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Estimation of JAK2 Serum Level and Gene Expression in Diabetes Type 2 Patients

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Abstract: Type 2 diabetes mellitus (T2DM) is a multifactorial not-communicable disease that is characterized by insulin resistance and chronic sub-clinical inflammation. This research aimed to study the role of biochemical and immunological marker (IL23) level its effect on the gene expression of JAKs in T2DM. The study divided participants into two groups: 60 with T2DM and 60 control aged 35-74 who attended Sheikh Zayed Hospital from Nov 2023 to Jan 2024. Participants underwent blood sampling to extract RNA for JAK2 transcript analysis using cDNA synthesis in RT-PCR, additionally the research also assessed the clinicopathological traits of diabetic patients and IL-23 concentration using ELISA. Type 2 diabetic patients showed significantly high JAK*2* expression. The study found a correlation between IL-23 concentration, *JAK2* expression level, and they may act as clinicopathological aspects like HBA1C and FBS levels. In conclusion, T2 DM had elevated *JAK2* and IL-23 levels Therapeutic targets of the disease linked to disease activity and pathophysiology may exist.

Keywords: Diabetes Mellitus Type2, *JAK2* gene, IL-23, RT-qPCR

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Introduction

Diabetes Mellitus is a chronic, inherited endocrine metabolic disorder characterized by elevated blood sugar levels, known as hyperglycemia (1). In Iraq, approximately 1.4 million individuals are affected by type 2 diabetes, with a prevalence range between 8.5% to 13.9% (2). Type 2 diabetes mellitus (T2DM) constitutes over 90% of diabetes cases and is characterized by inadequate insulin secretion from pancreatic islet β-cells, insulin resistance in tissues, and insufficient compensatory insulin release (3). The *JAK* subfamily consists of many subtypes, including *JAK1, JAK2, JAK3, and TYK2* (4) .Inflammatory processes often accompany T2DM symptoms. The *JAK*/*STAT* signaling pathway participates in inflammation and impacts the insulin signaling pathway through the *SOCS3* gene (5). Interleukins (ILs) were initially discovered in the 1950s, IL-1, interferon, and nerve growth factors were identified as early members of this family. Over the years, research has led to the recognition of more than 35 different types of ILs (6). Among these, the protein IL-23, which is released by macrophages it is essential for the treatment of autoimmune disorders, infections, and inflammation. It increases T cell activation by binding to IL-23 receptor on T cell surfaces. CD4+

T cells, γδ T cells, and iNKT cells are the main sources of IL-17, which affects the innate and adaptive immune responses. Numerous signaling pathways, including TGF-β-IL-IL-23, IL-23-IL-23R, and IL-1-IL1R, are made up of the two subunits p40 and p19(7).When IL-23 binds with IL23R, it enhances the proliferation of specialized Th17 cells. These cells produce IL-17A and other inflammatory cytokines, activate NK cells, and regulate antibody production (8) Changes in IL-23 levels are associated with inflammatory and autoimmune disorders. furthermore, both Th17 cells and IL-17 are implicated in development of type 2 diabetes mellitus (T2DM) (9). IL-23, contributes to the milder forms of inflammation associated with type 2 diabetes (10).

Materials and Methods

Study design and patient selection

This study compared sixty T2DM (31 men and 29 women) with sixty apparently healthy participants (31 men and 29 women) in the 35–74 years of age who visited Sheikh Zayed Hospital between November 2023 and January 2024. The criteria that were used to identify the healthy individuals were as the following: they had no autoimmune illnesses, were not taking any medications that were thought to affect serum glucose levels, and had no anomalies related to hormones.

Exclusion criteria

Individuals affected by type 1 diabetes typically require insulin therapy to manage their condition. Additionally, certain groups such as pregnant individuals, those with chronic or endocrine disorders, as well as individuals with liver, kidney, or thyroid conditions, may also be impacted by diabetes and require specialized care and management. All these cases were excluded from study.

Sample collection

Five milliliters of venous blood were collected from both patients with T2DM and healthy control individuals via venipuncture following a 12-hour overnight fasting. The blood samples were later divided into two tubes: the first tube contained EDTA and was used to quantify HbAlc and conduct molecular investigations. The second tube, placed in a gel tube, was used to collect serum and subsequently held at -20°C until it was required for measuring fasting blood sugar (FBS) interleukin levels using ELISA.

Estimation of hemoglobin A1C

The HbA1c was estimated by Siemens (DCA Vantage analyzer).

Estimation of Fasting blood sugar

The FBS was estimated by Fujifilm (fuji Dri-Chem NX600).

Measurement of serum (IL-23) concentration

Using an ELISA system (HumaReader HS-Human) and the Sandwich-ELISA kit (Cloud-Clone Corp.,USA), following the manufacturer's guidelines in carrying out the process. the serum IL-23 levels in the samples were determined.

Extraction of RNA

RNA was extracted from serum samples by using TRIzolTM Reagent, the samples underwent ethanol washing, isopropanol precipitation, chloroform extraction, and rehydration in nuclease-free water. The concentration and purity of the RNA were determined using a Quintus Fluorimeter and Quantifluor Dye for detection.

Reverse transcription

Total RNA was converted to complementary DNA (cDNA) using the Add Script Reverse Transcriptase Kit (Macrogen, Korea). The manufacturer's

instructions were followed, and the reaction took place in a volume of 20 µl, mRNA has been changed cDNA by this process. The program (heat cycle stages) of the cDNA reverse transcription process was finished following the data displayed in (Table 1).

Table (1): Program RT-PCR converted RNA to cDNA.

Step	temp °C	Time	Cycle
Annealing	25	05:00	1
Extension	42	60:00	1
Enzyme inactivation	82	15:00	1
Hold	4	10:00	1

Quantitative real-time PCR

For the conversion of RNA to cDNA, a real-time PCR reaction was used and the template this time is cDNA, RNA detection using RNA-specific primers (Table 2). According to Table 3, the component is made up of the reaction mix and its quantity. and Real-Time PCR program thermal cycling conditions for *JAK2* as shown in (Table 4). The expression of the GAPDH RNA was utilized as an endogenous control for data normalization. The compare RQ level (fold change) for *JAK2* expression among the patients besides the apparently healthy individuals was calculated by using the equation: $2^{-\Delta\Delta Ct}$.

Table (2): Primers for the *JAK2* and *GAPDH* gene expression systems.

Primer Name	Sequence (5′→3′d)	Reference
***JAK2* Forward**	ATATTGGTGGAGAACGAGAACAGAG	(9)
***JAK2* Reverse**	TCATACGCATAAATTCCGCTGGTG	
***GAPDH* Forward**	GAGTCAACGGATTTGGTCGT	
GAPDH Reverse	TTGATTTTGGAGGGATCTCG	

Table (3): Calculation of Components in Real Time PCR.

Components	20 µl
$Mgcl_2$ 1	1 µl
Forward Primer	1 µl
Reverse Primer	1 µl
c DNA	3 µl
Nuclease Free Water	4 µl
2.5 Reaction Mixture	10 µl

Table (4): RT-qPCR cycling program.

Steps	°C	Time	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:20	1
Annealing	(55-60)	00:20	45
Extension	72	00:20	45

Data analysis of RT-qPCR

The gene expression of (*JAK2* gene) was assessed by using *GAPDH* as a housekeeping gene using the following equations (9)

ΔCT =CT gene - CT House Keeping gene

ΔΔCT =ΔCT Treated or Control – Average ΔCT Average Con

Fold change $=2^{-\Delta\Delta CT}$

Statistical analysis

The Statistical Analysis System-SAS (2018) program was used to detect the effect of different groups (patients and control) on study parameters, T-test was used to compare between means. The chi-square test was used to compare between percentages (0.05 and 0.01 probability) in this study.

Results and discussion

In this study, sixty individuals aged 35 to 75 with good health and another sixty with type 2 diabetes were enrolled. According to the data presented in (Table 5), the serum levels of hemoglobin A1C and fasting blood sugar in patients showed a notable increase compared to the controls group (P≤0.01). That was found in another study (11) , underlining the association between elevated HbA1c and fasting blood sugar in individuals with type 2 diabetes mellitus.

The significant increase suggests potential factors such as compromised ß-cell function, inadequate insulin production, or escalating insulin resistance. That was found in another study (12, 13)

Table (5): Comparison between patients and control groups in HbA1c and FBS.

Group	HbA1c (%)	F.B.S. (mg/dl)
Patients	7.49 ±0.16	151.18 ±8.70
Control	5.46 ±0.03	99.30 ±1.04
P-value	0.0001	0.0001

(P≤0.01).

The ELISA technique was utilized in this study to quantify the concentration of interleukin 23 protein. The results revealed a highly significant difference in interleukin 23 protein levels (P≤0.01) between individuals with diabetes (507.05 ±16.87) and those without diabetes (153.90 ±11.31) shown in (Table 6).Persistent immune activation is known to trigger the chronic inflammation and interleukin 23 (IL-23), a pro-inflammatory cytokine that is essential to the survival of Th17 cells and the promotion of a milieu of inflammation in the immune system. It is plausible that IL-23 and its associated *JAK* gene contribute to the pathophysiology of type 2 diabetes mellitus (T2DM). That was found in another study (9).

Table (6): Comparison of the IL-23 Conc. patient and control groups.

Group	IL-23(pg/ml)
Patients	507.05 ±16.87
Control	153.90 ±11.31
P-value	0.0001

(P≤0.01).

The study observed a notable increase in the expression level of the *JAK2* gene among individuals with type 2 diabetes mellitus compared to healthy

individuals, with (fold changes of 3.77, 1), respectively (Table7). It suggests a possible link between the *JAK2* gene and insulin resistance. JAK-associated proteins can trigger cellular signaling pathways, which release certain chemicals that exacerbate inflammation. Adipose tissue inflammation can lower insulin sensitivity and raise the risk of diabetes development. These results align with previous study conducted by (14). found that *JAK2* expressed in fat and liver had opposite effects on lipid accumulation which related with (IR) in T2DM.

Table (7): Comparing the *JAK2* fold expression levels of patients and control groups.

Group	Means Ct of *JAK2* gene	Means CT of *GAPDH* gene	Means ΔCt (mean Ct of *JAK*-mean Ct of *GAPDH*	Mean ofΔΔCt	$2^{-\Delta\Delta Ct}$	experimental group/ control group	Fold change
Control	35.624315	32.550654	3.073661	-0.103546	1.074411015	1.07/1.07	1
Patients	36.177166	35.014853	1.162313	-2.014894	4.041508831	4.04/1.07	3.77

The study found that a correlation between IL-23 protein levels individuals with diabetes and control (Table 8), *JAK2* expression levels and clinicopathological aspects like (HBA1c and FBS) levels .These findings are consistent with another study (9).

Table (8): Correlation between IL-23, *JAK2* gene, (FBS and HBA1c) in diabetes and control.

Group	FBS	HBA1c	IL-23	*JAK2*gene
Control	99.30 ±1.04	5.46 ±0.03	153.90 ±11.31	1
Patients	151.18 ±8.70	7.49 ±0.16	507.05 ±16.87	3.77

Conclusion

Analysis of the gene expression revealed a marked increase in the levels of the *JAK2* gene and IL-23 level among individuals with Type 2 diabetes, suggesting a possible connection between these genes and insulin resistance and chronic inflammation in T2DM.

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