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Evaluation of the Genetic Association and Expressions of Notch-2 /Jagged-1 in Patients with Type 2 Diabetes Mellitus

Wajdy J. Al-Awaida[1], Wasan S. Hameed[2], Haider J. Al Hassany[2], Moh'd Mohanad Al-Dabet[3], Omar Al-bawareed[4], Najah R. Hadi[5]

[1]Department of Biology and Biotechnology, American University of Madaba, Madaba, Jordan

[2]Department of Microbiology, Faculty of Medicine, University of Kufa, Kufa, Iraq

[3]Department of Medical Laboratories, American University of Madaba, Madaba, Jordan

[4]Department of normal physiology, RUDN University, Moscow, Russia

[5]Department of Pharmacology and Therapeutics,Faculty of Medicine, University of Kufa, Kufa, Iraq

Corresponding author: Najah R. Hadi, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Kufa, Kufa, Iraq. E mails: drnajahiraq@gmail.com, drnajahhadi@yahoo.com. ORCID ID: https://www.orcid.org/0000-0001-9084-591X.

ABSTRACT

Background: Diabetes mellitus (DM) is the world's most common cause of chronic kidney diseases (CKD), with approximately 1 in 4 adults with DM having CKD and 1 out of 10 to 20% of DM patients die from CKD. **Objective:** The current study aims to investigate the correlation between Notch-2 and Jag-1expressions and specific inflammation biomarkers IL-1β and IL-6 with different stages of diabetic nephropathy. **Methods:** From August 2018 to January 2019, three hundred subjects were recruited for this study. One hundred and fifty subjects were healthy and age-matched to the diabetic group and selected as a control group. Another 150 patients with an established diagnosis of type 2 diabetes (T2DM) according to the criteria of the American Diabetes Association (ADA) were also recruited. Blood specimens were eventually used to identify the expressions Notch-2 and Jagged-1 and the levels of inflammatory biomarkers IL-1β and IL-6. **Result:** The current study shows a significant increase in gene expression and inflammatory biomarkers in patients with moderate and severe diabetic nephropathy compared to the control group. However, there was no significant difference between healthy control and mild diabetic nephropathy patients. This study shows a close association between the increase in the levels of inflammatory biomarkers IL-1β and IL-6 as well as the gene expressions levels of both Notch-2 and Jag-1 with human diabetic nephropathy. **Conclusion:** According to our findings, we emphasize the use of Notch-2 and Jag-1 expressions and IL-1β and IL-6 levels as potential biomarkers for different stages of diabetic nephropathy.

Keywords: Notch-2, Jag-1, IL-1β, IL-6; T2DM, diabetic nephropathy.

1. BACKGROUND

Diabetes mellitus (DM) is a chronic metabolic disease that occurs either when pancreatic beta cells are unable to make an appropriate amount of insulin or when the body is unable to make effective use of insulin, leading to glucose accumulation in the blood (1). Diabetic nephropathy (dNP) is a severe progressive renal microvascular complication of DM and may occur in type 1 and type 2 DM. DNP may lead to chronic renal failure, eventually requiring dialysis or renal transplantation. In the developed world, DM is the leading cause of adult renal failure (2). The first stage of dNP involves the hypertrophic and hyperfiltration characteristics of the kidneys. At this stage, the glomerular filtration rate shows normal or slightly elevated levels. The first phase may last for about five years from the onset of the disease. The size of the kidneys shows an increase of almost 20%, and the renal plasma flow increases by 10-15 percent without albuminuria or hypertension (3). Some morphological alterations, such as thickening of the glomerular basement membrane (GBM), glomerular hypertrophy, and tubulointerstitial expansion, are identified in the second or silent stage (4). The second stage starts approximately two years after the onset of the disease. Importantly, no clinical signs of disease present during the second stage; also, the glomerular filtration rate may also return to typical values. However, many patients remain at this stage until the end of their lives. In the third stage, which is also referred to as initial nephropathy, early clinical evidence of nephropathy appears to be low but abnormal levels (≥ 30 mg/day) of albumin in the urine, referred to as albuminuria. The conventional laboratory assessment of renal malfunction can not be carried out quickly in the third stage. More

sensitive methods must, therefore, be proposed and evaluated. In addition, blood pressure increases in the third stage, although it remains within the normal range, and the glomerular filtration rate (GFR) may begin to decrease (5). When the fourth stage reaches the stage of chronic kidney disease (CKD) with irreversible proteinuria (> 30 mg / day), GFR decreased below 60 ml/ min/1.73 m^2. In the end-stage renal disease, the kidneys are no longer able to function appropriately in response to daily life needs. End Stage Renal Failure (ESRD) is one of the most serious and life-threatening forms of kidney disease and has high costs associated with it (6). End-stage renal disease is the terminal stage of chronic renal failure, with progressive, irreversible renal damage and declining renal function. The human body is, therefore, unable to maintain the balance of fluids and electrolytes, which leads to uremia and reduced GFR. Histological staining at histopathological level shows that less than 10% of nephron function remains (7). Globally, there is a continuous rise in the percentage and prevalence of chronic kidney diseases (CKD). Some CKD patients will experience disease progression resulting in end-stage renal disease, which requires lifelong renal replacement therapy (RRT) or transplantation (8).

Genetic factors are crucial elements in the etiology and progression of type 2 diabetes, as shown by marked changes between diabetic and non-diabetic individuals in different ethnic groups and by studies in monozygotic twins where concordance rate for type 2 diabetes approach 100% (9). Insulin resistance, as illustrated by reduced glucose utilization in the muscle tissue, is common among people who have type 2 diabetes despite some inherited impaired insulin secretion or action (10). In 2005, a cancer-oriented study identified and suggested a strong association between the survival rate and the levels of JAG-1 expression (11, 12). Moreover, cytokines play a vital role in inflammatory diseases such as diabetes and are connected to JAG-1 and Notch expression and signaling pathways (13, 14). IL-1β is known to be secreted by damaged cells and inflamed tissues; factors released by damaged cells called Damaged Associated Molecular Patterns (DAMPs) are recognized by PRRs TLR,which in turn induce the production of IL-1β (Kim et al., 2020; Maedler et al., 2002).

Adhesion molecules and pancreatic B-cell destruction are promoted by IL-1β secretion (15). Anotherimportant inflammatory cytokine produced in tissue damage and diabetes is IL-6;the role of IL-6 in diabetes is illustrated through its ability to enhance proliferation, overexpression, and vascular permeability,which lead to the progression of DM, althoughserum IL-6 levels have been showing to be increased in patients with type 2 DM (16). On the other hand, IL-6 is a pro-inflammatory cytokine, a 26 kDain size protein.Fibroblasts, monocytes, endothelial cells, B cells, and T cells are capable of producing and secreting IL-6. Also, IL-6 affects the function of a broad spectrum of cell types involving differentiation of B cells and enhanced antibody production (17, 18).

This study investigates the correlation between Notch-2 and Jag-1expressions and certain inflammation biomarkers IL-1β and IL-6 with different stages of diabetic nephropathy.

2. OBJECTIVE

This study estimated potential biomarkers such as IL-1β and IL-6 as well as the gene expressions levels of both Notch-2 and Jag-1 for human diabetic nephropathy.

3. MATERIALS AND METHODS

Patients and sample collection

The current study was carried out at the Centre for Researches and Treatment of Diabetic Mellitus in AL-Sader Teaching Hospital and the practical place at the Department of Medical Microbiology College of Medicine, the University of Al-Kufa in Iraq, three hundred individuals were recruited and analyzed. All subjects were divided into two major groups: diabetic nephropathy and control groups.

Patients group

One hundred fifty patients were collected from the Centre for Researches and Treatment of Diabetic Mellitus in AL-Sader Teaching Hospital and the suitable place at the Department of Medical Microbiology College of Medicine, the University of Al-Kufa in Iraq. Male and female patients with established T2DM, according to the American Diabetes Association (ADA) were participated in this study. Age ranged between (23-70) years, with T2DM duration between 1-42 years. The patients were divided into three groups based on their creatinine/albumin ratio (ACR),as follow: 50 diabetic patients with stage A1 (normal to mild ACR < 3 mg/mmol), 50 patients with stage A2 (moderate ACR 3-30 mg/mmol) and 50 patients with stage A3 (severe ACR > 30 mg/ mmol) [19].

Control group

One hundred fifty non-diabetic control subjects (males and females) have been recruited in this study. All of the controlsubjects were free of DM and dNP as they were evaluated for diabetes and diabetic nephropathy as follows; fasting blood sugar (FBS) value< 100 mg/dl, level of glycated hemoglobin A1c(HbA1c) ≤ 5.8 %, and ACR < 3 mg/mmol, Participants' age ranged between 28-66 years.

Collecting blood samples

Five ml of blood were collected according to each subject's standard aseptic technique by puncturing the anti-cubital vein. The blood specimens were collected in ethylene diamine tetra-acetic acid (EDTA) tubes, then immediately used for Glycated hemoglobin A1 (HbA1) evaluation and RNA extraction.

Quantitative colorimetric determination of glycohemoglobin (HbA1) in the whole blood

HbA1 was quantified as instructed by the manufacturer (Glycohemoglobin HbA1 Fast Ion-Exchange Resin Separation Method, Human). Shortly, whole blood is mixed with a lysing reagent containing detergents and borate ions. The hemolysate is then diluted with a slightly binding exchange resin for 5 minutes. HbA0 (non glycatedHbA) binds to the resin during this time. The supernatant fluid containing the HbA1 (glycated HbA)

Primers		Primer sequence 5′ – 3′	Product size (base pair)
House-keeping gene	F	CAGCTCGTGTCGTGAGATGT	150 bp
	R	CGTAAGGGCCATGATGACTT	
Notch-2	F	GCAGGTAGCTCAGACCATTCT	301 bp
	R	ATCTCCAGCATTACTACCGAG	
Jagged-1	F	CGACCGTAATCGCATCGTAC	246 bp
	R	ATGCACTTGTCGCAGTACAG	

Table 1. Sequences of primers that use for geneexpression

is removed by a special resin separator. The HbA1c % of total hemoglobin was performed by estimating the absorbance of the glycohemoglobin and the total hemoglobin portion at 415 nm compared to standard glycohemoglobin carried through the test procedure.

Total RNA extraction

The TRIzol® reagent kit (Promega,USA) was used to extract the total RNA according to its instructions. The total RNA's quality was ensured on an agarose gel and by analyses of the A260/280 ratio.The reverse transcription reaction was conducted using 1 μg of total RNA. cDNA was amplified using specific primers as published before [20], the primers' sequence is summarized in Table1.

ELISA for Evaluation of Serum IL-6 and IL-1β Levels

The blood concentrations of human IL-6 and IL-1β were achieved using the company's ELISA technique (Elabsciencechina). This ELISA kit uses the method Sandwich. The optical density (OD) with a wavelength of 450 nm ± 2 nm can be measured by spectrophotometry. The OD value is proportional to the human serum IL-6, IL-1β concentration. The concentration of the samples can be determined by comparing the OD of the samples with the standard curve.

GoTaq® 1-Step RT-qPCR System

The expression analysis of Notch-2 and Jagged-1 was quantified using stem-1 RT-qPCR, which was further normalized with mRNA housekeeping genes. The procedure was performed based on a previously described method (21).

Data qRT-PCR analysis

qRT-PCR rawdata for the housekeeping gene, Notch-2 and Jagged-1, were examined with the assistance of relative quantification levels of expression gene. ΔCT procedure was performed while utilizing the reference, as previously described(Livak and Schmittgen 2001). The gene quantification depends on thefollowing equation: ratio (reference/target) = $2^{CT(reference) - CT(target).}$

Ethical Statement

All participants gave their written informed consent and received instructions for the blood collection procedure and possible risks. The study was conducted following the Declaration of Helsinki. The Ethical Committee approved the study of Clinical studies at the Faculty of Medicine-University of Kufa (IRB no. MC. 08).

Statistical Analysis

Statistical analysis was conducted by GraphPad Prism version 7.0 (GraphPad Software, Inc., La Jolla, California, USA).Data were presented as mean±SEM. Two-tailed Student t-tests were used to compare differences between the two groups.

The values were considered significantly different when the *P*-value at $P < 0.05$, *, **, ***,**** = $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively

4. RESULTS

Demographic data, clinical and biochemical characteristics of studied individuals

Biochemical parameters creatinineand urea are significantly higher (p<0.05) in the diabetic patient group (2.3±0.40 mg/dl and 77.09±21.19 mg/dl, respectively) compared to healthy control (0.79±0.16mg/dl and 22.7±6.75 mg/dl, respectively), high concentration of creatinine and urea were resulting from diminishing of glomerular filtration rate in the diabetic group. Also, the results of the study showed a statistically significant increase (p<0.05) in the level of HbA1c and FBS (8.03±0.81% and 191.3±31.52mg/dl, respectively) in the diabetic group compared to the control group (5.2±0.35 % and 97.4±11.26mg/dl) (Table 2).

Parameter	Control (n=150)	Patients (n=150)	*P*-value	*P*-value summary
Age/years	44.72± 5.24	48.14±7.93	P > 0.05	ns
Gender Male Female	 48% 52%	 48% 52%	P > 0.05	ns
FBS mg/dl	97.4±11.26	191.3±31.52	P < 0.05	*
HbA1C %	5.2±0.35	8.03±0.81	P < 0.05	*
Creatinine mg/dl	0.79±0.16	2.3±0.40	P < 0.05	*
Urea mg/dl	22.7±6.75	77.09±21.19	P < 0.05	*

Table 2. Comparison of demographic data, clinical and biochemical characteristics of studied individuals

The values were considered significantly different when the *P*-value at $P < 0.05$. *, **, ***, **** = $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

Serum IL-1β level between control and diabetic nephropathy patients

In this study, we estimated the IL-1β level in both control and diabetic patient group; our result shows that the level of IL-1β is significantly higher in the control group (0.7±0.35pg/ml, p< 0.01) compared to the diabetic patient group (19.8±3.58pg/ml), as shown in Figure1.

Serum IL-1β level in control with different diabetic patient groups

Our study showed that there is a significant difference between the three patients group. The concentration of IL-1β was highly elevated in patients with moderate and severe diabetic nephropathy compared to the control group. However, and there is no significant difference between a patient with mild diabetic nephropathy and the control group, as shown in Figure 2. those findings

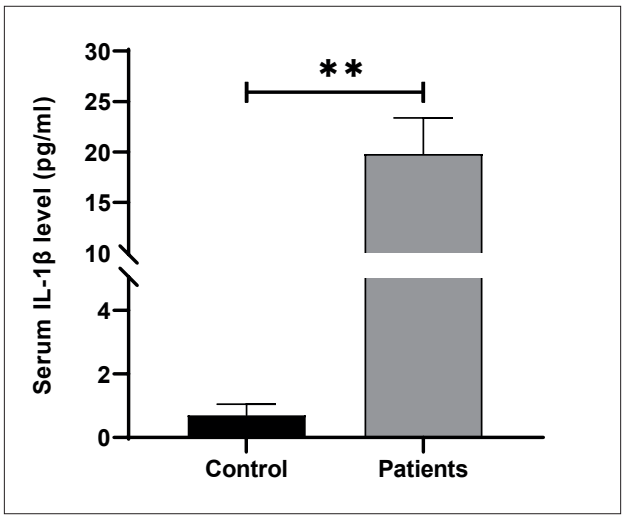

Figure 1. Comparison of serum IL-1β level between control and diabetic Patient group.. The values were considered significantly different when the P-value at P < 0.05. *, **, *, **** = P < 0.05, P < 0.01, P < 0.001, and P < 0.0001, respectively**

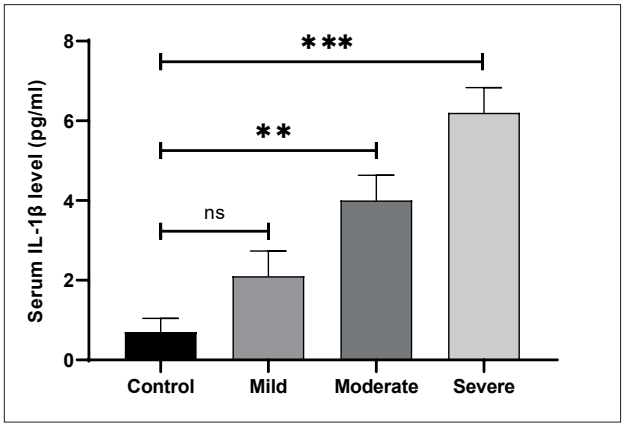

Figure 2. Comparison of serum IL-1β level in control with different diabetic Patient groups. The values were considered significantly different when the P-value at P < 0.05. *, **, *,**** = P < 0.05, P < 0.01, P < 0.001, and P < 0.0001, respectively**

agreed with the study performed by Wada and Makino (22).

Interleukin-6 (IL-6) levels among diabetic and control group

The level of IL-6 in diabetic patients (13.4 ± 1.59 pg/ml) was significantly higher (p< 0.0001) compared to the control group (2.9 ±1.44 pg/ml), as shown in Figure 3. These results have shown an increased inflammatory status in diabetic nephropathy patients.

Serum IL-6 level in control with different diabetic patient groups

The concentration of IL-6 was highly elevated in patient with moderate and severe diabetic nephropathy groups compared to the control group.

However, there is no significant difference between a patient with mild diabetic nephropathy and the control group, as shown in Figure 4. those findings agreed with the study performed by Flynn et al. (23).

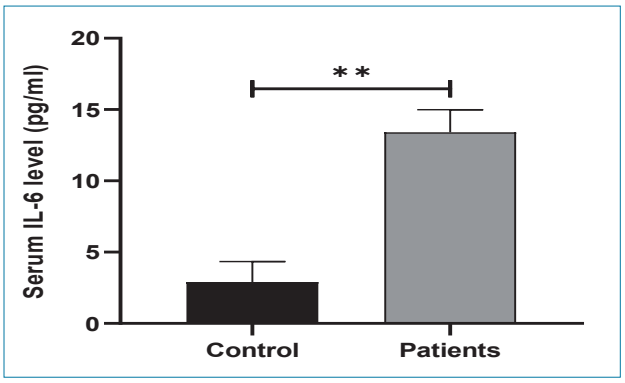

Figure 3. Comparison of serum IL-6 level between control and diabeticnephropathy Patients Group. The values were considered significantly different when the P-value at P < 0.05. *, **, *,**** = P < 0.05, P < 0.01, P < 0.001, and P < 0.0001, respectively**

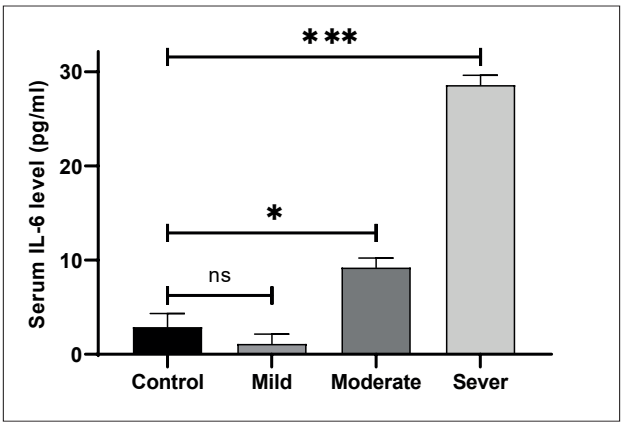

Figure 4. Comparison of serum IL-6 level in control with different diabetic nephropathy Patients. The values were considered significantly different when the P-value at P < 0.05. *, **, *,**** = P < 0.05, P < 0.01, P < 0.001, and P < 0.0001, respectively**

Notch-2 and Jag-1 gene expressions in diabetic patients and control groups

Gene expression of the notch-2 and Jagged-1 revealed a statistically significant increase (p < 0.0001) in the diabetic patient compared to the control group. In which, diabetic patient group showed Notch-2 (15.3±4.10) and Jagged-1 (27.3±3.52), compared to Notch-2 (1.4±1.15) and Jag-1 (1.3±0.98) in the control group, as shown in Figure 5.

Notch-2 and Jag-1 gene in control with different diabetic patient groups

The current study shows that there is a significant increase in gene expression in moderate and severe diabetic nephropathy patients as compared to mild diabetic nephropathy. The gene expression of Jag-1in moderate was 15.7±4.04, sever 54.2±4.04 and in mild 0.92±4.04 While Notch-2 expression shows 25.7±6.35 in moderate dNP, 50.2±6.35 in severe and0.9±6.58 in mild dNP patients. The difference was statistically significant between moderate and severe diabetic nephropathy patients, as shown in Figure 6. These findings are consistent with a previous study published by others (24).

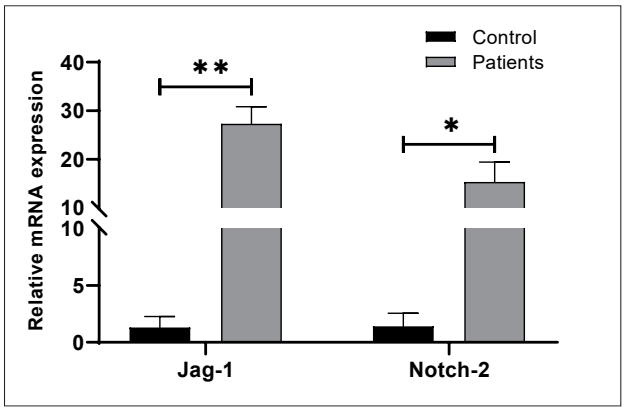

Figure 5. Notch-2 and Jag-1 gene expression in diabetic patients and control groups. The values were considered significantly different when the P-value at P < 0.05. *, **, *, **** = P < 0.05, P < 0.01, P < 0.001, and P < 0.0001, respectively**

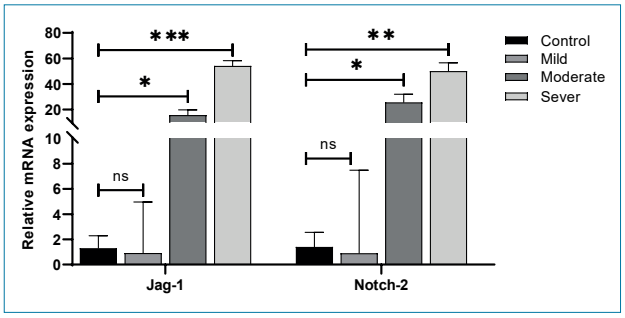

Figure 6.Comparison of Jag-1 and Notch-2 gene expression in different diabetic nephropathy patient Groups. The values were considered significantly different when the P-value at P < 0.05. *, **, *, **** = P < 0.05, P < 0.01, P < 0.001, and P < 0.0001, respectively**

5. DISCUSSION

In recent decades the global prevalence of diabetes mellitus (DM) in adults has increased significantly. In the 7th edition of the Diabetes mellitus (DM) Atlas, published in 2015, 415 million DM adults worldwide were reported, and by 2045 an estimated 693 million people are expected to live with DM (25). Failure to properly control high glucose levels leads to severe micro-and macrovascular complications. Cardiovascular disease (CVD) with a propensity to heart attacks and strokes are characteristic of macrovascular complications. Neuropathy, retinopathy, and diabetic nephropathy (DN) are among the microvascular complications. Diabetes mellitus is the world's most common cause of CKD, with approximately 1 in 4 adults with DM having CKD (26), and 1 out of 10 to 20% of DM patients die from CKD. This study estimated potential biomarkers such as IL-1β and IL-6 and the gene expression levels of both Notch-2 and Jag-1 for human diabetic nephropathy. The high concentration of creatinine and urea in our diabetic patient group resulted from the diminishing of glomerular filtration rate in the diabetic group. Also, the high level of both HbA1c and FBS in the diabetic group compared to the control group in our study was shown in many previous studies (27, 28). Hasegawa et al. al. (29) stated that inflammatory cytokine could play a role in diabetic nephropathy when he noticed that TNF- α and IL-1 found in glomerular basement membranes was high when compared between diabetic and non-diabetic rats. Also, it has been noticed that the glomerular basement membranes of diabetic rates contained a significantly increased amount of TNF- α and IL-1 when compared with non-diabetic rats (29, 30). A study by Mojtaba et al. (31) found that serum level of IL1 beta increase significantly in patients with type 2 diabetes compared to the non-diabetic patients.

Accumulating evidence indicates that metabolic syndrome diseases are characterized by abnormal cytokine production, including elevated serum IL-1β levels, increased acute-phase proteins, e.g., CRP and activation of inflammatory signaling pathways (32, 33). Niknami et al. (34) reported a high IL-1β concentration in diabetic patients with nephropathy (15.95 pg/ml), another study lead by Flynn MG. (16) found that the concentration of IL-1β in hemodialysis patients (9.63 pg/ml) is significantly higher than other stages of chronic kidney disease. Maedler et al. (35) recorded that high glucose-induced IL-1β production and secretion in human β cells leads to β cell apoptosis and dysfunction. He also observed that IL-1β–producing β cells in diabetic patients- studied the pathway by which hyperglycemia causes impairment and loss of insulin-producing cells. In addition his theory proposed that the proinflammatory cytokine IL-1β may be a crucial factor contributing to β cell glucotoxicity in the pathogenesis of type 2 diabetes. On the other hand, his results disagree with a study by Tripepi et al. (36), who found that the level of IL-1β in ESRD was at normal range (0.39 pg/ml), also results by Spranger et al. (37) recorded that there were no significant differences in serum IL-1β concentration between diabetic and non-diabetic patients. The result regarding IL-6 agrees with a study by George et al. (38), which reported a significant increase in IL-6 concentration than non-diabetic patients.Earlier studies found that circulating IL-6 levels in diabetic nephropathy patients were significantly higher than control groups (39-41). Bastard et al. (11) found that the circulating IL-6 level is highly correlated with insulin resistance observed in type 2 diabetes and inhibited glucose uptake. Another study found that IL-6 impairs the insulin signaling pathway in hepatocytes (42). Diabetes and hyperglycemia create a pro-inflammatory microenvironment that progresses to microvascular complications such as nephropathy, retinopathy, and neuropathy. A recent study investigated the role of IL-6 in the inflammatory process and insulin resistance pathogenesis among type 2 diabetes mellitus patients Senthilkumar et al. (18). based on IL-6 data suggested important role played by inflammatory .A review by Akbari and Hassan-Zadeh in 2018 was aimed to summarize their current knowledge about the role of IL-6 in the development of T2DM. They also discussed the importance of specifically blocked IL-6 trans-signaling rather than inhibiting both signaling pathways as a therapeutic strategy for the treatment of T2DM and its associated macrovascular complications. Beberashvili et al. performed a study of diabetic nephropathy patients, which found that chronic inflammation, as assessed by elevated serum IL-6 levels, is correlated with clinical

changes and diagnostic markers in patients with ESRD, showing IL-6 to be the strongest independent predictor of all-cause fatalities.

Our results regarding IL-6 similar to another two studies, which reported that the level of IL-6 in patients with ESRD was higher than its level in the control group. Fatima et al. (43) found that the concentration of IL6 in hemodialysis patients was significantly higher than their concentration in other stages of chronic kidney disease. Interestingly, previously published studies have linked the single nucleotide polymorphisms (SNP) of the Notch gene with the development of diabetes (44). The mechanism of this association is not yet evident in human studies. However, experimental studies imply that not only are pancreatic β cells affected by the gain or loss of the Notch gene (45) and the nephron endowment (46). On the other hand, the genetic deletion of the γ-secretase enzyme components (Presenilin 1, 2) or pharmacological inhibition of the γ-secretase complex is vital for the complete development of meta-nephric mesenchyme induced nephron formation. These studies indicate that the removal of Notch-2 or Jag-1 has a significant impact on renal growth and differentiation of nephron segments. However, Notch-1 did not affect the change in renal development. Experiments using mouse models show that Notch is a pathogenic sign in the adult glomerulus (47). Notch Intracellular Domain (NICD) expression in the mature podocytes causes disturbance, glomerulosclerosis, and cell-death, which led to albuminuria and gradual renal failure. The latter obstruction preserves the nephron and podocyte differentiation markers' expression, reduces pathological expression of VEGF, and ameliorates diabetes-induced cell-death and loss of podocytes. Therefore, it seems to be a highly potential therapeutic approach to suppress reactive Notch signaling in patients with diabetic nephropathy (48). On the other hand, the expression of notch-1 in podocytes causes albuminuria and glomerulosclerosis to develop. In another study, diabetic kidney diseases were ameliorated in the experimental diabetic nephropathy model by genetic deletion or pharmacological inhibition (49). In podocytes, Notch signaling interacts with the transforming growth factor (TGF)-pathway. This interaction appears to form a positive feedback loop: TGF transcriptionally upregulates Notch ligand Jagged-1 expression. Furthermore, Notch activation also increases TGF-expression. Given the potent pro-fibrotic activity of TGF in glomerular disease, this suggests that Notch is an important "chef regulator" of glomerulosclerosis pathomechanism. Consistent with the current findings, a previous study (50) strongly supports the view that Notch signaling in podocytes plays a critical role in albuminuria development.

Several other studies have shown that the Notch signaling pathway is also involved in regulating cell proliferation and cell death (51). In which, the expression of renal pathways of Jagged-1 / Notch in the mice mediate TGF-β1 may increase renal fibrosis. Furthermore, experiments confirmed the expression of Jagged-1, Notch-2, and Notch-4 is required to induce TGF-β1-mediated epithelial-mesenchymal transition (EMT). The activation of the Notch pathway was found to have a specific proteinuric renal disease pathogenesis, which was prevalent in glomerulosclerosis pathophysiology and tubulointerstitial fibrosis (52). It was observed in patients with diabetic nephropathy that the expression of Jagged-1 and Hes-1 was significantly upregulated in tubulointerstitial fibrosis. The studies mentioned before (26-28) suggest that regulation of Notch-1/Jagged-1 signaling and TGF-β may play a significant role in diabetic nephropathy progression, which strongly implies the significance of Notch in EMT progression of diabetic nephropathy. The current study agrees with the previous one, where the authors reported that Jagged-1 and Notch-1 expression are significantly increased in the disease model group compared to the healthy group. More interestingly, they significantly reduced upon the treatment with gliquidone. They are indicating that gliquidone might efficiently block the Notch-activation pathway. Collectively, the findings showed that the pathway of Jagged / Notch is crucial in the induction of renal interstitial fibrosis that might be therapeutically targeted to ameliorate the progression of diabetic nephropathy.

6. CONCLUSION

This study shows a close association between the increase in the levels of inflammatory biomarkers IL-1β and IL-6 and the gene expression levels of both Notch-2 and Jag-1 to human diabetic nephropathy. We, therefore, emphasize the use of Notch-2 and Jag-1 expressions and IL-1β and IL-6 levels as potential biomarkers for both moderate and severe stages of diabetic nephropathy

- **Pateint Consent Form**: The authors certify that they have obtained all appropriate patient consent forms.
- **Author's contribution**: All authors were involved in the preparation of this study. Final proofreading was made by the first author.
- **Availability of Data and Materials**: All data generated or analyzed during this study are included in this published article.
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