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RESEARCH ARTICLE

URINARY TGF-B1 PROFILE IN DIABETIC NEPHROPATHY: INSIGHTS FROM A SINGLE-CENTER STUDY IN INDIA

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Abstract

Introduction: Diabetic nephropathy (DN) is a major global cause of end-stage renal illness and chronic kidney disease, and it also greatly increases morbidity in individuals with diabetes. A key element in the development of diabetic kidney disease (DN) is transforming growth factor-beta 1 (TGF-β1), which stimulates fibrosis and renal damage. This study evaluated urinary TGF-β1 levels in patients with diabetic nephropathy compared to healthy controls and assess its correlation with proteinuria and kidney function.

Methods: This observational study included 130 people in total, 65 of whom were diabetic nephropathy patients (Group A) and 65 of whom were healthy controls (Group B). Flow cytometry was used to measure the amounts of TGF-β1 in the urine. The urine protein-creatinine ratio (UPCR) was used to measure proteinuria, and the estimated glomerular filtration rate (eGFR) was used to assess kidney function. T-tests, chi-square tests, and Pearson's correlation were used to analyse the data in order to determine the correlations between TGF-β1, UPCR, and eGFR.

Results: Patients with diabetic nephropathy had considerably greater urinary TGF-β1 levels (15.8 ± 4.3 pg/ml) than healthy controls (4.2 ± 1.9 pg/ml) ($p < 0.001$). Additionally, there were significant differences in UPCR and eGFR between the groups, with Group A exhibiting worse kidney function and increased proteinuria ($p < 0.001$). Urinary TGF-β1 and UPCR exhibited a substantial positive association ($r = 0.76$, $p < 0.001$), while eGFR showed a negative correlation ($r = -0.65$, $p < 0.001$) according to Pearson's correlation analysis.

Conclusion: Patients with diabetic nephropathy have significantly higher urinary TGF-β1 levels, which are strongly correlated with impaired kidney function and increased proteinuria. According to these results, urine TGF-β1 could be a non-invasive biomarker for the development of diabetic nephropathy.

Keywords: Diabetic nephropathy, TGF-β1, Proteinuria, eGFR, Kidney function, Biomarker.

BACKGROUND/INTRODUCTION

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One of the main microvascular consequences of diabetes is diabetic nephropathy (DN), which is also the primary cause of end-stage renal disease (ESRD) and chronic kidney disease (CKD) globally. DN affects nearly 30-40% of individuals with diabetes, making it one of the most prevalent contributors to kidney failure globally. The pathogenesis of DN is complex, involving metabolic and hemodynamic abnormalities such as hyperglycemia and hypertension, which lead to glomerular damage, proteinuria, and eventually, a progressive decline in kidney function [1].

Transforming Growth Factor-β1 is one of the major mediators involved in the development of diabetic nephropathy (TGF-β1). TGF-β1 plays a central role in the fibrosis and scarring that characterize diabetic nephropathy, primarily through its effects on extracellular matrix production, mesangial cell expansion, and glomerular basement membrane thickening. Increased TGF-β1 activity promotes the accumulation of fibrotic material, leading to progressive kidney dysfunction. This cytokine is also involved in epithelial-mesenchymal transition (EMT), which exacerbates kidney fibrosis by transforming tubular cells into myofibroblasts [2, 3].

Research into the signaling pathways of TGF-β1 has revealed its significant involvement in activating the SMAD family of proteins, which regulate gene transcription related to fibrosis. The activation of SMAD2 and SMAD3, in particular, contributes to kidney damage by upregulating fibrotic gene expression [4]. On the other hand, SMAD7, an inhibitory SMAD, has been found to counteract this process, providing a potential therapeutic target. Several experimental models have demonstrated that inhibition of TGF-β1 signaling can reduce fibrosis and slow the progression of DN, although clinical trials targeting TGF-β1 directly have shown limited success [5].

Given its pivotal role in diabetic nephropathy, urinary levels of TGF-β1 have been explored as potential biomarkers for early detection and disease monitoring. Elevated urinary TGF-β1 has been correlated with increased proteinuria and declining renal function, suggesting it could be a valuable non-invasive marker for assessing the severity of kidney damage in DN. Furthermore, its correlation with disease progression makes it a promising target for future therapeutic interventions [6].

The study aimed to evaluate urinary TGF-β1 levels in patients with diabetic nephropathy compared to healthy controls.

MATERIALS AND METHODS

Study Design

This was a prospective, single-center, observational study designed.

Study Setting

The study took place at a tertiary care hospital in India, involving the Nephrology Department during a

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period of one year. Recruitment, sample collection, and clinical evaluations were performed on-site, with laboratory analyses carried out in the institution's diagnostic laboratory.

Participants

A total of 130 participants were enrolled in the study, allotted into two groups:

- Group A (Cases): 65 patients diagnosed with diabetic nephropathy.
- Group B (Controls): 65 healthy individuals without any known renal diseases.

Inclusion Criteria

- Group A (Diabetic Nephropathy Cases):
  - Age ≥ 18 years.
  - Urinary protein-creatinine ratio (UPCR) > 0.5 mg/mg.
- Group B (Healthy Controls):
  - Age ≥ 18 years.
  - Normal kidney function with no known history of renal or systemic diseases.

Exclusion Criteria

- Were under 18 years of age.
- Had an active urinary tract infection at the time of the study.
- Were CKD Stage Vd patients on maintenance hemodialysis.
- Were renal transplant recipients.

Bias

To minimize bias, cases and controls were recruited from the same geographic region to control for environmental and demographic confounding factors. Both groups were subjected to the same tests, and laboratory personnel were blinded to participant group allocation during analysis. Multiple measurements (in triplicate) were performed to ensure accuracy.

Variables

Variables included urinary TGF-β1 levels measured in 24-hour urine samples, UPCR, complete blood count (CBC), eGFR (calculated using CKD-EPI 2021 equation), demographic characteristics (age, sex), and clinical history (duration of diabetes, co-morbidities).

Data Collection

Data were collected using a semi-structured questionnaire that included demographic details, clinical history, and laboratory results. Participants were interviewed, and physical examinations were conducted. Laboratory investigations included CBC, 24-hour urine collection for protein and creatinine estimation, and urine analysis for protein, creatinine, and TGF-β1 levels. Data were recorded in a pre-designed proforma and later entered into Microsoft Excel 2019 for analysis.

Procedure

All participants were briefed about the study's objectives and procedures before enrollment.

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- Urinary TGF-β1 Measurement:
    - Participants provided 24-hour urine samples, and the total volume was recorded.
    - 20 µl of urine was analyzed using flow cytometry (MACSQuant® Analyzer 10).
    - Urine was mixed with cytometric array capture beads, incubated for two hours, followed by centrifugation, and analyzed after a series of washing and detection steps.
    - The test was performed in triplicates, and the mean value was used for analysis.
- UPCR Estimation:
    - Urine protein and creatinine levels were estimated using pyrogallol red and alkaline picrate kinetic methods, respectively.
    - UPCR was calculated as urine protein (mg/dl) divided by urine creatinine (mg/dl).
- Other Laboratory Tests:
    - CBC was measured using DxH 800 Hematology Analyzer.
    - Routine urine analysis was done using UC-3500 fully automated urine chemistry analyzer.
    - eGFR was calculated using the CKD-EPI 2021 equation.

Statistical Analysis

Data was analysed using IBM SPSS Statistics for Windows 22.0. Data was presented using frequencies and percentages for non-continuous variables and mean ± standard deviation for continuous variables. Chi-square, student's t-test was used. P-values under 0.05 were statistically significant at 95% confidence intervals.

Ethical considerations

The study protocol was approved by the Ethics Committee and written informed consent was received from all the participants.

RESULTS

The study included 130 participants: 65 diabetic nephropathy patients (Group A) and 65 healthy controls (Group B). Group A participants had a mean age of 54.8 ± 10.2 years, whereas Group B participants had a mean age of 52.1 ± 9.4 years (p = 0.12), showing no significant age difference. Group A comprised 37 males (56.9%) and 28 females (43.1%), while Group B had 35 males (53.8%) and 30 females (46.2%) (p = 0.71), indicating no gender bias. Table 1 details demographics.

Table 1: Demographic Characteristics of Study Participants

Characteristics	Group A	Group B	p-value
Age (years, mean ± SD)	54.8 ± 10.2	52.1 ± 9.4	0.12
Gender (n, %)			

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- Male	37 (56.9%)	35 (53.8%)	0.71
- Female	28 (43.1%)	30 (46.2%)	0.71
Duration of Diabetes (years, mean ± SD)	12.5 ± 6.4	-	-

In diabetic nephropathy patients (Group A), urine TGF-β1 levels were considerably higher than in healthy controls (Group B). Group A had a mean urine TGF-β1 level of 15.8 ± 4.3 pg/ml, while Group B had 4.2 ± 1.9 pg/ml ($p < 0.001$). Significant correlation exists between high urine TGF-β1 levels and diabetic nephropathy. Table 2 presents the results.

Table 2: Comparison of Urinary TGF-β1 Levels

Group	Mean Urinary TGF-β1 (pg/ml) ± SD	p-value
Group A (Cases)	15.8 ± 4.3	< 0.001
Group B (Controls)	4.2 ± 1.9	

The urine protein-creatinine ratio (UPCR) was likewise considerably higher in Group A than Group B. Group A had a mean UPCR of 1.7 ± 0.6 mg/mg, while Group B had 0.1 ± 0.03 mg/mg ($p < 0.001$). Table 3 compares UPCR in the two groups.

Table 3: Comparison of Urinary Protein-Creatinine Ratio (UPCR)

Group	Mean UPCR (mg/mg) ± SD	p-value
Group A (Cases)	1.7 ± 0.6	< 0.001
Group B (Controls)	0.1 ± 0.03	

Diabetic nephropathy patients (Group A) had a considerably lower mean eGFR than healthy controls (Group B). Group A had a mean eGFR of 52.6 ± 12.7 ml/min/1.73 m^2, while Group B had 95.4 ± 9.8 ml/min/1.73 m^2 ($p < 0.001$), demonstrating reduced kidney function in diabetic nephropathy patients. Comparison of eGFR is in Table 4.

Table 4: Comparison of Estimated Glomerular Filtration Rate (eGFR)

Group	Mean eGFR (ml/min/1.73 m^2) ± SD	p-value
Group A (Cases)	52.6 ± 12.7	< 0.001
Group B (Controls)	95.4 ± 9.8	

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The study used Pearson's correlation analysis to assess the connection between urine TGF-β1 levels and clinical markers including UPCR and eGFR. Evidence suggests a strong positive correlation between urine TGF-β1 and UPCR ($r = 0.76$, $p < 0.001$), linking greater levels to increased proteinuria in diabetic nephropathy patients. There was a strong negative connection between urine TGF-β1 levels and eGFR ($r = -0.65$, $p < 0.001$), indicating that increased levels may decrease kidney function.

Table 5: Correlation Between Urinary TGF-β1 Levels and Clinical Parameters

Parameter	Correlation Coefficient (r)	p-value
UPCR	0.76	< 0.001
eGFR	-0.65	< 0.001

Complete blood count (CBC) and urine analysis were within normal limits in the control group (Group B). In Group A, diabetic nephropathy patients, there were mild variations in CBC values, but none of them reached statistical significance between the two groups.

DISCUSSION

The study involved 130 participants: 65 diabetic nephropathy sufferers (Group A) and 65 healthy controls (Group B). The groups were well-matched demographically, with no significant age or gender differences. The mean age of Group A participants was 54.8 years and Group B participants 52.1 years, with comparable gender distribution. Demographic similarities reduce confounding effects and strengthen group comparisons.

Patients with diabetic nephropathy had considerably higher urinary TGF-β1 levels (15.8 ± 4.3 pg/ml) compared to healthy controls (4.2 ± 1.9 pg/ml) ($p < 0.001$). Elevated TGF-β1 levels may indicate kidney damage in diabetic nephropathy. The data support the role of TGF-β1 in renal fibrosis and kidney failure in diabetes, as previously reported. Urinary TGF-β1 may be a useful biomarker for monitoring disease development or measuring kidney damage in diabetic nephropathy.

The UPCR, a proteinuria indicator, was substantially higher in Group A (1.7 ± 0.6 mg/mg) compared to Group B (0.1 ± 0.03 mg/mg) ($p < 0.001$). Diabetic nephropathy causes proteinuria, indicating glomerular injury. TGF-β1 levels are strongly correlated with increased UPCR ($r = 0.76$, $p < 0.001$), indicating its participation in exacerbated protein leakage from glomerular damage in diabetic nephropathy.

Diabetic nephropathy patients had significantly reduced estimated glomerular filtration rate (eGFR)

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(52.6 ± 12.7 ml/min/1.73 m²) compared to healthy controls (95.4 ± 9.8 ml/min/1.73 m²) ($p < 0.001$). In Group A, this eGFR decrease implies reduced kidney function, a sign of diabetic nephropathy. Urinary TGF-β1 is negatively correlated with eGFR ($r = -0.65$, $p < 0.001$), indicating that increased levels are linked to worsening kidney function.

Results show elevated urine TGF-β1 levels in diabetic nephropathy patients, linked to increased proteinuria and decreased kidney function. These data imply that urine TGF-β1 may be a key indicator for kidney damage severity in diabetic nephropathy and contribute to its pathogenesis. This findings could aid in designing tailored therapies to reduce TGF-β1-related kidney damage in diabetes.

A study investigated the TGF-β1/SMAD signaling pathway and found that TGF-β1 activation plays a significant role in renal fibrosis, which is a hallmark of DN. Their findings highlighted that targeting the SMAD2/3 signaling proteins could mitigate fibrotic responses in diabetic kidneys, offering potential therapeutic strategies. However, the study also indicated the complexity of TGF-β1's roles, noting that broad inhibition of TGF-β1 might not be sufficient due to its involvement in multiple biological processes [7].

A comprehensive review of TGF-β1's role as a master regulator in diabetic nephropathy was conducted. Their research underscored that TGF-β1 is central to the development of renal fibrosis by promoting extracellular matrix accumulation and mesangial cell expansion. They also examined how TGF-β1 activates both SMAD-dependent and SMAD-independent pathways, reinforcing its pivotal role in kidney damage. Notably, the study also discussed therapeutic approaches that target the downstream effects of TGF-β1 signaling, with some strategies showing promise in animal models [8].

Another study focused on how IL-15 counteracts TGF-β1-induced renal fibrosis, providing evidence of a novel regulatory mechanism that could potentially be exploited for therapeutic intervention. Their study demonstrated that IL-15 inhibits TGF-β1-mediated epithelial-mesenchymal transition (EMT), a process that contributes to fibrosis in diabetic nephropathy. By modulating this pathway, IL-15 was shown to significantly reduce fibrotic markers in vitro, suggesting a potential avenue for fibrosis management in DN patients [9].

An another important study linked oxidative stress to the progression of diabetic complications, including nephropathy. Their research showed that TGF-β1 not only contributes to fibrosis but also exacerbates oxidative stress in renal tissues, compounding the damage caused by hyperglycemia in diabetes. The study emphasized the role of TGF-β1 in the inflammation and fibrosis pathways, which are central to the progressive nature of DN, making it a critical target for future therapies [10].

A study examined the involvement of TGF-β1 in glomerular basement membrane thickening and extracellular matrix accumulation in diabetic

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nephropathy. The study found that TGF-β1 is a key mediator in these pathological processes, driving renal fibrosis and functional decline. Their results suggested that inhibiting TGF-β1 could help slow the progression of kidney damage in diabetic patients, especially by reducing fibrosis-related damage [11].

A study explored the relationship between TGF-β1 and oxidative stress in DN. They found that TGF-β1 not only contributes to renal fibrosis but also increases the production of reactive oxygen species (ROS), which exacerbates kidney damage. This dual role of TGF-β1 in promoting both fibrosis and oxidative stress suggests that therapies targeting this cytokine may offer a twofold benefit in managing diabetic nephropathy. The study supported the idea that TGF-β1 could be a key target for reducing both fibrotic and oxidative damage in DN patients [12].

A study was conducted on novel inhibitors targeting the TGF-β1/Smad3 signaling pathway in animal models of diabetic nephropathy. Their findings showed that inhibiting this pathway led to significant reductions in renal fibrosis and proteinuria. This indicates that the TGF-β1/Smad3 pathway plays a critical role in the progression of diabetic nephropathy and could be a promising target for future therapies. The study highlighted the potential for new drug developments that specifically target this signaling pathway to mitigate DN progression [13].

A study reviewed emerging therapeutic strategies targeting TGF-β1 in diabetic nephropathy, with a focus on the use of microRNAs and long non-coding RNAs (lncRNAs) to modulate TGF-β1 activity. They found that while direct inhibition of TGF-β1 had limited success in clinical settings, regulating its downstream effects through RNA-based therapies holds great promise. These findings suggest that targeting the TGF-β1 signaling pathway indirectly could offer more effective therapeutic options for diabetic nephropathy [14].

CONCLUSION

The study demonstrates that urinary TGF-β1 levels are significantly elevated in patients with diabetic nephropathy and are closely correlated with increased proteinuria and declining kidney function. These findings suggest that urinary TGF-β1 could serve as a valuable biomarker for assessing the severity and progression of diabetic nephropathy, providing potential insights for early diagnosis and targeted therapeutic interventions.

LIMITATION

The limitations of this study include a small sample population who were included in this study. Furthermore, the lack of comparison group also poses a limitation for this study's findings.

RECOMMENDATION

Further research should explore the therapeutic potential of targeting TGF-β1 signaling in diabetic nephropathy to slow disease progression. Larger multicenter studies are recommended to validate urinary TGF-β1 as a reliable biomarker for clinical use.

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CONFLICT OF INTEREST

The authors have no conflicting interests to declare.

LIST OF ABBREVIATION

DN – Diabetic Nephropathy

TGF-β1 – Transforming Growth Factor-beta 1

UPCR – Urinary Protein-Creatinine Ratio

eGFR – Estimated Glomerular Filtration Rate

ESRD – End-Stage Renal Disease

CKD – Chronic Kidney Disease

EMT – Epithelial-Mesenchymal Transition

SMAD – Sma and Mad Related Proteins (family of proteins involved in signaling)

CBC – Complete Blood Count

CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration (equation for eGFR calculation)

SD – Standard Deviation

pg/ml – Picograms per milliliter

ROS – Reactive Oxygen Species

IL-15 – Interleukin-15

lncRNAs – Long Non-Coding RNAs

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