Interleukin 37 Role in Diabetic Iraqi Patients: A comparison between patients treated with and without Insulin

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KEYWORDS
IL 37, DM, FBS, HOMO IR.

ABSTRACT

Background: Diabetes Mellitus (DM) is defined as a metabolic disorder that is characterized by some clinical features, such as chronic hyperglycemia accompanied by impairment of carbohydrates metabolism, proteins, and lipids. However, relative or absolute insulin deficiency is associated with the high blood glucose levels, which is attributed to the β-cells dysfunction, insulin resistance or could be both.

The anti-inflammatory cytokine interleukin-37 (IL-37) negatively regulates inflammation, therefore, reduces inflammation and alters metabolic pathways. It acts by suppressing inflammatory cytokines and chemokines, activation of IL-37 inhibits inflammation and obstructs the infiltration of pro-inflammatory cells, primarily neutrophils and eosinophils. Aim of Study: The estimation of IL 37 and other biochemical parameters in patients with Diabetes mellitus and study its role in the pathogenicity of the disease.

Materials and methods: A case-control study design was adopted to study the relationship between Interleukin 37 and insulin treatment in Nasiriyah in the Diabetes and Endocrinology Specialized Center between December 2023 and January 2024. It was conducted on patients with Diabetes Mellitus type 2 (60 patients) divided into two groups (30 patients treated with Insulin injection) and (30 patient treated with oral tab only). On the other hand, 30 people were taken as a control group without Diabetes Mellitus.

Result: Patients with diabetes mellitus have been noticed to have higher levels of IL-37 in comparison to the healthy control groups. In general, patients with diabetes mellitus have higher levels of IL-37 compared to three groups, G1, G2, and healthy control groups.

Conclusion We demonstrated that serum levels concentration of IL 1b and IL 37 significantly increased in our patients also FBS, HbA1c, Insulin Resistance, HOMO IR, TG, cholesterol, and decrease C- peptide as compared to healthy individuals.

1. Introduction

Diabetes Mellitus (DM) is a metabolic disease marked by persistently high blood sugar levels and varying degrees of impairment in the metabolism of proteins, fats, and carbs. (1) elevated blood glucose levels associated with insulin resistance, β-cell malfunction, or both, and resulting from an absolute or relative insulin deficit. (2) Micro- and macrovascular problems result from structural and functional abnormalities in the vasculature of the organ systems. Organ malfunction, damage, and eventually organ failure Describe how these issues impact the body's organs, especially the heart, kidneys, eyes, and nerves. Retinopathy, a condition linked to eye problems, progresses to blindness. Nephropathy and perhaps renal failure are caused by issues related to the kidneys. (3) Diabetes mellitus is considered as an independent risk factor for heart failure disease, which is one of the most prevalent consequences of diabetes. (4)

About 400 million people worldwide suffer from type 2 diabetes mellitus (T2DM), which has a significant negative impact on public health due to a variety of diabetic complications.(5) Compared to type 2 diabetes, a great deal more is understood about the pathophysiology of other types of diabetes mellitus. The autoimmune-mediated death of β cells is the etiology of type 1 diabetes mellitus (T1DM), which is thought to have a different pathophysiology from type 2 diabetes. Nonetheless, several uncommon types of diabetes mellitus, such as maturity-onset diabetes of the young (MODY), do exhibit clinical parallels with type 2 diabetes. (5) the most frequent pregnancy-related medical problem and the frequency of young women's untreated hyperglycemia and even overt diabetes is rising. Major GDM risk factors include maternal obesity and overweight, later age at childbearing, history of GDM in the past, family history of type 2 diabetes mellitus, and ethnicity.(6)

Hyperglycemia, glucosuria, acidosis, ketosis, polyuria, polydipsia, weight loss despite polyphagia, and, in more extreme situations, coma are examples of clinical indications.(7) Cytokines are essential for the host's defense

against infections and play a critical function as mediators of immune system communication. Apart from immune cells, a wide variety of cells can also produce or release cytokines in response to infection, as seen with interferons, or in response to cellular injury when cell integrity is weakened.[8]

The novel member of the IL-1 family, interleukin (IL)-37, has drawn more attention lately. [9] It has been demonstrated that IL-37, a recently discovered member of the IL-1 family, plays a significant role as an anti-inflammatory cytokine in the development of numerous inflammatory illnesses, autoimmune disorders, and malignancies. Human tissues include the thymus, lymph nodes, testis, and bone marrow as well as different cell types such as dendritic cells, peripheral blood monocytes (PBMCs), and epithelial cells all exhibit high levels of IL-37 expression. This cytokine can be expressed as five different splice variants: IL-37a, IL-37b, IL-37c, IL-37d, and IL-37e. Because it is the most stable isoform among them, IL-37b.[10] Nowadays, obesity is thought to be a chronic inflammatory illness. Patients with obesity overexpress inflammatory markers in their adipocytes, and the condition activates macrophages, chemokines, and other immune cells, resulting in chronic, long-lasting inflammation. Abnormal expression of immune components and the emergence and progression of chronic inflammation are directly linked to insulin resistance. [11] In this study we aimed to evaluate IL-37 and demonstrate it's role in pathogenicity of diabetes.

2. Material and method

Ninety people, ages ranging from 30 to 80, were examined in this study: 60 diabetes mellitus patients divided into two groups (30 patients treated with Insulin injection) (30 patients treated with oral tab only) and 30 controls (healthy). The patients were referred to the Nasiriyah in Diabetes and Endocrinology Specialized Center between December 2023 and January 2024, on patients with Diabetes Mellitus type 2. Using a brief questionnaire, clinical history data, demographics (age, height, duration, and weight), chronic illnesses, and the treatment plan were gathered.

Sample Collection:

Five milliliters were taken from each patient. It was divided into two parts. We put 3 ml in a test tube (10 ml Gel Tube), and 2 ml in an EDTA tube. The specimen for the Gel tube was separated by centrifugation at 3000 rpm for 10 minutes to get the serum.

FBS was done at the time of serum separation.

- The three ml serum was immediately divided into two small tubes (Eppendorf tube 2.0) to do these tests later.
- Three ml separated serum stored at –20 °C for the subsequent assay of IL 37 by ELISA, Insulin resistance, and C-peptide
- FBS; and Insulin resistance these analyses were measured by fully automated. Hemolysis samples were rejected.
- Tow ml whole blood into EDTA tube use for HbA1c test.

Ethical approval: Before participating in the study, all participants were asked to provide informed consent. They were informed of the study procedure and any associated risks to ensure they fully understood what was involved. The study protocol was reviewed and approved by the local ethics committee, including the permission form and subject data. The approval was documented using the reference number 796, dated 27/11/2023.

Results: In this study, a total of 90 individuals were included, 60 diabetes mellitus patients and 30 samples of normal cases as healthy controls. Therefore, to calculate the sample size, a specific formula was used to calculate the equation based on the reported prevalence report. Moreover, disease history and other relevant demographic information were collected from the patients through face-to-face interviews. Based on age, gender and disease history, patient's groups were subdivided into subgroups.

Clinical characteristics of the study groups, including demographic information and laboratory parameters are as shown in Table (1). The mean age level in the patients' group was (50.71±13.37), and the mean level of BMI (29.64±4.45). The age range of participants was (50%) (More than 56) years old, (31.7%) of patients were within (41 -56) years, while (18.3%) of the patients were with the age range of (25-40) years.

Also, the analysis of data illustrated that about (36.7 %) of patients had a disease duration of (More than 10)

years, (6.7%) had a duration of less than one year, and (31.7%) of the patients group had having duration (1-5) years, the analysis of data illustrated that most of the patients (55%) was having obesity, (33%) of patient was overweight, and (11.7%) of patient was normal weight.

Table 1: Study population demographic characteristics (N= 90).

Variables	Groups	Patient	Control
Age. (Years)	25-40 Years	11	8
	41-56 Years	19	20
	More than 56 Years	30	2
BMI. Groups	Normal weight	7	8
	Overweight	20	12
	Obesity	33	10
Sex	Male	30	16
	Female	30	14
Treatment type	Insulin(G1)	30	0
	Oral(G2)	30	0
	Control	/	30
Duration Disease	No	/	30
	Less than one Years	4	/
	1-5 Years	19	/
	6-10 Years	15	/
	More than 10 Years	22	/

Difference between the level of Immune marker IL -37 in the diabetes mellitus cases and control group:

|In comparison to healthy control group, diabetes patients shown an increased range of IL-37. Interestingly, results as shown in (Figure 1) the difference in IL-37 differed significantly between the studied groups. The IL -37 mean was (299.15±87.34) for the patient and (164.12±30.03) for the control.

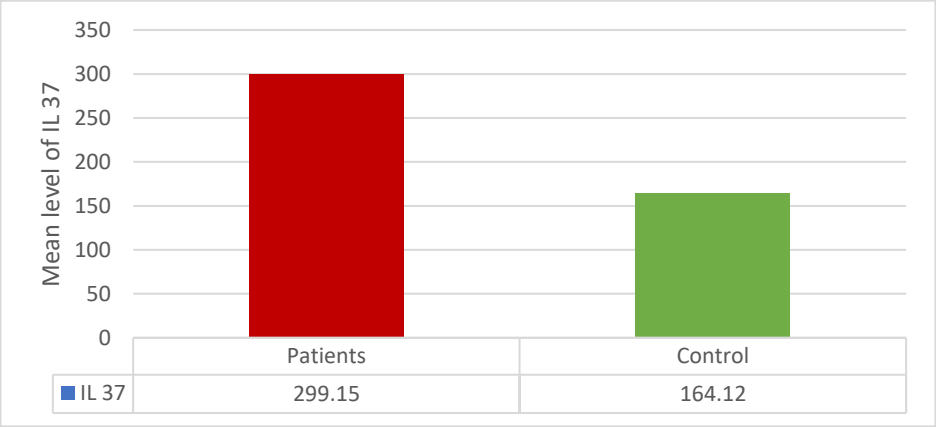

Figure 1: The figure shows the mean levels of IL-37 of treatment patients in comparison to the control group. Statistical analysis using T-test indicated that patients group showed a higher mean IL-37 level compared to the control group ($p \leq 0.05$)

Difference between the level of Diagnostic biomarker of diabetes (FBS and HOMO IR) in the diabetes mellitus cases and control group

Results indicated increasing and highly statistically significant differences in (FBS and HOMO IR) levels among patients and healthy groups. The mean levels of FBS and HOMO IR, were (213.02±76.97) and (7.26±6.23) respectively which were significantly higher than for the control group (88.10±10.94) and (1.71±0.20) respectively, ($p \leq 0.001$). The serum levels distribution of FBS, C-Peptide, HbA1c, IRI, and HOMO IR in patients in comparsion to the healthy control group was shown in Table (2).

SGP-130	Patients Mean±SD N=60	Control Mean±SD N=30	P value
FBS	213.02±76.97	88.10±10.94	<0.001[S]
HOMO IR	7.26±6.23	1.71±0.20	<0.001[S]
significant at $p \leq 0.05$ SD: standard deviation			

Difference between the level of Immune marker for diabetes mellitus Patients (Insulin-dependent (G1) and Non-Insulin dependent (G2)) compared to the control group

Diabetes mellitus patients showed a higher level of IL-37 in comparison with G1, G2 and the control group. Among the studied groups, IL-37 was significantly different, descriptive statistics related to it are as indicated by Figure 2. The IL -37 mean level was (251.74±69.50), (346.55±77.71), and (164.12±30.03) for the G1 respectively by using post-HOC (LSD) which was significantly higher for the G2 and Control ($p \leq 0.001$).

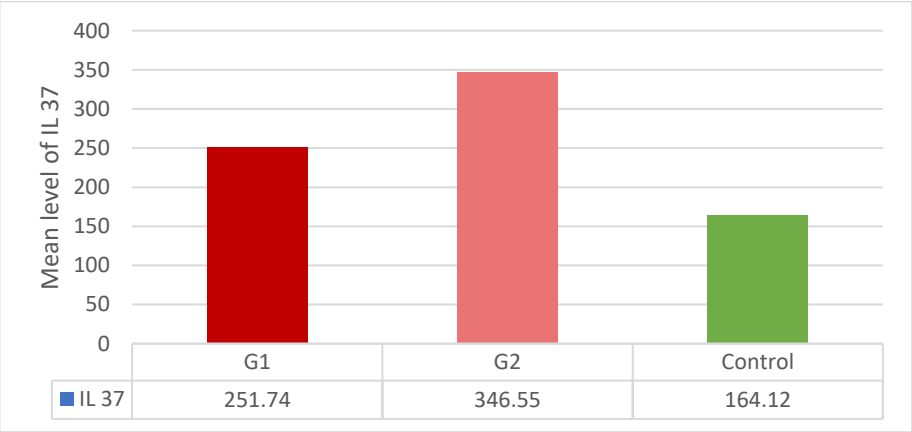

Table 2: The difference in IL 37 for diabetes mellitus disease (G1 and G2) and control

Difference between the level of Diagnostic biomarkers of diabetes for diabetes mellitus Patients (G1 and G2) compared to the control group.

Biomarkers	G1 Mean±SD N=30	G2 Mean±SD N=30	Control Mean±SD N=30	P value
FBS	232.63±88.37	193.40±58.70	88.10±10.94	<0.001[S]
HOMO IR	9.33±7.13	5.18±4.39	1.71±0.20	<0.001[S]
ANOVA -test was *: significant ($p \leq 0.05$), N: cases number; G1= Insulin, G2= Oral				

Difference between the levels of biomarkers for diabetes mellitus Patients G1 and G2

Interestingly, G2 diabetes mellitus group showed a higher level of IL-37 in comparison to G1, as depicted in (figure 3). The mean level of IL -37 was (251.74±69.50) for the G1 which was significantly less than G2 ($p \leq 0.001$).

Figure 3: Results of the analysis of basic diabetes mellitus for patients with control groups (G1= Insulin, G2= Oral).

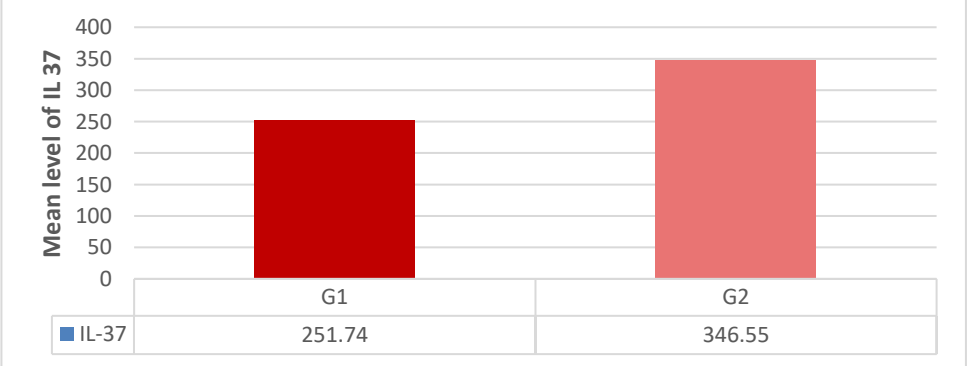

Table 4: The difference in biomarkers for diabetes mellitus disease compared G1 and G2

SGP-130	G1 Mean±SD N=30	G2 Mean±SD N=30	P value
FBS	213.02±76.97	88.10±10.94	<0.001[S]
HOMO IR	7.26±6.23	1.71±0.20	<0.001[S]
T-test was *: significant at $p \leq 0.05$, SD: standard deviation; S: significant; NS= Non-significant. G1= Insulin, G2= Oral			

8. Examination of the mean differences in the IL -37 levels according to the Sex groups

The results shown a significant difference in IL-37 levels in both males and females incomparsion to the control group, p values were <0.001.

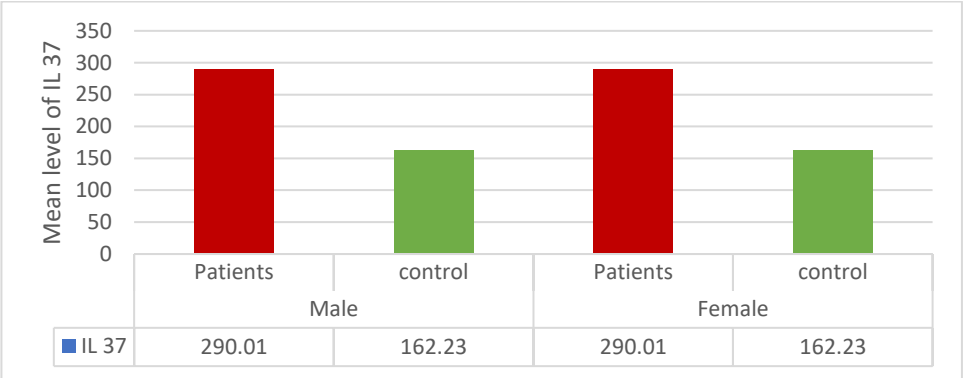

Figure 4: IL-37 differnces based based on gender

The effect of gender on the biochemical parameters according to the Patients and control groups

9. Examination of the mean differences in the IL -37 levels according to the BMI groups

A comparison of serum levels of IL -37 in different BMI groups see Figure (5). Both levels of IL -37 were increased within all the BMI ranges and were highly statistically significant, (p= <0.001).

10. correlation|

In this study, Spearman rank test analysis was utilized to analyze the relationship between the tested parameters in this experiment. Therefore, different related correlations have been noticed (figure 6).

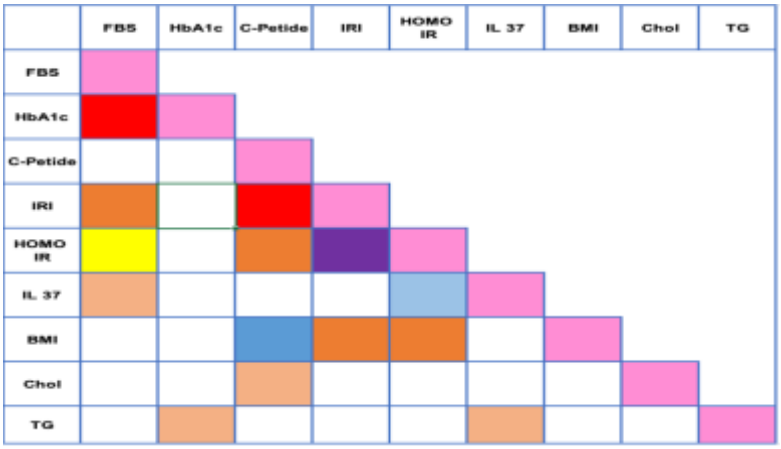

Figure 6:A heat map representation of Spearman rank test analysis for diabetes mellitus patients. Based on correlation (p>0.05), white boxes are not correlated, coloured boxes are correlated (direct or indirect) and statistically significant. The colour intensity is expressed as following: Yalow (r= 0.7); Red (r=6), Purple

(r=0.9) Orange (r=0.3); Light Orange (r=-0.3); Blue (r=-0.4); Light Blue (r=0.4);

3. Discussion

Diabetes mellitus is a metabolic disorder characterized by consistently elevated blood sugar levels. This condition can be caused by failed insulin function, secretion, or both.[12] The global prevalence of type 2 diabetes mellitus (T2DM) is on the rise, and it is closely linked to the increase in obesity. Several mechanisms have been proposed to explain this connection, which include insulin resistance (IR), beta cell dysfunction, reduced amounts of circulating insulin resulting in death, oxidative stress, and inflammation.[13]

Our latest study examined the expression levels and clinical importance of the IL-37 in patients with diabetes mellitus. Our findings revealed a significant negative correlation between the expression of IL-37 and fasting blood sugar levels, suggesting that IL-37 may inhibit the development of diabetes mellitus complications.

After conducting extensive research and analyzing the data, we found that the HOMO IR was high, whereas IL 37 was either normal or at the upper limit. The data also revealed a significant correlation between HOMO IR, FBS, and IL 37. Notably, the correlation between IL37 and HOMO IR was statistically significant in the negative direction, which supports our finding of Tianyi Li [14]. Insulin resistance conditions, such as type 2 diabetes and metabolic syndrome, are commonly associated with elevated plasma lipid levels and obesity. Insulin plays a critical role in obese individuals as adipocytes rely on insulin the most. The number of insulin receptors mainly regulates the cellular response to circulatory insulin. A reduction in this number significantly lowers insulin sensitivity. Insulin receptor function is necessary for both insulin binding and signal transduction. Therefore, any mutation in insulin receptors significantly impairs insulin binding and related functions. As GLUT-4 is essential for the uptake of glucose into insulin-dependent cells, any defects in the protein could prevent it from performing as intended. A single alteration to this transporter can significantly affect the uptake of glucose into cells and the resulting signaling cascades. [15]

The present study found that levels of IL-37 were increased in both group 1 and group 2 across all BMI ranges Fadwa E. Alhayali[16].

The study suggests that patients with type 2 diabetes who are overweight or not obese have higher levels of IL-37 as compared to healthy individuals. This indicates that IL-37 may play a role in the development of type 2 diabetes by contributing to minor changes. Furthermore, the study supports the theory that there is a strong correlation between insulin resistance and inflammatory cytokines induced by excess body fat. Dandona, Aljada [13]. Our study revealed that the level of IL-37 in patients undergoing insulin therapy (G1) was significantly lower compared to those taking oral medication (G2) for diabetes treatment. This indicates that insulin therapy can effectively reduce the inflammatory processes which may help to prevent complications associated with diabetes. Our findings are in line with the results of a previous study and add to our understanding of the mechanisms behind anti-diabetic medications. Moreover, our data showed that the treated group had a considerable reduction in the expression level of IL-37 in diabetic cases when compared to healthy individuals. This applies to what we found in the study by Abeer M. Abd El-Hameed [17].

Surprisingly, study results indicated that there was no relationship between the disease and control groups based on gender. However, it was observed that the levels of IL-37 were higher in the patient group for both males and females as compared to the control group. This finding is consistent with previous research on this topic Yinkun Yan [18]. which found no statistically significant sex difference.

4. Recommendation

More carefully monitored research is needed to better understand how chronic inflammation affects glucose metabolism and insulin resistance.

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