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# Unraveling the Inflammatory Symphony: Dissecting the Dysregulated *NF-κB*, *IL-6*, and *IFN-γ* in Nephropathy Patients

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## Abstract

**Background:** Diabetes patients have been known to activate the inflammasome pathway. The current research was carried out to assess the gene expression levels of nuclear factor-κappa B (*NF-κB*), Interleukin 6 (*IL-6*), and Interferon Gamma (*IFN-γ*) in PBMC samples from peripheral blood to find out whether its mRNA could be used as an early marker for renal function deterioration in diabetics.

**Methods:** This empirical study collected serum samples from 60 diabetic patients suffering from nephropathy and 60 healthy controls. Collected blood from the patients was isolated and preserved in the medium at a temperature of -20. Fasting blood sugar (FBS), total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, cholesterol/HDL-LDL, AST, ALT, alkaline phosphatase, and CBC were analyzed. Real-time PCR measured *NF-κB*, *IL-6*, and *IFN-γ* gene expression.

**Results:** Diabetic patients with nephropathy showed increased fasting plasma glucose levels compared to non-diabetic subjects, although there were no significant differences between them regarding cholesterol, triglyceride or other lipid levels, white blood cell count, and red blood cell count. Along with platelet count parameters, the levels of MCV, MCH, MCHC, RDW-CV, AST, ALT, and alkaline phosphatase did not change in the control group. The patient group showed a significant increase in *NF-κB* and *IL-6* gene expression at the level of (P-value <0.001) and also *IFN-γ* expression at the level of (P-value <0.01) compared to the healthy control group.

**Conclusion:** Due to the elevated level of these genes in nephropathic patients, it can be possibly concluded that *NF-κB*, *IL-6*, and *IFN-γ* are involved in nephropathy complications of diabetes.

**Keywords:** Diabetic nephropathy; *NF-κB*; *IL-6*; *IFN-γ*; PBMC

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## 1. Introduction

The general population worldwide is at risk of diabetes mellitus and its complications making it one of the major causes of mortality (1). In the vasculature, chronic hyperglycemia leads to severe harm by triggering numerous disorders, both micro and macro, like neuropathy, nephropathy, retinopathy, and cardiomyopathy (2,3). DN, a kidney disease linked to diabetes mellitus, is a kind of microvascular complication in diabetic patients and is the second highest cause of end-stage renal disease globally after glomerulonephritis only it is more prone to concurrent macrovascular events (4). DN, a grave consequence of diabetes, has been linked to several genetic and risk factors. This illness is characterized by proteinuria, edema, elevated blood pressure, and chronic renal failure, making it potentially fatal. According to studies on how diabetes affects people, the disease affects approximately 273-482 persons out of every 10,000. Death is a risk for patients whose risk for patients is also elevated, while others have kidney failure that completely prevents them from secreting urine (5).

The development of nephropathy is affected by renin-angiotensin-aldosterone system activation, which generates inflammation, cell stress, apoptosis, pyroptosis as well as autophagy (6). The first symptom of diabetic kidney disease is that the kidneys filter too much blood. After this, there is a loss of small amounts of protein in urine between 30mg/day and 300mg/day known as microalbuminuria (7,8). The diabetic patient is when sugar level cannot be maintained under normal range thus damaging the person's kidneys to a point that it might eventually reach an irreversible stage called Uremia. It arises from many genetic and environmental causes. Such components are significant in determining when the disease starts and how it is established. Currently, the actual biological mechanisms that precipitate DKD remain undefined. Genetic research aims to determine the underlying biological pathways of DKD to alleviate these problems (9).

Cytokines are a type of protein produced by multiple types of cells, mainly leukocytes, either constitutively or after activation; they serve as molecular mediators of innate and adaptive immunity, generally acting as intermediaries within and between these subsystems(10,11). Cytokines are classified into three families: chemokines, which drive chemotaxis; interleukins (including most lymphokines), which perform a variety of tasks such as immune cell maturation and proliferation; and interferons, which mostly resolve pathogenic presence (12,13).

The Nuclear factor-κappa B (*NF-κB*) pathway is a transcriptional factor involved in the diffuse neutrophil disease pathophysiology (DN). At the same time, it regulates gene expression that takes part in biological processes such as inflammation, innate and adaptive immunity as well as reactive oxygen species (ROS) overproduction. It is worth mentioning that diabetes presents *NF-κB* as one of its inflammatory markers, which is also tied to inflammation found in diabetic glomerulosclerosis. Research on the protein is crucial for early detection of diabetic glomerulosclerosis. *NF-κB* plays a pivotal role in renal disease progression by regulating genes like MCP-1 and RANTES. It is an emphasized importance." first respondent" to DN because it is constantly present in cells even when inactive. The Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway is one of the main pathways that respond to and transduce inflammatory signals. *NF-κB* is activated via JAK-STAT stimulates the transcription of proinflammatory cytokines, chemokines, and adhesion molecules in resident kidney cells (14,15).

Interleukin-6 (*IL-6*) is a cytokine whose functions include immune response regulation, and increased levels can be found in most patients with high blood sugar and heart disease. Some cells have the membrane-bound form, while others have the soluble form. Examples include kidney podocytes and leukocytes found in systemic circulation. Elevated *IL-6* levels are linked to unfavorable

cardiovascular outcomes and declining renal function in patients without type 2 diabetes. *IL-6* which directly acts on renal cells leads to damage and apoptosis of podocytes and stimulates mesangial cells to produce substances that attract inflammatory cells. Increased levels of *IL-6* are linked with decreased glomerular mass and mesangial expansion found in diabetes nephropathy. Therefore, targeting *IL-6* signaling might offer a possible alternative treatment for diabetes nephropathy. Also, *IL-6* contributes to metabolism, insulin resistance, energy balance, lipid metabolism, and other functions. The two major routes through which its actions are transmitted are classical and trans pathways (16–19).

*IFN-γ*, a kind of interferon, has effects on biological systems through the enhancement or inhibition of specific genes. It binds to some receptors found on the surfaces of most body cells, and the α chain plays a prominent role in combining with it, as the ϭ chain heightens its affinity. *IFN-γ* signaling activates gene expression through the JAK-STAT3 pathway. The receptor α chain dimerizes, JAK1 and JAK2 kinases are activated, and p-STAT1 forms as well. Transcription is also promoted by other genes, for example, IRF1 and CIITA by which *IFN-γ* exerts its effect. *IFN-γ* expression in IgA nephropathy is significantly affected by *IL-18* (20,21).

This experiment explores whether *NF-κB*, *IL-6*, and *IFN-γ* gene expression in the peripheral blood mononuclear cells (PBMCs) of diabetic patients with nephropathy indicates immune activation and inflammatory pathways involved in diabetes and its complications.

## 2. Materials and Methods

### 2.1. Study Participants

Before enrollment, all participants were given information about the study and gave signed informed consent. Sixty persons with DN (aged ≥28) were recruited. In addition, sixty healthy controls of the same age and gender were recruited. Inflammatory and systemic diseases, drug use and supplements, smoking, alcohol consumption, and pregnancy were all evaluated six weeks before the trial began. Pregnant women and those with kidney impairment were also excluded from the trial. Exclusion criteria included a history of kidney disease, inflammatory diseases during the last two months, other renal abnormalities, active autoimmune disease, and prior or current use of immunosuppressive drugs or steroids.

### 2.2. Sampling

Venous blood (20 mL) was collected into EDTA tubes (Sigma-Aldrich®). Within 3 hours of blood collection, PBMC was extracted using Ficoll-Paque density gradient centrifugation (HB705 Behsan laboratory) (temperature of 18 to 20 degrees Celsius, 2500 g, 15 to 20 minutes). To the portioned Buffy Coat sample, 10 mL of PBS (Sigma-Aldrich®) was put. After that, it was centrifuged for 5 minutes at 1400 rpm and a temperature of 4-6°C in a refrigerated centrifuge. This step was done many times. Afterward, the precipitate cells are moved into 1.5 ml microtubes. Fasting blood sugar, total cholesterol, triglycerides, HDL and LDL cholesterol, cholesterol/HDL LDL ratio, AST, ALT, alkaline phosphatase, WBC, RBC, platelets, HGB, HCT, MCV, MHC, HHC, and RDW-CV were measured using an Autoanalyzer.

### RT-PCR analysis

The genes (*NF-κB*, *IL-6*, and *IFN-γ*) were evaluated for expression using Real-Time PCR in each tissue group. After designing the primers, total RNA was extracted from the PBMC following company protocol and converted into cDNA using a cDNA Synthesis Kit. Subsequently, the expression of the abovementioned genes was assessed after cDNA was amplified using PCR. A comparison method of the Threshold Cycle: CT was used to evaluate the expression ratios of the genes investigated in this work. The PCR primers listed in Table 1 were utilized for RT-PCR.

### 2.3. Statistical Analysis

We used different types of statistical methods like one-way ANOVA, Tukey post-hoc test, non-

parametric Mann-Whitney test, and GraphPad Prism for the research while ascertaining the relationship between these two variables by using Pearson's correlation method. It had been considered statistically significant if a p-value was below 0.05.

Table 1: Primer Blast of genes under investigation and standard (NCBI).

| Gene | Primer sequence | Length | Tm | GenBank Code |
|---|---|---|---|---|
| ***NF-κB*** | F: **5'** –GCAGCACTACTTCTTGACCACC- **3'** | 130 | 61.44 | **XM-054350118.1** |
| | R:**5'** –TCTGCTCCTGAGCATTGACGTC- **3'** | | 62.60 | |
| ***IFN-γ*** | F: **5'** –GAGTGTGGAGACCATCAAGGAAG- **3'** | 124 | 60.62 | **NM-00619.3** |
| | R:**5'** –TGCTTTGCGTTGGACATTCAAGTC- **3'** | | 62.79 | |
| ***IL-6*** | F: **5'** – TTCTGCCAGTGCCTCTTTGCTG **- 3'** | 132 | 61.67 | **XM-054358146.1** |
| | R: **5'** –GGGTTGTGTTGGTTGTAGAG- **3'** | | 63.60 | |
| ***GAPDH*** | F: **5'** –GTCTCCTCTGACTTCAACAGCG **- 3'** | 131 | 60.92 | **NM-001357943.2** |
| | R: **5'** –ACCACCCTGTTGCTGTAGCCAA **-3'** | | 64.41 | |

## 3. Results

### 3.1. Gender and Age

The study took on the blood of participants equally according to gender (30 men and 30 women), both in the group of DN patients and in the healthy control group. Additionally, the individuals whose blood plasma was tested ranged in age from 28 to 34.

### 3.2. Analysis of blood factors

The following biochemical and hematological factors were examined including Fasting blood sugar test (FBS), total cholesterol and triglycerides, HDL and LDL cholesterol, WBC, RBC, platelets, HGB, HCT, M.C.V, M.C.H, M.C.H.C., RDW-CV, ALT, AST, Alkaline Phosphatase (Table 2).

Our study found that the patient group's fasting blood sugar levels increased compared to the control group (p=0). The peripheral blood samples from the two groups of individuals with DN and the healthy group did not exhibit any statistically significant differences in terms of cholesterol and triglyceride levels (total cholesterol (P-value = 0.1473) and triglyceride (P-value = 0.00289). For HDL, the normal and ideal range is 50–59 mg/dL, and for LDL, it is less than 100 mg/dL. The LDL (P-value = 0.13139) and HDL levels (P-value = 0.18262) did not significantly differ across the groups under study. The analysis of the rate of bad cholesterol (LDL)/good cholesterol (HDL) showed no remarkable differences (P=0.92942). Remarkable differences were not observed in white blood cell count between WBCs of patients with DN and healthy controls, so they are all well in health along with each other (P=0.03158 The survey found that red blood cell levels inside the bodies belonging to persons suffering from DN do not vary significantly from those contained in the control group (P = 0.30453). Data collected implied that while both categories of individuals had values between 150-450*103/uL our patients had way too many platelets than their counterparts who are considered normal (P = 0.00016). The study results showed that no significant differences were found in MCV (P-value = 0.07377), MCH (P-value = 0.16777), MCHC (P-value = 0.40494), and RDW-CV (P-value = 0.22395) levels between peripheral blood samples taken from patients diagnosed with DN and those taken from non-diabetes patients. All subjects were healthy. Moreover, patients had normal levels of Hemoglobin (HGB) and Hematocrit (HCT) compared to the controls. The peripheral blood levels of AST or ALT did not show a significant difference in DN patients when compared with the control group according to the study's findings (P-value = 0.70697 and P-value = 0.64543, respectively). Also, the patient and healthy groups did not exhibit any significant difference between them on alkaline phosphatase levels (P-value = 0.68873).

### 3.3. mRNA Expression Level of *NF-κB, IFN-γ and IL-6*

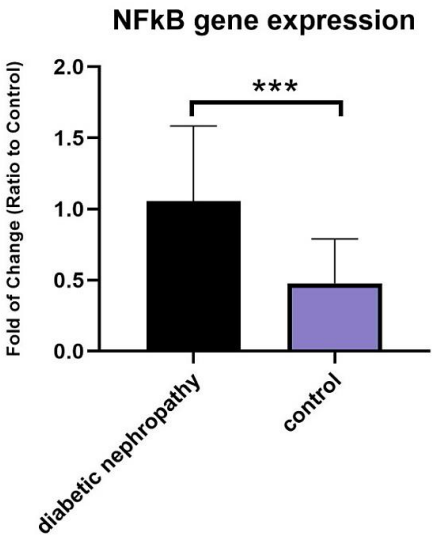
A substantial increase at the P<0.001 level was seen in the *NF-κB* gene expression analysis between the patient group and the control group. The level of *IFN-γ* expression in the patient group was significantly increased compared to the healthy control group at the level of P<0.01. The level of *IL-6* expression in the patient group was significantly increased compared to the healthy control group at the level of P<0.001 (Figures 1 & 2).

GAPDH also known as glyceraldehyde-3-phosphate dehydrogenase is a gene that is frequently used for reference purposes for certain gene expressions. Throughout all organisms, it has been referred to as a housekeeping gene because its expressional levels remain constant.

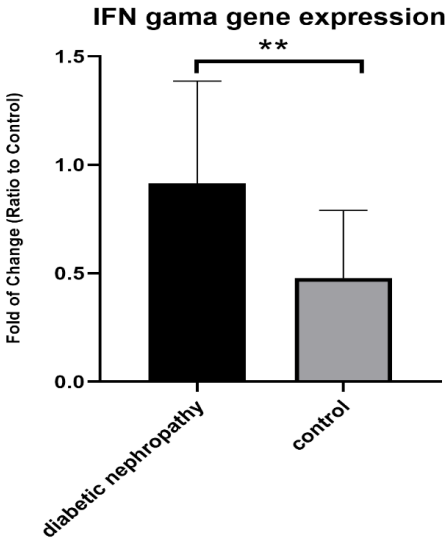
Table 2: Biochemical and hematological biomarkers in patients and healthy groups:

| Serum factor | Average healthy Group | Average Patient Group | P-value |
|---|---|---|---|
| **Fasting blood sugar test (FBS) (mg/dL)** | 96.86667 | 162.5483871 | 0 |
| **Total Cholesterol (mg/dL)** | 177.5172 | 186.0968 | 0.1473 |
| **Triglycerides(mg/dL)** | 160.4 | 187.7419 | 0.00289 |
| **HDL Cholesterol (mg/dL)** | 46.96667 | 49.3 | 0.18262 |
| **LDL Cholesterol (mg/dL)** | 107.8333 | 117.7333 | 0.13139 |
| **LDL/ HDL** | 2.404181 | 2.420287 | 0.92942 |
| **WBC ($10^3$/uL)** | 5.656667 | 6.266667 | 0.03158 |
| **RBC ($10^3$/uL)** | 5.132 | 4.981667 | 0.30453 |
| **Platelets ($10^3$/uL)** | 256.5333 | 323.7667 | 0.00016 |
| **HGB (g/Dl)** | 14.39 | 14.76 | 0.29614 |
| **HCT (%)** | 44.67 | 46.64 | 0.08511 |
| **M.C.V. (Fl)** | 88.33333 | 90.87667 | 0.07377 |
| **M.C.H. (pg)** | 28.85667 | 29.81667 | 0.16777 |
| **M.C.H.C. (g/dL)** | 33.19333 | 33.51667 | 0.40494 |
| **RDW-CV (%)** | 13.30667 | 12.96 | 0.22395 |
| **S.G.O.T. (AST) (U/L)** | 22.56667 | 23.23333 | 0.70697 |
| **S.G.P.T. (ALT) (U/L)** | 22 | 20.76667 | 0.64543 |
| **Alkaline Phosphatase (IU/L)** | 170.2333 | 174.5333 | 0.68873 |

A)




B)




C)

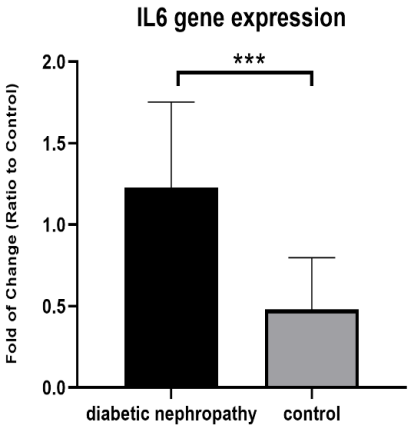



Figure 1: Examining changes in A) NF-Kb, B) IFN-γ, C) IL-6 expression in the patient group compared to the healthy control group (* has a significant difference at P<0.05, ** has a significant difference at P<0.01, and *** has a significant difference at P<0.001).

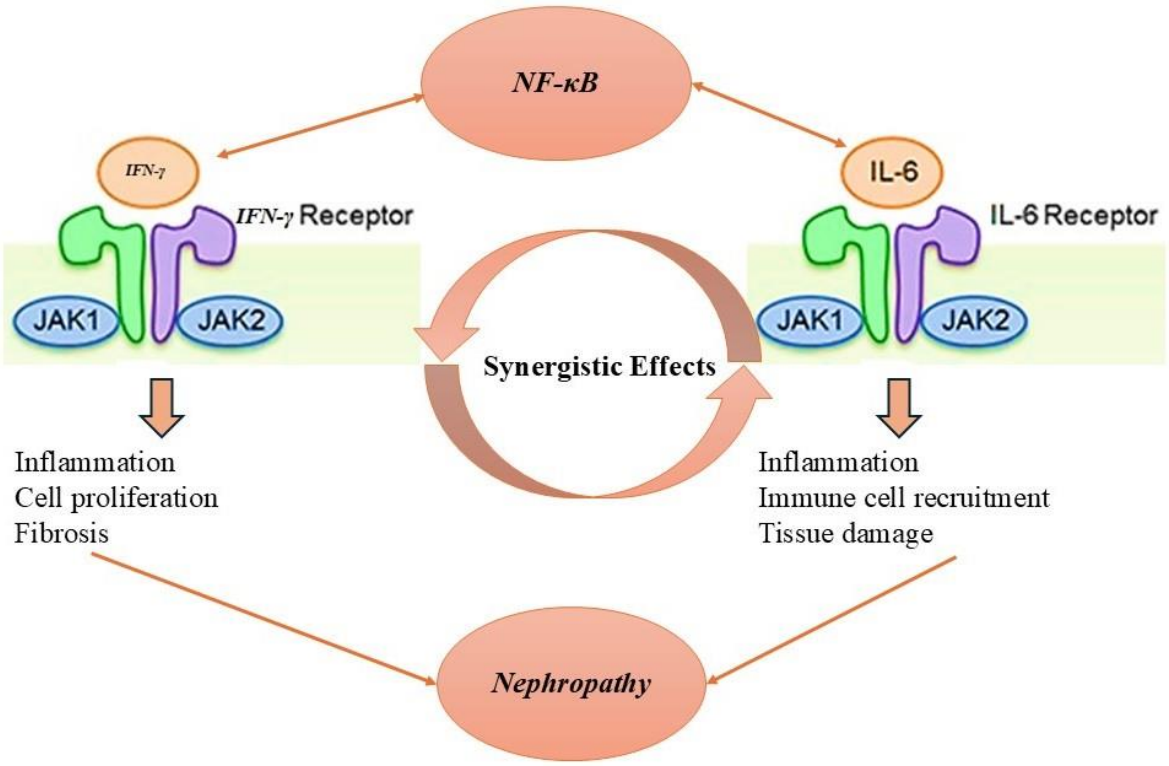



*Figure 2: NF-κB signaling pathway. Pro-inflammatory cytokines such as* IL-6 *and IFN-γ, released upon NF-κB activation, bind to their receptors to stimulate the JAK/STAT pathway. JAK/STAT pathway leads to nephropathy as it causes inflammation, cell division, fibrosis, immune cell attraction, and tissue destruction, as shown by the upregulation of* IL-6 *and IFN-γ in the nephropathy. The self-sustaining cycle of NF-κB activation and* IL-6 *and IFN-γ production contributes to nephropathy progression, with potential synergistic effects in the context of nephropathy.*

## 4. Discussion

This study intended to compare *NF-κB*, *IL-6*, and *IFN-γ* expressions at the gene level in peripheral blood mononuclear cells (PBMC) among nephropathic and normal people with diabetes. Examining the connection between the *NF-κB*, *IL-6*, and *IFN-γ* gene expression and the clinical outcome was another consideration for diabetic patients with nephropathy. One of the main objectives of the planned study was to examine how *NF-κB*, *IL-6*, and *IFN-γ* function in the onset and progression of nephropathy in diabetic patients and how to create an effective treatment plan.

Poloni and Rotta (2022) point out that advanced diabetic kidney disease is the leading cause of serious renal impairment in individuals suffering from diabetes mellitus; it therefore constitutes a microvascular disease requiring immediate immunosuppression hormonal treatment for reversing the condition and gradually enhancing renal performance (22). The urinary protein which has been considered a conventional marker in diagnosing DN may however be an underestimation of the initial renal injury as a result of dietary patterns, and metabolic utilization in patients (23). Premature kidney damage is detected by elevated proinflammatory cytokines in serum markers like

*IL-6*, *NF-κB*, and *IFN-γ*, offering a new approach to managing DN (24).

Immune cell formation or delayed proliferation can result from *NF-κB* inhibition that is prolonged, but inflammation may arise when it is excessively activated. Hence, the transformation of *NF-κB* which is usually located in cytoplasm while attached to inhibitory proteins activated through extracellular signals translocates to the nucleus where it binds to DNA sequences at κB sites regulating various genes responsible for inflammation, immune response regulation, cell death inhibition or promotion including those that support the division of cells (25).

According to the research, interleukin-6 (IL6) is overproduced in the kidney tissue of DN patients, which is linked to para-endothelial permeability of renal glomerular capillaries, hyperplasia of the mesangial cells, as well as thickening of basal membranes. This has been observed in several animal models with diabetes mellitus type I or II exhibiting increased levels of circulating or urine *IL-6*, especially in those that develop nephrotic syndrome (25).

One of the type II interferons, *IFN-γ*, is a cytokine that promotes inflammation and is made in natural killer cells as well as activated T cells. For instance, the inflammation may become worse when *NF-κB* is triggered by it. DN severity is related to increased levels of *IFN-γ* in renal tissues. In this way, enhanced responsiveness in these cells might be a result of their priming by *IFN-γ* whereas their sensitization could have been possible due to *NF-κB*'s presence (26). Both innate and adaptive immune system cells release *IFN-γ* protein which activates macrophages and an antigen presentation. The major function of this protein is to enhance the immune response by promoting cell activation. The nature of the cellular environment and other cofactors determines if *NF-κB*, a transcription factor can either stimulate or inhibit *IFN-γ* expression. The transcription factors are indirectly influenced by *NF-κB* concerning *IFN-γ* expression (27).

The study found that people who have DN had higher fasting blood sugar levels (184-201 mg/dL) than those without diabetes however, lipid profile in peripheral blood samples did not differ much. The research did not show any spacious disparities in LDL and HDL levels together with the ratio of LDL/HDL or WBC levels among people suffering from DN as against healthy subjects. Such findings reveal how these patients were just faring alright. There was not much difference between the red cell count (RBC) and hemoglobin concentrations, including hematocrit volumes, MCVs, or MCHs rather than RDWCVs whatsoever vis-a-vis those patients suffering from diabetes nephropathy if we compared them with their controls. The hemoglobin and hematocrit levels of both groups were normal, with no main differences in MCV, MCH, MCHC, or RDW-CV. DN patients had the same levels of AST as well as ALT compared to the control group.

The results of gene expression analysis showed that there is a significant increase in *NF-κB* gene expression between the patient group and the control group at the level of $P<0.001$. In addition, the patient group showed a significant increase ($P<0.01$) in the expression level of *IFN-γ* compared to the healthy control group. As expected, there was a significant increase in *IL-6* expression in the patient group compared to the healthy control group, with a P value of less than 0.001.

The study aimed to investigate how the *NFKB1* gene-94 ATTG Insertion/Deletion Polymorphism relates to susceptibility to DN among normoglycemic Asian Indians and subjects with type 2 diabetes, also referred to as T2DM and DN. A total of three hundred subjects were involved. Heightened UEMCP-1 and *TNF-α* concentrations were noticed in those possessing the -94 ATTG ins allele of the *NFKB1* gene, consequently raising the possibility for the occurrence of DN by almost twice (28). The researchers examined levels of an important gene in

urine and blood samples from 35 patients with diabetes as well as healthy control subjects. It was found that diabetic patients had elevated levels of *NF-κB* in both samples resulting in raised microalbuminuria, serum creatinine, urea, and BMI. In addition, this study proved that increased production of *NF-κB* is responsible for DN development while its genetic makeup can always be identified using liquid sampling (29). The current investigation indicates that nephropathy-affecting *NF-κB* significantly influences kidney damage as well as inflammation, especially diabetic kidney disease among other metabolic kidney conditions. Consequently, treatment of this could involve fibrosis reduction hence decreasing inflammation.

Navarro found out that in diabetic rats renal cortical mRNA levels of pro-inflammatory cytokines *TNF α*, *IL-1*, and *IL-6* are much higher than in non-diabetic rats. Nevertheless, enalapril significantly decreases PTF-administered rats' over-expression resulting in a reduction of kidney wet weight and albuminuria which means the involvement of inflammatory mechanisms in renal diabetic rats' injury (30). The participants in a study with DN discovered that *TNFα* is crucial in inducing the harmful alterations that result in nephropathy. According to the results, there was a significant increase in the levels of interleukin-6 and *TNF-α* in the serum between the various groups. According to the study, *TNF-α* and *IL-6* may serve as potential new therapeutic targets for patients with DNs; thereby developing improved preventive and therapeutic options for the disease (31,32). n Bali last year, a survey was done to determine the association between type 2 diabetes *IL-6* gene polymorphism rs1800796 and DN. A case-control method was employed to choose sixty people. It was found that the prevalence of DN is not affected much by genotype or allele distribution. While the risk of DN was increased in those with the CG-GG genotype, it was not statistically significant (33). As shown by these outcomes, this research also supported a noteworthy rise in *IL-6* and *IFN-γ* expression within the DN cohort compared with that in controls with no such disorder. The synthesis of these pro-inflammatory factors is presumably involved in establishing or advancing micro- and macrovascular complications of DKD due to several effects, e.g., inflammation triggering, promotion of oxidative stress, fibrosis induction, etc. Both studies show a remarkable increase in *IL-6* levels. In the progress of DN, *IL-6* is involved in numerous deranged processes, including provoking inflammation pathways, disrupting endothelial function, and releasing extracellular matrix elements, all resulting in glomerular and tubular damage. The considerable increase *in IFN-γ* detected in our research is also characterized by great importance, given that this particular cytokine can aggravate inflammation and pave the way for kidney fibrosis. Thus, treating DN could target specific inflammatory pathways involving *IL-6* and *IFN-γ*. In this way, it can be interpreted that the JAK/STAT pathway is activated by the binding of *IL-6* to its receptor and inflammatory, proliferative, and fibrotic genes can be transcribed through this pathway, which are all pathological features of DN. Furthermore, *IL-6* likely manipulates the WNT signaling pathway, which has significant effects on cell fate, differentiation, and extracellular matrix. Therefore, it is thought that disruption of this pathway may lead to DN, and thus the interaction between *IL-6* and WNT may worsen the pathological progression.

In Eastern India, Behera et al. did a study on the correlation between *NFkB1* gene polymorphism and Inflammatory markers Urinary Monocyte Chemoattractant Protein 1 (UMCP1) and Tumor Necrosis Factor alfa (*TNF-α*) among patients with diabetes mellitus. A thing that the study indicated is that there was an elevation of *NFkB1* gene expression, *UMCP1* & *TNF-α* levels in patients with DN. In these diabetics, it seems that the danger of developing this condition is more than twice as high if they have an insertion/insertion polymorphism in the *NFkB1* gene (34). In a study by Sevak et al. in 2022, it was observed that Nrf2 mRNA expression

increased in DN and non-diabetic patients, while *NF-κB* expression was significantly elevated in all groups (35). In the same year, another study by Dias et al. demonstrated that the *NF-κB* gene expression and *TNF* level were higher in such persons with type-II diabetes mellitus who also had nephropathy due to diabetes compared with healthy persons, showing a high level of sensitivity to N6-carboxymethyl lysine (36). It can be seen that the rise in *NF-κB* gene expression among patients with DN is superior to what is obtainable in healthy individuals, which is similar to the observation made in our study. This is a pointer to the importance of *NF-κB* signaling towards the development of diabetic kidney disease as given by different researchers. The *NF-κB* pathway is crucial for inflammation and immune responses. Translation to numerous genes related to inflammation, oxidative stressors, growth factors of the cell multiplication process, and fibrotic changes are typical features of chronic kidney damage from diabetes; therefore, it could be transcribed from this source.

DN rats showed increased Scr and BUN levels as well as elevated secretion of *TGF-β1*, *TNF-α*, and *IFN-γ* while chemerin and VEGF expressions were overexpressed suggesting their critical participation in DN. Chemerin and VEGF expression were overexpressed in DN rats, suggesting they play crucial roles in DN. Chemerin, a fat cell factor, regulates Inflammation, while VEGF promotes cell proliferation and differentiation (37). Li An demonstrated that elevated levels of *IFN-γ* and SCF were linked to a higher risk of DN, but elevated levels of MIP1b and *IL-16* were probably related to a lower risk of DN. In the next study, An and colleagues (2014) discovered that levels of plasma and urine complement, and inflammatory biomarkers were much greater in patients with T2DM than in those who did not have it. Urinary MBL (mannan-binding lectin) and *IFN-γ* were identified as independent risk factors for DM in T2DM patients. The study suggested that urinary *IFN-γ* and MBL levels are independent risk factors with high predictive power for DM in T2DM patients (38). The pathogenesis of DN is heavily influenced by numerous inflammatory cytokines and signaling molecules. In the rat model of DN, the marked secretion of *TGF-β1*, *IFN-γ*, and *TNF-α*, together with higher levels of serum creatinine and blood urea nitrogen, provides evidence for an imbalanced development of these inflammation pathways that might be pivotal in causing this complication. We detected a substantial induction of the expression of *IFN-γ* in patients with DN compared to normal subjects. These findings correlate with the previous studies. Altogether, the present results demonstrate that *IFN-γ* plays a significant role as an inflammatory mediator in the development of DN. The powerful *IFN-γ* induces the JAK/STAT signaling pathway that results in the transcription of genes implicated in inflammation, cell proliferation, and fibrosis. The implication of this pathway for the onset and progression of DN is extensive mainly because it results in increased deposition of matrix outside cells, promotes proliferation of mesangial cells, and attracts immune system cells to the site. Our study found a co-dependence between *IFN-γ* and the *NF-κB* signaling cascade. This study also found the upregulation of *NF-κB* in the same experiment. Additionally, communication crosstalk among inflammatory signaling networks exacerbates kidney damage, prolonging inflammation, increasing oxidative stress, and leading to DN.

## 5. Conclusion

Analyzing the expression of *NF-κB*, *IL-6*, and *IFN-γ* genes in the peripheral blood mononuclear cells (PBMCs) could introduce biomarkers linked to disease progression and outcome. These signs can work as practical markers of disease seriousness and risk prediction for patients with diabetic kidney disease when they are estimated from peripheral blood mononuclear cells (PBMCs) *NF-κB*, *IL-6*, *IFN-γ* levels in blood monocytes can be used as indicators for evaluating whether anti-inflammatory

therapies targeting these pathways are effective considering how important are they during inflammatory processes underlying diabetic nephropathy. Studying these factors' expression might help us to understand the pathogenetic mechanisms underlying DN. Considering the significant increase in the expression of the mentioned genes in patients with DN in this study, it can be said that the low levels of these biomarkers can be a factor for more promising treatment.

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(I) Conception and design: Nazanin Jabbari, Parisa Gheibi, Yousif Badr Makki, Zahra Azizi

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(VI) Manuscript writing: All authors

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## Data Availability

All data generated or analyzed during this study are included in this manuscript.

## Declaration of interests

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

## Ethics approval and consent to participate

All procedures in this research were carried out by the rules of the Iran National Research Ethics Committee (IR.TUMS.TIPS.REC.1401.137), Tehran, Iran.

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