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# Determination A Role of Some Immunological Markers for Diabetic Patients in Diyala Province

**Athraa Tariq Ahmed[1] and Mohammed Abdul-Daim Saleh[2*]**

[1]Department of Biology – College of Science – University of Diyala
[2]Department of Biotechnology – College of Science – University of Diyala

abedmostafa64@yahoo.com

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## Abstract

Diabetes is a metabolic disease that results from a defect in the action of insulin or its secretion or both, or for secondary reasons that are due to a defect in the endocrine function, in which the blood sugar (glucose) level is higher than normal levels. There are three main types of this disease: type 1 diabetes, type 2 diabetes and gestational diabetes. This study was conducted in the Baquba Teaching Hospital - Diyala Health Department, in the period from the beginning of October 2021 to the end of January 2022.Which aimed to evaluate some immunological indicators for patients infected with diabetes type 2 which included assessment levels of complements proteins C3 and C4 in DM patients and healthy population. For the current investigation, 90 blood samples were collected and divided into the following groups: The first group: - Approval of (60) blood samples from diabetics who have been medically diagnosed at the Baquba Teaching Hospital. Within my age range of (21-70) years, there were (28) males and (32) females, who were split into age-related groups. The second group, which serves as a control group, consists of 30 samples of persons of both sexes who did not have any chronic illnesses at the time the research was being conducted. There were 15 males and 15 females in this group who were between the ages of (21- 70).

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The findings of our study indicate that there are no non-significant differences (p>0.05) among patients in terms of gender or diseases. The (41-50) and (51-60) age groups scored higher percentages (25.60% and 28.90%) than the (21-30) and (31-40) age groups, which scored lowest percentages (11.10% and 15.60%), according to our study, which contrasted with this. The current study's findings also revealed lower median levels of C4 complement parameters in patients (23.00) compared to healthy individuals (40.00), with a significant difference for C4 complement (p0.05). In contrast our study found significantly higher levels of C3 complement in patients (150.00) compared to healthy individuals (120.00).

**Keywords:** Diabetes, C3, C4, Blood glucose.

## تحديد دور بعض الواسمات المناعية لدى مرضى السكري في مدينة بعقوبة

**عذراء طارق احمد[1] و محمد عبد الدايم صالح[2]**

[1]قسم علوم الحياة – كلية العلوم – جامعة ديالى

قسم التقانة الاحيائية – كلية العلوم – جامعة ديالى

### الخلاصة

السكري هو مرض ايضي استقلابي ينتج عن خلل في عمل الانسولين او افرازه او كليهما او لأسباب ثانوية تعود الى خلل في وظيفة الغدد الصماء وتكون نسبة سكر الدم (الكلوكوز) اعلى من المستويات الطبيعية وهناك ثلاث انواع رئيسية من هذا المرض وهي السكري النوع الاول، السكري النوع الثاني، سكر الجمل. تضمنت الدراسة جمع 90 عينة دم وتم تقسيمهم الى مجموعتين: تضمنت المجموعة الاولى (60) عينة من اشخاص مصابين بالسكر النوع الثاني بعد التشخيص من قبل الطبيب الاخصائي في العيادة الاستشارية في مستشفى بعقوبة التعليمي، وكان عدد الذكور (28) وعدد الاناث (32) تراوحت اعمارهم ما بين (21-70) حيث تم تقسيمهم الى ستة مجاميع عمرية. اما المجموعة الثانية: تتكون من (30) عينة من اشخاص اصحاء من كلا الجنسين غير مصابين بأمراض مزمنة خلال فترة اجراء البحث كمجوعة سيطرة، اذ بلغ عدد الذكور (15) وعدد الاناث (15) تراوحت اعمارهم ما بين (21-70). اظهرت نتائج الدراسة عدم وجود فوارق معنوية بين المرضى (p>0.05) من حيث الجنس والاضطرابات، وعلى العكس كشفت الدراسة وجود فوارق معنوية بين المجاميع العمرية، حيث سجلت المجموعة العمرية (41-50) و (51-60) اعلى نسبة (28.90%, 25.60%) بينما سجلت المجموعة العمرية 21-30) و (31-40) اقل نسبة (11.10%,(15.60)%. وكشفت الدراسة ايضا انخفاض في مستويات بروتين المتمم C4 في المرضى

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(23.00) على التوالي مقارنة مع الاشخاص الاصحاء حيث كانت (40.00) على التوالي مع وجود فارق معنوي (p <0.05) بالنسبة لبروتين المتمم C 4. واظهرت نتائج هذه الدراسة ارتفاع في مستوى بروتين المتمم C3(150.00) مقارنة مع الاشخاص الاصحاء (120.00) بفارق معنوي.

**الكلمات المفتاحية: مرض السكري، C3،C4، سكر الدم.**

## Introduction

Diabetes mellitus (DM) is a metabolic condition characterized by increased blood glucose levels. The hormone insulin moves glucose from the blood into the cells to be stored or used for energy, untreated high blood glucose can damage the nerves, eyes, kidneys and others organs. There are several types of diabetes mellitus include: Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) are the two primary subtypes of DM, and both are typically brought on by defects in insulin secretion (T1DM) and/or action (T2DM) [1]. T1DM presents in children or adolescents, while T2DM is thought to affect middle-aged and older adults who have prolonged hyperglycemia due to poor lifestyle and dietary choices [2].

Prediabetes and type 2 diabetes are prevalent, affecting, respectively, roughly 34% and 13% of all US people in 2018 [3]. According to the most recent data, diabetes mellitus (DM) is still a serious worldwide health concern and is expected to increase significantly over the next few decades. This will have a considerable impact on healthcare spending, especially in emerging nations [4]. Unfortunately, in diabetes, the host's immune response is disrupted. In addition to the risk of natural barrier damage due to neuropathy, T2D can also affect cellular immunity. This is caused by insulin deficiency and hyperglycemia [ 5].

## Materials and Methods

### Samples collection

90 blood samples were obtained from the consultative clinic - Baquba Teaching Hospital - Diyala Health Department, within the period from the beginning of October 2021 to the end of January 2022. The samples were classified into groups as follows: 60 blood samples from

diabetes mellitus and medically diagnosed by specialized doctors in the Baquba Teaching Hospital Consultation, where the number of males was 28 and the number of females 32 within age range 21-70 years. Addionally, 30 samples of apparently healthy people of both sexes were adopted and used as a control group, where the number of males was 15 and the number of females 15 within the age range 21-70 years and they did not suffer from any chronic or acute illness at the time of sample collection. 5 ml of blood samples were collected by drawing venous using plastic medical syringes and left 10 minutes at room temperature for coagulation, then the serums are separated by centrifugation for 5 minutes and at a rate of 3000 cycles | min. and the serum is divided into equal quantities 250 µl by Eppendorf tube set and stored at ̊-20 °C until use, then it was divided into a group of Eppendorf tubes.

The complement proteins (C3, C4) were then measured in the serum of diabetic patients by single radial immunofluorescence assay (SRIA) [6].

**Statistical analysis**

The C3, C4 complements proteins levels parameters were first tested for normality (Kolmogorov-Smirnov and Shapiro-Wilk test). Parameters that fit both tests (no significant difference) were given as mean ± standard deviation (SD), and the parameters that did not fit the normality tests (significant difference) were given as median and range, and significant difference between median was assessed by Mann-Whitney (for comparison between two groups). The sperman correlation was employed to understand the correlation between certain parameters. The statistical package SPSS version 25.0 and Graph pad prism version 6 were employed to carry out these analyses [7].

## Results and discussion

Results of our study show non-significant different ($p>0.05$) among patients according to gender and disorders. In contrast, our study revealed significant different among age groups, where the 41-50 and 51-60 years scored highest percentage (25.60% and 28.90%) respectively than 21-30 and 31-40 that scored lower percentage (11.10% and 15.60%) (Table 1).

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**Table 1:** Demographic characters of patients.

| | | Count | Percent | P value |
|---|---|---|---|---|
| Gender | males | 43 | 47.80% | P >0.05 |
| | females | 47 | 52.20% | |
| Age groups (years) | 21-30 | 10 | 11.10% | p<0.001*** |
| | 31-40 | 14 | 15.60% | |
| | 41-50 | 23 | 25.60% | |
| | 51-60 | 26 | 28.90% | |
| | >60 | 17 | 18.90% | |
| Disorders | Eye | 15 | 25.00% | P >0.05 |
| | Renal | 27 | 45.00% | |
| | Foot DM | 18 | 30.00% | |

The current study indicates that gender has no appreciable influence on diabetes. The results of the study are consistent with those found, which show that DM increases as people age [8]. However, with time, both the effects of aging and the progression of the disease could become more pronounced, leading to early complications and even death in those with type 2 diabetes who were diagnosed at a younger age. To give an example, a person with type 2 diabetes who is diagnosed at age 30 has a lower absolute risk of complications than a person who is diagnosed at age 50, but by the time they both reach age 60, the person who was diagnosed earlier has a higher relative and absolute risk because of the effects of aging, which are exacerbated by the effects of having had diabetes for a longer period of time [9]. One in nine fatalities among adults between the ages of 20 and 79 is thought to be caused by diabetes. Diabetes and associated consequences must be prevented, especially in middle-income nations [10]. The present study showed the diabetes is increased with age progression due to impaired immune status, chronic diseases, and organ dysfunction. demonstrate the presence of renal problems in DM patients, and these findings are consistent with our findings. End-stage renal disease is most frequently brought on by diabetic kidney disease (DKD) (ESRD). The current investigation found conditions like obesity, CKD, and retinal inflammation that are linked to diabetes, and these findings are consistent with previous findings [11].

Results of current study mentioned the decreased median levels of C4 complement parameters in patients (306.72) than healthy (548.94) with significant different ($p<0.05$). In contrast, our study mentioned increase levels of C3 complement in patients (150.00) than healthy (120.00) with significant different (table 2).

**Table 2:** Comparative complements proteins between study groups

| Groups | | C3 | C4 |
|---|---|---|---|
| Patients | Median (range) | 150.00 (26.00- 311.00) | 23.00 (8.00-60.00) |
| Controls | Median (range) | 120.00 (90.00-151.00) | 40.00 (20.00-50.00) |
| P value | | P<0.05* | P<0.05* |

The present study showed high levels of C3 complement in DM than healthy because of the complement protein are triggered in metabolic diseases due to occurrence inflammation. The current study shows Low levels of C4 complement in patients with DM, and these findings were consistent with previous research [12]. There are distinct methods by which chronic complement activation could result in cardiovascular disease, type 2 diabetes, and the metabolic syndrome. Additionally, genetic research indicates that C3 polymorphisms and C4 copy number variations may put people at higher risk for metabolic and cardiovascular disease [12].

Our results show there is non-significant difference between C3 complement and C4 complement between patients gender ($P>0.05$). (table 4-4).

**Table 3:** Logistic regression analysis of complements proteins

| | | gender | | Median | p value | Odd ratio Confidence intervals (95%) |
|---|---|---|---|---|---|---|
| | | Males | Females | (range) | | |
| C3 | > Median | 13 | 16 | 150.00 (26.00- 311.00) | p>0.05 | 0.86 (0.3138 to 2.394) |
| | <= Median | 15 | 16 | | | |
| C4 | > Median | 12 | 12 | 23.00 (8.00-60.00) | p>0.05 | 1.25 (0.4437 to 3.522) |
| | <= Median | 16 | 20 | | | |

Innate and adaptive immune responses differ between males and females, as do their immunological reactions to self- and foreign antigens. It is possible that both genes and

hormones are at play because some immunological sex differences are present throughout life while others become obvious only after puberty and before reproductive senescence. Additionally, early environmental exposures affect the immune system and the microbiota in a sex-dependent manner. Importantly, these sex-based immunological differences affect how susceptible people are to infectious infections, how they react to immunizations, and how frequently they develop autoimmune conditions and cancer [13].

Study findings the complement system exhibits significant sex and age-related variations. When researching complement-related disorders, these modifications should be taken into consideration.

The findings of the study suggested that cytokine levels may be influenced by sex. This is in line not just with sex variations in infection susceptibility, but also with the higher cardiovascular risk that men demonstrate when compared to women. Nevertheless, hormone levels can only partially account for this connection [14].

Our results show there is non-significant difference between C3 complement and C4 complement among patients age groups (P>0.05). (table 4-5).

**Table 4:** Distribution of complements protein test levels (> median and ≤ median) in patients according to age groups

| | | Age groups (years) | | | | | Median (range) | P value |
|---|---|---|---|---|---|---|---|---|
| | | 21-30 | 31-40 | 41-50 | 51-60 | >60 | | |
| C3 | > Median | 4 | 3 | 6 | 10 | 6 | 150.00 (26.00- 311.00) | P>0.05 |
| | <= Median | 2 | 2 | 9 | 9 | 9 | | |
| C4 | > Median | 4 | 3 | 4 | 9 | 4 | 23.00 (8.00-60.00) | P>0.05 |
| | <= Median | 2 | 2 | 11 | 10 | 11 | | |

The immune system is one of the major biological systems that ages, making it more susceptible to infectious diseases and less efficient in immunizing against them as a result of age-related changes in immunity. Additionally, one of the primary signs of aging—inflammaging—the rise in low-grade inflammation—is caused by the innate immune system [15]. The causes and effects of aging-related inflammation, the effects of senescence on immunity, age-related changes in immune function the effects of decreased immune function on infection and

vaccination, and methods to counteract aging-related immunity are the five research challenges that need to be addressed. All of them are crucial now more than ever because aging is a significant risk factor for the emergence of serious consequences from infectious diseases as COVID-19, influenza, and bacterial pneumonia. [15]

Our results show there is non-significant difference between C3 complement and C4 complement among patients disorders (P>0.05). (table 4-6).

**Table 5:** Distribution of complements protein test levels (> median and ≤ median) in patients according to disorders

| | | Disorders | | | Median (range) | P value |
|---|---|---|---|---|---|---|
| | | Eye | Renal | Foot DM | | |
| C3 | > Median | 6 | 13 | 10 | 150.00 (26.00- 311.00) | p>0.05 |
| | <= Median | 9 | 14 | 8 | | |
| C4 | > Median | 2 | 12 | 10 | 23.00 (8.00-60.00) | p>0.05 |
| | <= Median | 13 | 15 | 8 | | |

Complement activation is related to both the presence and the severity of diabetes nephropathy, indicating that the complement system may play a role in the emergence of renal pathology in diabetic patients and represents a promising target for inhibiting and/or preventing diabetes nephropathy in these individuals [16]. Diabetes nephropathy is the most common cause of end-stage renal disease and is a microvascular consequence that impacts 20% to 40% of diabetic people. A growing body of research indicates that the development of DN may be influenced by complement activation via classical complement pathways [17]. In a sizable cohort of patients with diabetes, both those with and without diabetes nephropathy, they looked at the deposition of complement proteins. Researchers discovered a correlation between the complement activation marker C4d and DN, as well as between DN severity, microvascular and interstitial lesions, and reduced eGFR in individuals with DN, indicating that complement activation may be involved in the onset of diabetes nephropathy [18]. Via reducing inflammatory reactions and T-cell adaptive immunity, C3aR loss may lessen the effects of diabetes on the kidneys. This may be accomplished by altering the cytokines released by macrophages. C3aR may therefore be a possible therapeutic target for DN [18].

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