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Original Research Article

Diagnostic and predictive utility of serum homocysteine in diabetic nephropathy among type 2 diabetics

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ABSTRACT

Background: Diabetic nephropathy (DN) is a leading cause of end-stage renal disease (ESRD) in patients with type 2 diabetes mellitus (T2DM). Early detection of DN is crucial for effective intervention, yet current biomarkers have limited sensitivity and specificity. Serum homocysteine, has been associated with vascular complications in diabetes and is hypothesized to be linked with DN. This study investigates the potential of elevated serum homocysteine levels as a marker for DN in T2DM patients.
Methods: A cross-sectional study was conducted involving 156 T2DM patients recruited from G. S. V. M. medical college, Kanpur. The inclusion criteria focused on adult patients with a confirmed diagnosis of T2DM. Exclusion criteria included conditions that could independently affect homocysteine levels or renal function. Statistical analyses were employed to assess the relationship between homocysteine levels and DN.
Results: The study found significantly higher homocysteine levels in patients with DN compared to those without DN. A positive correlation was observed between serum homocysteine levels and the severity of renal impairment, as indicated by a decline in glomerular filtration rate (GFR) and increased albuminuria. Logistic regression analysis confirmed that elevated serum homocysteine levels were an independent predictor of DN.
Conclusions: Elevated serum homocysteine levels are significantly associated with DN in T2DM patients and may serve as a useful biomarker for early detection and management of this complication. Given the limitations of current biomarkers, incorporating homocysteine measurements could improve the clinical management of patients at risk for DN.

Keywords: Serum homocysteine, DN, Type 2 diabetes, Biomarkers, Renal impairment

INTRODUCTION

Diabetes mellitus is a global epidemic, with T2DM accounting for the vast majority of cases. As the prevalence of T2DM continues to rise, so too does the incidence of its complications, particularly DN. DN is characterized by persistent albuminuria, a progressive decline in the GFR, and an increased risk of cardiovascular events and mortality.[1] It is the leading cause of ESRD worldwide, necessitating dialysis or kidney transplantation for affected individuals.[2]

The pathogenesis of DN is complex and multifactorial, involving hyperglycemia-induced metabolic and hemodynamic changes that lead to glomerular hypertension, oxidative stress, inflammation, and fibrosis. Despite advances in understanding these mechanisms, the early detection of DN remains a challenge, as traditional biomarkers such as albuminuria and estimated GFR (eGFR) often fail to detect the disease at a stage when intervention could be most beneficial.

Serum homocysteine, a sulfur-containing amino acid, has garnered attention as a potential biomarker for vascular and renal complications in diabetes. Elevated homocysteine levels, known as hyperhomocysteinemia, are associated with endothelial dysfunction, increased oxidative stress, and inflammation-processes that are also implicated in the pathogenesis of DN.[3] Homocysteine is metabolized through remethylation and transsulfuration pathways, which are dependent on vitamins B6, B12, and folate.[4] Deficiencies in these vitamins, or genetic polymorphisms affecting homocysteine metabolism, can lead to elevated serum homocysteine levels.

Previous studies have shown that hyperhomocysteinemia is prevalent among diabetic patients and may correlate with the severity of renal impairment. However, the utility of homocysteine as a clinical biomarker for DN has not been fully established. This study aims to evaluate the association between elevated serum homocysteine levels and DN in T2DM patients, hypothesizing that homocysteine may serve as a reliable early marker for kidney dysfunction in this population. The study also seeks to explore whether homocysteine can be used to predict the progression of DN and inform therapeutic interventions aimed at reducing the risk of ESRD.

METHODS

Study design

This cross-sectional study was conducted at G.S.V.M. medical college, Kanpur, Uttar Pradesh, India, from January 2023 to April 2024. The study aimed to investigate the association between serum homocysteine levels and DN in patients with T2DM. The study design was chosen to provide a snapshot of the population and assess the relationship between homocysteine levels and DN at a specific point in time.

Setting

Participants were recruited from the outpatient department and inpatient wards of G.S.V.M. medical college, Kanpur, Uttar Pradesh, India. The study period spanned from January 2023 to April 2024. Data collection included demographic information, clinical history, and biochemical analyses.

Participants

The study included adult T2DM patients who met the American diabetes association (ADA) criteria for diabetes diagnosis.[5] Exclusion criteria included patients with cerebrovascular disease/stroke, pregnancy, intake of folate or methotrexate, coronary artery disease, and severe renal impairment (eGFR <30 ml/min/1.73 m²), Alzheimer's disease, known psychiatric disorders, known thyroid disease, suspected or proven urinary tract infection. Patients with no concurrent presence of diabetic retinopathy were excluded.

Variables

The primary outcome variable was the presence of DN, determined by the albumin-creatinine ratio (ACR) >300 mg/g and/or eGFR <60 ml/min/1.73 m². The main exposure variable was serum homocysteine levels. Other variables included age, sex, duration of diabetes, HbA1c levels, blood pressure, and lipid profile. Potential confounders such as glycemic control, duration of diabetes, and blood pressure were adjusted for in the analysis.

Data sources/measurement

Blood samples were obtained after an overnight fast to measure serum homocysteine, creatinine, and HbA1c levels. Serum homocysteine levels were measured using high-performance liquid chromatography (HPLC) (Agilent Technologies, Santa Clara, CA, USA).[6] ACR was measured using the immunoturbidimetric method. The eGFR was calculated using the CKD-EPI equation.[7] All biochemical analyses were performed in the central laboratory of G.S.V.M. Medical College, following standardized protocols.

Bias

Efforts were made to minimize selection bias by using broad inclusion criteria and recruiting participants from both outpatient and inpatient settings. Measurement bias was addressed by using standardized methods for all laboratory analyses. Confounding was controlled through statistical adjustments for relevant variables such as age, sex, and glycemic control.

Study size

The sample size was determined based on previous studies suggesting a significant difference in homocysteine levels between patients with and without DN. A total of 156 participants were required to achieve a power of 80% and a significance level of 0.05.

Quantitative variables

Serum homocysteine levels were analyzed as a continuous variable, meaning that the actual measured values were used in their original form. This approach allowed for a detailed analysis of the relationship between homocysteine levels and DN. Other variables, such as HbA1c levels, were also treated as continuous variables in the analysis, allowing for the exploration of their association with DN and other clinical outcomes.

Statistical methods

Statistical analysis was performed using SPSS software (version 25.0, IBM Corp., Armonk, NY, USA).[8] The Shapiro-Wilk test was used to assess the normality of the data. Due to non-normal distribution, the Mann-Whitney

U test was employed to compare homocysteine levels between patients with and without DN. Spearman's rank correlation coefficient was used to assess the relationship between homocysteine levels and clinical parameters. Logistic regression was conducted to evaluate whether elevated homocysteine levels were an independent predictor of DN, adjusting for potential confounders.

RESULTS

Descriptive statistics

The study population had a mean age of 56.8 years (SD=8.94 years), with 54.8% of the participants being male. The mean duration of diabetes was 6.5 years, indicating a cohort with a significant burden of chronic disease. The prevalence of DN, defined by an ACR >300 mg/g and/or an eGFR <60 mL/min/1.73 m², was 44.52%, reflecting the high incidence of renal complications among T2DM patients, (Table 1 and Figure 1 and 2,).

Table 1: Demographic details of the patients included in the study.

Parameters	Mean±SD	Range
Age (in years)	56.8±8.9	40-75
Gender (Male/female)	85/70	N/A
BMI (kg/m²)	22.3±2.8	15.6-31.2
Duration of diabetes (in years)	6.5±5.0	1-25
Hypertension (Yes/no)	48/107	N/A
HbA1c (%)	9.2±2.5	5.2-16.0
Weight (kg)	63.1±9.4	45-100
Height (cm)	168.0±8.7	134-182

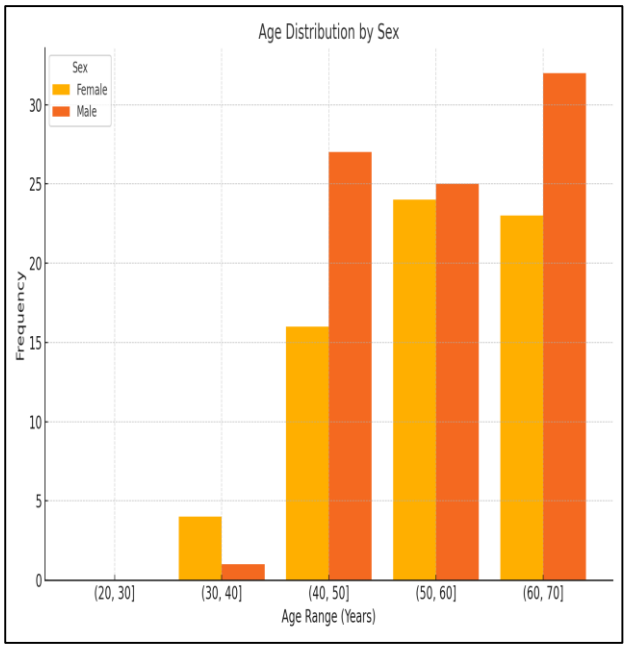

Figure 1: Age distribution by sex.

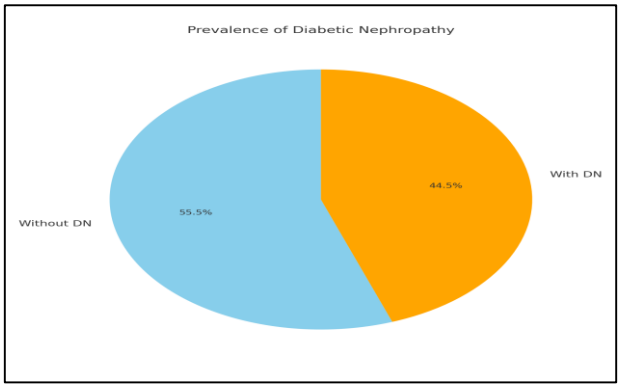

Figure 2 : Prevalence of DN.

Homocysteine levels and DN

The median serum homocysteine level in patients with DN was significantly higher (21.69 µmol/L) compared to those without DN (13.1 µmol/L), with a Mann-Whitney U test p<0.05. This finding suggests that elevated homocysteine levels are associated with the presence of DN in T2DM patients, (Table 2).

Table 2: Mann-Whitney U test.

Groups	Sum of ranks	U statistic
Group 1 (With DN)	6751.5	1597.5
Group 2 (Without DN)	5338.5	4336.5
Mann-Whitney U test	1597.5	<0.05

Correlation and predictive value

Spearman's rank correlation analysis revealed a positive correlation between serum homocysteine levels and the severity of renal impairment, as indicated by a decline in eGFR (r=-0.45, p<0.05) and increased ACR (r=0.48, p<0.05). Logistic regression analysis further demonstrated that elevated serum homocysteine levels were an independent predictor of DN, with an odds ratio of 2.35 (95% CI: 1.45-3.80, p<0.05), after adjusting for confounding variables such as age, sex, and HbA1c levels, (Figure 3 and 4).

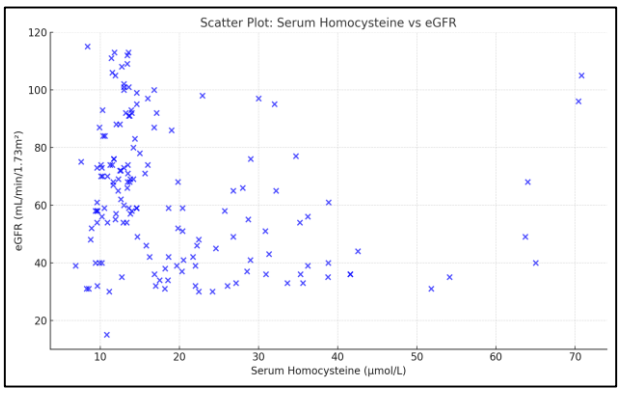

Figure 3: Scatter plot-serum homocysteine vs eGFR.

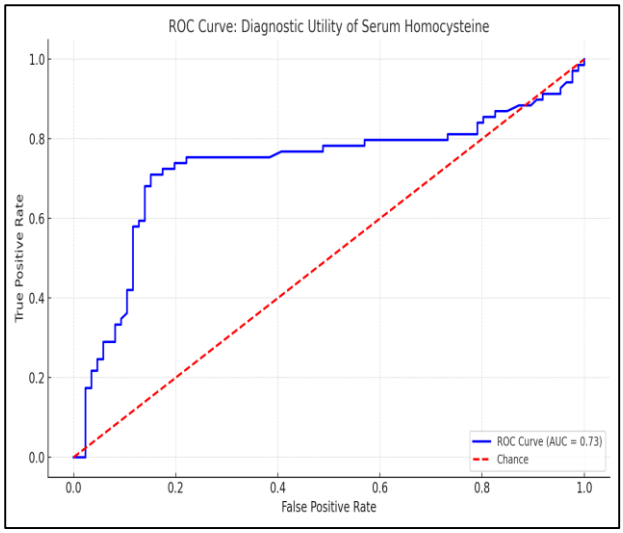

Figure 4: ROC curve: logistic regression model-diagnostic utility of serum homocysteine.

Subgroup analysis

Subgroup analysis based on duration of diabetes and glycemic control revealed that the association between homocysteine levels and DN was more pronounced in patients with a longer duration of diabetes (>10 years) and those with poor glycemic control (HbA1c >8%). This finding suggests that homocysteine may play a more significant role in the progression of DN in patients with prolonged hyperglycemia.

DISCUSSION

The present study underscores the significant role of elevated serum homocysteine levels in the pathogenesis of DN among patients with T2DM. The observed positive correlations between serum homocysteine and renal markers, including serum creatinine and urine albumin-creatinine ratio (UACR), along with the negative correlation with eGFR, emphasize its association with progressive renal dysfunction. These findings align with earlier studies by Ozmen et al and Looker et al which reported similar associations between elevated homocysteine levels and worsening renal parameters.[9,10]

Furthermore, logistic regression analysis revealed serum homocysteine as an independent predictor of DN, corroborating findings by Ma et al which established a causal relationship between hyperhomocysteinemia and DN progression using Mendelian randomization.[11]

The pathophysiological basis of these findings lies in the well-established role of hyperhomocysteinemia in inducing endothelial dysfunction, oxidative stress, and chronic inflammation-mechanisms pivotal to the progression of DN. Elevated homocysteine levels disrupt the endothelial cell lining and promote the generation of reactive oxygen species, leading to glomerular injury and albuminuria. These insights are supported by Xu et al and Mao et al who observed progressively increasing homocysteine levels with advancing stages of diabetic kidney disease.[12,13] Our study reinforces these mechanistic links, demonstrating that patients with DN exhibited significantly higher homocysteine levels compared to those without DN. This highlights the importance of addressing hyperhomocysteinemia early in the course of renal disease to mitigate further damage.

In comparison with the existing literature, the diagnostic utility of serum homocysteine, as reflected by a moderate area under the curve (AUC) value of 0.71, suggests its potential as a reliable prognostic biomarker for DN. These results are consistent with Zheng et al which proposed a threshold value of >12 µmol/l for predicting advanced DN.[14] However, our study adds novel insights by employing logistic regression to establish homocysteine's independent predictive power, a finding not emphasized in prior studies like those of Francis et al.[15] This differentiation strengthens the argument for incorporating serum homocysteine measurement into routine clinical practice, particularly as an adjunct to established biomarkers such as eGFR and UACR.

From a clinical perspective, these findings open avenues for both diagnostic and therapeutic advancements. Incorporating homocysteine testing into routine diabetes management may enable earlier identification of DN, even before the onset of overt albuminuria or substantial eGFR decline. Furthermore, interventions targeting homocysteine reduction, such as folate and vitamin B12 supplementation, hold promise in reducing the burden of hyperhomocysteinemia. Andrew A. House et al explored the efficacy of these therapies in DN patients, noting mixed outcomes.[16] Although our study did not evaluate therapeutic strategies directly, it provides a strong foundation for future research investigating the potential renoprotective effects of homocysteine-lowering interventions.

The implications of this study extend beyond diagnostics and therapeutics to broader research priorities. Longitudinal studies are crucial to establish the temporal relationship between hyperhomocysteinemia and DN progression. Additionally, randomized controlled trials assessing the impact of targeted homocysteine-lowering interventions on renal outcomes could provide definitive evidence for incorporating such strategies into clinical guidelines. Our findings also underscore the need for a multi-marker approach to DN diagnosis, integrating homocysteine with other established biomarkers to improve accuracy and predictive value.

Despite its strengths, including a robust sample size and advanced statistical modeling, this study has certain limitations. The cross-sectional design precludes the establishment of causality, and potential confounders such as dietary folate intake and genetic polymorphisms influencing homocysteine metabolism were not assessed.

Future multi-center, prospective studies should address these gaps to validate and generalize these findings.

In conclusion, this study highlights the pivotal role of serum homocysteine in DN, demonstrating its diagnostic and prognostic utility in T2DM patients. These findings add to the growing body of evidence supporting hyperhomocysteinemia as both a biomarker and a therapeutic target, emphasizing its potential to improve early detection and management of DN. Further research is warranted to translate these insights into actionable clinical interventions.

CONCLUSION

The findings of this study highlight the potential of serum homocysteine as a biomarker for DN in patients with T2DM. Elevated homocysteine levels were significantly associated with both the presence and severity of DN, suggesting that homocysteine may contribute to the pathogenesis of renal impairment through mechanisms involving oxidative stress, endothelial dysfunction, and inflammation. As a result, measuring homocysteine levels could provide valuable information for the early detection and management of DN. Given the limitations of current biomarkers, such as albuminuria and eGFR, homocysteine represents a promising alternative that could enable earlier intervention and potentially improve renal outcomes in diabetic patients. Future studies should focus on longitudinal designs to establish the temporal relationship between homocysteine levels and DN progression, as well as randomized controlled trials to evaluate the efficacy of homocysteine-lowering therapies in preventing or delaying the onset of DN. The integration of homocysteine measurement into clinical practice could enhance the risk stratification of T2DM patients and guide personalized treatment strategies aimed at reducing the burden of DN. As our understanding of the complex interplay between homocysteine and renal function continues to evolve, it is likely that homocysteine will emerge as a key player in the fight against diabetic kidney disease.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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