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Study the Relationship of Biochemical and Immunological (IL-23) Level and *SOCS3* Expression in Diabetes Type 2 Patients

[1]Aya H. Saleh, [2]Mohammed I. Nader

[1]Al-Alawyia Pediatric Teaching Hospital.
[2]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.

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Abstract: diabetes mellitus, a metabolic disorder distinguished by chronically high blood glucose levels, includes thirst, weight loss, and excessive urination. In diabetic patients, there is a correlation between the overexpression of the cellular signaling inhibitor *SOCS3* and both insulin resistance and glucose levels. The present research aims to examine the impact of IL-23 on the expression of the *SOCS3* gene. In the present study, 120 individuals were Involved between November 2023 and January 2024, 60 of whom were diagnosed with Type 2 Diabetes Mellitus (T2DM) treated with Glucophage and Amaryl, excluding patients who use sitagliptin as a treatment `and the remaining 60 were apparently healthy. To evaluate the level of *SOCS3* Each and every individual had blood samples taken and RNA was extracted. cDNA synthesis was performed, and real-time PCR was used for analysis. Compared to healthy individuals, *SOCS3* gene expression was upregulated in T2DM patients (fold change: 3.75). Moreover, T2DM patients had elevated IL-23 levels, suggesting a correlation with disease activity.in conclusion, our findings indicate that *SOCS3* expression and IL-23 are positively linked with insulin resistance indicators in a diabetic environment.

Keyword: Type 2 diabetes mellitus, SOCS3, Interleukin-23, gene expression.

Corresponding author: (Email: ayah.Hazem2300m@ige.uobaghdad.edu.iq).

Introduction

Type 2 diabetes (T2DM) is a multifactorial chronic disease that is initiated by the interplay of genetic and environmental influences. It has a global impact, impacting more than 425 million individuals (1). Insulin resistance and beta-cell deficiency are both contributory factors in the pathogenesis of disease (2). Although there is currently no known treatment for diabetes, previous medical data suggests that its effects can be effectively managed through routine medical examinations and appropriate health management. Elevated blood sugar levels are characterized by increased thirst, appetite, and frequency of urination (3). In contemporary countries, DM is widely regarded as a prevalent ailment. Approximately 90-95% of individuals diagnosed with diabetes mellitus have type 2 diabetes mellitus. It is more commonly observed in adults, especially in persons who are overweight (4). The IL-23 is a heterodimeric cytokine that belongs to the IL-12 family of cytokines. The protein is comprised of two subunits, p40 (which is also found in IL-12) and

p19 (a component linked to IL-12 p35). It is released by many immune cells, including natural killer cells and dendritic cells. The protein is composed of 189 amino acids and has a molecular mass of 20730Da. Its gene is located on human chromosome 12q13.3 (5). IL-23 contributes to the inflammation associated with low levels of T2DM. During persistent infections, antigens activate dendritic cells and macrophages, leading to the synthesis of IL-23. This, in turn, stimulates the production of IL-17. In addition, IL-23 stimulates the synthesis of IL-6, IL-1, and tumor necrosis factor (TNF)-α through the autocrine/paracrine route. IL-23 is involved in the pathogenesis of T2DM (6). The suppressors of cytokine signaling (*SOCS*) family consists of molecules that play a role in the inhibition of cytokine-induced signaling. They play a role in reducing the activity of insulin signaling pathways. *SOCS1* and *SOCS3* specifically target insulin receptor substrate (IRS) 1 and 2 molecules in the insulin signaling pathway for destruction by the proteasome. Consequently, elevated levels of *SOCS1* and *SOCS3* may cause impairments in insulin signaling, leading to the development of hyperglycemia (7).

Materials and method

Five milliliters of blood were obtained from 60 individuals diagnosed with T2DM (31 men and 29 women) who were treated with Glucophage and Amaryl, excluding patients who use sitagliptin as a treatment as well as from individuals who are in good health (31 men and 29 women) *via* venipuncture following a 12-hour overnight fast.in the age group of 35 – 74 years who attended Sheikh Zayed Hospital in the period between November 2023 and January 2024. The blood samples were later divided into two tubes: the first tube contained EDTA and was used to quantify HbA1c and conduct molecular investigations. The second tube, placed in a gel tube, was used to collect serum and subsequently held at -20˚C until required for measuring fasting blood sugar interleukin levels using ELISA.

Fasting Blood Glucose measurement

FBG was determined using the Fuji Dri-Chem NX600 (Fuji film-Jaban) Reference number: 06489205.

Hemoglobin A1C measurement

The measurement of HbA1c was determined using the DCA Vantage analyzer (Siemens- Germany) serial number: 17223119.

The concentration of IL-23 measurement in Serum

The IL-23 levels in the samples were measured using a sandwich ELISA kit (cloud-clone corp.- USA) with the ELISA system (Huma Reader HS- Germany). The procedure was carried out in compliance with the instructions provided by the manufacturers.

Molecular examinations

RNA extraction

RNA was isolated from EDTA samples using Triazole TM Reagent. The samples were then subjected to ethanol washing, isopropanol precipitation, chloroform extraction, and rehydration in nuclease-free water. The RNA content and purity were assessed using a Quintus Fluorimeter and Quanti fluor Dye for detection.

Reversed transcription

The reaction was carried out in a volume of 20 µl according to the manufacturer's instructions as shown in Table 1. Macro gen Korea's Add Script Reverse Transcriptase Kit was used to convert total RNA to complementary DNA (cDNA). RNA was detected using

RNA-specific primers as listed in Table (2). The lyophilized form of these primers was provided by Microgen Company. The lyophilized primers were reconstituted in nuclease-free water to provide a stock solution with a final concentration of 100pmol/µl. To create a functional solution of these primers, 10µl of the primer stock solution (kept in a freezer at -20 C) was combined with 90µl of nuclease-free water. This resulted in a functioning primer solution with a concentration of 10pmol/µl. The data that were used to complete the program (heat cycle stages) of the cDNA reverse transcription process as listed in table (3).

Table (1): Components of reaction Real-time.

Constituents	20µl
2.5 Reaction Mixture	10µl
Mgcl2	1µl
Forward primer	1µl
Reverse primer	1µl
Nuclease Free Water	4µl
Complementary DNA (cDNA)	3µl

Table (2): Primers utilized to express the *SOCS3* and *GAPDH* genes.

Primer	Sequence (5′→3′ direction)	Primer size bp	Annealing Temp (˚C)	Reference
***SOCSs3* Forward**	GCACCAAGCCAGCCCACAG	19	66.7	(8)
SOCSs3 Reverse	CAGCAGTCCAGCCTCTCCAATG	22	*66.7*	
***GAPDH* Forward**	GAGTCAACGGATTTGGTCGT	20	58.5	(8)
***GAPDH* Reverse**	TTGATTTTGGAGGGATCTCG	20	58.5	

SOCS: suppressor of cytokine signaling, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

Table (3): PCR program that convert in to RNA into cDNA.

Step	Temp (C˚)	Time(minute)	Number of Cycle
Annealing	25	05:00	1
Extension	42	60:00	1
Enzyme inactivation	82	15:00	1
Hold	4	10:00	1

Real-time quantitative PCR

In a real-time PCR reaction with cDNA as the template to convert RNA to cDNA. The component is made up of the reaction mix, whose quantity is listed in Table 1, and the thermal cycling parameters for the Real-Time PCR Program for *SOCS3*, which are displayed in Table 4. For data normalization, the expression of *GAPDH* RNA was used as an endogenous control. Using the $2^{-\Delta\Delta C}$t method, the comparative RQ level (fold change) for *SOCS3* expression among the patient and the healthy controls were determine.

Table (4): Program for real-time polymerase chain reaction (PCR).

Step	Temp (˚C)	Time minute: second	Number of Cycle
Primary denaturation	95	05:00	1
Denaturation	95	00:20	1
Annealing	(55 – 66)	00:20	45
Extension	72	00:20	45

Statistical analysis

The SAS (2018) program was utilized to identify the impact of distinct groups (patients and control) on the study parameters. A T-test was employed to statistically compare the means and determine if there was a significant difference. In this investigation, the chi-square test was employed to compare percentages with a significance level of 0.05 and 0.01 likelihood.

Result and Discussion

Distribution of patients and controls according to age ranges between 35-74. In the present study, the average age of T2DM patients was 57.02±1.22 while the average of healthy individuals was 54.18±1.39 as shown in Table (5).

Table (5): Age comparison between the patient and control groups.

Factor	Patients	Control	P-Value
Age	57.02±1.22	54.18±1.39	0.130

***P≤0.05: Non-significant.**

The results indicate that there is no statistically significant correlation between the patients and the control group in the age group, as depicted in table (5). This is consistent with the findings of the prior investigation (9).

The distribution of patients based on sex groups revealed that sex does not have a significant impact on the occurrence of T2DM among both male and female individuals in the patients and controls included in the current investigation, as indicated in Table (6).

Table (6): Distribution of sample study according to sex in patients and control.

Factor		Patients	Control	P-Value
Sex	**Male**	31(51.67%)	31(51.67%)	1.00 NS
	Female	29(48.33%)	29(48.33%)	1.00 NS
	P-Value	0.841NS	0.841 NS	

NS: Non-significant.

The result in Table (6) showed that sex has no significant contribution on the incidence of T2DM among the 60 diabetic patients involved in the present study. This study is consistent with a previous study (10).

The findings from T2DM patients indicated a rise in the levels of (FBG) and (HbA1c) biomarkers, with values of 151.18±8.70 mg/dl and 7.49±0.16% respectively. In comparison, the control group had FBG and HbA1c levels of 99.30±1.04 mg/dl and 5.46±0.03% respectively. The table (7) demonstrates a notable distinction between the patients and the control group.

Table (7): FBS and HbA1c levels in a comparison between T2DM patients and healthy control groups.

Parameters	Type 2 Diabetes Mellitus	Healthy control	p-value
FBG	151.18±8.70 mg/dl	99.30±1.04 mg/dl	0.0001*
HbA1c	7.49±0.16%	5.46±0.03%	0.0001*

***P≤0.01: highly significant difference.**

Patients with T2DM had highly significantly elevated glucose levels in comparison to the control group, according to the study's results. This increase may be attributed to the weakened β-cell, reduced insulin production and/or activity, and increased insulin resistance (11). These findings are consistent with prior reports (12).

T2DM patients had substantially higher serum glucose concentrations than healthy controls. The elevated HbA1C levels observed in T2DM patients indicate inadequate management of blood sugar levels in comparison to individuals without diabetes. The HbA1c is a valuable instrument that is considered a benchmark in monitoring the glycemic status of patients with T2DM. Other studies have confirmed similar findings, indicating a notable increase in the level of HbA1c (12).

Serum Level of IL-23

The findings indicate a substantial rise in the IL-23 concentration among individuals in the T2DM group (507.05+16.87 Pg/ml) compared to the control group (153.90+11.31 Pg/ml), with a statistically significant difference (P=0.0001) as demonstrated in table (6).

Table (8): Comparative analysis of IL-23 concentration in patients and control groups.

Group	Mean ± SE of IL-23 9 (Pg/ml)
Patients	507.05±16.87
Control	153.90±11.31
P-value	0.0001

***P≤0.01: highly significant difference.**

There is an evidence that diabetic pancreatic islets experience increased levels of numerous inflammatory cytokines, which lead to oxidative stress and endoplasmic reticulum (ER) stress in beta cells. Specifically, interleukin-23 (IL-23) and interleukin-24 (IL-24) are implicated in this process (13). These findings are consistent with earlier investigations (8).

Gene Expression of *SOCS3*

The findings of this study indicate that the expression of the *SOCS3* gene is elevated in patients with T2DM compared to healthy individuals. The fold change in expression was 3.75, as shown in Table (7).

Table (9): The levels of *SOCS3* gene expression were measured in the various groups under study using the $^{2-\Delta\Delta Ct}$ method.

Group	Means Ct of *SOCS3*	means of GAPDH-F	Mean of ΔΔCt	$2^{-\Delta\Delta Ct}$	Fold of gene
Control	36.191154	33.100443	-0.760739	1.69	1
Patients	39.183866	37.998567	-2.666151	6.34	3.75

Previous studies have shown that *SOCS* causes insulin resistance and decreases insulin signaling, indicating a significant connection between *SOCS* and DM. Cytokinese initiate *JAK/STAT* signaling, which enhances the production of *SOCS*, a gene located farther downstream. The *JAK/STAT* pathway is regulated by a negative feedback loop to maintain dynamic equilibrium due to the accumulation of *SOCS*. These findings are consistent with prior reports (14).

IL-23 is an immune system cytokine that promotes inflammation and is essential for the development and maintenance of Th17 cells. This study examined the levels of IL-23 in the supernatant, as well as the gene expressions of *SOCS3*, and their relationships in patients with T2DM. The study shows a correlation between IL-23, FBG and HbA1c with the *SOCS3* gene expression as shown in table 10. The role of pro-inflammatory cytokines in the immunopathogenesis of insulin resistance and T2DM with *SOCS3* gene has been studied and show a relationship with insulin resistance. these finding are consistent with previous study (8).

Table (10): The correlation between the expression of the *SOCS3* gene and the levels of IL-23, HbA1c and FBG in individuals with type 2 diabetes

Parameters	Correlation coefficient with *SOCS3*	P-Value
IL-23	-0.66**	0.0004
HbA1c	-0.55**	0.0052
FBG	0.60**	0.0021

****P≤0.01 highly significant**

Conclusion

In conclusion, our findings indicate that *SOCS3* expression are positively linked with insulin resistance indicators in a diabetic environment. *SOCS* is strongly linked to B-cell functional abnormalities. As a result, *SOCS* has emerged as a novel therapeutic target for T2DM.also the study find a positive correlation between SOCS3 and IL-23 in patients with T2DM.

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