Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
Al-Qadisiyah Journal of Pure Science

The role of TGF-Beta in diabetic nephropathy

Ahmed Issa Abdulhussein[1] *Dr. Wijdan Thamer Mahdi*[2]

[1]Department of Biology, College of Science, University of AL-Qadisiyah, AL-Diwaniyah, Iraq. [2]Department of Biology, College of Science, University of AL-Qadisiyah, AL-Diwaniyah, Iraq. Email: sci.bio.phd.23.2@qu.edu.iq

Received: 16/04/2026; Revised: 25/04/2026; Accepted: 26/04/2026

ABSTRACT:

Objectives: The primary objective of this study was to investigate the role of certain biomarkers, including transforming growth factor beta 1(TGF-β1), interleukin-6 (IL-6), Mammalian target of rapamycin (mTOR), and total antioxidants, in the development of diabetic nephropathy, alongside routinely used biochemical parameters.

Materials and Methods: Patients were screened and enrolled using a convenient non-probability sampling technique at Diwaniyah Teaching Hospital. A total of 100 individuals participated in the study, divided into two groups: 50 patients with Type 2 Diabetes Mellitus (T2DM) (26 males and 24 females) and 50 healthy subjects (28 males and 22 females) as the control group. Five mL of venous blood was drawn from the antecubital vein of each participant. Biomarkers such as TGF-β1, interleukin-6 (IL-6), mTOR, and total antioxidants were assessed in the serum of both patient and control groups using standard laboratory techniques.

Results: The mean values of biochemical parameters (HbA1c, serum creatinine, TGF-β1, IL-6, and mTOR) were found to be higher, while serum albumin, total antioxidant levels, and estimated glomerular filtration rate (eGFR) were lower in the nephropathy group compared to the control group.

Conclusions: Elevated levels of TGF-β1 and mTOR, along with impaired antioxidant defenses, may contribute to the progression of diabetic nephropathy. Additionally, increased levels of inflammatory mediators such as IL-6 are responsible for enhancing inflammation in patients with diabetic nephropathy.

1-INTRODUCTION

Diabetic nephropathy (DN) is one of the most common and severe complications of diabetes mellitus (DM), contributing to higher morbidity and mortality among diabetic patients(1). The global prevalence of diabetes is rapidly increasing, particularly in developing nations(2). Consequently, the prevalence of DN is also expected to rise unless the clinical strategies for its prevention are improved immediately(3,4).

Several pathways and mediators contribute to the onset and progression of DN(5), including oxidative stress, angiotensin II (Ang-II), inflammatory processes, and metabolic pathway disorders caused by persistent hyperglycemia(6). Elevated glucose levels in blood vessels play a major role in the development of DN. Hyperglycemia causes metabolic dysfunctions in mitochondria and the glucose metabolic pathway through the excessive production of reactive oxygen species (ROS)(7). High glucose concentrations lead to the glycation of plasma proteins, forming covalent adducts. One significant consequence of this is the production of advanced glycation end products (AGEs), which are a major risk factor for diabetes complications(8).

Chronic exposure to hyperglycemia can cause abnormalities in podocytes, which are crucial components of the glomerular filtration barrier. The damage to podocytes is one of the earliest glomerular morphological alterations and plays a key role in the development of DN. Clinically, DN is characterized by impaired kidney function and proteinuria(9,10,11). Pathologically, kidney enlargement, thickening of the basement membrane, deposition of extracellular matrix proteins, glomerular sclerosis, and interstitial fibrosis are commonly observed in DN(12).

The pathogenesis of diabetic nephropathy (DN) is significantly influenced by various mediators and signaling mechanisms, particularly the cytokine transforming growth factor beta (TGF-β).TGF-β1 is a well-known prosclerotic and profibrotic cytokine that contributes to tissue fibrosis, including the development of DN, by inducing the expression of extracellular matrix (ECM) components(13). Clinically, patients with DN exhibit elevated levels of plasma and urinary TGF-β1, and these levels positively correlate with the severity of the disease, including progressive renal fibrosis(14,15,16,17).

This review discusses several biomarkers of DN, particularly total antioxidants, IL-6, and mTOR. This study attempted to identify potentially diagnostic and predictive biomarkers to stratify the risk of DN progression.

2-METHODS

Patients and samples

Study participants (N=100) were recruited from Diwaniyah Teaching Hospital between October 2024 and August 2025. Subjects were categorized accordingly into two groups: 50 patients with T2DM (26 male, 24 female) and 50 normal healthy subjects (28 male, 22 female) as control group. The mean age of patients was 62.06 ± 8.55 and that of control subjects was 59.80 ± 8.86 years.

Blood pressure measurements were taken immediately following completion of the recruitment questionnaire. Estimated glomerular filtration rate and HbA1c were also recorded. Exclusion criteria included participants <18 years of age with autoimmune disease or who had been diagnosed with a condition or illness that could impact kidney function, e.g., hepatitis B or C, polycystic kidney disease and/or stones.

Participant Sample Collection

Venous blood (5 ml) was collected from both diabetic nephropathy (DN) patients and the control group. The blood samples were kept at room temperature for 30 to 60 minutes to allow for clot formation. The vacutainers were then centrifuged at 1300 g for 10 minutes at 4°C. The serum was carefully decanted and aliquoted into pre-labeled cryovials, which were stored at -80°C until analysis. The study parameters were measured using standard laboratory techniques; IL-6, mTOR, and TGF-β1 were quantified using ELISA, while total antioxidant capacity was measured using the T-AOC assay. Biochemical assays included albumin measurement using the BCG method and creatinine measurement using the Jaffe method.

Statistical analysis

The present study enrolled 50 patients with diabetic nephropathy (DN) and 50 healthy control subjects, as shown in figure (3-1). Data were collected, summarized, analyzed and presented using the Statistical Package for the Social Sciences (SPSS) version 26 and Microsoft Office Excel 2010. Numeric data were presented as mean, standard deviation after performance of **Kolmogorov-Smirnov normality test** and making a decision about normally and non-normally distributed variables.

3-RESULTS

A total of 50 patients and 50 healthy control subjects were enrolled in the recent study. A comparison of HbA1c, GFR, creatinine and albumin between diabetic nephropathy (DN) patients and the control group was conducted, with the results displayed in Table 1.

Mean serum TGF-β1 levels were significantly higher in DN patients at 4.25 ± 0.72 ng/ml compared to 1.27 ± 0.19 ng/ml in healthy controls ($P < 0.001$) (Fig.1).

Mean levels of total antioxidants were 0.85 ± 0.07 mmol/L in DN patients and 1.47 ± 0.06 mmol/L in the healthy control group, demonstrating that antioxidant levels were significantly lower in the patients ($P < 0.001$) (Fig.2).

Mean serum mTOR levels were 1.90 ± 0.19 ng/mL in DN patients and 0.39 ± 0.07 ng/mL in the healthy control group, with mTOR levels significantly higher in the patients ($P < 0.001$) (Fig.3).

Mean serum IL-6 levels were 20.66 ± 4.8 pg/mL in DN patients and 4.94 ± 1.76 pg/mL in the healthy control group, revealing that IL-6 levels were significantly higher in the patients ($P < 0.001$) (Fig.4).

Table 1: Comparison of the biochemical and genetic variables study groups

Variables	Control group	DN group
Age (years)	**59.80** ± 8.86	**62.06** ± 8.55
HbA1c (%)	**3.67** ± 0.55	**8.53** ± 1.33
Creatinine (mg/dl)	**0.69** ± 0.11	**2.15** ± 0.52
GFR(mg/min/1.73m^2)	**106.6** ± 9.39	**32.25** ± 5.58
Albumin (g/dL)	**4.35** ± 0.17	**2.97** ± 0.25
TGF-β1(ng/ml)	**1.27** ± 0.19	**4.25 ± 0.72**

Total Antioxidants (mmol/L)	**1.47** ± 0.06	**0.85 ± 0.07**
mTOR (ng/mL)	**0.39** ± 0.07	**1.90 ± 0.19**
IL-6 (Pg/mL)	**4.94** ± 1.76	**20.66 ± 4.8**

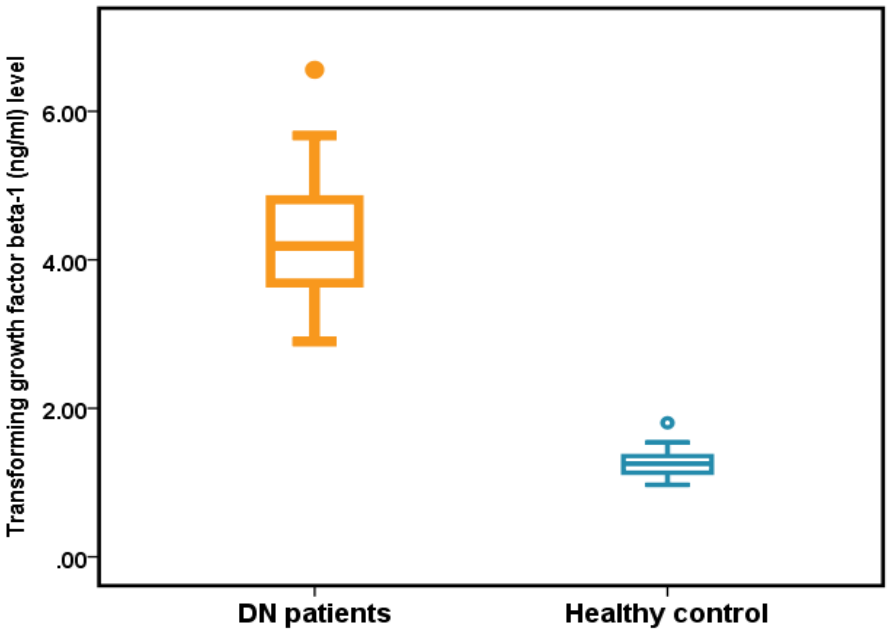

Figure (1): Mean serum TGF-β1 level of patients and healthy controls .

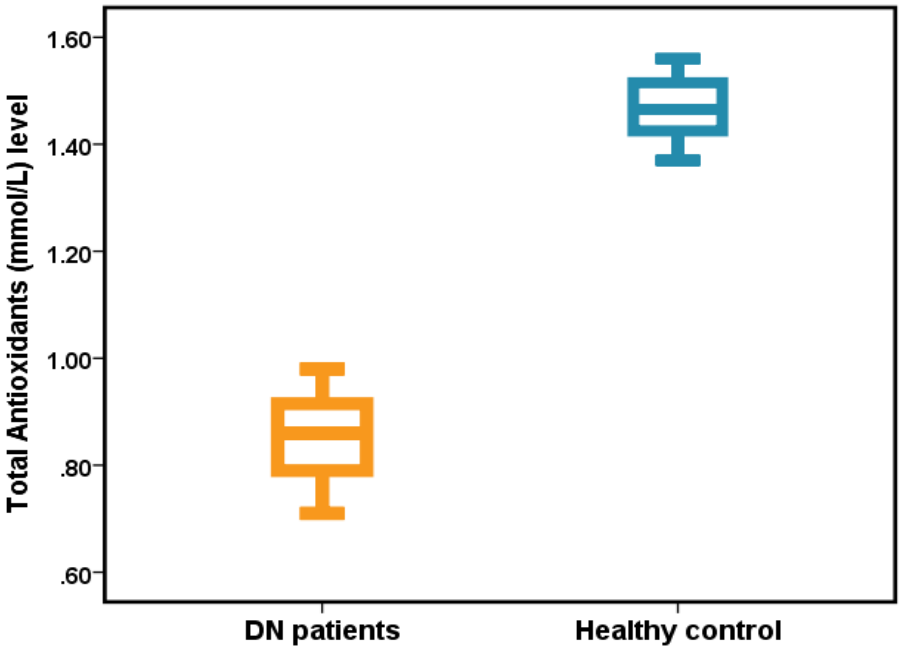

Figure (2): Mean Total Antioxidants of patients and healthy controls .

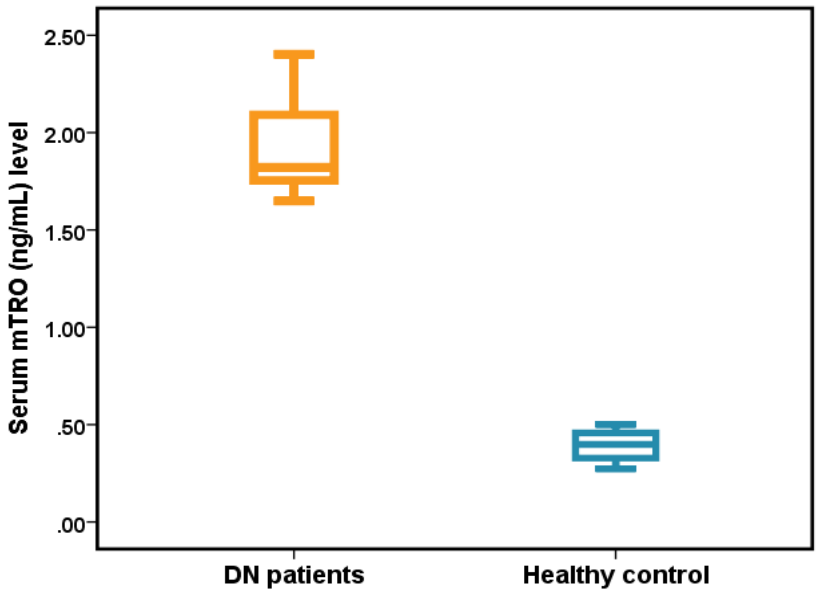

Figure (3): Mean Serum mTOR level of patients and healthy controls .

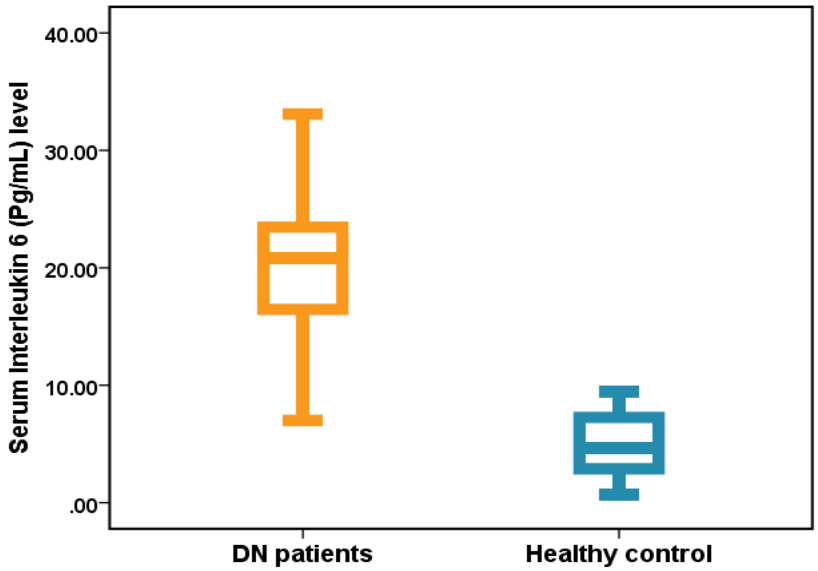

Figure (4): Mean serum IL-6 level of patients and healthy controls .

4-DISCUSSION

A significant increase in serum TGF-β1 was observed in patients with diabetic nephropathy (DN) compared to the control group ($P < 0.001$).

TGF-β1 levels are significantly elevated in the blood serum, urine, and kidneys of patients with diabetic nephropathy (18). Studies indicate that TGF-β1 not only promotes mesenchymal cell proliferation, mesenchymal matrix deposition, and glomerular sclerosis but also leads to interstitial tubular fibrosis (19).

Elevated glucose levels enhance TGF-β signaling and promote the transcriptional activity of fibronectin promoter and luciferase structures containing Smad-binding elements (SBEs) in the parenchyma cell (20). Indeed, elevated glucose levels stimulate Tgfb1 transcription in various kidney cell types, including parenchyma cells (21,22), fibroblasts(23), and proximal tubular cells(24). In addition to promoting Tgfb1 expression, elevated glucose levels also enhance TGF-β1 activity by upregulating thrombospondin 1 (TSP1), which can activate latent TGF-βs (25,26). Furthermore, elevated glucose levels have been

found to increase Tgfbr2 transcription in mouse parenchyma cells independently of TGF-β stimulation (27). Therefore, elevated glucose levels can activate TGF-β signaling during the development of diabetic neuropathy.

Our study observed that diabetic nephropathy (DN) patients had a mean IL-6 level significantly higher than that of the control group. There was a statistically significant association. Similarly, Sanchez-Alamo *et al*. (28) discovered a strong correlation between the development of kidney illness in DN patients and elevated blood IL-6 levels. Their research demonstrates that higher IL-6 levels are linked to a greater risk of advancing to ESKD, reinforcing IL-6 as a critical biomarker for DN.

The development of DN is significantly impacted by inflammatory responses, which are facilitated by IL-6. (29). IL-6 trans-signaling exacerbates tissue damage and fibrosis in DN by attracting and activating more inflammatory cells in the kidney. IL-6 primarily affects mesangial cells, a kind of kidney cell involved in the formation and function of the glomerulus. Mesangial cell proliferation and the synthesis of extracellular matrix components are caused by elevated IL-6 levels, which also contribute to glomerulosclerosis and mesangial expansion (30).

Both our research and that of Reddy *et al*. (31) highlight the potential of IL-6 as a noninvasive biomarker for identifying and tracking the development of disease, suggesting that monitoring IL-6 levels may offer important insights into the course of the disease and direct therapeutic measures.

The mTOR signaling pathway plays an essential role in regulating various pathological processes associated with diabetic nephropathy (DN). Dysregulation of mTOR can influence the expression of other molecules, either individually or in conjunction with each other, leading to the formation of complexes that modulate the mechanistic pathway. This dysregulation induces pathological

changes in four different types of kidney resident cells as well as immune cells. Consequently, this aberrant regulation contributes to the onset and progression of DN(32).

According to Maity *et al*.,(33), transforming growth factor beta (TGF-β) can enhance mTOR activity, promoting the proliferation and hypertrophy of mesangial cells by inhibiting the mTOR regulatory protein, deptor, through the mediation of miR-181a targeting. Additionally, TGF-β can activate mTOR by phosphorylating the platelet-derived growth factor receptor beta (PDGFR-β), which encourages mesangial cell hypertrophy and leads to the deposition of matrix proteins(34). Furthermore, mTOR activation can increase the production of reactive oxygen species by upregulating antioxidant enzymes and downregulating the activities of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. Consequently, this process may lead to apoptosis in glomerular mesangial cells (GMCs) and impair the glomerular filtration barrier(35).

Hyperglycemia-induced diabetic nephropathy (DN) and multi-organ damage are associated with a reduction in cellular antioxidant mechanisms and the depletion of antioxidants in cells through both enzymatic and non-enzymatic processes. Numerous studies have demonstrated that DN is linked to an imbalance between reactive oxygen species (ROS) generation and antioxidant scavenging in cells(36). Additionally, elevated glucose levels promote the auto-oxidation and glycosylation of proteins, leading to the production of free radicals. This increase in reactive oxygen species ultimately reduces antioxidant activity, which is a critical factor in oxidative stress(37).

Endogenous antioxidant systems, such as thioredoxin, glutathione, and peroxidases, play a vital role in maintaining ROS at optimal levels. However, under hyperglycemic conditions, these antioxidant systems become

dysfunctional, and ROS-dependent signaling pathways are activated(38). The increase in ROS levels, due to the failure of antioxidant mechanisms, contributes to inflammation, kidney scarring, and other pathophysiological changes. This process results in a further decline in glomerular filtration rate (GFR) and the progression of diabetic nephropathy(39).

5-CONCLUSION

In conclusion, four biomarkers were studied: TGF, mTOR, IL-6 and antioxidant to have clinical usefulness for differentiating patients with T2D from healthy people. A combination of these indicators may be used to identify patients who are at risk of developing kidney disease, enabling earlier, and more efficient management intervention. longitudinal investigations involving T2D's and their potential progression to DN would need to be validate Our results.

6-REFERENCES

1-Valencia, W. M., & Florez, H. (2017). How to prevent the microvascular complications of type 2 diabetes beyond glucose control. *Bmj*, *356*.

2-Gheith, O., Farouk, N., Nampoory, N., Halim, M. A., & Al-Otaibi, T. (2015). Diabetic kidney disease: world wide difference of prevalence and risk factors. *Journal of nephropharmacology*, *5*(1), 49.

3-Stenvinkel, P. (2010). Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. *Journal of internal medicine*, *268*(5), 456-467.

4-Arora, M. K., & Singh, U. K. (2013). Molecular mechanisms in the pathogenesis of diabetic nephropathy: an update. *Vascular pharmacology*, *58*(4), 259-271.

5-Kopel, J., Pena-Hernandez, C., & Nugent, K. (2019). Evolving spectrum of diabetic nephropathy. *World journal of diabetes*, *10*(5), 269.

6-Tavafi, M. (2013). Diabetic nephropathy and antioxidants. *Journal of nephropathology*, *2*(1), 20.

7-Nishikawa, T., Edelstein, D., Du, X. L., Yamagishi, S. I., Matsumura, T., Kaneda, Y., ... & Brownlee, M. (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*, *404*(6779), 787-790.

8-Singh, V. P., Bali, A., Singh, N., & Jaggi, A. S. (2014). Advanced glycation end products and diabetic complications. *The Korean journal of physiology & pharmacology: official journal of the Korean Physiological Society and the Korean Society of Pharmacology*, *18*(1), 1.

9-Lewko, B., & Stepinski, J. (2009). Hyperglycemia and mechanical stress: targeting the renal podocyte. *Journal of cellular physiology*, *221*(2), 288-295.

10-Eid, A. A., Gorin, Y., Fagg, B. M., Maalouf, R., Barnes, J. L., Block, K., & Abboud, H. E. (2009). Mechanisms of podocyte injury in diabetes: role of cytochrome P450 and NADPH oxidases. *Diabetes*, *58*(5), 1201-1211.

11-Williams, M. E. (2005). Diabetic nephropathy: the proteinuria hypothesis. *American journal of nephrology*, *25*(2), 77-94.

12-Cao, Z., & Cooper, M. E. (2011). Pathogenesis of diabetic nephropathy. *Journal of diabetes investigation*, *2*(4), 243-247.

13-Meng, X. M., Tang, P. M. K., Li, J., & Lan, H. Y. (2015). TGF-β/Smad signaling in renal fibrosis. *Frontiers in physiology*, *6*, 82.

14-Ibrahim, S., & Rashed, L. (2007). Estimation of transforming growth factor-beta 1 as a marker of renal injury in type II diabetes mellitus. *Saudi medical journal*, *28*(4), 519-523.

15-Shaker, Y. M., Soliman, H. A., Ezzat, E., Hussein, N. S., Ashour, E., Donia, A., & Eweida, S. M. (2014). Serum and urinary transforming growth factor beta 1 as biochemical markers in diabetic nephropathy patients. *Beni-Suef University Journal of Basic and Applied Sciences*, *3*(1), 16-23.

16-Jakuš, V., Sapák, M., & Kostolanská, J. (2012). Circulating TGF-β1, Glycation, and Oxidation in Children with Diabetes Mellitus Type 1. *Journal of Diabetes Research*, *2012*(1), 510902.

17-Murakami, K., Takemura, T., Hino, S., & Yoshioka, K. (1997). Urinary transforming growth factor-β in patients with glomerular diseases. *Pediatric Nephrology*, *11*(3), 334-336.

18-Gao, P., Li, L., Yang, L., Gui, D., Zhang, J., Han, J., ... & Liu, J. (2019). Yin Yang 1 protein ameliorates diabetic nephropathy pathology through transcriptional repression of TGFβ1. *Science translational medicine*, *11*(510), eaaw2050.

19-Meng, X. M., Nikolic-Paterson, D. J., & Lan, H. Y. (2016). TGF-β: the master regulator of fibrosis. *Nature Reviews Nephrology*, *12*(6), 325-338.

20-Isono, M., Chen, S., Hong, S. W., Iglesias-de La Cruz, M. C., & Ziyadeh, F. N. (2002). Smad pathway is activated in the diabetic mouse kidney and Smad3 mediates TGF-β-induced fibronectin in mesangial cells. *Biochemical and biophysical research communications*, *296*(5), 1356-1365.

21-Wolf, G., Sharma, K., Chen, Y., Ericksen, M., & Ziyadeh, F. N. (1992). High glucose-induced proliferation in mesangial cells is reversed by autocrine TGF-β. *Kidney international*, *42*(3), 647-656.

22-Hoffman, B. B., Sharma, K., Zhu, Y., & Ziyadeh, F. N. (1998). Transcriptional activation of transforming growth factor-β1 in mesangial cell culture by high glucose concentration. *Kidney international*, *54*(4), 1107-1116.

23-Han, D. C., ISONO, M., HOFFMAN, B. B., & ZIYADEH, F. N. (1999). High glucose stimulates proliferation and collagen type I synthesis in renal cortical fibroblasts: mediation by autocrine activation of TGF-β. *Journal of the American Society of Nephrology*, *10*(9), 1891-1899.

24-Rocco, M. V., Chen, Y., Goldfarb, S., & Ziyadeh, F. N. (1992). Elevated glucose stimulates TGF-β gene expression and bioactivity in proximal tubule. *Kidney international*, *41*(1), 107-114.

25-Wang, S., Skorczewski, J., Feng, X., Mei, L., & Murphy-Ullrich, J. E. (2004). Glucose up-regulates thrombospondin 1 gene transcription and transforming growth factor-β activity through antagonism of cGMP-dependent protein kinase repression via upstream stimulatory factor 2. *Journal of Biological Chemistry*, *279*(33), 34311-34322.

26-Murphy-Ullrich, J. E., & Suto, M. J. (2018). Thrombospondin-1 regulation of latent TGF-β activation: A therapeutic target for fibrotic disease. *Matrix Biology*, *68*, 28-43.

27-Isono, M., Mogyorósi, A., Han, D. C., Hoffman, B. B., & Ziyadeh, F. N. (2000). Stimulation of TGF-β type II receptor by high glucose in mouse mesangial cells and in diabetic kidney. *American Journal of Physiology-Renal Physiology*, *278*(5), F830-F838.

28-Sanchez-Alamo, B., Shabaka, A., Cachofeiro, V., Cases-Corona, C., & Fernandez-Juarez, G. (2022). Serum interleukin-6 levels predict kidney disease progression in diabetic nephropathy. *Clinical nephrology*, *97*(1), 1.

29-Su, H., Lei, C. T., & Zhang, C. (2017). Interleukin-6 signaling pathway and its role in kidney disease: an update. *Frontiers in immunology*, *8*, 405.

30-Suzuki, D., Miyazaki, M., Naka, R., Koji, T., Yagame, M., Jinde, K., ... & Sakai, H. (1995). In situ hybridization of interleukin 6 in diabetic nephropathy. *Diabetes*, *44*(10), 1233-1238.

31-Reddy, V. K. K., Shiddapur, G., Jagdale, N., Kondapalli, M. P., & Adapa, S. (2024). Investigating Interleukin-6 levels in type 2 diabetes mellitus patients with and without diabetic nephropathy. *Cureus*, *16*(8), e67014.

32-Shi, J., Liu, X., Jiao, Y., Tian, J., An, J., Zou, G., & Zhuo, L. (2025). mTOR pathway: A key player in diabetic nephropathy progression and therapeutic targets. *Genes & Diseases*, *12*(2), 101260.

33-Maity, S., Bera, A., Ghosh-Choudhury, N., Das, F., Kasinath, B. S., & Choudhury, G. G. (2018). microRNA-181a downregulates deptor for TGFβ-induced glomerular mesangial cell hypertrophy and matrix protein expression. *Experimental cell research*, *364*(1), 5-15.

34-Maity, S., Das, F., Kasinath, B. S., Ghosh-Choudhury, N., & Ghosh Choudhury, G. (2020). TGFβ acts through PDGFRβ to activate mTORC1 via the Akt/PRAS40 axis and causes glomerular mesangial cell hypertrophy and matrix protein expression. *The Journal of biological chemistry*, *295*(42), 14262–14278.

35-Lu, Q., Zhou, Y., Hao, M., Li, C., Wang, J., Shu, F., ... & Yin, X. (2018). The mTOR promotes oxidative stress-induced apoptosis of mesangial cells in diabetic nephropathy. *Molecular and Cellular Endocrinology*, *473*, 31-43.

36-Hernandez, L. F., Eguchi, N., Whaley, D., Alexander, M., Tantisattamo, E., & Ichii, H. (2022). Anti-oxidative therapy in diabetic nephropathy. *Frontiers in Bioscience-Scholar*, *14*(2), 14.

37-Aghadavod, E., Khodadadi, S., Baradaran, A., Nasri, P., Bahmani, M., & RAFIEIAN, K. M. (2016). Role of oxidative stress and inflammatory factors in diabetic kidney disease.

38-Munteanu, C., Rotariu, M., Turnea, M. A., Anghelescu, A., Albadi, I., Dogaru, G., ... & Onose, G. (2022). Topical reappraisal of molecular pharmacological approaches to endothelial dysfunction in diabetes mellitus angiopathy. *Current Issues in Molecular Biology*, *44*(8), 3378-3397.

39-Nezu, M., & Suzuki, N. (2020). Roles of Nrf2 in protecting the kidney from oxidative damage. *International journal of molecular sciences*, *21*(8), 2951.