutrophil cells ^[58] , that the high level of sugar prevent the migration of neutrophils because of its effect on the process of chemotactic or gets direct interaction between the sugar glucose and receptors on the surface of neutrophils ^[59] , or perhaps get the breakdown of some blood proteins and when the correlation of these proteins with the surface receptor neutrophil cells get inhibition for chemotactic and inhibition neutrophil migrate ^[60] , that the high level of sugar has a detrimental effect on the functions of neutrophils in several important stages (chemotactic , adhesion, phagocytosis) ^[61] , because it causes cross the amounts of sugar across the cell membrane of neutrophil by diffusion without the mediation of insulin and this in turn causes disorder in the process of glycolysis ^[62] , because the enzymes of glycolysis independent on insulin like (PFK) PhosphoFructoKinase and Pyruvate kinase enzyme and become ineffective which requires the occurrence of alteration in glucose metabolism, causing weakness of the process of glycolysis and decrease in energy production necessary for the neutrophil cells ^[63,64] . As the decrease in the ability of neutrophil adhesion and failing to increase its ability to kill bacteria

due to the effects of direct and indirect to the hormone insulin ^[65]. Showed the results of the current study, high significant difference ($p \leq 0.001$) in the concentration of immunoglobulins (IgM, IgG, IgA) in a patients group with diabetes type I compared a healthy control group, where the concentration of IgG (1562.385 ± 458.127) of the patients, while the healthy control is (972.420 ± 223.375), and IgA (359.560 ± 148.779) for patients and (129.420 ± 62.809) for healthy control, as well as IgM (303.540 ± 79.618) for patients and (186.090 ± 39.061) for healthy control.

The results of this study agree with the results of a studies each of ^[66,67,68], as well as the results of this study are agree with the results of a study ^[69], which was carried in India, as well as with the study ^[70], that was carried in Saudi Arabia. These studies all indicated to the high level of immunoglobulins in chronic diseases such as type I diabetes and bacterial infections.

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As well as the study carried out by each of ^[71,72], which indicated a high level of immunoglobulins in patients with diabetes . The IgA increase with diabetes patients and those who suffer from complications in kidney and arteries, high blood pressure and bacterial infections and acute and chronic diabetic patients with inflammatory of urinary tract ^[73] . The IgG increase in patients with diabetes patients , particularly type I more prepared for infections than individuals' natural ^[74,75], it is likely that diabetes effects on the body's immunity through inhibition of the effectiveness of immunoglobulin without reduce its concentration and this is happening in the process of phagocytosis ^[76] . And diabetic patients suffering from acute and chronic injuries, and this corresponds with a high level of IgG antibody, which is basic in chronicinfections and the level of IgM in acute injuries ^[77] .

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الخلاصة :

وقد أجريت الدراسة الحالية في مركز السكري والغدد الصماء في مديرية الصحة في محافظة ذي قار، خلال الفترة من أكتوبر 2013 إلى مايو 2014. تهدف دراسة وتقييم الحالة المناعية لمرضى داء السكري النوع الأول عن طريق قياس المستويات المناعية (الجلوبيولينات المناعية IgG, IgM, IgA) من خلال طريقة الانتشار المناعي الاشعاعي المفرد (SRID)، وشملت الدراسة اختبار الخلايا البلعمية على البلعمة (معامل البلعمة) وقياس المعايير الدموية (عد خلايا الدم الحمراء، الهيموجلوبين، حجم كريات الدم المذغوظة وعد التفرقي لخلايا الدم البيضاء). وشملت الدراسة ما مجموعه 100 المرضى الذين يعانون من الإصابة بداء السكري النوع الأول (51 ذكور) و (49 إناث) و تتراوح أعمارهن بين 1-40 عاماً. تم قياس مستويات المناعية (قياس تركيز IgG, IgM, IgA) في المصل لمدة 20 عينة من مرضى داء السكري النوع الأول، وكذلك درست 10 عينات من الأشخاص الأصحاء في المجموعة السيطرة. أظهرت نتائج التحليل الإحصائي زيادة معنوية عالية ($P \leq 0.001$) في مستوى الجلوبيولينات المناعية (IgG, IgM, IgA) في مرضى داء السكري النوع الأول مقارنة مع مجموعة السيطرة. وكان انخفاض معامل البلعمة معنويًا ($P \leq 0.001$) في جميع المرضى الذين يعانون من مرض داء السكري النوع الأول مقارنة مع مجموعة السيطرة، أما فيما يتعلق بالمعايير الدموية فقد أوضحت نتائج الدراسة الحالية، عدم وجود فروق معنوية ($P \leq 0.05$) في معدلات عد كريات الدم الحمراء والهيموجلوبين وPCV في كل مرضى داء السكري النوع الأول بالمقارنة مع مجموعة السيطرة، كما زاد معدل عد كريات الدم البيضاء و الخلايا للمفاوية، والحمضة، والقعدة، والوحيدة ($P \leq 0.001$) في المرضى الذين يعانون من مرض السكري من النوع الأول مقارنة مع الأشخاص الأصحاء في المجموعة السيطرة.