Al-Esraa University College Journal for Medical Sciences

Volume 5 | Issue 7 Article 5

September 2024

Evaluate Levels of Nephrin and Kidney Injury Molecule in Diabetic Nephropathy Patients

Ahmed Malik Hassan
College of Applied Medical Sciences, University of Karbala, Karbala, Iraq,
ahmad.malik@s.uokerbala.edu.iq

Hassan Ali Al-Saadi
College of Applied Medical Sciences, University of Karbala, Karbala, Iraq

Radhwan Mohammed Hussein
College of Pharmacy, University of Ahl Al Bait, Kerbala, Iraq

Recommended Citation

Hassan, Ahmed Malik; Al-Saadi, Hassan Ali; and Hussein, Radhwan Mohammed (2024) "Evaluate Levels of Nephrin and Kidney Injury Molecule in Diabetic Nephropathy Patients," *Al-Esraa University College Journal for Medical Sciences*: Vol. 5: Iss. 7, Article 5.
DOI: https://doi.org/10.70080/2790-7937.1004

AL-ESRAA UNIVERSITY COLLEGE JOURNAL OF MEDICAL SCIENCES 2024;5(7):34–41

RESEARCH ARTICLE

Evaluate Levels of Nephrin and Kidney Injury Molecule in Diabetic Nephropathy Patients

Ahmed Malik Hassan [a,*], Hassan Ali Al-Saadi [b], Radhwan Mohammed Hussein [c]

[a] College of Applied Medical Sciences, University of Karbala, Karbala, Iraq
[b] College of Applied Medical Sciences, University of Karbala, Karbala, Iraq
[c] College of Pharmacy, University of Ahl Al Bait, Kerbala, Iraq

ABSTRACT

Introduction: Diabetes, particularly when uncontrolled for an extended length of time, was already related with a higher risk of complications and progression of both macrovascular and microvascular problems. Diabetic nephropathy is one of the complications of diabetes can be a result from micro vascular effect of diabetes, kidney disease can occur in both type one or type two diabetes patients kidney disease usually affects young and middle-aged patients. Kidney injury molecule and Nephrin is a biomarkers used to diagnosis of early biomarkers in diabetic nephropathy. **Objective:** The aim was effectiveness of KIM-1 and nephrin for diagnosis of diabetic kidney disease and compares with other markers as blood urea and s.cr and eGFR. **Materials and methods:** A case-control study included 90 participant that divided into three groups: 30 diabetic mellitus DM,30 diabetic nephropathy (DN), and 30 healthy control groups, the study starter in November 2023 until March 2024. Laboratory tests were performed on all patients and controls. These included measurements of fasting blood sugar, blood urea, serum creatinine, and by full automated analyzer and KIM-1, and Nephrin by ELISA method. **Results:** The biomarkers KIM-1, Nephrin showed high significant increase in diabetic nephropathy group, and the correlation between KIM-1 and Nephrin was positive and, the ROC analysis of KIM-1 show high sensitivity and specificity (93.3%, 99.3%). **Conclusion:** kim-1 and nephrin used for diagnosis diabetic nephropathy disease and more sensitivity and specificity from other markers and used to detect kidney disease without clear clinical signs.

Received 20 June 2024; accepted 20 July 2024.
Available online 3 September 2024

* Corresponding author.
E-mail address: ahmad.malik@s.uokerbala.edu.iq (A. Malik Hassan).

https://doi.org/10.70080/2790-7937.1004

Introduction

Diabetic nephropathy (DN) refers to Diabetic patients may experience kidney illness due to microvascular difficulties stemming from their diabetes, concurrent kidney disease of another origin, or a mix of both. Patients with type 1 diabetes are more likely to experience microvascular disease [10]. Numerous studies have confirmed that kidney disease in type 2 diabetes may be a more complex entity than that found in type 1 diabetes, and that individuals with type 2 diabetes are frequently older at the time of diagnosis and are more likely to develop renal disease from causes other than diabetes. End-stage renal disease (ESRD) is the final stage of diabetic nephropathy, which progresses through multiple clinical stages including hyperfiltration, microalbuminuria, macroalbuminuria, and nephrotic proteinuria (Elsayed et al., 2022).

KIM-1 is a cell surface receptor found on lymphoid/myeloid and epithelial cells. It functions as an entrance receptor for the hepatitis A and Ebola viruses as well as a scavenger receptor for oxidized LDL and phosphatidylserine [17]. In proximal tubules from AKI and CKD patients, Kim-1 expression is noticeably elevated. The Federal Drug Administration and the European Medicines Agency have qualified the urinary excretion of the KIM-1 ectodomain for preclinical assessment of nephrotoxicity and for case-by-case clinical evaluation. is a transmembrane protein that, following ischemia injury, is up-regulated in renal tubular cells [9].

While KIM-1 is primarily utilized in relation to drug-induced acute kidney injury (AKI), newer studies indicate that KIM-1 might also be useful in forecasting the course of chronic kidney disease (CKD) [7]. While KIM-1 is not expressed in healthy kidneys, it is expressed in many different types of kidney disorders in humans, mainly in the proximal tubular cells' apical membrane. A significant factor in the development of autosomal dominant polycystic kidney disease (ADPKD) is cyst growth, and the degree of compression that developing cysts impose on neighboring renal tubules may elevate KIM expression [1].

The transmembrane proteins known as kidney injury molecule-1, which have Ig-like and mucin domains in their ectodomain, were only recently found. In addition to being expressed by injured kidney proximal tubules, TIM-1 regulates CD4+ T-cell responses [15].

Renal failure and progressive fibrosis are caused by persistent KIM-1 expression, and the cause of this is thought to be oxidized lipids. By means of the PI3K pathway, KIM-1 can initiate signalling. It was found that in cases of acute ischemia and toxic damage, KIM-1-mediated phagocytosis functions suppress innate immune responses and inflammation. According to theory, tubular interstitial damage is influenced by KIM-1. It is believed that urine concentrations of KIM-1 reflect the expression of tubular KIM-1, which is unique to continuous tubular cell injury and dedifferentiation. Additionally, in certain forms of renal illness, KIM-1 is linked to renal interstitial fibrosis and inflammation [16].

Nephrin is a transmembrane protein of the immunoglobulin superfamily. It is a 180 KD and 1242- amino-acid, It is has been localized to the slit membrane between adjacent podocytes of the glomerulus [13]. Comprises a fibronectin type III-like domain, eight extracellular immunoglobulin-like modules, and a cytosolic C-terminal tail. It is a transmembrane protein with a single pass that interacts with other trans and cis nephrin proteins both within and outside of cells. (Kondapi et al., 2018).

Kidney diseases are frequently caused by persistently elevated blood sugar. Research suggests that a significant number of patients with diabetes mellitus may experience renal impairment over an extended period. For an extended period without any symptoms, diabetic nephropathy is thought to be a silent illness. The many types of kidney cells that are subsequently impacted by chronic hyperglycemia eventually develop progressive

fibrosis, glomerular and tubular damage, and renal failure. (Chen et al., 2018). These indicators typically capture a single mechanism of the disease process, such as glomerular or tubular damage, inflammation or oxidative stress. These findings suggest that podocyte destruction may occur in DM patients prior to the development of microalbuminuria (Dumont et al., 2017). Several studies showed that nephrinuria was associated with higher urine albumin concentrations and diabetes status, thus, given that hyperglycemia is likely to cause further damage to renal vasculature and glomerular filtration barrier over time [11].

1. Materials and methods

1.1. Study design

A case control study was carried at AL-Imam AL Sadiq general teaching Hospital in Hilla city during the period from December 2023 to March 2024. An endocrinologist and nephrologist diagnosed all diabetic patient that included in this study based on clinical findings and measurements of FBS, B.urea, S.cr. All participants in this study divided into three group each group consist of 30 person, diabetic mellitus patients without any complication, diabetic nephropathy and 30 person as control group (apparently healthy). sample size was determined according to G power program for sample size determination.

Inclusion criteria: Patients group whose age varied from 18 to 80 years, patient with and who have undergone a clinical examination by endocrinologist and nephrologist. The control group was selected as apparently healthy individuals.

Exclusion criteria: Cancer Patients, patients less than 18 years old, pregnancy, congestive heart failure, systemic lupus erythematous, acute kidney injury, and, incomplete data were excluded from the study.

1.2. Blood sampling

Venous blood was drawn from all participant using disposable syringes. The blood was discharged into plain tubes, where the blood was allowed to clot before being centrifuged to separate the serum into two parts. The first was used right away for a routine test that included serum FBS, B. urea, and S. creatinine. The second was kept in a deep freezer to be analyzed using the ELISA method for nephrin and KIM-1.

1.3. Statistical analysis

Statistical program (SPSS version 22.0) was used. A one-way analysis of variance (ANOVA) test was used to analyze the differences in variable means between the control and patient groups. The results were expressed as mean ± standard deviation (SD). Using Pearson's correlation coefficient (r), correlations between all of the variables under study were assessed, and data analysis methods included linear regression analyses. Statistics were deemed significant when the P-value was less than 0.05. The study variables' cut-off values, sensitivity, specificity, and area under the curve were displayed using the receiver operating characteristic (ROC) curve.

Table 1. The mean ± SD of Ssrum levels of nephrin for both control group and patient groups (DM and DN).

	Control No = 30	DM No = 30	DN No = 30
KIM-1(ng/ml)	0.54 ± 0.04	0.71 ± 0.11$^{a=ns}$	5.4 ± 0.4$^{a▲***b▲***}$
Nephrin(ng/ml)	5.03 ± 0.66	8.2 ± 1.2$^{a▲*}$	25.7 ± 2.4$^{a▲***b▲***}$

N: Number, SD: Standard deviation, significant increase, ▼: significant decrease, *(P < 0.05), **(P < 0.01), ***(P < 0.001) ns = non-significant, a = ANOVA test between control, DM and DN groups, b = ANOVA test between DM and DN, groups.

2. Results

The results of mean ± SD of serum KIM-1 in control group and patients groups (DM, DN) were (0.54 ± 0.04), (0.71 ± 0.11), (5.4 ± 0.4) respectively. There were significant increase (P < 0.05) in mean of serum KIM-1 in DM group when compared with the control group. There were increased high significantly (P < 0.001) in mean of serum KIM-1 in (DN) group when compared with control group, also there were increased high significantly (P < 0.001) in mean serum KIM-1 found in(DN) groups when compared with DM group as shown in Table 1.

The results of the mean ± SD of nephrin for control group and patient's groups (DM, DN) were 5.03 ± 0.66, 8.2 ± 1.2, 25.7 ± 2.4 respectively. There were increases significantly (P < 0.05) in mean serum nephrin in the patients group (DM) compared with the control group, also there were high significant increases (P < 0.001) in mean serum nephrin found in (N) group when compared with control group and this results clearly demonstrated in Table 1.

These results significant increase (P < 0.05) in mean serum levels of B. urea between control groups and DM group, also there were very high increase significantly (P < 0.001) in mean of B. urea between control group and diabetic nephropathy group.

There were no statistical differences (P > 0.05) in mean serum levels of creatinine between control group and DM group, in contrast very high significant increases (P < 0.001) in mean of S. creatinine in diabetic nephropathy group when compared with control group. The level of eGFR in DM and DN groups high significant decreases (P < 0.001) when compared with control group. These results clearly illustrated in Table 2.

The correlation of the KIM-1 values of DN group showed positive significant correlation with B. urea (r = 0.675, p < 0.05) and positive correlation with S. creatinine (r = 0.626 p < 0.05), as well showed positive significant correlation with FBS (r = 0.453, p < 0.05), KIM-1 showed positive significant correlation with nephrin (r = 0.867, p < 0.01) and showed in Fig. 1.

Table 2. The mean ± SD levels of biomarkers (B. urea, S. creatinine, eGFR, and FBS).

Parameters	Control mean ± SD n = 30	DM mean ± SD n = 30	DFU&CKD mean ± SD n = 30
B. Urea (mg/dl)	28.7 ± 5.6	34.4 ± 2.2$^{a▲*}$	100.9 ± 18.6$^{a▲***b▲***}$
S. Creatinine (mg/dl)	0.76 ± 0.13	0.84 ± 0.10$^{a=ns}$	2.9 ± 0.38$^{a▲***b▲***}$
eGFR (ml/min/1.73m2)	109.6 ± 10.2	95.7 ± 10.4$^{a▼**}$	21.4 ± 4.2$^{a▼***b▼***}$
FBS (mg/dl)	102.3 ± 9.8	256.7 ± 29.6$^{a▲***}$	363.7 ± 28.3$^{a▲***b▲***}$

N: Number, SD: Standard deviation, ▲; significant increase, ▼: significant decrease, (P < 0.05), (P < 0.01), (P < 0.001) ns = non-significant, a = ANOVA test between control, DM, DFU and DFU &CKD groups, b = ANOVA test between DM and DFU, DFU & CKD groups, c = ANOVA test between DFU and DFU & CKD groups.

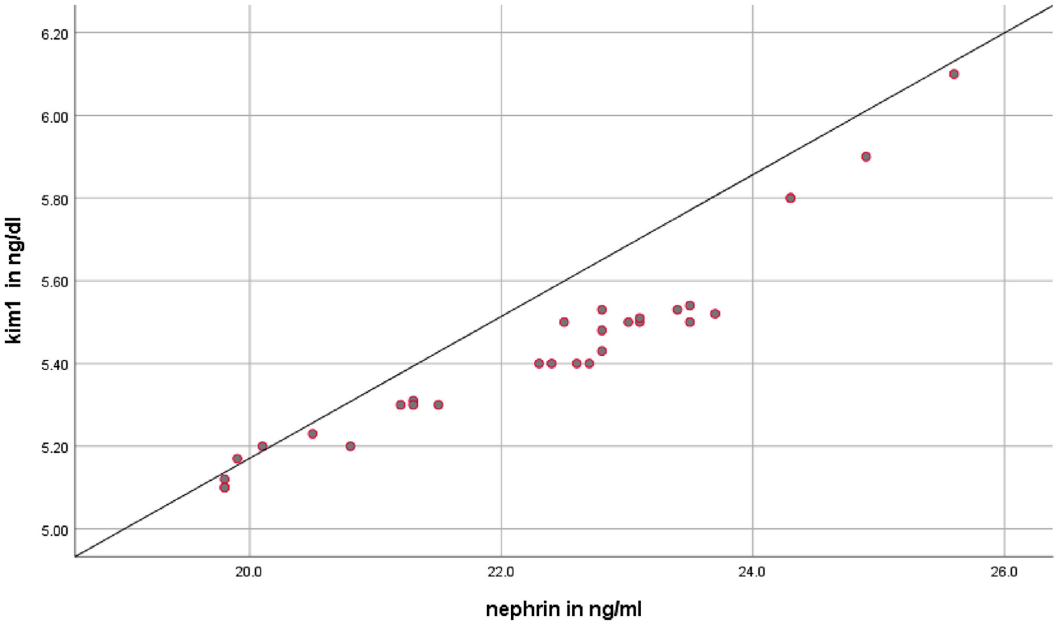

Fig. 1. The correlation between serum levels of KIM-1 (ng/ml) with nephrin (ng/ml) in the Diabetic nephropathy group (P value = 0.01), (R = 0.867).

Table 3. The ROC results of KIM-1 biomarkers.

Test Variable positive actual critical state	AUC	P value	Cut-off points ng/dl	Specificity	Sensitivity
KIM-1	0.939	0.001	0.66	99.3%	93.3%
Nephrin	0.94	0.001	9.4	83.3 %	99.6%

When compared to the Control group, the ROC analysis data showed that KIM-1 had a superior ability to predict DN in the diabetic patients (Table 3), which included groups DM and DN. This conclusion was attained by investigations that looked at the test's sensitivity and specificity values, area under the curve (Fig. 2).

KIM-1 and nephrin had a strong capacity to distinguish and predict DN patients from healthy individuals (AUC = 0.93, 0.94, respectively). The P value in terms of prior probability was discovered to be 0.001. Nephrin exhibits high sensitivity and specificity values of 99.6 percent and 83.3 percent, respectively, while KIM-1 displays very high values of 99.3 percent and 93.3%, respectively. It suggests that this marker has an equal role in both confirming and ruling out illness. The optimal threshold for KIM-1 (0.66 ng/ml) and nephrin (9.44 ng/ml) was determined by analyzing the ROC curve (Fig. 2). When comparing the patients to normal individuals, a value of KIM-1 >0.66 ng/ml and nephrin >9.4 ng/ml suggests that the patients most likely have DN.

3. Discussion

Results from this study indicate that diabetes mellitus (DM) not only raises blood levels of urea and creatinine but also causes weight loss. Additionally, the results demonstrated a rise in the mean KIM-1 in diabetic nephropathy. This is because DM causes oxidative stress in the kidney tissue, which boosts serum KIM-1 levels and renal expression. KIM-1

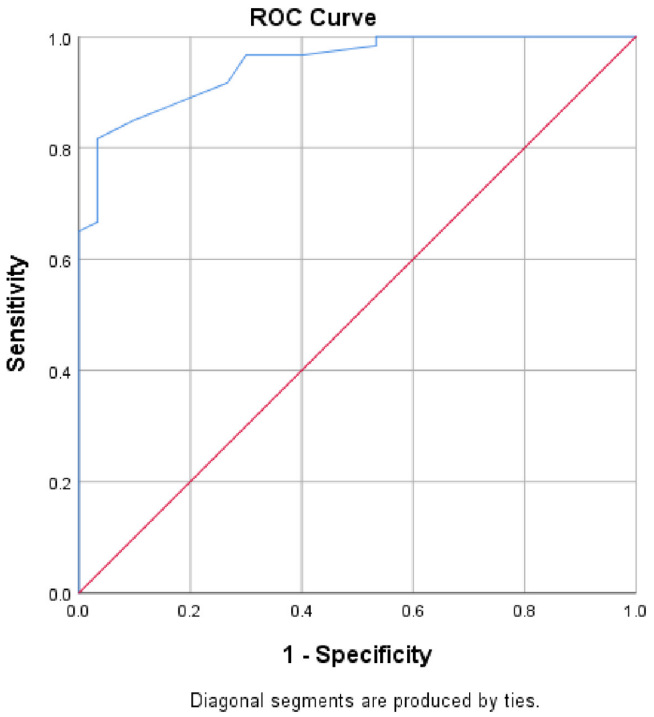

Fig. 2. Receiver operatng characteristic curve for Nephrin showing sensitivity and specificity.

is a phosphatidylserine receptor that identifies apoptotic cells and guides them to lysosomes, where it changes kidney proximal epithelial cells into semi-professional phagocytes. Al-Bataineh et al. [2] showed significantly increase in serum KIM-1 in chronic kidney disease patients.

Due to its function in facilitating the removal of dead cells by the tubular cells that are still alive. Via mechanisms aided by apoptotic cell uptake, KIM-1 protects the kidney in the early stages following damage [5].

Due to proximal cell inflammation in diabetic nephropathy, KIM-1 is expressed in chronic kidney disease [9]. Hypoxia is a potent inducer of proximal tubular cells' increased KIM-1 expression, which may lead to the development of persistent interstitial inflammation. In the context of signaling connections between the cells of injured renal proximal tubules and macrophages serving as autocrine-paracrine factors in relation to the epithelial and stromal cells, both membrane-bound and free KIM-1 were thought to be implicated [17] that increase KIM-1 in DN groups.

Corresponding to this finding [6] was the finding that insulin resistance in elderly people was associated with a considerably higher KIM-1 level. Through the promotion of mutagenic and fibrotic processes via various pathophysiologic pathways, including activation of insulin-like growth factor1, transforming growth factor-b, endothelin-1, and the renin–angiotensin–aldosterone system, impaired insulin sensitivity and compensatory hyperinsulinemia have been suggested to contribute to the development of renal injury [3].

Pro-inflammatory cytokines, adipokines, and oxidative stress are strongly linked to insulin resistance and may potentially exacerbate kidney damage. However, the converse scenario is also conceivable: persistent kidney damage could lead to increased inflammatory activity, which would reduce insulin sensitivity [14]. Although not all diabetes

patients with nephrinuria go on to develop kidney disease, nephrin may offer an early warning sign of renal impairment. The current study was consistent with a previous study that indicated nephrin loss significantly and redistribution in the glomeruli of diabetic patients with microalbuminuria. It also revealed that patients with diabetes and nephropathy have structural changes to the glomerular filtration unit, such as increased width of podocyte foot processes and filtration slits [18]. As a result, one can infer that nephrin can be found in the systemic circulation or that nephrin secreted by podocytes while passing through the nephron can be reabsorbed in the renal tubular system and discovered in the serum. Nephrin, a protein exclusive to podocytes found in serum, indicates only podocyte injury, unrelated to the other two elements of the glomerular filtration barrier.Because podocyte damage is assumed to exist before to the onset of microalbuminuria and proteinuria, podocyte proteins, including nephrin, are regarded as more accurate and early indicators of diabetic kidney disease (DN) [12]. In keeping with the findings of the present investigation (Jim, et al., 2022) that reported increase of serum and urine nephrin in early stage of diabetic nephropathy.

4. Conclusion

Kidney injury molecule-1 as biomarker for kidney proximal tube specific damage and increase in diabetic nephropathy complications of diabetes. Nephrin protein is specific for podocyte cell damage in kidney and increase in DN. This study suggested that serum level kim-1 and nephrin associated with the patients with diabetic nephropathy inflammation. The measurement of kim-1 and nephrin level was value importance for screening out the DM patients with more risk of diabetic nephropathy and use to predictor disease in early stage.

References

1. F. Abid Khan, S. S. Fatima, G. M. Khan, and S. Shahid, “Evaluation of kidney injury molecule-1 as a disease progression biomarker in diabetic nephropathy: KIM-1 and Diabetic Nephropathy,” *Pakistan Journal of Medical Sciences*, vol. 35, no. 4, pp. 992–996, 2019.
2. M. M. Al-Bataineh, C. L. Kinlough, Z. Mi, E. K. Jackson, S. M. Mutchler, D. R. Emlet, and R. P. Hughey, “KIM-1-mediated anti-inflammatory activity is preserved by MUC1 induction in the proximal tubule during ischemia-reperfusion injury,” *American Journal of Physiology-Renal Physiology*, vol. 321, no. 2, pp. F135–F148, 2021.
3. R. H. Aljorani, E. S. Saleh, and K. G. H. Al Mohammadawi, “Correlation of kidney injury molecule-1 and nephrin levels in Iraqi patients with diabetic nephropathy,” *Al-Rafidain Journal of Medical Sciences (ISSN 2789-3219)*, vol. 5, pp. 99–104, 2023.
4. S. Chen, H. Chen, Q. Liu, and Q. Ma, “Effect of simvastatin on the expression of nephrin, podocin, and vascular endothelial growth factor (VEGF) in podocytes of diabetic rat,” *International Journal of Clinical and Experimental Medicine*, vol. 8, no. 10, p. 18225, 2019.
5. P. M. C. De Silva, T. D. K. S. C. Gunasekara, S. D. Gunarathna, P. M. M. A. Sandamini, R. A. I. Pinipa, E. M. D. V. Ekanayake, and N. Jayasundara, “Urinary biomarkers of renal injury KIM-1 and NGAL: reference intervals for healthy pediatric population in Sri Lanka,” *Children*, vol. 8, no. 8, p. 684, 2021.
6. R. Dong and Y. Xu, “Glomerular cell cross talk in diabetic kidney diseases,” *Journal of Diabetes*, vol. 14, no. 8, pp. 514-523, 2022.
7. N. E. El-Ashmawy, E. A. El-Zamarany, N. F. Khedr, A. I. Abd El-Fattah, and S. A. Eltoukhy, “Kidney injury molecule-1 (Kim-1): an early biomarker for nephropathy in type II diabetic patients,” *International Journal of Diabetes in Developing Countries*, vol. 35, pp. 431–438, 2015.
8. M. S. Elsayed, A. El Badawy, R. Omar, and A. Mohamed, “Serum cystatin C as an indicator for early detection of diabetic nephropathy in type 2 diabetes mellitus,” *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, vol. 13, no. 1, pp. 374-381, 2019.

9. H. Ghasemi, B. Einollahi, N. Kheiripour, S. R. Hosseini-Zijoud, and M. F. Nezhad, “Protective effects of curcumin on diabetic nephropathy via attenuation of kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) expression and alleviation of oxidative stress in rats with type 1 diabetes,” *Iranian journal of basic medical sciences*, vol. 22, no. 4, p. 376, 2019.
10. T. Feng, S. Ciardullo, and G. Perseghin, “Soluble α-Klotho levels, glycemic control and renal function in US adults with type 2 diabetes,” *Acta diabetologica*, vol. 59, no. 6, pp. 803–809, 2022.
11. B. Jim, M. Ghanta, A. Qipo, Y. Fan, P. Y. Chuang, H. W. Cohen, and J. C. He, “Dysregulated nephrin in diabetic nephropathy of type 2 diabetes: a cross sectional study,” *PloS one*, vol. 7, no. 5, p. e36041, 2012.
12. K. Kondapi, N. L. Kumar, S. Moorthy, and S. Silambanan, “A study of association of urinary nephrin with albuminuria in patients with diabetic nephropathy,” *Indian journal of nephrology*, vol. 31, no. 2, pp. 142–148, 2021.
13. I. Kostovska, K. Tosheska-Trajkovska, S. Topuzovska, S. Cekovska, G. Spasovski, O. Kostovski, and D. Labudovic, “Urinary nephrin is earlier, more sensitive and specific marker of diabetic nephropathy than microalbuminuria,” *Journal of medical biochemistry*, vol. 39, vol. 1, p. 83, 2020.
14. G. Mansoor, M. Tahir, T. Maqbool, S. Q. Abbasi, F. Hadi, T. A. Shakoori, and I. Ullah, “Increased expression of circulating stress markers, inflammatory cytokines and decreased antioxidant level in diabetic nephropathy,” *Medicina*, vol. 58, no. 11, p. 1604, 2022.
15. S. Sadar, D. Kaspate, and N. Vyawahare, “Protective effect of L-glutamine against diabetes-induced nephropathy in experimental animal: Role of KIM-1, NGAL, TGF-β1, and collagen-1,” *Renal failure*, vol. 38, no. 9, pp. 1483–1495, 2016.
16. B. Satirapoj, “Tubulointerstitial biomarkers for diabetic nephropathy,” *Journal of diabetes research*, vol. 2018, no. 1, p. 2852398, 2018.
17. K. Siddiqui, S. S. Joy, T. P. George, M. Mujammami, and A. A. Alfadda, “Potential role and excretion level of urinary transferrin, KIM-1, RBP, MCP-1 and NGAL markers in diabetic nephropathy,” *Diabetes, Metabolic Syndrome and Obesity*, pp. 5103–5111, 2020.
18. G. Veluri and M. Mannangatti, “Urinary nephrin is a sensitive marker to predict early onset of nephropathy in type 2 diabetes mellitus,” *Journal of Laboratory Physicians*, vol. 14, no. 04, pp. 497–504, 2022.