Research Article

Role of Inflammatory and oxidative stress biomarkers with albuminuria: A cross sectional analysis in type 2 Diabetes Mellitus patients

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Article Info

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Keywords

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Abstract

Background: Diabetic nephropathy (DN) is a prevalent and severe complication of type 2 diabetes mellitus (T2DM), contributing to kidney disease and cardiovascular risks. Oxidative stress and inflammation play crucial roles in DN pathogenesis. This study investigates the association of inflammatory cytokines, oxidative stress markers, and cortisol with albuminuria in T2DM patients.

Methods: A cross-sectional study was conducted on 150 T2DM patients categorized into normoalbuminuria, microalbuminuria, and macroalbuminuria groups. Blood samples were analyzed for total antioxidant capacity (T-AOC), pro-inflammatory cytokines (TNF-α, IL-1, IL-6, IL-8), high-sensitivity C-reactive protein (hsCRP), and cortisol levels. Statistical analyses included ANOVA and logistic regression.

Results: T2DM patients with albuminuria exhibited significantly lower T-AOC ($P=0.027$) and elevated TNF-α ($P=0.006$), IL-1 ($P<0.001$), IL-6 ($P<0.001$), IL-8 ($P<0.001$), hsCRP ($P<0.001$), and cortisol ($P<0.001$). High tertiles of TNF-α, IL-6, and hsCRP were strongly associated with increased albuminuria risk, particularly in overweight and hypertensive patients.

Conclusion: The findings highlight the interplay of oxidative stress, inflammation, and metabolic dysregulation in DN progression. Elevated inflammatory markers and cortisol levels correlate with albuminuria severity, emphasizing their potential as biomarkers for early DN detection. Targeting inflammatory pathways may offer therapeutic strategies to mitigate DN progression in T2DM patients.

Introduction

Diabetic nephropathy (DN) is a common and serious complication in patients with type 2 diabetes mellitus (T2DM) and is recognized as the foremost cause of kidney disease and a major cardiovascular risk factor in the T2DM population [1]. Approximately 40% of individuals with T2DM eventually progress to DN [2]. DN is characterized by a range of abnormalities, including metabolic and hemodynamic dysregulation, aggravation of oxidative stress and the renin-angiotensin-aldosterone system, and the development of fibrosis. These factors collectively contribute to increased intraglomerular and systemic pressure, leading to symptoms indicative of renal failure, such as albuminuria, glomerular hypertrophy, and diminished glomerular filtration rate (GFR) [3]. The involvement of these diverse factors highlights the intricate and multifactorial process. DN risk can be effectively assessed by measuring albuminuria in random urine samples [4]. Thus, albuminuria is a crucial parameter for identifying diabetic nephropathy at its incipient stage and is a known risk factor for the development of overt renal failure.

Oxidative stress and the activation of inflammatory pathways are widely acknowledged as critical mediators in the initiation and development of DN. Disruption of the oxidative balance, whether through heightened oxidant production or diminished antioxidant activity, precipitates oxidative stress, resulting in renal tissue damage and injury. Oxidative damage within glomerular capillaries compromises the integrity of the glomerular filtration barrier by disrupting all of its layers. This process begins with the disruption of the functional relationship between glomerular endothelial cells and their glycocalyx layer, extending to podocytes. Consequently, this leads to significant extracellular matrix accumulation, predominantly characterized by increased production and secretion of collagen, particularly type IV collagen [5]. Additionally, research indicates that the progressive increase in free radical formation, coupled with a deficiency in antioxidants in T2DM, significantly contributes to the onset of DN [6]. These findings highlight the necessity to evaluate antioxidant levels to better understand and manage this condition. Thus, the prominent role of oxidative stress in DN underscores the intricate relationship between oxidative stress and microvascular damage, highlighting the pivotal influence of oxidant species in DN progression.

Furthermore, the involvement of immune and inflammatory pathways in the onset and progression of renal damage in T2DM has gained substantial recognition [7]. Activation of the innate immune response engages multiple cellular elements, such as macrophages and neutrophils, and triggers various acute phase responses. Furthermore, Macrophages, monocytes, and endothelial cells release pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α), which triggers the liver to produce acute-phase proteins such as complement, C-reactive protein (CRP), and fibrinogen [8]. CRP enhances the immune response by binding to immune-complex parasites and bacteria, thereby facilitating their recognition and destruction. Additionally, it activates the classical complement pathway and induces monocytes to secrete additional tissue factors and pro-inflammatory cytokines, thereby perpetuating the inflammatory process [9].

In individuals with T2DM complicated by nephropathy, IL-6 levels are significantly elevated. Likewise, mRNA expression of IL-6 is markedly increased in renal cells, including kidney-infiltrating cells, in those with DN, compared to individuals with T2DM. Importantly, the expression levels of IL-6 mRNA are directly correlated with the extent of mesangial expansion, a defining histological feature of DN [10.11]. Furthermore, elevated IL-18 levels in DN were found to regulate the synthesis of various pro-inflammatory cytokines such as IL-1, interferon gamma (INF-γ), TNF-α, and transforming growth factor beta (TGF-β) and enhance the expression of chemokine receptors in mesangial cells, further amplifying inflammatory responses within the renal environment [12].

Cortisol, a glucocorticoid synthesized by the adrenal cortex, plays a pivotal role in the regulation of glucose, protein, and lipids metabolism [13]. Dysregulation leading to hypercortisolism has been implicated in various pathological conditions, including T2DM and cardiovascular disorders (CVDs) [14]. The incidence of hypercortisolism was also reported to be prevalent in patients with T2DM, and it was positively correlated with glycated hemoglobin (HbA1c) and albuminuria, independent of antidiabetic medications [15,16]. However, the association between serum cortisol level and cardiometabolic factors remains controversial. Some investigations, particularly those focusing on individuals with lower BMI rather than those who are overweight or obese, have yielded contradictory findings [17].

The rationale for this study is based on the hypothesis that inflammation, potentially instigated by factors beyond hyperglycemia, may significantly contribute to the pathogenesis of microvascular dysfunction, such as DN. However, the exact biological mechanisms underlying this relationship remain to be elucidated. Given this context, it is essential to investigate the total antioxidant capacity and circulating levels of various pro-inflammatory cytokines (IL-1, IL-6, IL-8, and TNF-α), acute phase protein (hsCRP), and serum cortisol in patients with T2DM. This study aims to elucidate their potential association with albuminuria, thereby advancing our understanding of the inflammatory contributions to DN.

Materials and methods

Study population

This cross-sectional study was conducted on patients aged greater than 18 years diagnosed with T2DM. The participants were systematically recruited from the outpatient department. The study included 150 participants, categorized into three groups: 50 individuals with T2DM with normoalbuminuria, 50 individuals with T2DM with microalbuminuria, and 50 individuals with T2DM with macroalbuminuria. Albuminuria was assessed using a single random urine sample, and the albumin-to-creatinine

ratio (ACR) was calculated to categorize participants as having normoalbuminuria (UACR < 30 mg/g), microalbuminuria (UACR between 30–300 mg/g), or macroalbuminuria (UACR > 300 mg/g). The American Diabetes Association (ADA) criteria, as described below, were used for the selection of T2DM cases: fasting plasma glucose (FPG) levels of ≥ 126 mg/dL on more than two occasions, 2-hour plasma glucose level of ≥ 200 mg/dL measured during an oral glucose tolerance test (OGTT) on one occasion, or random plasma glucose concentration of ≥ 200 mg/dl accompanied with classic symptoms of hyperglycemia or during a hyperglycemic crisis. A diagnosis of diabetes was confirmed if any of these criteria were met. The diabetes status of each participant was also confirmed through their medical records, indicating the use of oral hypoglycemic agents and/or insulin therapy. We meticulously documented the participants' medical histories, anthropometric measurements, and medicine usage. Hypertension was defined based on JNC 8 guidelines. Participants were classified as hypertensive if they had a systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg, as measured during the study visit, aligning with the JNC 8 recommendations for initiating treatment. Additionally, individuals were classified as hypertensive if they were currently using antihypertensive medications, regardless of their blood pressure readings at the time of the study, ensuring the identification of those with well-controlled hypertension due to medication use. By incorporating these criteria, we aimed to capture both diagnosed and treated hypertension, as well as undiagnosed or uncontrolled cases, for a comprehensive assessment of hypertension status in our study population.

Blood investigations

Fasting blood samples from the antecubital vein were collected by venipuncture after at least 8 hours of fasting from each patient during their examination visit. The samples were centrifuged at 2000 × g for 10 min. The plasma or serum obtained was aliquoted into polypropylene tubes for further analysis. Blood samples were analyzed for total antioxidant capacity (T-AOC) by standard ELISA method, pro-inflammatory cytokines (TNF-α, IL-1, IL-6, IL-8), high-sensitivity C-reactive protein (hsCRP), and cortisol levels. Blood samples for cortisol measurement were collected in the morning, specifically between 8:00 AM and 10:00 AM, to account for the diurnal variation in cortisol levels. This timing aligns with the typical peak cortisol levels observed in the morning, ensuring consistency and reliability in our measurements. Additionally, the time of blood collection was standardized across all participants to minimize variability and ensure comparability of results.

Ethical Consideration

The study adhered to the ethical principles outlined in the Declaration of Helsinki (1964) and its subsequent revisions concerning biomedical research involving human subjects. This study was approved by the Institutional Human Research Ethics Committee. Prior to participation, all participants provided informed consent after being thoroughly briefed on the study protocol and objectives.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 17. Continuous variables were presented as means and standard deviations, while categorical variables were expressed as numbers. Comparisons between groups were conducted using one-way Analysis of Variance (ANOVA) test. The odds ratio (OR) for detecting albuminuria risk was determined using conditional logistic regression. A p-value of less than 0.05, determined from two-sided tests was considered to denote statistical significance in all analyses.

Results

Table 1 provides a detailed overview of the analysis of the baseline clinical characteristics of the study participants and the statistical analysis. First findings indicated that age (P=0.104), height (P=0.131), weight (P=0.255), and BMI (P=0.788) were not significantly different among the three groups. However, systolic blood pressure (SBP) [P <0.001], diastolic blood pressure (DBP) [P=0.002], hip circumference (HC) [P=0.003], fasting blood glucose (FBS) [P <0.001], creatinine [P <0.001], total cholesterol (TC) [P <0.001], triglycerides (TG) [P <0.001] were significantly higher, and high-density lipoprotein (HDL) [P <0.001] levels were significantly higher in T2DM patients with microalbuminuria and macroalbuminuria than in those with normoalbuminuria.

Table 1: Baseline characteristic of the participants.

Parameters	T2DM with normoalbuminuria	T2DM with microalbuminuria	T2DM with macroalbuminuria	P value
Age (years)	42.85 ± 6.50	46.0 ±7.64	45.78 ± 10.07	0.1
Height (m)	1.56 ± 0.09	1.57 ± 0.05	1.59 ± 0.08	0.13
Weight (kg)	64.72 ± 12.61	62.70 ± 7.66	61.41 ± 9.24	0.25
BMI (kg/m2)	26.08 ± 4.70	25.55 ± 5.18	26.13 ± 3.98	0.78
SBP (mm/Hg)	127.0 ± 5.55	133.90 ± 6.52	136.35 ± 6.97	< 0.001
DBP (mm/Hg)	78.0 ± 5.88	81.67 ± 2.94	82.65 ± 5.17	0.002
WC (inches)	33.26 ± 3.10	33.56 ± 3.83	34.35 ± 2.21	0.19
HC (inches)	36.86 ± 5.01	37.74 ± 4.54	36.95 ± 2.75	0.51
FBS (mg/dl)	140.92 ± 11.28	156.21 ± 36.8	168.21 ± 41.8	<0.001
Creatinine (mg/dl)	0.94 ± 0.14	1.93 ± 0.72	3.9 ± 0.92	< 0.001
TC (mg/dl)	172.98 ± 18.88	197.33 ± 22.42	203.33 ± 22.31	<0.001
TG (mg/dl)	145.26 ± 2.28	173.52 ± 34.46	177.69 ± 32.05	<0.001
HDL (mg/dl)	47.72 ± 4.06	43.04 ± 1.11	43.75 ± 0.96	<0.001

Table 2 provides a comprehensive overview of the mean and standard deviation of total antioxidant capacity (ELISA based assay), circulating levels of inflammatory cytokines, hsCRP, and serum cortisol levels across different groups of study participants. Our analysis revealed that the total antioxidant capacity (T-AOC) was significantly lower in T2DM patients with microalbuminuria and macroalbuminuria than in those with normoalbuminuria (P=0.027). Furthermore, we observed significantly elevated levels of the pro-inflammatory cytokines α-TNF (P = 0.006), IL-1 (P < 0.001), IL-6 (P < 0.001), and IL-8 (P < 0.001) in T2DM patients with both microalbuminuria and macroalbuminuria, relative to those with normoalbuminuria. This indicates a heightened inflammatory state in patients with increased urinary albumin excretion, suggesting that inflammation plays a critical role in the progression of diabetic nephropathy.

Table 2: Total antioxidant capacity and circulating levels of inflammatory cytokines, hsCRP, and serum cortisol level in study participants.

Parameters	T2DM with normoalbuminuria	T2DM with microalbuminuria	T2DM with macroalbuminuria	P value
T-AOC (mmol/l)	2.10 ± 0.92	1.84 ± 0.69	1.71 ± 0.52	0.027
α-TNF (pg/mL)	11.97 ± 5.26	14.15 ± 2.42	15.17 ± 3.35	0.006
hsCRP (mg/l)	1.99 ± 0.78	3.37 ± 1.18	3.47 ± 0.70	< 0.001
IL-1 (pg/ml)	4.06 ± 0.84	5.94 ± 1.99	6.49 ± 2.18	< 0.001
IL-6 (pg/ml)	7.17 ± 1.69	9.30 ± 3.40	11.90 ± 4.31	< 0.001
IL- 8 (pg/ml)	10.29 ± 3.66	12.73 ± 4.58	14.27 ± 4.35	< 0.001
Cortisol (mmol/l)	357.64 ± 61.09	448.55 ± 135.46	465.55 ± 140.39	< 0.001

Similarly, high-sensitivity C-reactive protein (hsCRP) (P < 0.001) and cortisol (P < 0.001) levels were significantly elevated in T2DM patients with both microalbuminuria and macroalbuminuria compared with those with normoalbuminuria. This finding reinforces the association between stress, inflammation, and kidney dysfunction in patients with T2DM.

An in-depth analysis of the association between α-TNF tertiles (Table 3), hsCRP tertiles (Table 4), IL-1 tertiles (Table 5), IL-6 tertiles (Table 6), IL-8 tertiles (Table 7), and cortisol tertiles (Table 8) with clinical characteristics demonstrated the stratified association of analytes with albuminuria in T2DM. All analytes were categorized into low, mid, and high tertiles. This stratification allows for a nuanced examination of how different analyte levels are associated with albuminuria and other variables, providing insights into the influence of analytes on albuminuria progression. The high tertile of α-TNF was significantly associated with the occurrence of microalbuminuria and macroalbuminuria in T2DM patients with a BMI > 25 kg/m², yielding odds ratios (OR) of 2.16 (95% CI: 0.65–7.19, p-trend = 0.01) and 2.4 (95% CI: 0.74–7.74, p-trend = 0.01), respectively. Additionally, there was a significant association with hypertension, with an OR of 4.16 (95% CI: 0.93–18.71, p = 0.0005) when compared to the low and mid tertiles.

Table 3: Association of α-TNF tertiles with clinical characteristics and albuminuria progression in T2DM patients.

α-TNF (pg/mL)	Low tertile < 12.9	Mid tertile 12.9-15.9	High tertile > 15.9	OR (95% CI)
Age ≤ 45 years				
Controls	20	4	5	1.0 (ref.)
T2DM with microalbuminuria	5	5	8	OR: 1.33; CI: 0.36 – 4.94
T2DM with macroalbuminuria	5	5	5	OR: 0.517; CI: 0.13 – 2.07
P value	0.06			
Age > 45 years				
Controls	14	3	4	1.0 (ref.)
T2DM with microalbuminuria	12	11	9	OR: 1.23; CI: 0.42 – 3.57
T2DM with macroalbuminuria	9	18	8	OR: 0.83; CI: 0.22 – 3.1
P value	0.05			
BMI ≤ 25 kg/m²				
Controls	13	4	5	1.0 (ref.)
T2DM with microalbuminuria	8	11	6	OR: 1.05; CI: 0.28 – 3.94
T2DM with macroalbuminuria	6	6	11	OR: 2.1; CI: 0.63 – 7.04
P value	0.05			
BMI > 25 kg/m²				
Controls	18	4	5	1.0 (ref.)
T2DM with microalbuminuria	6	9	10	OR: 2.16; CI: 0.65 – 7.19
T2DM with macroalbuminuria	8	7	12	OR: 2.4; CI: 0.74 – 7.74
P value	0.01			
Hypertension				
No				
Controls	13	4	8	1.0 (ref.)
T2DM with microalbuminuria	10	12	10	OR: 0.97; CI: 0.34 – 2.83
T2DM with macroalbuminuria	10	14	12	OR: 1.04; CI: 0.37 – 2.91
P value	0.23			
Hypertension				
Yes				
Controls	19	3	3	1.0 (ref.)
T2DM with microalbuminuria	3	8	7	OR: 3.24; CI: 0.74 – 14.26
T2DM with macroalbuminuria	3	4	7	OR: 4.16; CI: 0.93 – 18.71
P value	0.0005			

Table 4: Association of hsCRP tertiles with clinical characteristics and albuminuria progression in T2DM patients.

hsCRP (mg/L)	Low tertile< 2.8	Mid tertile 2.8-3.6	High tertile>3.6	OR (95% CI)
Age ≤ 45 years				
Controls	14	9	3	1.0 (ref.)
T2DM with microalbuminuria	6	6	5	OR: 2.54; CI: 0.54– 12.08
T2DM with macroalbuminuria	5	5	11	OR: 4.53; CI: 1.11 – 18.41
P value	0.04			
Age > 45 years				
Controls	13	6	5	1.0 (ref.)
T2DM with microalbuminuria	9	10	14	OR: 2.03; CI: 0.645 – 6.42
T2DM with macroalbuminuria	6	7	16	OR: 2.64; CI: 0.85 – 8.28
P value	0.04			
BMI ≤ 25 kg/m²				
Controls	16	5	3	1.0 (ref.)
T2DM with microalbuminuria	3	6	10	OR: 4.21; CI: 1.014 – 17.48
T2DM with macroalbuminuria	4	5	14	OR: 4.86; CI: 1.23 – 19.19
P value	0.0007			
BMI > 25 kg/m²				
Controls	16	6	4	1.0 (ref.)
T2DM with microalbuminuria	10	6	5	OR: 1.54; CI: 0.36 – 6.49
T2DM with macroalbuminuria	5	6	16	OR: 3.85; CI: 1.13 – 13.05
P value	0.005			
Hypertension				
No				
Controls	20	5	3	1.0 (ref.)
T2DM with microalbuminuria	4	3	4	OR: 3.39; CI: 0.65 – 17.69
T2DM with macroalbuminuria	4	3	8	OR: 4.97; CI: 1.14 – 21.59
P value	0.02			
Hypertension				
Yes				
Controls	14	6	2	1.0 (ref.)
T2DM with microalbuminuria	8	8	10	OR: 4.23; CI: 0.83 – 21.39
T2DM with macroalbuminuria	6	6	23	OR: 7.22; CI: 1.54 – 33.72
P value	0.0004			

Table 5: Association of IL-1 tertiles with clinical characteristics and albuminuria progression in T2DM patients.

IL-1 (pg/mL)	Low tertile< 4.5	Mid tertile 4.5-6.0	High tertile> 6.0	OR (95% CI)
Age ≤ 45 years				
Controls	20	8	2	1.0 (ref.)
T2DM with microalbuminuria	5	5	6	OR: 5.62; CI: 1.01 – 31.14
T2DM with macroalbuminuria	5	8	8	OR: 5.71; CI: 1.1 – 29.65
P value	0.01			
Age > 45 years				
Controls	11	7	2	1.0 (ref.)
T2DM with microalbuminuria	12	8	14	OR: 2.6; CI: 0.71 – 9.5
T2DM with macroalbuminuria	5	7	17	OR: 3.51; CI: 1.04 – 11.86
P value	0.01			
BMI ≤ 25 kg/m²				
Controls	14	6	4	1.0 (ref.)
T2DM with microalbuminuria	8	5	10	
T2DM with macroalbuminuria	4	6	12	
P value	0.04			
BMI > 25 kg/m²				
Controls	13	8	5	1.0 (ref.)
T2DM with microalbuminuria	5	8	14	OR: 2.69; CI: 0.85 – 8.55
T2DM with macroalbuminuria	5	8	15	OR: 2.78; CI: 0.88 – 8.74
P value	0.02			
Hypertension				
No				
Controls	18	8	2	1.0 (ref.)
T2DM with microalbuminuria	4	2	9	OR: 8.4; CI: 1.6 – 43.9
T2DM with macroalbuminuria	4	2	10	OR: 8.75; CI: 1.7 – 45.0
P value	0.0008			
Hypertension				
Yes				
Controls	9	9	4	1.0 (ref.)
T2DM with microalbuminuria	9	9	9	OR: 1.83; CI: 0.49 – 6.76
T2DM with macroalbuminuria	5	10	19	OR: 3.07; CI: 0.92 – 10.24
P value	0.04			

Table 6: Association of IL-6 tertiles with clinical characteristics and albuminuria progression in T2DM patients.

IL-6 (pg/mL)	Low tertile < 7.0	Mid tertile 7.0-12.0	High tertile > 12.0	OR (95% CI)
Age ≤ 45 years				
Controls	12	16	2	1.0 (ref.)
T2DM with microalbuminuria	5	7	5	OR: 4.41; CI: 0.77 – 25.24
T2DM with macroalbuminuria	4	7	10	OR: 7.14; CI: 1.41 – 35.91
P value	0.02			
Age > 45 years				
Controls	7	10	3	1.0 (ref.)
T2DM with microalbuminuria	9	15	9	OR: 2.0; CI: 0.48 – 8.22
T2DM with macroalbuminuria	4	10	15	OR: 3.7; CI: 0.97 – 14.74
P value	0.07			
BMI ≤ 25 kg/m²				
Controls	9	10	3	1.0 (ref.)
T2DM with microalbuminuria	5	13	5	
T2DM with macroalbuminuria	3	13	8	
P value	0.19			
BMI > 25 kg/m²				
Controls	9	15	4	1.0 (ref.)
T2DM with microalbuminuria	5	17	5	OR: 1.29; CI: 0.31 – 5.34
T2DM with macroalbuminuria	3	11	12	OR: 3.23; CI: 0.92 – 11.29
P value	0.03			
Hypertension				
No				
Controls	10	14	2	1.0 (ref.)
T2DM with microalbuminuria	3	15	3	OR: 1.85; CI: 0.28 – 12.16
T2DM with macroalbuminuria	3	7	7	OR: 5.35; CI: 0.99 – 28.9
P value	0.02			
Hypertension				
Yes				
Controls	7	15	2	1.0 (ref.)
T2DM with microalbuminuria	7	17	5	OR: 2.06; CI: 0.36 – 11.63
T2DM with macroalbuminuria	3	16	14	OR: 5.09; CI: 1.05 – 24.52
P value	0.02			

Table 7: Association of IL-8 tertiles with clinical characteristics and albuminuria progression in T2DM patients.

IL-8 (pg/mL)	Low tertile < 11.0	Mid tertile 11.0-14.0	High tertile > 14.0	OR (95% CI)
Controls	17	5	5	1.0 (ref.)
T2DM with microalbuminuria	5	5	7	OR: 2.22; CI: 0.6 – 8.14
T2DM with macroalbuminuria	5	5	11	OR: 2.82; CI: 0.85 – 9.4
P value	0.04			
Age > 45 years				
Controls	14	4	5	1.0 (ref.)
T2DM with microalbuminuria	10	10	13	OR: 2.09; CI: 0.66 – 6.56
T2DM with macroalbuminuria	4	11	14	OR: 2.06; CI: 0.64 – 6.62
P value	0.01			
BMI ≤ 25 kg/m²				
Controls	16	4	4	1.0 (ref.)
T2DM with microalbuminuria	6	5	8	OR: 2.52; CI: 0.65 – 9.67
T2DM with macroalbuminuria	5	8	7	OR: 2.1; CI: 0.53 – 8.21
P value	0.04			
BMI > 25 kg/m²				
Controls	13	8	5	1.0 (ref.)
T2DM with microalbuminuria	5	15	11	OR: 1.84; CI: 0.56 – 5.99
T2DM with macroalbuminuria	6	10	14	OR: 2.42; CI: 0.76 – 7.65
P value	0.02			
Hypertension				
No				
Controls	16	14	4	1.0 (ref.)
T2DM with microalbuminuria	6	8	5	OR: 2.23; CI: 0.53 – 9.34
T2DM with macroalbuminuria	3	8	10	OR: 4.04; CI: 1.12 – 14.46
P value	0.02			
Hypertension				
Yes				
Controls	14	6	4	1.0 (ref.)
T2DM with microalbuminuria	10	9	12	OR: 1.16; CI: 0.41 – 3.28
T2DM with macroalbuminuria	6	8	15	OR: 1.55; CI: 0.56 – 4.27
P value	0.04			

Table 8: Association of cortisol tertiles with clinical characteristics and albuminuria progression in T2DM patients.

Cortisol (nmol/L)	Low tertile < 350.0	Mid tertile 350.0-450.0	High tertile > 450.0	OR (95% CI)
Age ≤ 45 years				
Controls	11	14	5	1.0 (ref.)
T2DM with microalbuminuria	5	6	7	OR: 2.33; CI: 0.64 – 8.45
T2DM with macroalbuminuria	5	6	12	OR: 3.13; CI: 0.96 – 10.14
P value	0.1			
Age > 45 years				
Controls	7	8	5	1.0 (ref.)
T2DM with microalbuminuria	8	16	8	OR: 1.00; CI: 0.28 – 3.48
T2DM with macroalbuminuria	6	8	13	OR: 1.92; CI: 0.59 – 6.28
P value	0.12			
BMI ≤ 25 kg/m²				
Controls	10	9	3	1.0 (ref.)
T2DM with microalbuminuria	4	10	8	OR: 2.66; CI: 0.62 – 11.39
T2DM with macroalbuminuria	2	11	10	OR: 3.18; CI: 0.77 – 13.14
P value	0.03			
BMI > 25 kg/m²				
Controls	12	13	4	1.0 (ref.)
T2DM with microalbuminuria	6	14	8	OR: 1.57; CI: 0.41 – 5.9
T2DM with macroalbuminuria	3	10	14	OR: 1.83; CI: 0.49 – 6.76
P value	0.01			
Hypertension				
No				
Controls	11	14	3	1.0 (ref.)
T2DM with microalbuminuria	4	15	5	OR: 1.94; CI: 0.42 – 8.95
T2DM with macroalbuminuria	2	9	6	OR: 3.29; CI: 0.72 – 14.93
P value	0.1			
Hypertension				
Yes				
Controls	9	10	3	1.0 (ref.)
T2DM with microalbuminuria	4	15	7	OR: 1.97; CI: 0.45 – 8.56
T2DM with macroalbuminuria	5	16	12	OR: 2.66; CI: 0.67 – 10.55
P value	0.1			

In continuation of our findings, the analysis demonstrated that elevated levels of hsCRP (Table 4), IL-1 (Table 5), and IL-8 (Table 7), were significantly associated with an increased risk of both microalbuminuria and macroalbuminuria in T2DM patients, regardless of age, BMI, and blood pressure. Notably, the risk of macroalbuminuria is higher than that of microalbuminuria.

Moreover, for patients aged ≤ 45 years and with a BMI greater than 25 kg/m², the high tertile of IL-6, in contrast to the mid and low tertiles, showed a significant association with an increased risk of both microalbuminuria and macroalbuminuria, irrespective of their blood pressure (Table 6). In addition, a high tertile of cortisol was significantly associated with an increased risk of both microalbuminuria and macroalbuminuria in patients with T2DM, irrespective of their BMI (Table 8).

Discussion

DN is characterized by a gradual decrease in GFR, the presence of albuminuria, and an increase in arterial blood pressure [17]. It has emerged as the leading cause of chronic kidney disease (CKD) in India, with matching trends observed in Western societies. It is one of the most serious long-term consequences in patients with diabetes, with considerable morbidity and mortality rates. In India, diabetes accounts for a significant proportion of end-stage renal disease (ESRD) cases, demonstrating the urgent need to understand the mechanism of its occurrence [18].

Our analysis revealed that baseline characteristics such as age, height, weight, BMI, WC, and HC were evenly distributed among the study groups (Table1), decreasing the possibility that our findings were influenced by demographic and anthropometric variables. As a result, any relationship between inflammatory biomarkers and microalbuminuria can be more firmly assigned to the clinical processes under study rather than variations in these underlying traits. However, patients with increased urinary albumin excretion exhibited more pronounced cardiometabolic risk factors, such as SBP, DBP, TC, TG, HDL, and creatinine (Table 1), indicating a higher likelihood of cardiovascular complications and renal impairment. These observations underscore the importance of the early detection and management of albuminuria in patients with T2DM to mitigate the associated risks and improve clinical outcomes.

T2DM patients with microalbuminuria and macroalbuminuria exhibited significantly lower T-AOC than those with normoalbuminuria (P=0.027). These findings emphasize the potential significance of oxidative stress in the progression of kidney damage in patients with T2DM, which is consistent with the literature stressing oxidative stress as a major contributor to diabetic sequelae, specifically, nephropathy. Oxidative stress in type 2 diabetes is caused by persistent hyperglycemia, which accelerates the production of reactive oxygen species (ROS) via mechanisms such as glucose auto-oxidation and the generation of advanced glycation end products (AGEs) [19]. Microalbuminuria, an early hallmark of diabetic nephropathy, is caused by this, which intensifies renal inflammation and fibrosis and activates vital signaling pathways like NF-κB and upregulates pro-inflammatory genes like IL-6 and TNF-α. This genetic modification of the inflammatory and fibrotic pathways reveals the importance of oxidative stress in diabetic kidney injury [20]. Various studies have reported a direct relationship between declining kidney function and increasing T-AOC reduction in patients with DN, connecting oxidative stress biomarkers to the advancement of DN [21,22]. An additional study provided more evidence that reduced antioxidant defenses, especially in the serum, are important in hastening the start of microalbuminuria by encouraging oxidative damage in renal tissues [23].

The noteworthy increase in pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, and IL-8, in T2DM patients exhibiting microalbuminuria and macroalbuminuria suggests that individuals with DN are experiencing an elevated inflammatory state. The role of chronic inflammation in DN pathogenesis is well documented, with cytokines such as TNF-α, IL-1, IL-6, and IL-8 contributing to endothelial dysfunction, glomerular inflammation, and renal fibrosis [12]. The significant association between high TNF-α levels and an increased risk of microalbuminuria and macroalbuminuria in T2DM patients with BMI > 25 kg/m², as well as with hypertension, reveals the role of TNF-α in promoting inflammation and vascular dysfunction. Elevated TNF-α, for instance, has been shown to induce podocyte apoptosis, disrupt the glomerular filtration barrier, and promote albuminuria by increasing glomerular permeability [24]. IL-6 is another critical player, as it has been implicated in promoting mesangial cell proliferation and matrix expansion, both of which contribute to glomerulosclerosis, a hallmark of DN [25]. The significant association between high IL-6 levels and increased risk of both microalbuminuria and macroalbuminuria, particularly in younger T2DM patients (≤ 45 years) with a BMI >25 kg/m², irrespective of blood pressure, underlines the role of IL-6 in mediating inflammatory responses that contribute to early kidney damage. These findings suggest that elevated IL-6 levels may serve as a biomarker for early renal impairment in younger overweight individuals with T2DM, independent of hypertension status. This is consistent with previous evidence linking IL-6 with obesity-related inflammation and the progression of kidney disease in diabetes [26]. Similarly, IL-1 and IL-8 have been associated with the recruitment of immune cells, further amplifying inflammation and oxidative stress, leading to dysfunction of proximal tubules and podocyte damage in patients with T2DM [27].

These elevated cytokine levels suggest a sustained inflammatory response that correlates with albuminuria severity, indicating a progressive decline in renal health. This finding aligns with previous research showing that inflammation is a central mechanism in the progression from microalbuminuria to overt nephropathy in T2DM. Targeting these inflammatory pathways may offer a therapeutic strategy for preventing or delaying the progression of kidney damage in patients with diabetes. Anti-inflammatory agents, particularly those that inhibit IL-6 and TNF-α, have shown promise in preclinical models and warrant

further investigation in the context of DN [28].

Elevated cortisol levels in DN (Table 2) may reflect the chronic stress state and altered hypothalamic-pituitary-adrenal (HPA) axis regulation commonly observed in patients with T2DM. Chronic hyperglycemia and insulin resistance exacerbate HPA axis activity, leading to sustained increases in cortisol secretion. Hypercortisolemia not only contributes to metabolic dysregulation, but also exacerbates inflammation, oxidative stress, and endothelial dysfunction, all of which are key factors in the progression of DN. Recent evidence also suggests that cortisol may directly influence kidney function by promoting glomerular hyperfiltration, increasing renal sodium retention, and stimulating the production of angiotensin II, thereby accelerating the development of nephropathy in T2DM patients [29].

Similarly, hsCRP, a marker of systemic inflammation, is consistently elevated in patients with diabetic nephropathy, reflecting ongoing inflammatory processes that contribute to the progression of renal impairment. hsCRP is produced by the liver in response to pro-inflammatory cytokines such as IL-6, and its elevated levels in T2DM patients with nephropathy suggest a state of chronic low-grade inflammation. Inflammation is known to play a critical role in endothelial dysfunction, promoting atherosclerosis and further compromising renal blood flow. Moreover, high hsCRP levels have been associated with increased albuminuria, suggesting a direct link between systemic inflammation and glomerular injury in diabetic nephropathy [30]. The combined elevation of cortisol and hsCRP levels in DN may emphasize the interplay between stress, inflammation, and metabolic dysregulation in the pathogenesis of renal complications in T2DM. Therapeutic strategies aimed at reducing cortisol levels, possibly through stress management techniques or pharmacological interventions, alongside anti-inflammatory approaches targeting hsCRP and related cytokines, could potentially mitigate DN progression. Further research is warranted to explore the efficacy of such interventions in clinical settings.

This cross-sectional study has several limitations that should be considered when interpreting the results. First, the design of the study limits the ability to establish causal relationships between elevated inflammatory biomarkers, oxidative stress markers, and the progression of kidney disease in T2DM patients. Longitudinal studies are needed to clarify the temporal sequence of these associations. Second, although the study was adjusted for key confounders such as BMI, age, and blood pressure, unmeasured factors such as dietary habits, physical activity, and genetic predispositions may have influenced the observed relationships. Additionally, reliance on single-point measurements of biomarkers and albuminuria might not fully capture the dynamic changes in these parameters over time, leading to potential misclassification. The generalizability of the findings is also a concern, as the study sample may not represent the broader population of T2DM patients and ethnic, geographical, or socioeconomic differences could impact the biomarkers and outcomes studied. Lastly, potential measurement biases in the assessment of biomarkers and albuminuria due to variability in laboratory methods or sample handling may affect the accuracy and reliability of the results. These limitations highlight the need for further research to validate and extend these findings.

Conclusion

The study findings indicate that pro-inflammatory cytokines (TNF-α, IL-1, IL-6, IL-8), hsCRP, and cortisol, coupled with a reduction in total antioxidant capacity (T-AOC), were significantly associated with albuminuria in patients with T2DM, highlighting their potential roles in the progression of DN. This study revealed the intricate interplay between metabolic dysregulation, chronic inflammation, and oxidative damage in the pathogenesis of DN.

Competing interests

The authors declare that they have no competing interests to disclose.

Declaration of conflict of interest

The authors affirm that this study is free from any conflicts of interest.

Ethical Approval

This investigation was conducted in accordance with the ethical guidelines of the Declaration of Helsinki on biomedical research on humans and was approved by the Institutional Human Research Ethical Committee.

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Deepak Parchwani: Conceptualization, Methodology, Review, Editing.

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Sagar Dholariya: Original Draft, Data curation, editing.

Ashishkumar Agravatt: Analysis, Validation, review and editing.

Ragini Singh: Original draft, software, data curation, and validation.

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References

1. Sagoo MK, Gnudi L. Diabetic Nephropathy: An Overview. Methods Mol Biol. 2020;2067:3-7. doi: 10.1007/978-1-4939-9841-8_1.
2. Natesan V, Kim SJ. Diabetic Nephropathy - a Review of Risk Factors, Progression, Mechanism, and Dietary Management. Biomol Ther (Seoul). 2021;29(4):365-372. doi: 10.4062/biomolther.2020.204.
3. Sinha SK, Nicholas SB. Pathomechanisms of Diabetic Kidney Disease. J Clin Med. 2023 Nov 27;12(23):7349. doi: 10.3390/jcm12237349.
4. Christofides EA, Desai N. Optimal Early Diagnosis and Monitoring of Diabetic Kidney Disease in Type 2 Diabetes Mellitus: Addressing the Barriers to Albuminuria Testing. J Prim Care Community Health. 2021;12:21501327211003683. doi: 10.1177/21501327211003683.
5. Wang N, Zhang C. Oxidative Stress: A Culprit in the Progression of Diabetic Kidney Disease. Antioxidants (Basel). 2024;13(4):455. doi: 10.3390/antiox13040455.
6. Duni A, Liakopoulos V, Roumeliotis S, Peschos D, Dounousi E. Oxidative Stress in the Pathogenesis and Evolution of Chronic Kidney Disease: Untangling Ariadne's Thread. Int J Mol Sci. 2019;20(15):3711. doi: 10.3390/ijms20153711.
7. Yang M, Zhang C. The role of innate immunity in diabetic nephropathy and their therapeutic consequences. J Pharm Anal. 2024;14(1):39-51. doi: 10.1016/j.jpha.2023.09.003.
8. Winter L, Wong LA, Jerums G, Seah JM, Clarke M, Tan SM, Coughlan MT, MacIsaac RJ, Ekinci EI. Use of Readily Accessible Inflammatory Markers to Predict Diabetic Kidney Disease. Front Endocrinol (Lausanne). 2018;9:225. doi: 10.3389/fendo.2018.00225.
9. Stanimirovic J, Radovanovic J, Banjac K, Obradovic M, Essack M, Zafirovic S, Gluvic Z, Gojobori T, Isenovic ER. Role of C-Reactive Protein in Diabetic Inflammation. Mediators Inflamm. 2022;2022:3706508. doi: 10.1155/2022/3706508.
10. Su H, Lei CT, Zhang C. Interleukin-6 Signaling Pathway and Its Role in Kidney Disease: An Update. Front Immunol. 2017;8:405. doi: 10.3389/fimmu.2017.00405.
11. Donate-Correa J, Ferri CM, Sánchez-Quintana F, Pérez-Castro A, González-Luis A, Martín-Núñez E, Mora-Fernández C, Navarro-González JF. Inflammatory Cytokines in Diabetic Kidney Disease: Pathophysiologic and Therapeutic Implications. Front Med (Lausanne). 2021;7:628289. doi: 10.3389/fmed.2020.628289.
12. Donate-Correa J, Martín-Núñez E, Muros-de-Fuentes M, Mora-Fernández C, Navarro-González JF. Inflammatory cytokines in diabetic nephropathy. J Diabetes Res. 2015;2015:948417. doi: 10.1155/2015/948417.
13. Bauerle KT, Harris C. Glucocorticoids and Diabetes. Mo Med. 2016;113(5):378-383.
14. Reynolds RM, Labad J, Strachan MW, Braun A, Fowkes FG, Lee AJ, Frier BM, Seckl JR, Walker BR, Price JF; Edinburgh Type 2 Diabetes Study (ET2DS) Investigators. Elevated fasting plasma cortisol is associated with ischemic heart disease and its risk factors in people with type 2 diabetes: the Edinburgh type 2 diabetes study. J Clin Endocrinol Metab. 2010;95(4):1602-1608. doi: 10.1210/jc.2009-2112.
15. Lehrer HM, Dubois SK, Maslowsky J, Laudenslager ML, Steinhardt MA. Hair cortisol concentration and glycated hemoglobin in African American adults. Psychoneuroendocrinology. 2016;72:212-218. doi: 10.1016/j.psyneuen.2016.06.018.
16. Zhang X, Deng X, Zhou J, Qiu K, Deng M, Lin Z, Mosha SS, Li W. The Association Of Serum Cortisol Level With Microalbuminuria In Patients With Type 2 Diabetes And Prediabetes. Int J Med Sci.;17(18):2998-3004. doi: 10.7150/ijms.48742.
17. Gariani K, Marques-Vidal P, Waeber G, Vollenweider P, Jornayvaz FR. Salivary cortisol is not associated with incident insulin resistance or type 2 diabetes mellitus. Endocr Connect. 2019;8(7):870-877. doi: 10.1530/EC-19-0251.
18. Kumar V, Yadav AK, Sethi J, Ghosh A, Sahay M, Prasad N, Varughese S, Parameswaran S, Gopalakrishnan N, Kaur P, Modi GK, Kamboj K, Kundu M, Sood V, Inamdar N, Jaryal A, Vikrant S, Nayak S, Singh S, Gang S, Baid-Agrawal S, Jha V. The Indian Chronic Kidney Disease (ICKD) study: baseline characteristics. Clin Kidney J. 2021;15(1):60-69. doi: 10.1093/ckj/sfab149.
19. Bhatti JS, Sehrawat A, Mishra J, Sidhu IS, Navik U, Khullar N, Kumar S, Bhatti GK, Reddy PH. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. Free Radic Biol Med. 2022;184:114-134. doi: 10.1016/j.freeradbiomed.2022.03.019.
20. Sinha SK, Nicholas SB. Pathomechanisms of Diabetic Kidney Disease. J Clin Med. 2023;12(23):7349. doi: 10.3390/jcm12237349.
21. Shao N, Kuang HY, Wang N, Gao XY, Hao M, Zou W, Yin HQ. Relationship between Oxidant/Antioxidant Markers and Severity of Microalbuminuria in the Early Stage of Nephropathy in Type 2 Diabetic Patients. J Diabetes Res. 2013;2013:232404. doi: 10.1155/2013/232404.
22. Medina-Navarro R, Corona-Candelas I, Barajas-González S, Díaz-Flores M, Durán-Reyes G. Albumin antioxidant response to stress in diabetic nephropathy progression. PLoS One. 2014;9(9):e106490. doi: 10.1371/journal.pone.0106490.

23. Darenskaya M, Kolesnikov S, Semenova N, Kolesnikova L. Diabetic Nephropathy: Significance of Determining Oxidative Stress and Opportunities for Antioxidant Therapies. Int J Mol Sci. 202;24(15):12378. doi: 10.3390/ijms241512378.
24. Barutta F, Bellini S, Gruden G. Mechanisms of podocyte injury and implications for diabetic nephropathy. Clin Sci (Lond). 2022;136(7):493-520. doi: 10.1042/CS20210625.
25. Su H, Lei CT, Zhang C. Interleukin-6 Signaling Pathway and Its Role in Kidney Disease: An Update. Front Immunol. 2017;8:405. doi: 10.3389/fimmu.2017.00405.
26. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. Immunity. 2022;55(1):31-55. doi: 10.1016/j.immuni.2021.12.013.
27. Milas O, Gadalean F, Vlad A, Dumitrascu V, Velciov S, Gluhovschi C, Bob F, Popescu R, Ursoniu S, Jianu DC, Matusz P, Pusztai AM, Secara A, Simulescu A, Stefan M, Patruica M, Petrica F, Vlad D, Petrica L. Pro-inflammatory cytokines are associated with podocyte damage and proximal tubular dysfunction in the early stage of diabetic kidney disease in type 2 diabetes mellitus patients. J Diabetes Complications. 2020;34(2):107479. doi: 10.1016/j.jdiacomp.2019.107479.
28. Nicholas SB. Novel Anti-inflammatory and Anti-fibrotic Agents for Diabetic Kidney Disease-From Bench to Bedside. Adv Chronic Kidney Dis. 2021;28(4):378-390. doi: 10.1053/j.ackd.2021.09.010.
29. Sagmeister MS, Harper L, Hardy RS. Cortisol excess in chronic kidney disease - A review of changes and impact on mortality. Front Endocrinol (Lausanne). 2023;13:1075809. doi: 10.3389/fendo.2022.1075809.
30. Tang M, Cao H, Wei XH, Zhen Q, Liu F, Wang YF, Fan NG, Peng YD. Association Between High-Sensitivity C-Reactive Protein and Diabetic Kidney Disease in Patients With Type 2 Diabetes Mellitus. Front Endocrinol (Lausanne). 2022;13:885516. doi: 10.3389/fendo.2022.885516.