



## Serum Evaluation of Complement Proteins (C3 And C4) and Antibodies (Igg And Igm) in Patients with Diabetic Foot Ulcers

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Abstract

Diabetic foot ulcers are a significant chronic health issue, leading to high morbidity and mortality rates. A study aimed to assess complement proteins C3 and C4, as well as antibodies IgG and IgM, in the serum of patients aged 25 to 65 with diabetic foot ulcers. The findings could help understand the immune response in diabetic foot ulcer patients and develop targeted therapies. The study also aimed to identify biomarkers for early ulcer detection and management. The Radial Immuno Diffusion Single method was used to measure serum levels in 90 individuals. The study analyzed patients hospitalized with ulcer symptoms, bleeding, or pain at Baghdad Teaching Hospital in Al-Mansour, Baghdad Governorate. Results displayed a significant increase in average levels of C3, C4, IgG, and IgM in patients compared to healthy individuals, with levels of  $38.1 \pm 8$ ,  $13.1 \pm 3.1$ ,  $448.9 \pm 107.3$ , and  $52.1 \pm 10.5$ , respectively. The results indicate that the significant increase in the levels of complement proteins C3 and C4 and antibodies IgG and IgM in diabetic foot ulcer patients may reflect a clear immunological and inflammatory role in the progression of the disease. Therefore, the study recommends the importance of using these indicators as auxiliary factors in the immunological assessment of patients with diabetic foot ulcers, which contributes to improving therapeutic response and reducing complications. It is also recommended to conduct more extensive studies to confirm the relationship between these indicators and the severity of the ulcers.

Keywords: Protein C3, Protein C4, IgG, IgM, Diabetic foot ulcers.

تقييم مصلي لبروتينات المتممة (C3 و C4) والأجسام المضادة (IgG و IgM) لدى مرضى قرحة القدم السكرية

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## الخلاصة

تُعد قرحة القدم السكرية من المشكلات الصحية المزمنة الخطيرة، إذ ترتبط بارتفاع معدلات المراضة والوفيات بين مرضى السكري. هدفت هذه الدراسة إلى تقييم بروتينات المتممة C3 و C4، إضافةً إلى الأجسام المضادة IgG و IgM في مصل المرضى الذين تتراوح أعمارهم بين (25–65) سنة ممن يعانون من قرحة قدم سكرية. تسهم هذه النتائج في فهم الاستجابة المناعية لدى المرضى المصابين بقرحة القدم السكرية وتطوير علاجات موجهة، كما تهدف إلى تحديد مؤشرات حيوية تساعد في الكشف المبكر عن القرحة وإدارتها. استُخدم أسلوب الانتشار المناعي الشعاعي المفرد (Radial Immuno Diffusion Single) لقياس تراكيز هذه المؤشرات في مصل الدم لـ (90) فرداً. شملت العينة مرضى راقدين في مستشفى بغداد التعليمي في منطقة المنصور بمحافظة بغداد، ممن ظهرت عليهم أعراض قرحة القدم، كالنزف أو الألم أو الالتهابات الموضعية. أظهرت النتائج ارتفاعاً معنوياً في المتوسط الحسابي لتركيز بروتينات المتممة C3 و C4 والأجسام المضادة IgG و IgM لدى المرضى مقارنةً بالأشخاص الأصحاء، إذ بلغت القيم (8±38.1)، (3.1±13.1)، (107.3±448.9)، و(10.5±52.1) على التوالي. تشير هذه النتائج إلى أن الارتفاع الملحوظ في مستويات بروتينات المتممة والأجسام المضادة لدى مرضى قرحة القدم السكرية يعكس دوراً مناعياً والتهابياً واضحاً في تطور المرض. وعليه، توصي الدراسة بضرورة اعتماد هذه المؤشرات كعوامل مساعدة في التقييم المناعي للمرضى المصابين بقرحة القدم السكرية، لما لها من أهمية في تحسين الاستجابة العلاجية وتقليل المضاعفات. كما تُوصي بإجراء دراسات أوسع لتأكيد العلاقة بين هذه المؤشرات وشدة القرحة.

الكلمات المفتاحية: بروتين C3، بروتين C4، IgG، IgM، قرحة القدم السكرية.

## Introduction

Diabetic foot ulcers (DFUs) are a common and serious complication of diabetes, and they are among the utmost widespread chronic wounds [1]. Management of diabetic foot ulcers still lacks the required effectiveness in clinical use. Presently free healing procedures such as arsenic operative debridement and force ease set not comprehensively work the progressive upset of the incendiary reaction to the hurt which associated with an increased likelihood of amputation [2]. Due to the difficulty in diagnosing and treating diabetic foot ulcers and the resulting increase in complication and mortality rates they represent a heavy burden that negatively influences the types of patients' life and increases pressure on healthcare systems [3].

The complement system which is an essential portion of the innate immune system consists of more than 50 organized and soluble proteins in serum or associated with the cell membrane. They act in concordance to pass microbes and dead cells without harming healthy cells. It can be activated through protein receptors that recognize molecules such as specific antibodies C1q and IgM as well as C-reactive protein (CRP). These elements lead to initiating the activating reductions which includes protein C3 as one of the important parts of the body



system [4]. Complement factor 3 (C3) is a principal part of the innate immune system and is essential for activating the three the complement system pathways: classical alternative and lectin pathways. C3 is mainly synthesized in the liver and it is similarly expressed and cast in the adipose tissue of human. Current evidence indicates that intracellular C3 activity has important physiological roles. C3 is categorized as an acute-phase indicator, with its serum levels up in reaction to infection and inflammation while decreasing upon immune complex deposit [5].

Studies have indicated that the effectiveness of component C3 in the diabetic patients' serum is very importantly lower in terms of its ability to eliminate bacteria compared to healthy individuals. This decrease in antimicrobial action is potentially attributed to weakness in the classical pathway of the complement system, which is based away the importantly levels of component C4. This elevation may reflect either a decrease in its consumption or a disturbance in other parts of the complement system. The evidence also suggests that hyperglycemia combined with diabetes get lead to the damage of full complement system roles done the non-enzymatic glycation of proteins [6 7].

Diabetic foot ulcers (DFU) treatment a condition that leads to high rates of disability and mortality, costs money and imposes a substantial physical, psychological and economic burden on patients, their families and society as a whole. The development of this disease is attributed to the important role of immunoglobulin G (IgG) in its role and suppression of inhibition of inflammatory response when its binding to glycosyl molecules, as demonstrated by evidence. Therefore, studying the binding pattern of IgG to glycosyl is decisive for understanding the immunological and inflammatory foundations of DFU. It deserves noting that IgG constitutes around 75%–80% of the unit immunoglobulins in serum of human, with a concentration the blood of healthy individuals of around 10–15 mg/ cc [8].

Immunoglobulin (IgM) is the first antibody to be produced and expressed during the immune response and its main roles include activating the complement system and immune agglutination. As well as, both IgM and IgA are important parts of humoral immunity, because they work to neutralize and eliminate pathogens during primary and secondary immune responses [9]. They improve inflammatory response and the removal of immune complicated by activating the complement system stimulating natural killer cells, macrophages, and mast cells and regulating the activity of cytotoxic cells [10]. The isotype IgM, present in the



autoantibodies complicated in type I diabetes, has been shown in studies to have different roles in the disease [9].

#### Material and methods

We conducted this research from October 2024 to November 2024, collecting 90 blood samples from both genders. The ages of the study participants ranged between 18 and 65 years, and they were all residents of Baghdad Governorate. The samples were obtained from the surgical emergency department and Baghdad Teaching Hospital in the City of Medicine, and the patients' cases were clinically diagnosed by specialized doctors.

**Blood sample collection:** 4–5 ml of venous blood was drawn from the participants using sterile medical syringes with 70% ethanol, and the blood was collected in test tubes containing clotting activators (gel and clot activator tube). After numbering the samples, they were stored in an icebox.

The levels of complement proteins C3, C4, and immunoglobulins (IgG, IgM) in the serum of both infected and healthy individuals were measured using the Single Radial Immuno Diffusion (RID) method.

**Principle of test:** (5 µL) of serum from both infected and healthy individuals were placed in each well of the agarose gel designated for complement proteins C3, C4, and antibodies (IgG, IgM). If complement proteins or these antibodies are present in the sample, an immune complex will form a precipitin ring around the well after 72 hours of incubating the (C3, C4, IgG) samples. The antibody sample (IgM) was incubated for 96 hours in a moist chamber, and then the diameter of the precipitin ring around the well was measured using a magnifying lens. These results were compared with the standard tables provided by the supplier to determine the concentrations.

#### Results and Discussion

Results of complement protein C3 level:

The results of the current study showed an increase in serum C3 levels in persons with diabetic foot ulcers (225.5±23.9).

Table 1: The comparison between the average serum complement protein C3 concentration in diabetic foot ulcers and the control group.

The test	P value	Mean ± Standard Deviation	The indicator	
Spiritual	0.00*	225.5±23.9	Patient	The case
		38.1±8	Control group	

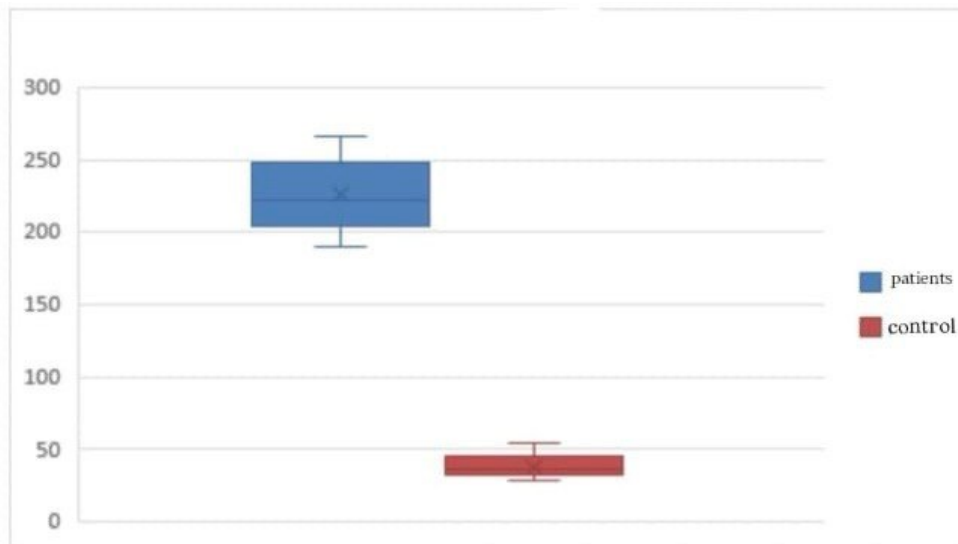


Figure 1: The comparison of complement protein C3 and control group serum concentration.

Serum C4 levels increased in individuals with diabetic foot ulcers (80.8 10), according to the current study results.

Table 2: Comparing the average serum complement protein C4 concentration between patients with diabetic foot ulcers and a control group.

The test	P value	Mean ± Standard Deviation	The indicator	
Spiritual	0.00*	80.8±10	Patient	The case
		13.1±3.1	Control group	

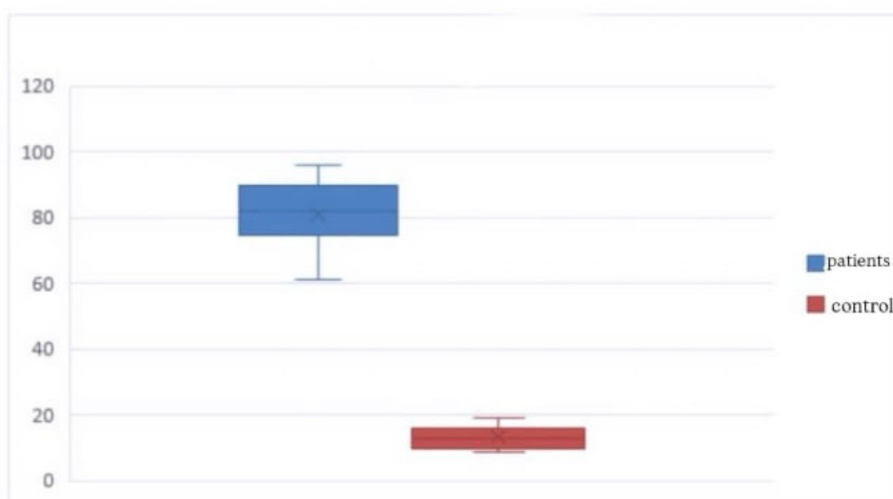


Figure 2: A graph illustrating that the complement protein C4 concentration in the serum of diabetic foot ulcer patients and the control group is similar.



The serum IgG antibody levels in patients with diabetic foot ulcers were found to increase significantly in the current study, resulting in a significant increase of 1888.3468.7.

Table 3: Comparing the average serum IgG antibody concentration among patients with diabetic foot ulcers to the control group.

The test	P value	Mean ± Standard Deviation	The indicator	
Spiritual	0.00*	1888.3±468.7	Patient	The case
		448.9±107.3	Control group	

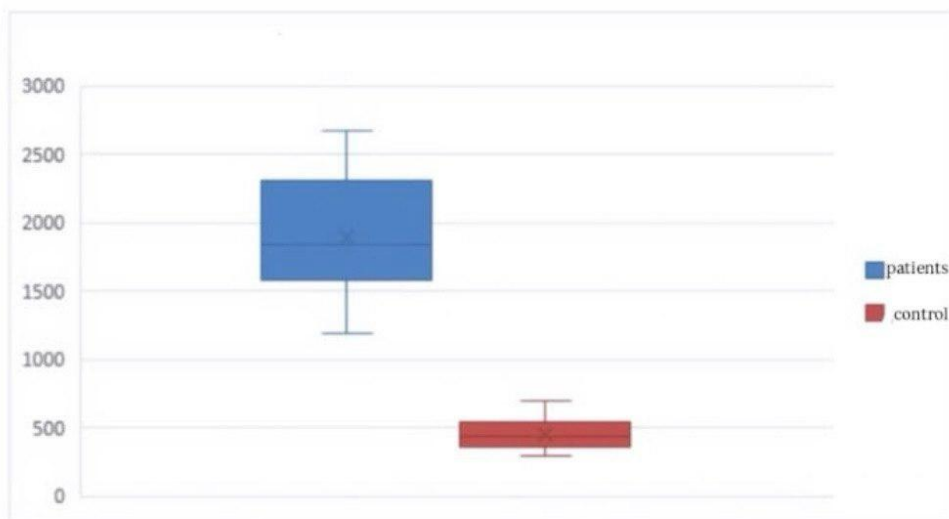


Figure 3: A graph displaying the average serum concentration of IgG antibodies in those who have diabetic foot ulcers and those who do not.

The study's results demonstrated a significant elevation in serum IgM antibody levels in patients with diabetic foot ulcers (337.372.3).

Table 4: Comparing the average serum IgM antibody concentration in diabetic foot ulcer patients and the control group.

The test	P value	Mean ± Standard Deviation	The indicator	
Spiritual	0.00*	337.3±72.3	Patient	The case
		52.1±10.5	Control group	

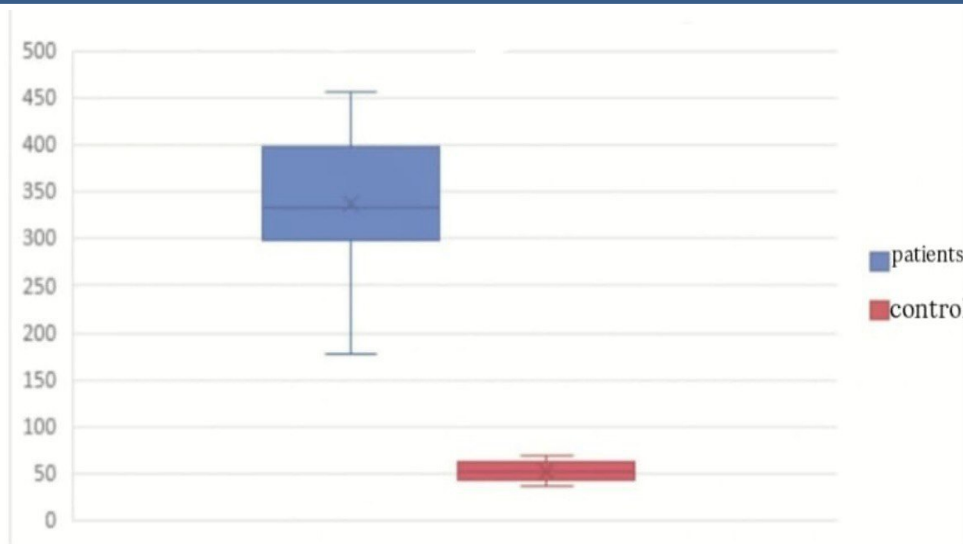


Figure 4: A comparison between the average serum concentration of IgM antibodies in diabetic foot ulcer patients and the control group.

In diabetic foot ulcer patients, the level of complement proteins C3 and C4 increased very significantly compared to healthy person according to the current study results. Previous study has shown that elevated serum C3 levels are connected to obesity, weight gain, high blood pressure, insulin resistance and metabolic syndrome and this gain in complement proteins in line with this determination. C3 and diabetes have been linked, and it has been proposed that the complement activation product (C3a) made by the proteolytic cleavage of C3 may have insulin-like effects, which may partly explain the relationship between C3 and diabetes [5].

The results of another study indicated that the significant increase in C4 levels might be due to either decreased consumption or a malfunction in other parts of the complement system. It is believed that the raise of glucose levels combined with diabetes get negatively affect complement functions through the non-enzymatic glycation of glucose [7].

The serum levels of IgG and IgM antibodies were found to increase significantly in the current study's findings. Several studies have shown that the increase was caused by changes in the humoral immune system's humoral branch, specifically an increase in serum levels of immunoglobulins IgG and IgA. Furthermore, there were significant distinctions in the IgG subclasses observed in DFU patients as opposed to the control group of diabetic patients. Particularly, there was an increase in IgG1 and IgG3 levels, which suggests immune activation induced by inflammation. Their elevated values in cases of infection are not surprising because both molecules are complement activators that bind to the pathogen's specific protein antigen [11]. Type 2 diabetes has been shown to have changes in



the humoral component of the adaptive immune system, which can be seen in the serum of patients with cardiovascular complications, due to an increase in total IgA and IgG levels [12]. In patients with diabetic foot ulcer infections, particularly in those with immunoglobulin deficiency, the response to bacterial infections may change, even though it hasn't been described before in DFU patients with chronic infections [11]. During the IgM analysis, this study's results are consistent with those of prior research, which demonstrated an increase in IgM levels in the serum of diabetic foot ulcer patients. As the condition progresses, patients with acute infections show elevated IgM levels in the early stages, according to the study [13]. Another study confirmed that IgM is the first antibody to rise during the primary immune response, especially in acute bacterial infections. Therefore, the increase in IgM in this study may be attributed to the samples being taken from patients in the early stage of the infection, before it developed into a chronic stage [14]. Nonetheless, these outcomes contradict a previous analysis that found a decrease in IgM levels in the serum of patients with diabetic foot ulcers that were connected to infection, suggesting that impaired humoral immunity is caused by the progression and chronic recurrence of the infection. This discrepancy could be explained by the fact that the individuals in the earlier study were in more advanced stages of the disease or had chronic immune response deficiency, which may not be true for the individuals in the present study who could have been in the early stages of inflammation [15]. IgG, IgM immunoglobulins, and complement C3 accumulate in the nervous system after a period of stimulation and varying degrees of insulin loss in diabetic patients [16].

#### Conclusion

This study concluded that there is a significant increase in the C3, C4, IgG, and IgM levels in the serum of patients with diabetic foot ulcers compared to the control group. These results indicate a clear activation of both innate and adaptive immunity in the context of the inflammatory response resulting from localized or chronic foot infections.

The complement system activating is signified by the increase in C3 and C4 which is a key indicator of defensive against pathogens and indication the severity of inflammation. Alternatively, elevated IgG indicates the stimulation of acquired immunity against a persistent or previous infection while elevated IgM is attributed to an active primary immune response which may reflect an early stage of infection in some patients.



The significance of the immune response in the development of diabetic foot ulcers is underlined by these results and allows for the use of certain biomarkers to indicate infection progression or assess treatment effectiveness. The study recommends conducting prospective search on larger scale to set the precise relationship between these immune indicators and the progression of the disease and its clinical complications

Source of Funding

No funding

Conflict of Interest

No conflict interest

Ethical Clearance

The study was approved by the Ministry of Health's Scientific Research Ethics Committee in Baghdad, Iraq (Reference No. 37692) after complying with the guidelines established by the latest version of the Declaration of Helsinki Ethics.

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