دراسة التغيرات الحاصلة في جين (IL-6) وبعض المعايير الدموية لدى مرضى السكري من النوع الثاني

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Study of changes in IL-6 gene and some hematological   parameters in patients type 2 diabetic mellitus

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الملخص:

يعتبر مرض السكري (DM) مشكلة صحية كبيرة، ويصف مجموعة من الاضطرابات الأيضية والفيزيولوجية المرضية الشائعة التي تتميز بارتفاع مستوى السكر في الدم، والذي يفرز من قبل الغدد الصماء في البنكرياس– جزيرات لانكرهانز (خلايا بيتا البنكرياسية المنتجة للأنسولين)، اذ ان فقدان كتلة خلايا بيتا ووظيفتها بسبب الالتهاب وموت الخلايا المبرمج هو عامل رئيسي يسهم في الإصابة بمرض السكري.

اجريت الدراسة الحالية على ٢٠ عينة من دم البالغين المصابين بمرض السكر من النوع الثاني ، تم تقدير مستويات بعض العوامل الدموية (كريات الدم الحمراء RBC، الهيموغلوبين Hb، حجم كريات الدم الحمراء (PCV) و متوسط حجم كريات الدم الحمراء MCV) والمصلية المتضمنة الانترلوكين–٦ (IL-6) في الدم لدى مرضى السكر من النوع الثاني   (DM-Type-1)، والتي اظهرت ارتفاعاً واضحاً عند مقارنتها مع الاشخاص الاصحاء والذي تمثل بمجاميع السيطرة التي كان عددها(١٠) اشخاص.

الكلمات المفتاحية: مرض السكري النوع الثاني، جين الانترلوكين–٦ (IL-6) ، كريات الدم الحمر، الهيموغلوبين، حجم كريات الدم الحمراء المكدسة، متوسط حجم كريات الدم الحمراء

Abstract

Diabetes is a major health problem and a common metabolic and pathophysiological disorder characterized by hyperglycemia. Within the islets of Langerhans, the endocrine powerhouse of the pancreas are the insulin-producing

pancreatic beta cells. Loss of beta cell mass and function due to inflammation and apoptosis is a major contributing factor to the development of diabetes.

This study aimed to determine the severity of type 2 diabetes in adults and to estimate the levels of some hematological parameters (RBC count, hemoglobin, PCV, and MCV) and serum (IL-6) levels, which were found to be significantly elevated above normal levels in healthy individuals.

Key words: DM-Type2, interleukin-6(IL-6), RBC, Hb, PCV, MCV).

1. Introduction

Diabetes is a disease that affects people and causes an increase in blood sugar levels. It becomes chronic when the pancreas in the body fails to produce the required amounts of the hormone insulin as a result of the immune system attacking the pancreas.[1] It affects children and in this case it is called type 1 diabetes. It is called type 2 diabetes when it affects adults and is also chronic. The body becomes resistant to insulin, so sugar accumulates in the blood as a result of it not being absorbed by insulin. Symptoms of this disease include thirst and frequent urination with the presence of ketones, which are by-products of the breakdown of fats and muscles when there is not enough insulin in the body. Complications of diabetes include diabetic ketoacidosis, cardiovascular disease, kidney failure, nerve damage, diabetic foot, and stroke.[2,3] In 2019, statistics indicated that the death rate from diabetes was about 4.2 million. Chronic diabetes is of two types: DM type 1 and DM type 2. Curable diabetes is represented by gestational diabetes, which usually disappears after childbirth, and prediabetes, which occurs when the blood sugar level increases slightly above the normal level. Type 1 diabetes affects children (age group under 12 years), while type 2 diabetes affects adults and occurs due to the body's failure to regulate sugar and use it to provide the body with the necessary energy. This type of sugar is called glucose, which usually plays a major role in causing the disease. Glucose

comes from food and the liver, after entering the cells via insulin, the liver stores and produces it, thus maintaining the normal level of glucose, as happens when you do not eat for a long time. The infection occurs a result of the accumulation of this type of sugar in the blood for long periods, which leads to disturbances in blood circulation. The nervous and immune systems, as well as type 1 and type 2 diabetes, are caused by a group of genetic factors.[4,5]

The risk factors for the disease lie in knowing the patient's family medical history, as cases of type 1,2 diabetes increase when there were forming immune cells against diabetic (autoantibodies), and people who are overweight or obese, normal measurements of blood components are affected by diabetes, as the hemoglobin A1c (HbA1C) increases, which leads to hardening of the arteries, as their elasticity decreases, which in turn leads to increase in blood pressure. The normal blood level hemoglobin blood (Hb) and the number of red blood cells decrease with people, with many changes occurring in the normal serum blood indicators such as D-dimer, Vit. D, IL-6 and ferritin.[6,7]

There are many proteins (cytokines) that are involved in the development of diabetes because they play a major role in making the body respond to inflammation and insulin resistance, thus tissue damage. Therefore, when they are controlled, it is possible to reduce and improve the sensitivity of cells to insulin, as most of the cytokines' rise with the increase in the symptoms of diabetes in the patient, including (IL-1β, TNF-α, IL-6, IL-18 MCP).[8] The focus of our research in this research will be on IL-6 because it is one of the most important cytokines, whose increase type 2 diabetes, which was studied in this research, so that, its irregular production has been associated with this disease, especially in cases of high blood pressure, and is associated with changes in the levels of interleukin 6 expression in people with diabetes.[9,10]

Objectives of study

Estimate the levels of some hematological parameters (RBC, hemoglobin Hb, PCV and MCV) in blood of type 2 DM in adults.

Estimate the levels of IL-6 in serum of type 1 diabetes.

Materials & methods

Sample Collection

30 samples were collected from patients DM type1 between (18 -50) age years by AL-Jarah hospital in Dujail city, as follows:

10 healthy people as a control group.

20 type 2 DM as patients group.

Collection of blood Samples

3ml of blood samples were taken from each patient of DM type 2, in the morning in a fasting state ,2ml of fresh blood were placed in EDTA tubes for the purpose of placing them in a shaker for mixing 5 minutes and then (Hb, RBC_2 , PCV, MCV) was measuring them by using CBC device. ,Complete Blood Counts (CBC).[11]

Genetic Study

Add 0.75 μL each of the following: forward & reverse inner primer, forward & reverse outer primer, 5 μL of DNA , then added 12 μL of free water nuclease. From 1 to 6 were added to a Premix tube. These tubes were placed in device and placed in a PCR. After the extraction process, electrophoresis used to measure the size of the PCR interaction's outcome bundle on the agarose gel or to identify the PCR interaction's outcome when standard DNA is present, and then prepared of the agarose Gel, then staining was applied, 70 V electric current was applied for a more than one hours, after was being immersed in a pool containing 0.5 distal water per 30 L of a red-safe nucleic acid, then gel was measured by 336 nm of

ultra violate .[12] Then DNA isolated by PCR technique,[13,19] the primers used according to the table(1).

Table (1)ARMS-PCR primers with their sequence and size

primer	Sequence (5'-3')	Product size
G. allele Wild type primer	CTGACACTGGGAAAACACCG	
A allele Mutant type primer	CTGACACTGGGAAAACACCA	205 bp
Common Reverse primer	CTCACTTCCCCATCGCCTAC	

The sequence of work steps was as follows:

Sample collection

↓

Hematological tests

↓

PCR test for IL-6 polymorphism
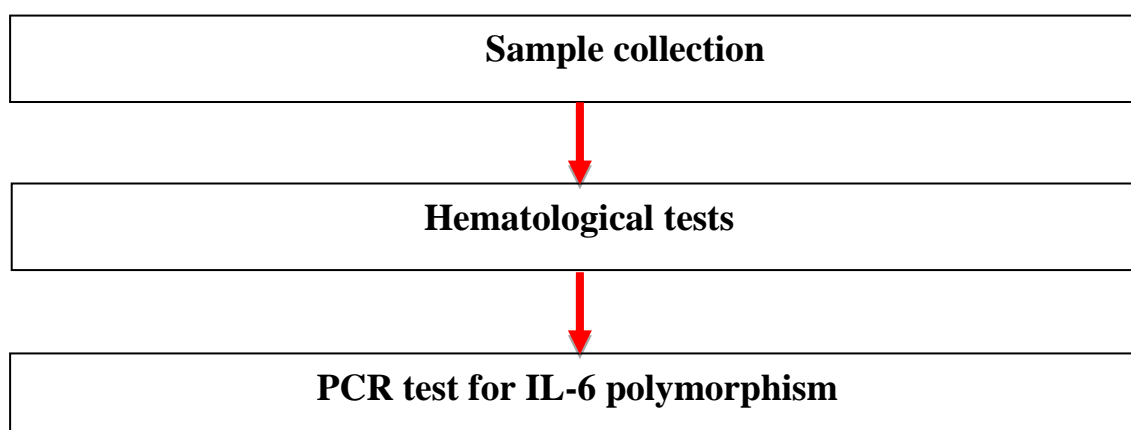
3- Result

Hematological parameters

The hematological parameters of T2DM patients, where (RBC) red blood cell count, (Hb) hemoglobin blood , packed cell volume (PCV), MCV (mean corpuscular volume), demonstrated significant ($P < 0.05$) reduce in patients (3.492 ± 0.122) , (9,330 ± 1.911), (30,080 ± 5.160), (82.120 ± 6.320) respectively, compared to control group (5.001 ± 0.112), (14.940 ± 1.45), (42.120 ± 4.85), (83.650 ± 7.49) respectively, as shown in table (2).

Table (2), average hematological parameters in control & patient

Groups Parameter	Control (50)	Patients (100)	P-Value
RBC	5.001 ± 0.112	3 .492 ± 0.122*	> 0.05
Hb	14.940 ± 1.45	9.330 ± 1.911*	> 0.05
PCV	42.120 ± 4.85	30.080 ± 5.160*	> 0.05
MCV	83.650 ± 7.490	82.120 ± 6.320	> 0.05

*Significant differences at p=0.5

The serum study of IL-6 concentrations in of T2DM patients, the IL-6 value was rising in patients (4.880 ± 0.25), while the value of healthy people or control group (2.50±0.70), at Significant value (P<0.05) table (3).

Table (3), IL-6 in control & patient

Groups Parameter	Control (50)	Patients (100)	P-Value
IL-6 (ng/ml)	2.550 ± 0.60	4.990 ± 0.25*	0.05

*Significant differences at p=0.5

IL6 gene polymorphism 174 C/G

Figure (1): Electroporation of the IL6 gene to 174 C/G rs1800795 shows the C and G alleles, where 1 to 10 samples are from diabetic patients, and 11 to 20 are control samples, The IL-6 gene 174 C/G polymorphism in T2DM patients showed that the CC allele was 1(1%), the CG allele was 15 (17%) and the GG allele was 85 (82%) in 100 patients, while in the group of healthy people

(control), the percentage of the CC allele was 3(6%), the percentage of the CG allele was 21(42%), and the percentage of the GG allele was 26(52%). Thus, the results showed that the G allele is more common in T2DM patients compared with healthy people.
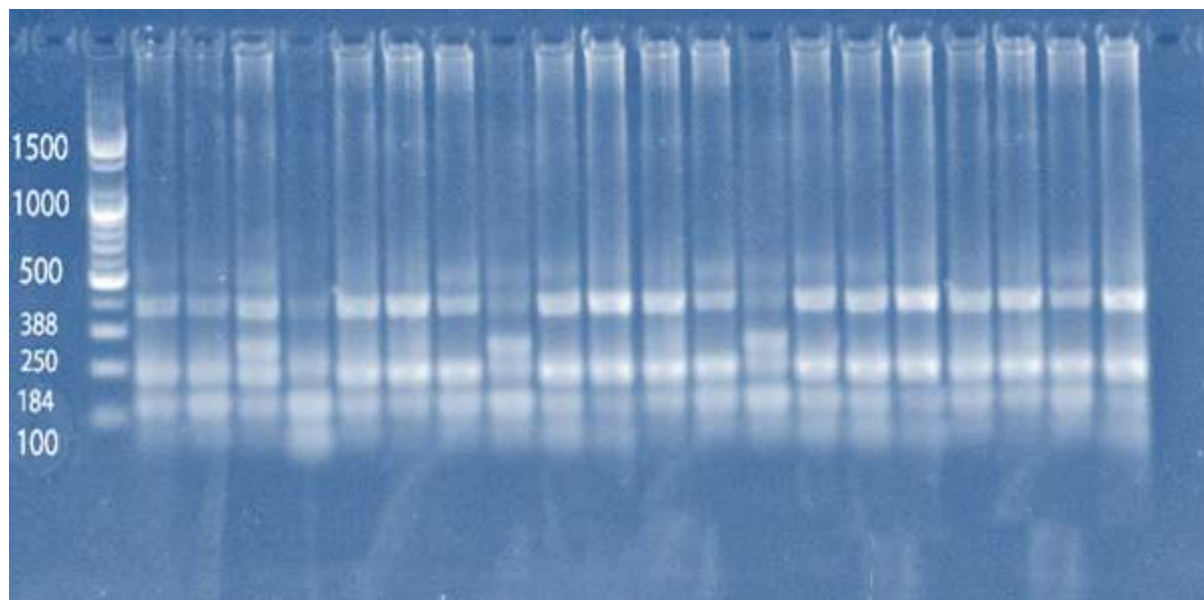

Figure 1: Figure (1): electroporation of the IL6 gene to 174 C/G

discussion

Many prior studies, mentioned that type I diabetes mellitus caused significant decrease in RBCs,[14,15] while increase in RBCs indices in diabetic adults.[16] Less of RBC in type I DM as diabetes is the most common cause of kidney disease which lead to decreasing in erythropoietin level, and cause un obvious renal anemia.[17,18] While the results of the current study were slightly higher than what was reached by Mohmmed, (2024) when he conducted a study on the occurrence of some serum and blood variables in patients with type 1 diabetes, the reason may be that type 2 diabetes affects children, which leads to a greater decrease in the body's standard criteria because its immunity is lower, especially blood changes.[19]

This study agreement with Yasar et al (2006) regarding the IL-6 levels, as it showed a high percentage in people with type 1 and type 2 diabetes as differences

significant when compared with healthy group, explained the percentage be higher in the case of people had been infected more severely or for long period, and individuals carrying the G allele of IL-6 compared to people carrying the C allele, was higher in patients than people of controls.[20]

While Gupta and others (2017) explain that allele frequencies of polymorphisms for cytokine gene IL-6 found no statistically difference between T2DM and control groups .[21]

Conclusion

Decrease in the hematological parameters (RBC, Hb, PCV, MCV) in patients have T2DM compared with healthy people (control), So there were a positive correlation between IL-6 polymorphism and type 2 DM, and the IL-6 polymorphism in patient individuals carrying the (G of IL-6 -174G / C) allele was increasing in patients than control group.

Recommendations

T2DM patients continue to use iron supplements due to complications caused by T2DM in terms of low blood parameters.

Conduct more studies on gene polymorphism in TIDM & T2DM patients and study the relationship between genetic mutations and TIDM.

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