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INTERLEUKIN-40 AS A PREDICTIVE TOOL OF RESPONSE TO TREATMENT FOR INSULIN RESISTANCE IN PATIENTS WITH TYPE 2 DIABETES

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Abstract

Background: *Type 2 diabetes mellitus (T2DM) is a prevalent chronic metabolic disorder primarily characterized by impaired insulin secretion from pancreatic β-cells or insulin receptor dysfunction. Both defects are linked to dysregulated cytokine production. A complex interplay between pro-inflammatory and anti-inflammatory cytokines is hypothesized to contribute to T2DM development and its complications. The present investigation sought to establish a novel biochemical association between IL-40 and type 2 diabetes mellitus (T2DM) and to assess IL-40 levels in serum samples obtained from T2DM patients and healthy controls.* ***Materials and Methods****: A study involving 90 participants investigated the levels of interleukin-40 in the blood. The participants were divided into two main groups: patients with a condition and healthy individuals: The patient group was further divided into three subgroups: Group 1 (G1) consisted of 20 patients (equal numbers of males and females) receiving only insulin-deficient medications. Group 2 (G2) included 20 patients (balanced male and female representation) who were treated with both insulin-deficient medications and medications to lower insulin resistance. Group 3 (G3) comprised 30 patients (15 males and 15 females) who solely received insulin resistance-lowering medications. The healthy control group included 20 individuals (10 males and 10 females) between 30 and 60 years of age who showed no signs of the condition. A technique called Sandwich-ELISA was used to measure the amount of interleukin-40 in the blood samples collected from all participants.* ***Results:*** *The study shows that there is significant statistical difference* ***(p=0.000)*** *in interleukin-40 levels when comparing the group of type 2 Diabetes disease patients to the healthy group. Statistically significant differences were observed in interleukin-40 levels between diabetic patients treated exclusively with insulin deficiency medications, the group of patients taking medications to improve insulin resistance with treatment for insulin deficiency* ***(p=0.008)*** *and the third group of patients taking medications to improve insulin resistance only* ***(p=0.043)****. In addition, statistical discrepancies in interleukin 40 levels were identified between group 1 and healthy controls* ***(p=0.022)****. the study identified no significant variations in interleukin 40 levels between males and females within each group (healthy individuals or diabetics). The only exception was the second group of patients who were administered both insulin deficiency drugs and medications to improve insulin resistance. In this particular group, a significant difference in interleukin 40 levels between the sexes was observed* ***(P=0.003).****The same trend holds true for comparisons between females within the type 2 diabetes (T2DM) groups, with one exception. Within the T2DM group, females in (G1) had significantly higher interleukin-40 levels compared to females in (G2) and (G3). This difference was also observed when compared to the healthy control group. Statistically significant differences were found between G1 females and G3 females* ***(p=0.015)****, between G1 females and G5 females* ***(p=0.000),*** *and between G1 females and the control group* ***(p=0.000)****. "Similarly, no significant difference in interleukin-40 levels was observed when comparing males within T2DM and with control groups. However, interleukin-40 levels in G2 males were significantly higher compared to males in G4* ***(p=0.000)****, and males in G6* ***(p=0.000)*** *And the males in the control group (G8),* ***(p=0.003). Conclusions****.Interleukin-40 is a good parameter for evaluating the T2DM patients respond to treatment in the three disease groups, regardless of the type of treatment used*

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Introduction

Cytokines, particularly interleukin 40 (IL-40), play a pivotal role in the immune response. IL-40, a recently discovered cytokine, is intricately involved in the regulation of B-cell homeostasis and mixed immune responses[1]. Additionally, it plays a critical role in the development of B-cells. IL-40 was expresses by activated human B-cells, in addition to human lymphoid B-cell tumors[2]. Notably, stimulation of B-cells with IL-40 and TGF-β enhances IL-40 secretion[3]. IL-40 belongs to a class of cytokines known as "orphan cytokines." These cytokines lack significant sequence homology with established cytokine families due to their unique structural features[4]. The C17orf99 gene encodes IL-40, a novel cytokine derived from B cells.IL-40 expression is typically elevated in activated B cells residing in the fetal liver and bone marrow[5].Emerging evidence suggests that IL-40 plays a pivotal role in various human diseases, including lymphoid tumors, autoimmune conditions, and type 2 diabetes[6].

Type 2 diabetes mellitus (T2DM) is a prevalent chronic metabolic disorder primarily characterized by impaired insulin secretion from pancreatic β-cells or insulin receptor dysfunction. Both defects were links to dysregulated cytokine production. A complex interplay between pro-inflammatory and anti-inflammatory cytokines was hypothesizes to contribute to T2DM development and its complications. Consequently, cytokines like interleukin-40 (IL-40)[7].

Materials and Methods

The Study Population: The current study involved 90 individuals, divided into four groups, three of which (70 cases) were previously diagnosed with type 2 diabetes mellitus (T2DM). The first patient group (G1) included 20 patients (10 males and 10 females) who treated with insulin-deficient medications only. Group two (G2) also included 20 patients (10 males and 10 females) who were treated with insulin-deficient medications in addition to insulin resistance-lowering medications. While the third patient group (G3) included 30 patients (15 males and 15 females) who were treated with insulin resistance-lowering medications only. The last group included 20 healthy individuals (10 males and 10 females). Demographic information indicated that all study patients treated to manage the symptoms of type 2 diabetes, while members of the control group did not take any medication during the sampling period or before the start of the study, with the emphasis that individuals in this group do not suffer from any type of inflammatory diseases.

Samples Collection: **Type 2 diabetes mellitus (T2DM) cases recruited from the Diabetes and Endocrinology Center at Al-Sadr Medical City in Najaf Governorate, Iraq. Healthy control samples were collected from the study community environment, including homemakers, postgraduate students, and hospital workers. Matched patient samples also collected.**

The current study required the exclusion of the following cases:

- All patients who experienced complications resulting from the progression of type two diabetes mellitus.
- Participants (type either two diabetes mellitus patients or healthy individuals) who suffered from chronic diseases, namely; liver, kidney, thyroid, cardiovascular, and autoimmune diseases, hypertension, and morbid obesity were not allowed to participate in the current study.
- Cases that underwent surgery within 5 years, smokers, and alcohol consumers also excluded.

The study involved collecting 5 milliliters of blood from the vein of each participant (both patients and healthy individuals). These samples collected in tubes containing a gel separator. After spinning the samples in a centrifuge at 5,000 times gravity for 5 minutes, the clear liquid portion, called serum,

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separated. This serum was then transfers to Eppendorf tubes and stored frozen at -20°C until it was needed for further analysis.

Assessment of interleukin-40 in the study groups: The Sandwich ELISA technique was employed to quantify the concentration of interleukin-40 within the serum samples collected from the participants of the study.

Statistical analysis of the data: This study's findings were analyzed using two software programs: IBM SPSS Statistics version 26 and Microsoft Excel. SPSS was used for the statistical analysis. Descriptive statistics were employed to summarize the data, including mean, standard error (SE), minimum, maximum, frequencies, percentages, and cumulative percentages. A rock curve was generated to illustrate the sensitivity of the measured parameters.

Inferential statistical analysis was conducted to assess potential differences between the evaluated biochemical. One-way analysis of variance (ANOVA) was used for this purpose. A *p-value* less than 0.05 was considered statistically significant, indicating a probable difference between the groups compared to the control. Additionally, sensitivity and specificity percentages were calculated based on established biomedical statistical methods.

Results and Discussion

Levels of serum Interleukin-40 were measured in the four studied groups; three of whom (70 participants) had been diagnosed with type 2 diabetes mellitus (T2DM) prior to the study. The first group (G1) consisted of 20 patients (10 males and 10 females) who received treatment solely with insulin deficiency drugs. The second group (G2) also included 20 patients (balanced between males and females) who were administered both insulin deficiency drugs and medications to improve insulin resistance. The third group (G3) comprised 30 patients (15 males and 15 females) who were given drugs to enhance insulin resistance. For comparison, the fourth group a healthy control group of 20 participants (10 males and 10 females). **Table 1**shows that there is significant statistical difference **(p=0.000)** in interleukin-40 levels when comparing the group of type 2 diabetes disease patients to the healthy group.

Table 1: Interleukin-40 Levels in the Sera Samples of Patients with Diabetes Disease <and Controls Groups

Parameter	Subjects (N)	Mean ± S.D.	Minimum-Maximum Range	*p-value*
Interleukin-40 (ng/L)	***Patients(70)***	**4.339±0.853**	**2.90 – 6.96 4.06**	**0.000 For G1 *vs* G2**
	Controls(20)	**4.080±0.540**	**2.70 – 4.80 2.10**	

The difference is considered significant at p<0.05.

From the statistical data in **Table 2**, the study determined that there were statistically significant differences in interleukin 40 levels between G1 patients with type 2 diabetes and those taking treatment for insulin deficiency when compared with the rest of the G2 and G3 patient groups, as well as when compared with the healthy control group. Statistically significant differences were found between G1 and G2 **(p=0.008)**, between G1 and G3 **(p=0.043)**, and between G1 and the control group **(p=0.022)**. No statistically significant differences in interleukin-40 levels were observed between the second patient group (receiving medications for insulin deficiency and insulin resistance) and group G3, nor between the second group and the healthy control group, or between group G3 and the control group."

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Based on the statistical analysis presented in Table 3, no significant differences in interleukin-40 levels were observed between male and female participants within each group (healthy controls and diabetic patients). The only exception to this finding was the second group of diabetic patients who received a combination of insulin deficiency medications and insulin resistance therapies. In this particular group, a significant difference in interleukin 40 levels between the sexes was observed **(p=0.003).**

Table 2: Levels of Interleukin-40 (ng/L) in the Sample of the Study Individuals

Subjects (n)	Interleukin-40 (pg/mL) Mean ± SD	Minimum-Maximum	p-value
G1 Patients (20)	**4.587±0.611**	**2.988-6.960**	**0.008 For G1 vs G2**
G2 Patients (20)	**3.819±0.913**	**0. 8528-5.323**	**0.043 For G1 vs G3**
G3 Patients (30)	**4.241±0.406**	**3.862-6.635**	**0.022 For G1 vs C**
Controls (20)	**4.080±0.540**	**2.700-4.800**	0.143 For G2 vs G3 0.344 For G2 vs C 0.427 For G3 vs C

G1: Patients taking medications to address insulin deficiency, G2: Patients receiving medications for both insulin deficiency and insulin resistance, G3: Patients on medications to improve insulin sensitivity (reduce resistance),Controls: Healthy individuals. The difference is Considered Significant at p<0.05.

The same trend holds true for comparisons between females within the type 2 diabetes (T2DM) groups, with one exception. Within the T2DM group, females in (G1) had significantly higher interleukin-40 levels compared to females in (G2) and (G3). This difference was also observed when compared to the healthy control group. Statistically significant differences were found between G1 females and G3 females **(p=0.015)**, between G1 females and G5 females **(p=0.000),** and between G1 females and the control group **(p=0.000)**."Similarly, no significant difference in interleukin-40 levels was observed when comparing males within T2DM and with control groups. However, interleukin-40 levels in G2 males were significantly higher compared to males in G4 **(p=0.000)**, and males in G6 **(p=0.000)** and the males in the control group (G8), **(p=0.003).**

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Table 3: Levels of Interleukin-40 (ng/L) in the Sample of the Study Individuals

Subjects (n)	*Sex (n)*	*Il-40 (ng/L) Mean ± SD*	*Minimum-Maximum Range*	*p-value*
G1 Patients (20)	Female 10	5.218±1.015	3.540-6.960 3.420	0.442For 1*vs*2 0.003For 3*vs* 4 0.978For 5*vs* 6 0.892For 7*vs* 8 0.015For 1*vs* 3 0.000For 1*vs* 5 0.000For 1*vs*7 0.087For 3*vs*5 0.146For 3*vs*7 0.901For 5*vs*7 0.000For 2*vs*4 0.000For 2*vs* 6 0.003For 2*vs* 8 0.121For 4*vs* 6 0.092For 4*vs*8 0.765For 6*vs* 8
	Male 10	4.992±0.983	3.540-6.440 2.900	
G2 Patients (20)	Female 10	4.489±0.474	4.100-5.700 1.600	
	Male 10	3.602±0.622	3.000-4.720 1.720	
G3 Patients (30)	Female 15	4.027±0.446	2.900-4.600 1.700	
	Male 15	4.020±0.484	3.100-4.600 1.500	
Controls	Female 10	4.060±0.582	2.200-4.800 1.600	
	Male 10	4.100±0.525	2.700-4.500 1.800	

When: 1, 3, 5, 7 are refer to Female Subgroups in the G1, G2, G3 and Control Groups; respectively, while:2, 4, 6,8 are refer to Male Subgroups in the G1, G2, G3 and Control Groups; respectively. The mean difference is significant at the 0.05 level.

Cytokines are proteins with low molecular weights that are produced by a group of cells, and they are involved in inflammation and immunity[8].There are many factors that affect the level of cytokines, including age, the treatment used, as well as demographic and clinical variables [9].Cytokines contribute to cancer, infection, inflammation, and autoimmunity[10]. The recent identification of interleukin-40 as a B cell-associated cytokine with roles in both humoral immune responses and B cell homeostasis and the pivotal role of B cells in autoimmune diseases prompted us to study the function of interleukin-40 in type 2 diabetes. In a previous study in 2022 by S. W. Nussrat and A. H. Ad'hiah, significant differences in interleukin-40 (IL-40) levels were observed between patients with type 2 diabetes mellitus (T2DM) and healthy controls. IL-40 was proposed as a biomarker to discriminate between patients and controls, without considering the type of treatment patients were receiving[7]. This study aims to assess the body's response to treatment and prevent complications of T2DM by evaluating IL-40 levels in patients receiving different types of treatment.

In addition, a previous study conducted by Rasha and Zainab in 2023 found that interleukin 17 (IL-17) also plays a role in the development of T2DM in treated patients. They noted that IL-17 levels were higher in the groups receiving treatment for insulin resistance compared to other groups of patients receiving different treatments and healthy controls. What our study found was that levels of IL-40 were higher in the group of patients treated with insulin compared to other treatment groups and healthy controls [16].

A previous study conducted by A Navrátilová and colleagues in 2021 looked at the relationship between interleukin 40 (IL-40) and rheumatoid arthritis (RA). They found statistically significant differences in IL-40 levels, with higher levels observed in RA patients and lower levels after treatment. This suggests

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a role for IL-40 in the development of inflammation and tissue destruction in rheumatoid arthritis. Our study also supports this idea by showing a role for IL-40 in destroying pancreatic beta cells, the cells responsible for insulin secretion.The results of A Navrátilová and colleagues in 2021 and 2023 also showed that IL-40 levels are elevated in rheumatoid arthritis and decrease after B-cell depletion therapy. The association of IL-40 with autoantibodies and chemokines may indicate its possible involvement in the development of rheumatoid arthritis[3, 11]. Furthermore, IL-40 regulates the secretion of chemokines and MMP-13 by synovial fibroblasts, suggesting its role in regulating inflammation and tissue destruction in rheumatoid arthritis. This is what ZAG Al Ghuraibawi and his colleagues reached in Egypt in 2022, when it was revealed that the increased level of serum IL-40 and its potential diagnostic role in rheumatoid arthritis[12]. Another study by C Rizzo, ML Pizzo, L Mohammadnezhad, and others in 2023 reported a significant increase in IL-40 levels in patients with primary Sjögren's syndrome (pSS). In addition, the parotid glands of patients with pSS-associated non-Hodgkin lymphoma showed significantly increased IL-40 expression[13].Another study by the same investigators in 2022, the first evidence of IL-40 expression at the kidney level in SLE-associated nephritis, pointed to an active role of IL-40 in SLE, with a particular focus on active kidney disease. The study results also highlighted the potential use of IL-40 as a marker of active GN, although its specific mechanism of action needs further clarification[14].This was demonstrated by W. L. Abdullah and R. M. Abed in 2023 in Iraqi women, where they indicated serum levels of IL-37 were decreased in the serum of female SLE patients. While the level of interleukin 40 in the blood serum of females with lupus erythematosus increased compared to the control group. Thus, these interleukins can be considered as biomarkers in the pathogenesis of SLE[15].It is important to note that the relationship between interleukin 40 and type 2 diabetes is still only partially understood. More research is needed to fully understand the role of interleukin 40 which plays a major role in many diseases and has potential to be used as a biomarker.

Efficiency of interleukin-40 in the diagnosis of Type 2 Diabetes disease: In order to illustrates the diagnostic ability of interleukin-40 for Type 2 Diabetes disease , ROC-AUC curve was tested. Table 3 illustrated that the sensitivity of interleukin-40 was 89% when 70 of T2DM cases were illustrated interleukin-40 levels higher than mean of these criteria recorded in the healthy controls, while the specificity of inerleukin-40 was 72% in T2DM group.

Table 4: Receiver Operating Characteristic Analysis of the interleukin-40 as Diagnostic Markers for Diabetes

IL-40	AUC	SE	p-value	Cutoff value	Sensitivity%	Specificity%	CI (95%)
	0.594	0.047	0. 000	2.724	89	72	0.422-0.685

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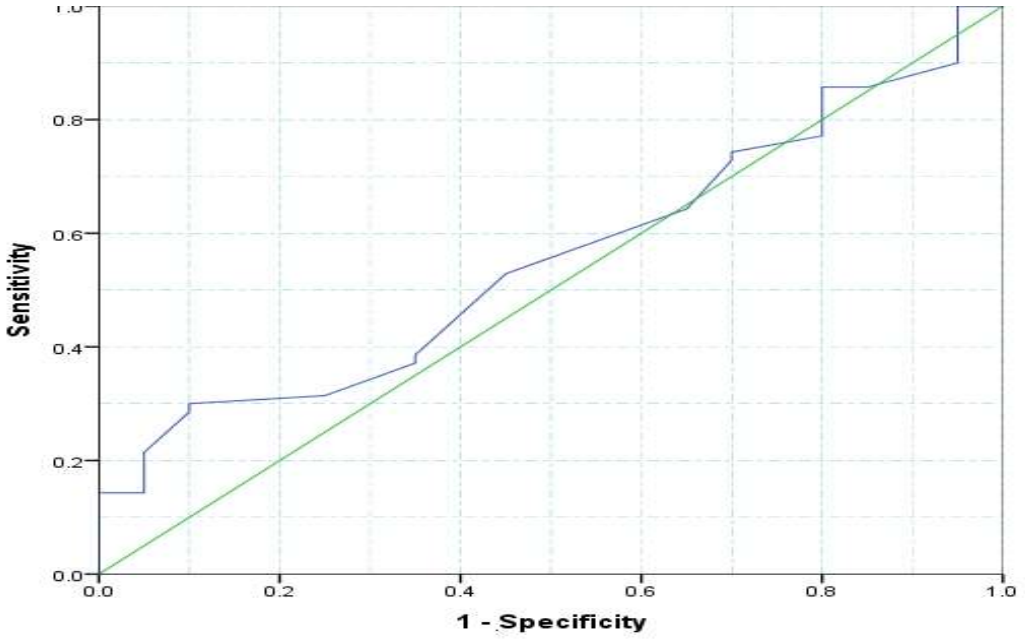

Figure 1: Receiver Operating Characteristic Curve of interleukin-40

Conclusions

Our study suggests that IL-40 may serve as a novel and effective biomarker to distinguish individuals with type 2 diabetes taking insulin from healthy individuals.

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