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Evaluation of Immunological Levels of IL-37, IL-38, and IL-17A in Iraqi Patients with Diabetic Foot Ulcers

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Abstract

Diabetes mellitus, a significant cause of mortality around the globe, can result in several secondary complications, including diabetic foot syndrome, which is brought on by diabetic neuropathy and ischemia. Approximately 15% of diabetic patients suffer from diabetic foot complications, which results in high rates of morbidity and mortality of people with Diabetes mellitus. The study's objective is to measure serum levels of IL-37, IL-38 & IL-17A in diabetic mellitus (type 1 & 2) patients with and without diabetic foot ulcer complications and in the control group as well as the possibility of using them as early biomarkers for diagnosis of diabetic mellitus complications & future prevention. Overall, of 193 participants included in this case-control study, they were divided into three groups: the first one contains patients with type 1 diabetes mellitus (29 with diabetic foot ulcer DFU+ 35 non-diabetic foot), the second group includes type 2 diabetes mellitus patients (41 with DFU + 38 Non). The third group includes (50) as apparently healthy controls. Serological techniques of sandwich ELISA did laboratory tests for specific serum human IL-37, IL-38, and IL-17A. The study revealed that IL-37 and IL-17A levels were significantly high ($P<0.01$) in all diabetic groups compared to the control healthy group. The results of IL-38 show substantially higher levels of T1DM and T2DM ($P<0.05$) compared to the control group. In addition, the DFU group of T2DM illustrated higher levels of IL-37, IL-38, and IL-17A compared with other diabetic groups.

In conclusion, Iraqi DM subjects with and without complications had higher values of interleukins, IL-37, IL-38, and IL-17A, than healthy controls, which suggests an inflammatory state in these patients. In addition, the DFU of T2DM patients expressed higher levels of interleukins than other diabetic groups. This can be focused on using them as novel therapeutic targets for preventing and treating DM complications. As well as the possibility of using them as markers of inflammation and progression for complications in DM patients.

تقييم مستويات المناعة لـ IL-37 و IL-38 و IL-17A في المرضى العراقيين الذين يعانون من قرح القدم السكرية

الملخص

يمكن أن يؤدي داء السكري، وهو أحد الأسباب الرئيسية للوفيات في جميع أنحاء العالم، إلى العديد من المضاعفات الثانوية، بما في ذلك متلازمة القدم السكرية، والتي تنتج عن الاعتلال العصبي السكري ونقص التروية. يعاني حوالي ١٥٪ من مرضى السكري من مضاعفات القدم السكرية، ويؤدي ذلك إلى ارتفاع معدلات الإصابة بالمرض والوفيات بين الأشخاص المصابين بداء السكري .

الهدف من الدراسة هو قياس مستويات IL-37 و IL-38 و IL-17A في مصل الدم لمرضى السكري من النوع الأول والثاني الذين يعانون من مضاعفات قرحة القدم السكرية او بدونها وفي المجموعة الضابطة وكذلك إمكانية استخدام هذه الحركيات كمؤشرات حيوية مبكرة لتشخيص مضاعفات مرض السكري والوقاية منها في المستقبل.

إجمالي ١٩٣ مشاركًا ادرجوا في هذه الدراسة؛ تم تقسيمهم إلى ثلاث مجموعات: المجموعة الأولى تضم مرضى السكري من النوع الاول ٢٩ مصابين بقرحة القدم السكرية و ٣٥ غير مصابين بقرحة القدم السكري، والمجموعة الثانية تضم مرضى السكري من النوع الثاني ٤١ مصابين بقرحة القدم السكرية و ٣٨ غير مصابين، أما المجموعة الثالثة فقد ضمت (٥٠) مشارك وهي مجموعة السيطرة.

تم إجراء الاختبارات المعملية بواسطة التقنيات المصلية لELISA ، وتم اختبار مصل الدم المحدد IL-37 ، IL-38و IL-17A. أظهرت نتائج الدراسة أن مستويات IL-37 و IL-17A كانت ذات دلالة عالية ($P<0.01$) في جميع مجموعات مرضى السكري مقارنة بمجموعة السيطرة. بينما أظهرت نتائج IL-38زيادة معنوية مع النوع الأول والثاني للسكري مقارنة بمجموعة السيطرة. بالإضافة إلى ذلك، أظهرت مجموعة المصابين بالقدم السكري للنوع الثاني مستويات أعلى من IL-37 و IL-38 و IL-17A بالمقارنة مع مجموعات مرضى السكري الأخرى.

في الاستنتاج؛ كان لدى الأشخاص العراقيين الذين يعانون من مرض السكري مع أو بدون مضاعفات قيم أعلى للحركيات؛ IL-37، وIL-38، و-IL 17A، مقارنة بمجموعة السيطرة التي تشير إلى وجود حالة التهابية لدى هؤلاء المرضى. بالإضافة إلى ذلك، أظهرت مجموعة المصابين بالقدم السكري من النوع الثاني مستويات أعلى من الحركيات مقارنة بمجموعات مرضى السكري الأخرى، يمكن أن يركز هذا على استخدامها كأهداف علاجية جديدة للوقاية من مضاعفات مرض السكري وعلاجها. وكذلك إمكانية استخدامها كعلامات للالتهاب وتطور المضاعفات لدى مرضى السكري.

1. Introduction

Diabetes mellitus (DM) is a significant health problem worldwide. High blood glucose levels indicate this metabolic disease due to insufficient insulin production or action. The immune response to high blood glucose levels and the presence of inflammatory mediators produced by adipocytes and macrophages in fat tissue causes an inflammatory response. A low and chronic state of inflammation damages pancreatic beta cells, resulting in insufficient insulin production and hyperglycemia. Diabetes hyperglycemia is thought to cause immune response dysfunction, which fails to control the spread of invading pathogens in diabetic subjects. As a result, diabetic patients are known to be more susceptible to infections ((AL-Sahi, AL-Hasnawi and Ali, no date), (Soto-Chávez *et al.*, 2022)).

The most commonly encountered micro-vascular complication of diabetes is diabetic neuropathy, with a prevalence of 50-60%; neuropathy may cause decreased nerve functions and nerve blood perfusion with persistent nerve damage. Diabetic peripheral neuropathy increases the development of foot ulceration risk (Barua *et al.*, 2022).

Interleukin IL-37 is also known as IL-1 family member 7 (IL-1F7). It is a novel anti-inflammatory cytokine with immunomodulatory effects in three ways, i.e., by reducing the synthesis of pro-inflammatory cytokines, lowering the expression of transcriptional cytokines, and inhibiting the activation of kinase signaling (Tan *et al.*, 2022). IL-37 is widely expressed in multiple human tissues and organs, including the skin, heart, kidney, gut, lymph node, thymus, bone marrow, lung, testis, placenta, and uterus (Tian *et al.*, 2022).

However, the expression of distinct subtypes differs according to the specific tissues and organs involved. Under physiological conditions, IL-37a is mainly found in the lymph nodes, thymus, bone marrow, placenta, colon, lung, testicles, and brain, whereas IL-37b is primarily found in the peripheral blood, lymph nodes, placenta, colon, lung, testicles, and kidney. IL-37c is mainly expressed in the lymph nodes, placenta, colon, lung, testis, and heart, whereas IL-37d is predominantly expressed in the testis, bone marrow, blood system, umbilical cord tissue, and adipose tissue mesenchymal stem cells ((Țiburcă *et al.*, 2022), (Tille, 2015), (Tirichen *et al.*, 2021)).

Interleukin (IL)-38 is a recently discovered, novel anti-inflammatory cytokine that belongs to the IL-1β family and inhibits subsequent signaling pathways, thereby regulating the differentiation and function of T cells, peripheral blood mononuclear cells, macrophages, and dendritic cells (Tola, Regassa and Ayele, 2021). Generally, IL-38 is expressed in the human heart, thymus, etc., but not in T cells in the tonsil. Additionally, IL-38 binds to the receptors via nuclear factor kappa-B (NF-κB), activating protein-1 (AP-1) and c-Jun N-terminal kinase (JNK) signaling pathways to regulate the inflammatory cytokines generation ((Turns, 2011), (Ubeid, 2020)). This data indicated that IL-38 might be related to autoimmune diseases. Furthermore, IL-38 may affect the mechanism of autoimmune diseases in regulating the balance of anti-inflammatory and pro-inflammatory. A study by Aravindhan et al. (2022) showed significantly decreased serum levels of IL-38 in diabetes subjects (Aravindhan *et al.*, 2022).

IL-17 secreted by Th17 cells initiates the secretion of pro-inflammatory factors and further amplifies the inflammatory response in inflammatory and autoimmune diseases. Therefore, Th17 and IL-17 might be involved in the pathogenesis of DM (Parhi *et al.*, 2019). The pathogenicity of IL-17 has been well-recognized in several diseases, including psoriasis, rheumatoid arthritis, multiple sclerosis, cancer, and diabetes. Studies (Ma *et al.*, 2022) showed elevated plasma IL-17 levels compared to healthy individuals in patients with diabetes (Țiburcă *et al.*, 2022).

The goal of the present study is to compare the levels of IL-37, IL-38, and IL-17A in diabetic mellitus patients without diabetic foot ulcers (DFUs), diabetic patients with DFUs, and healthy people using (a control group) using the ELIZA technique. To assess any immunological association between IL-37, IL-38 & Il-17A serum levels and diabetic foot ulcer. In addition, it is possible to use them as early biomarkers for diagnosis of DFUs & future prevention.

2. Materials and Methods

2.1 Ethical Approval

Patients involved in this study were informed about the detailed aim of the study, and verbal agreement was obtained from each one before samples were collected. This study was approved by the Scientific Council of Karbala Medical College with reference no. 64.

Experimental design

A case-control study was conducted for six months, from August 2022 to January 2023. All patients enrolled in the study were from Imam AL-Hussain Medical City.

The total number of participants was 193 subjects; they were divided into three groups: the first one includes patients with type 1 diabetes mellitus (29 with diabetic foot ulcer + 35 Non-diabetic foot), the second group includes type 2

diabetes mellitus patients (41 with diabetic foot ulcer + 38 Non-diabetic foot ulcer), and the third group includes (50) as apparently healthy control.
Serological techniques of sandwich ELISA were used to do laboratory tests for specific serum human IL-37, IL-38, and IL-17A. Cat. No. (E-EL-H2571, E3276Hu and E-EL-H0105, respectively).
Inclusion Criteria: The study included any case with a clinical diagnosis of type 1 and type 2 diabetes, with and without Diabetic foot ulcers (DFUs) complications and their duration.
Exclusion criteria include: if a different diagnosis was documented, Pregnancy, patients with multiple autoimmune diseases and a history of current inflammation and infection, patients with cardiovascular disease (CVD), patients taking immunosuppressive therapy, renal failure patients, Liver failure patients, and patients with an age group below 18 years.
Blood Sample collection: Five milliliters of venous blood were taken from each participant. The blood sample was immediately transformed into a gel tube and left to clot for 15 minutes at room temperature (20-25) °C. Then, after collecting, it was centrifuged at (3000 rpm) for approximately (15) minutes to obtain serum. The isolated serum of samples was distributed into three aliquots (0.5ml) in tightly closed Eppendorf tubes. Then, the tubes were stored at -20 C until ELIZA assayed them for immunological markers testing.

3. Results

3.1. IL-37 Level in Diabetes Groups

Comparison of IL-37 among the studied groups revealed a highly significant difference (P<0.001) in the mean level of IL-37 across the studied groups of T1DM and T2DM, as in Table (1.1).

Table 1: IL-37 Levels Among Different Groups

Parameter	**Group**				**Control (N=50)**	
	T1DM (N=64)		**T2DM (N=79)**			**P. value**
	DFU(N=29)	**Non(N=35)**	**DFU(N=41)**	**Non(N=38)**		
IL-37 pg/mL	**Mean± SD**	**Mean± SD**	**Mean± SD**	**Mean± SD**	**Mean± SD**	
	28.20±8.05	23.29±7.06	30.94±9.95	25.02±7.74	16.09±4.10	**<0.01 Sig.**
Multiple pairwise comparisons./ least significant difference (LSD) post hoc test						

Subgroups	**P. value**		
	DFU	**Non**	**DFU vs Non**
T1DM			**<0.05**
T2DM			**<0.05**
T1DM vs. T2DM	**<0.01**	**<0.01**	
T1DM vs. controls	**<0.01**	**<0.01**	
T2DM vs. controls	**<0.01**	**<0.01**	

SD; Standard Deviation of mean, sig: significant**.** P. value (≤ 0.05), (≤ 0.01).

*** SD; Standard Deviation of mean, sig: significant. P. value (≤ 0.05), (≤ 0.01)**

The mean IL-37 was 28.20 and 23.29 in the DFU and non-DFU groups of T1DM, respectively. On the other hand, T2DM had 30.94 and 25.02 mean in the DFU and non-DFU groups of T2DM patients, while the mean of the control group was 16.09. Generally, in all comparisons, the results illustrated a higher level of IL-37 in the T1DM and T2DM patient groups compared with the control (Table 1.1).

As shown in the table above, there were many significant differences when compared with study groups. There are significant differences (p <0.05) when compared between DFU and Nongroups in T1DM and T2DM. In addition, there are highly significant differences (p <0.01) when comparing comparing T1DM and T2DM with the control group. As well as, there are highly significant differences (p <0.01) when compared among DFU and Nongroups T1DM and T2DM diabetic groups.

3.2. IL-38 Level in Diabetic and Healthy Control Groups

A comparison of IL-38 among the studied groups revealed that all groups of T1DM and T2DM had significantly higher levels of IL-38 than control groups. The DFU group of T2DM patients had a higher mean level of IL-38 (3.57) than the DFU group of T1DM (3.05); on the other hand, there was a significant difference (P <0.05) in IL-38 between the studied groups Table 2.

Table 2: IL-38 Levels in Studied Groups

Parameter	**Group**				**Control (N=50)**	
	T1DM (N=64)		**T2DM (N=79)**			**P. value**
	DFU(N=29)	**Non(N=35)**	**DFU(N=41)**	**Non(N=38)**		
	Mean± SD	**Mean± SD**	**Mean± SD**	**Mean± SD**		
IL-38 ng/L	3.05±1.90	2.13±1.87	3.57±1.66	2.55±1.75	1.13±0.40	**<0.05**
Multiple pairwise comparisons./ least significant difference (LSD) post hoc test						
Subgroups	**P. value**					
	DFU	**Non**	**DFU vs Non**			
T1DM			**<0.01**			
T2DM			**<0.01**			
T1DM vs. T2DM	**0.13 ns**	**0.24 ns**				
T1DM vs. controls	**<0.01**	**<0.01**				
T2DM vs. controls	**<0.01**	**<0.01**				
SD; Standard Deviation of mean, sig: significant**.** P. value (≤ 0.05), (≤ 0.01). NS: not significant.						

3.3. IL-17A Level in Studied Groups

The results summarized in Table (3.6) indicated a statistically significant difference (P<0.01) in levels of IL-17A in all diabetes case groups compared to a healthy control group. On the other hand, the level of IL 17 was highest in the group with chronic complication (DFU) with a mean value (37.44), followed by
DFU of T1DM with a mean level (of 25.19), then the Non-group of T2DM with a mean level (of 23.87), and lastly Non-group of T1DM (21.05). While the mean level of apparent control is (6.12). However, there are significantly higher differences (P<0.01) when compared between studied diabetic groups (Table 1.3).

Table 3: Mean Levels of IL-17A in Different Groups

Parameter	Group				Control	P. value
	T1DM (N=64)		T2DM (N=79)			
	DFU(N=29)	Non(N=35)	DFU(N=41)	Non(N=38)		
	Mean± SD	Mean± SD	Mean± SD	Mean± SD		
IL-17A pg/mL	25.19±10.84	21.05±8.19	37.44±10.90	23.87±11.24	6.12±3.80	**<0.01**
Multiple pairwise comparisons. / Least significant difference (LSD) post hoc test						
Subgroups	**P. value**					
	DFU	**Non**	**DFU vs Non**			
T1DM			**<0.05**			
T2DM			**<0.01**			
T1DM vs. T2DM	**<0.01**	**<0.05**				
T1DM vs. Controls	**<0.01**	**<0.01**				
T2DM vs. Controls	**<0.01**	**<0.01**				
SD; Standard Deviation of mean, sig: significant**.** P. value (≤ 0.05), (≤ 0.01).						

4. Discussion

The IL 37 is of the IL 1 family. It has anti inflammatory properties. It appears widely. IL 37 is usually expressed in granule cells and T cells, with the best degree of statement in regulatory T cells (Treg cells) (Alhayali, Yücel and Ashoor, 2021). In this study, the comparison of İL 37 level between different experiment groups, IL-37 was significantly higher (P<0.01) in patients with T1DM and T2DM when compared with the control group. This finding is similar to the findings of Alhayali et al., who found that the mean of IL-37 was higher in patients with DM than in control healthy (Alhayali, Yücel and Ashoor, 2021). In our study, significant high differences were found between the study groups examined in terms of serum IL 37 levels (P < 0.01). Serum IL 37 levels were highest in DFU of T2DM patients and lowest in the healthy control group; the reason for this can be explained by a study in the literature showing that IL 37 administration, at least by reducing local and systemic inflammation, correct the established metabolic disorders caused by diabetic and thus contributes to improved systemic insulin sensitivity. In addition, IL 37 causes a decrease in proinflammatory cytokines. In addition, in diabetics, the production of pro-inflammatory cytokines and chemokines increases, which can attract and activate macrophages and other immune cells; this causes chronic low grade inflammation and promotes diabetic complications (Jia, Liu and Han, 2018).

In the present study, comparing IL-38 among the studied groups revealed that T1DM and T2DM patients had higher levels of IL-38 than controls. Likewise, the level of IL-38 was higher in DFU of T2DM compared with other diabetic groups. These results are consistent with a study (Gurău *et al.*, 2021), which found that plasma IL-38 was more elevated in T2DM patients.

On the other hand, this study's findings contrast with those reported by (Zhao *et al.*, 2020). These findings illustrated that Serum IL-38 levels in T2DM patients were significantly lower than those in controls.

Another study by (Yu *et al.*, 2017) in studied on gestational diabetes showed that IL-38 was increased 3.3, 2.6, or 2.6 fold in chorionic villi (P<0.01), umbilical artery (P<0.05), umbilical vein (P<0.05) from GDM women, respectively, compared to that from non-GDM women and herein, IL-38 produced in the chorionic villi and umbilical

cords may be a response to local inflammation during the development of GDM. Thus, such a dysregulated micro-environment may contribute to the development of GDM via an immune-mediated mechanism.

IL-17, a pro-inflammatory cytokine, has been studied in the development of diabetes. The present data revealed a highly significant increase in IL17A serum levels in all diabetic group patients compared with the healthy group. It is believed that IL17A had a crucial role in the development of diabetic mellitus and its complications, such as DFU. The DFU group of T2DM also showed higher mean levels. These findings are consistent with the studies of Parhi et al., which showed an increase in the level of IL-17 in newly diagnosed diabetes than the healthy controls (Parhi *et al.*, 2019), and one of the possible mechanisms for that is the binding of IL17A with its receptor may enhance activation of metalloproteinase, hypertensive and vascular dysfunction. Another mechanism is the activation of the JAK/STAT pathway that leads to hepatic insulin resistance, beta and liver cell apoptosis, and downregulation of gluconeogenesis-related molecules (Yousefidaredor *et al.*, 2014).

Likewise, the level of IL 17A was even higher in the group of patients with diabetic complications (DFU); this finding is in corroboration with Yousefidaredor et al., who found that IL 17 plays a vital role in the development of T2DM and its complications via the up-regulation of several inflammatory molecules including angiotensin II type I receptor and JAK 2 STAT 3 pathway related molecules ((Yang and Jiang, 2022), (Yuan *et al.*, 2022)). Thus, higher IL17A levels in patients may cause inflammation in the ulceration, deterioration of skin integrity, and various types of bacteria causing infection (Kaleli *et al.*, 2019). These results indicate that IL17A is a contributory factor to the inflammatory process in T2DM and its complications (Kaminski *et al.*, 2019).

5. Conclusion

Iraqi DM subjects with and without complications had higher values of interleukins, IL-37, IL-38, and IL-17A, than healthy controls, which suggests an inflammatory state in these patients. Also, the DFU of T2DM patients illustrated higher levels of interleukins than other diabetic groups, so they might be using them as novel therapeutic targets for preventing and treating DM and preventing organ damage. However, these novel markers (IL-37, IL-38, and IL-17A) might be associated with the progression of diabetes mellitus and may be used as markers of inflammation, progression, and complications in patients with DM.

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