Analysing The Diagnostic Value Of Microalbuminuria As A Bio-Marker For Diabetic Nephropathy By Correlating With Fasting Blood Sugar, Serum Creatinine And Hba1c In Type 2 Diabetes Mellitus: A Case-Control Study

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Keywords
Type 2 Diabetes Mellitus, Diabetic Nephropathy, Microalbuminuria, HbA1c, Serum Creatinine, Glycemic Control.

Abstract
Objective: This study aimed to evaluate the diagnostic value of microalbuminuria as a biomarker for diabetic nephropathy in type 2 diabetes mellitus (T2DM), correlating it with fasting blood sugar (FBS), serum creatinine, and HbA1c levels. The study also examined the relationship between diabetes duration and kidney dysfunction to highlight the importance of early detection of microalbumin to reduce microvascular complications.
Material and Methods: A case-control study was conducted with 100 participants: 50 T2DM patients (cases) and 50 non-diabetic controls, aged 30-60 years. Fasting blood samples were collected to measure HbA1c, FBS, and serum creatinine levels, and spot urine samples were analyzed for microalbuminuria.
Results: A strong positive correlation was found between HbA1c and microalbuminuria (RS = 0.7608, p = 0.001), indicating that poor glycemic control is linked to kidney damage. Serum creatinine also showed a moderate correlation with microalbuminuria (RS = 0.5024, p = 0.001). A positive correlation was observed between diabetes duration and serum creatinine (RS = 0.5557, p = 0.001), emphasising the adverse effects of prolonged diabetes on kidney function. No significant correlation was found between FBS and microalbuminuria (RS = -0.271, p = 0.10). All biomarkers were significantly higher in the diabetic group ($p < 0.0001$).
Conclusion: Microalbuminuria is a valuable biomarker for early renal impairment in T2DM. Regular monitoring of HbA1c, serum creatinine, and microalbuminuria is essential for early detection and management of diabetic nephropathy, aiding in preventing kidney damage.

Introduction

Type 2 diabetes mellitus (T2DM) has emerged as a global health crisis, with its prevalence steadily increasing worldwide. Persistent hyperglycemia in T2DM patients can lead to severe complications, including cardiovascular disease, diabetic nephropathy, neuropathy, and retinopathy, contributing significantly to morbidity and mortality (Yu et al., 2024). Among these complications, diabetic nephropathy is particularly concerning, as it often progresses silently and can lead to end-stage renal disease (ESRD) if left untreated (Thipsawat et al., 2021). Effective management of blood glucose levels is crucial to prevent or delay the onset of these complications; therefore, early detection, monitoring, and intervention in individuals with T2DM are critical (Ullah et al., 2020).

Diabetic nephropathy is one of the most common microvascular complications of diabetes. Its early detection is critical for preventing irreversible kidney damage. Microalbuminuria, defined as a subclinical increase in urinary albumin excretion, is one of the earliest signs of kidney damage in diabetic patients (Ali & Al Lami, 2016). Patients are classified on the basis of their urinary Albumin to Creatinine Ratio (ACR) as Normal albuminuria (<30 mg/g), Microalbuminuria (30 – 299 mg/g) and Macroalbuminuria (>300 mg/g). Current guidelines of the American Diabetes Association recommend annual screening for microalbuminuria in diabetic patients to facilitate early intervention and delay the progression of diabetic kidney disease (DKD) (Sana et al., 2020). American Diabetes Association in 2010 also authenticates HbA_1C as diagnostic criteria for diabetes at a cutoff of ≥6.5%, pre-diabetes between 5.7% - 6.4% and normal <5.7%. Microvascular and macrovascular complications in T2DM are an active area of diabetic research. The findings of our study will be a valuable addition to this current body of evidence and will aid future research efforts exploring diabetes and nephropathy.

Materials and Methods

***Study Setting and Design*:** This case-control study was conducted in the Department of Biochemistry, Don Bosco Hospital, North Paravur, Kerala, over a one-year period from 2023 to 2024. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The ethical approval was obtained from the Srinivas University Ethics Committee (SUEC), and informed consent was collected from all participants (Protocol no: 25/AHS/2023).

***Study Population*:** The study included 100 participants, comprising 50 T2DM patients (cases) and 50 non-diabetic individuals (controls). Participants were aged between 30 and 60 years, with cases selected based on a confirmed diagnosis of T2DM and no other severe comorbidities. Controls were age- and sex-matched to the cases and had no significant renal or systemic complications. Samples from 25 male patients and 25 female patients were classified in both the groups of cases and controls to maintain gender homogeneity. Patients with type 1 diabetes, pregnancy, or major comorbidities were excluded from the study.

***Sample Collection and Processing*:** Fasting venous blood samples were collected from all participants; 2 ml of blood were placed in EDTA bottles, and the level of HbA1c was determined by using an Auto analyser - The Afinion™ 2, Abbott.
The other 2 mL was collected in fluoride oxalate bottles for the detection of fasting blood glucose estimation, which was performed on the same day by collecting fluoride plasma. Additionally, 5 mL of blood was collected into plain serum bottles, allowed to clot and retract, then centrifuged to separate the serum. The serum was then analysed immediately on the same day for the detection of serum creatinine, if any delay occurred, the samples were aliquoted, properly labelled and kept -20, within the analyte’s storage stability limits. Spot Urine sample was collected for microalbumin in a universal sterile urine bottle, The Erba EM-200 autoanalyzer were used to detect all biochemical parameters.

***Procedure*:** The tests were performed such as HbA1c, FBS, Serum Creatinine, and urine microalbumin. The HbA1c was analysed by using the colourimetric enzymatic method, fasting blood sugar (FBS) by using the glucose oxidase peroxidase (GOD-POD) method, serum creatine by using the Jaffe method, microalbuminuria measurement using immunoturbidimetric methods, all these tests were conducted in semi auto-analysers.
In summary, all blood samples were processed for HbA1c, fasting blood glucose, and serum creatinine levels. Spot urine samples were collected for microalbuminuria measurement. All biochemical analyses were performed using standardized methods and automated analyzers.

***Statistical Analysis*:** All the renal and glycaemic parameters between cases and controls were compared, and obtained the significance by calculating the P-value using one-way ANOVA. According to the results of the Shapiro-Wilk normality test, the data for both the control and diabetic groups did not follow a normal distribution (alpha < 0.05). Therefore, it is appropriate to use the Kruskal-Wallis one-way ANOVA, a non-parametric test, for comparing the medians between these two groups. This test is particularly suitable as it does not rely on the assumption of normality and provides a robust method to evaluate whether there are statistically significant differences in the studied parameters such as HbA1c, Serum Creatinine, and Microalbuminuria, between the control and diabetic populations.
Spearman's Rank Correlation was performed to assess the relationships between various parameters within the case group. To conduct the statistical analyses, the Spearman Correlation Calculator (provided by the Barcelona Field Studies Centre, Barcelona, Spain) and Jamovi software was employed for all statistical analyses.

Observation and Results

***Demographic Characteristics*:** The study included 100 participants, comprising 50 diabetic patients (T2DM cases) and 50 non-diabetic controls. The control group consisted of 50 samples, with 25 females and 25 males, having a median age of 47 years and an age range of 31 to 58 years. The T2DM group also included 50 samples, with 25 females and 25 males, a median age of 44 years, and an age range of 31 to 60 years, ensuring homogeneity in age and gender distribution between the groups. Regarding diabetes duration, the control group had no diabetes diagnosis, making this parameter inapplicable for them. In contrast, the T2DM group had a median diabetes duration of 3 years, with a range of 1 to 12 years, indicating significant variability in the time since diagnosis. This variability allowed for a detailed analysis of the significance and correlation of biological parameters, such as microalbuminuria, serum creatinine, and HbA1c, to understand the influence of diabetes duration on the development and progression of diabetic nephropathy (See Table-1).

Table 1: Demographic characteristics of the groups

Group characteristics	**Control N=50 (25 Female and 25 Males)**	**Diabetes Mellitus (DM) N=50(25 Female and 25 Males)**
Age in years (Median with range)	47 (31-58)	44 (31-60)
Duration of Diabetes Diagnosed in Years (Median with range)	0	3 (1-12)

Table 1: Represents the group characteristics of the Control and T2DM

The median FBS level in the control group was 90 mg/dL (range: 79–123 mg/dL), while in the diabetic group, it was significantly higher at 136 mg/dL (range: 88–227 mg/dL), with a p-value of <0.00001, indicating a statistically significant difference between the two groups. For HbA1c, the control group had a median of 5.1% (range: 4.6–5.4%), compared to 7.4% (range: 5.9–11.8%) in the diabetic group, with a p-value of <0.00001, reflecting a significant elevation in the diabetic population. Serum creatinine levels were also higher in the diabetic group, with a median of 1.24 mg/dL (range: 0.67–2.1 mg/dL), compared to 0.85 mg/dL (range: 0.6–1.02 mg/dL) in the control group, showing a statistically significant difference (p-value < 0.00001). Additionally, microalbuminuria levels were markedly increased in the diabetic group, with a median of 212.45 mg/L (range: 28–279.7 mg/L), compared to 31 mg/L (range: 12–89 mg/L) in the control group, demonstrating a significant difference with a p-value of <0.00001. These findings highlight the metabolic and renal changes associated with diabetes, with all parameters showing statistically significant differences between the diabetic and control populations (See Table 2).

Table 2: Comparison of all biomarkers analysed

STUDY PARAMETERS	**CONTROL** Median and Range	**CASES** Median and Range	**Kruskal Wallis P- value**
FBS (mg/dL)	90 (79 – 123)	136 (88-227)	< .00001
HbA1c (%)	5.1 (4.6 – 5.4)	7.4 (5.9 – 11.8)	< .00001
Serum Creatinine (mg/dL)	0.85 (0.6 – 1.02)	1.24 (0.67 – 2.1)	< .00001
Microalbuminurea (mg/L)	31 (12- 89)	212.45 (28- 279.7)	< .00001

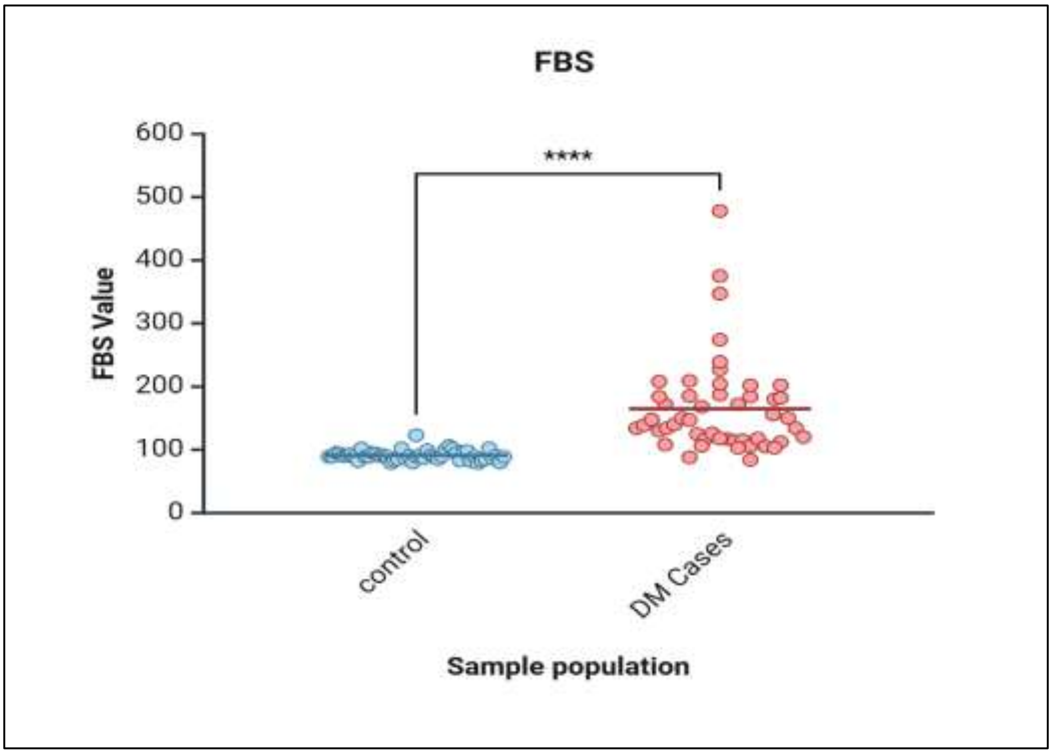

Chart 1: Distribution of FBS values of both cases and controls

According to the results of Kruskall-Wallis test between the values of control and diabetic population, there is a statistically significant difference between both the group. However, some FBS values in Diabetic patients comes within the normal level as because many of them are controlling their sugar level by medication.

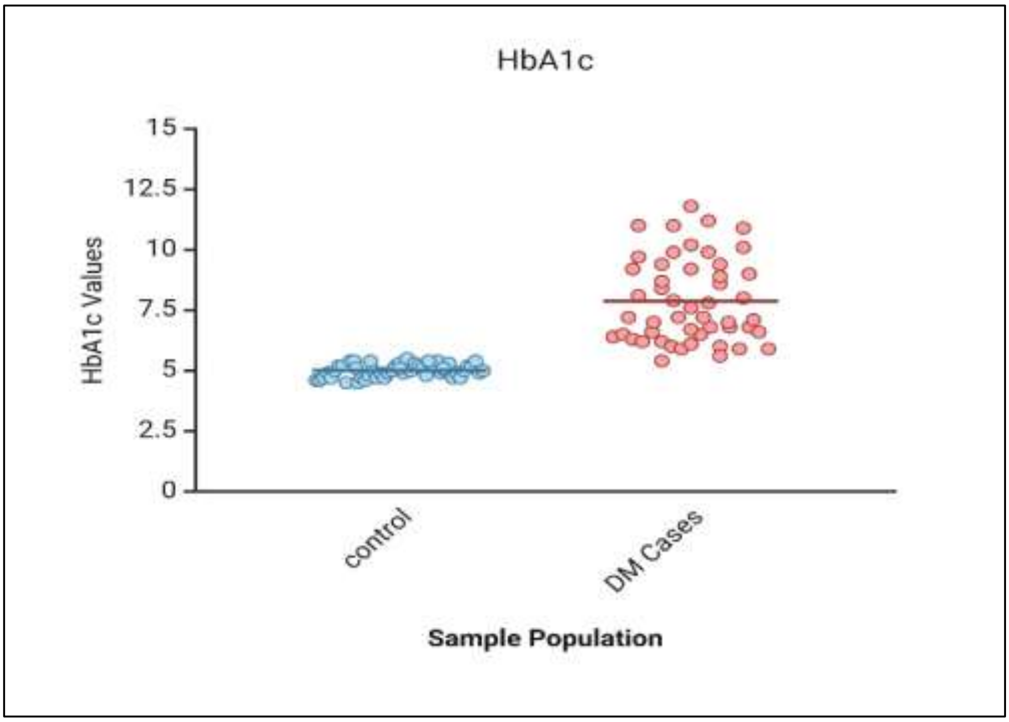

Chart 2: Distribution of HbA1c Values between Cases and Controls

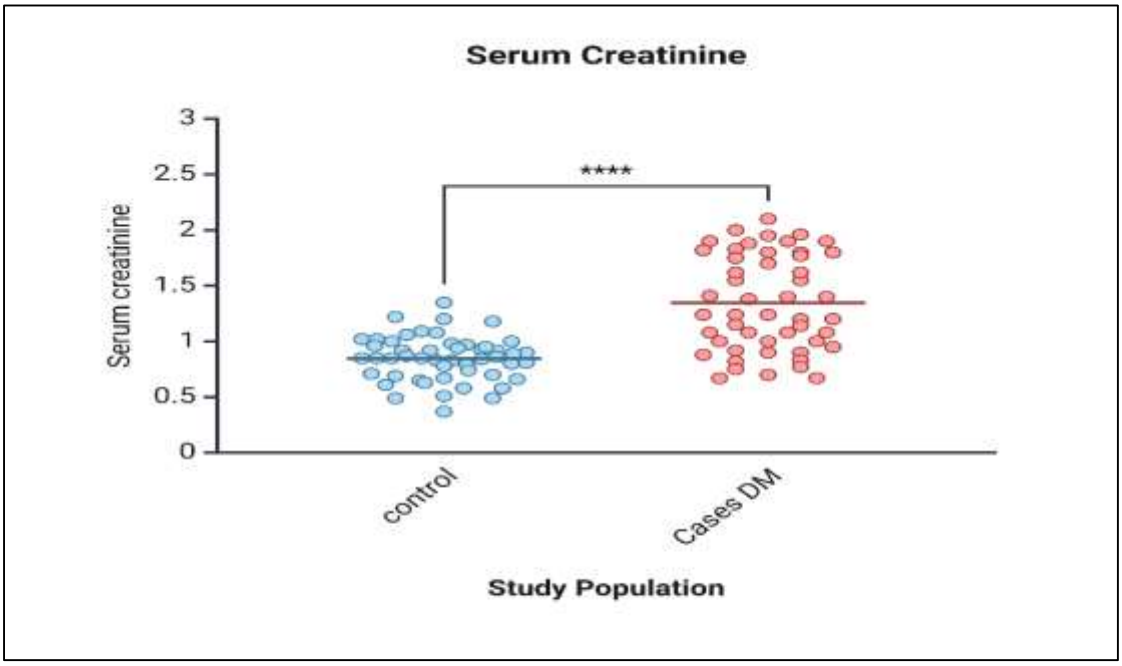

Chart 3: Distribution of Serum creatinine Values Between Both Cases and Control

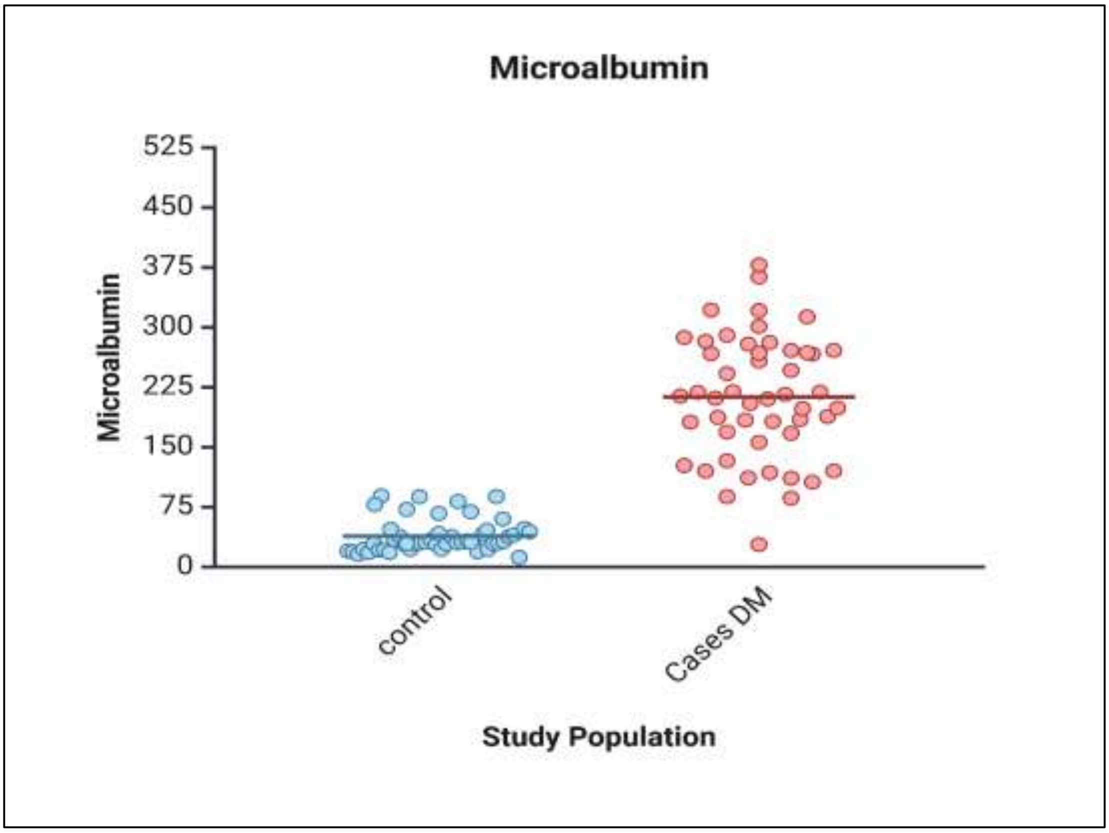

Chart 4: Distribution of Microalbumin values among case-control groups

According to the data represented in Chart 4, there is a strong significant difference in the values of microalbuminuria between the healthy controls and the diabetic population. The data shows a significant elevation in the majority of the microalbuminuria values among diabetic patients, indicating that microalbuminuria can be a reliable biomarker for early detection and monitoring of kidney damage in diabetic individuals. This highlights its potential as a crucial diagnostic and prognostic tool in managing diabetes-related complications.

Spearman's rank correlation Test

Table 3:Spearman's rank correlation Test

Parameters	**R_S**	**P Value**	**Result**
HbA1c and MA	0.7608	0.001	Significant correlation
FBS and microalbuminurea	-0.271	0.10	No significant Correlation
Serum Creatinine and MA	0.5024	0.001	A moderate correlation
Duration of diabetes and Microalbumin	0.5557	0.001	A moderate correlation

Among all tested markers, HbA1c, serum creatinine, and duration of diabetes showed a significant positive correlation with microalbuminuria. However, fasting blood sugar (FBS) did not show a correlation because some patients are managing their blood sugar levels effectively, resulting in FBS values that are not consistently elevated. In contrast, kidney markers such as serum creatinine and microalbuminuria tend to increase with rising HbA1c levels and show a moderate correlation with the duration of diabetes. This suggests that the duration of diabetes increases, the risk of kidney damage also rises. Microalbuminuria serves as an effective early detection marker for kidney damage as per the statistical test.

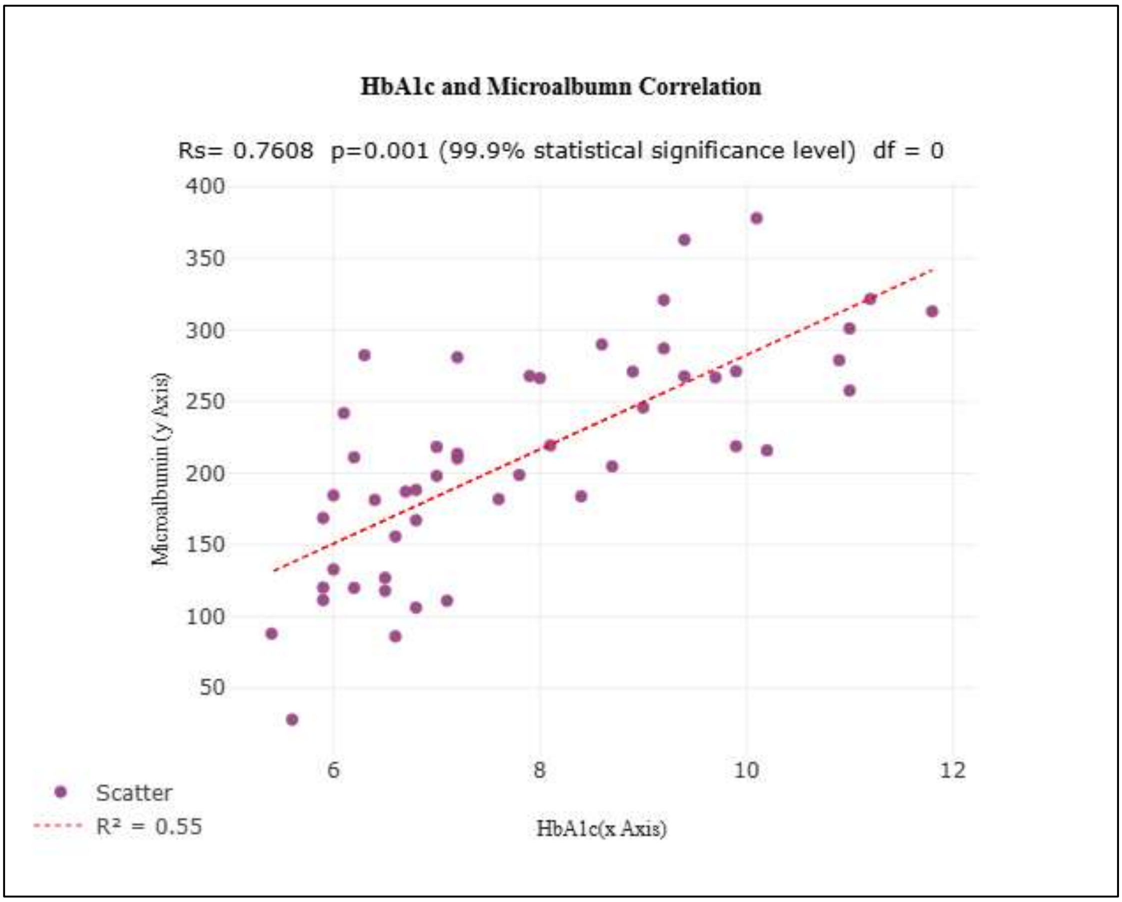

Chart 5: The correlation between Microalbuminuria and HbA1c

The correlation coefficient of Microalbuminuria and HbA1c detected as (Rs value +0.7608) and this appears to be a strong positive correlation between these values, the trendline shows that the HbA1c levels increases, microalbuminuria levels also tend to increase significantly. indicating that the poor glycaemic controls associated with higher levels of microalbuminuria.

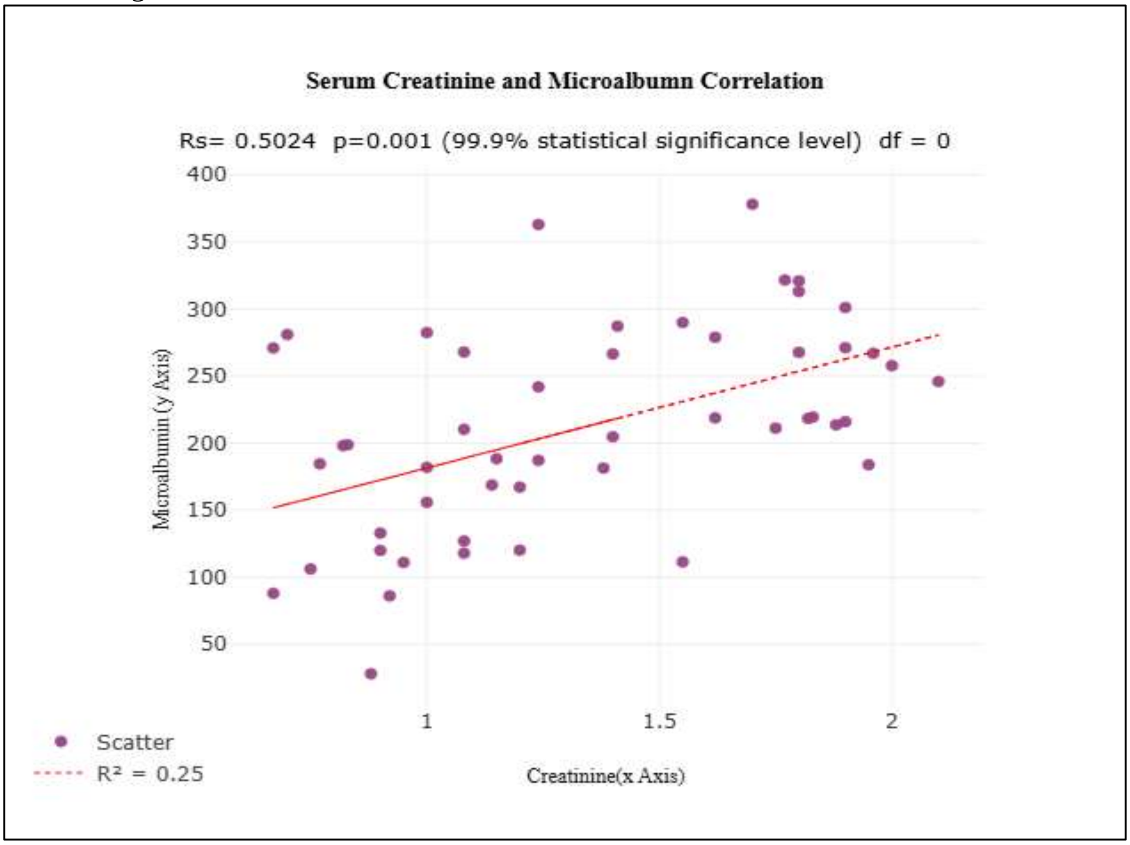

Chart 6: The correlation between Serum Creatinine and Microalbuminuria

As per the chart 6, There appears to be a moderate positive correlation Rs value (+0.5557). so that this rejects the null hypothesis that there is no correlation.It indicates a slight but significant increase in creatinine and microalbuminuria levels in diabetic patients.

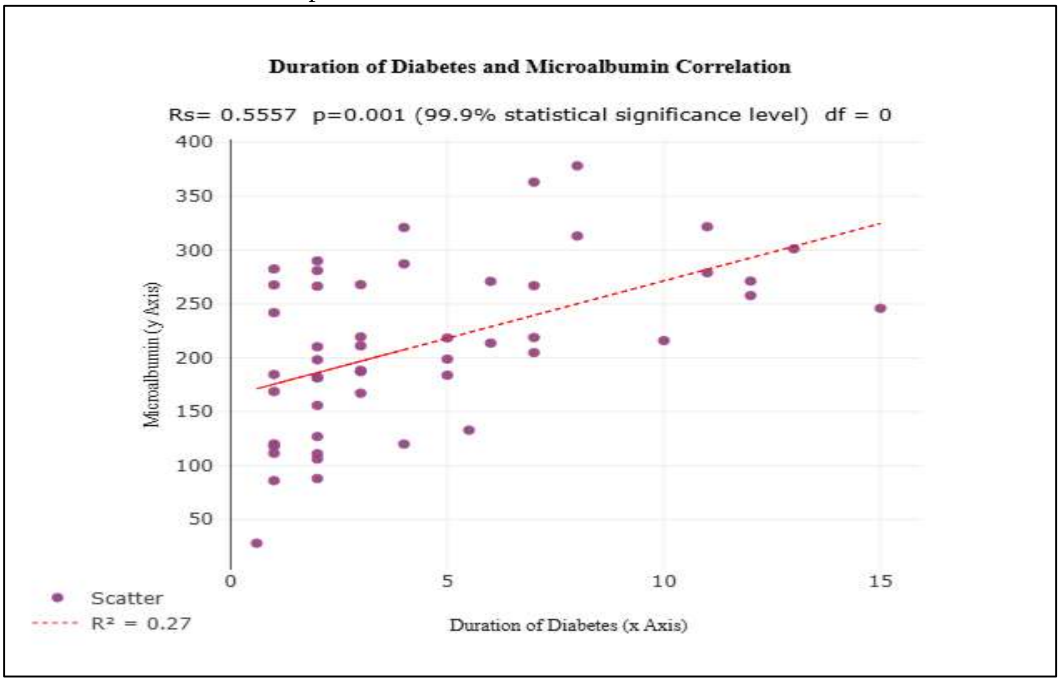

Chart 7: Correlation of Diabetic Duration and Microalbuminuria

Chart 7 indicates an increase in the values of microalbumin with the duration of diabetes.

Discussion

In developing countries like India, diabetes mellitus often leads to both microvascular and macrovascular complications, including diabetic neuropathy and nephropathy. Microalbuminuria plays a crucial role as a biochemical marker in assessing the risk of microvascular complications in individuals with T2DM. This is a trending area of research where, every day, studies are recognizing new markers that are useful in diabetes and its management (S et al., 2024).

This study aimed to examine the association between clinical parameters and kidney function indicators in diabetic patients compared to a control group. Notably, diabetic patients exhibited significantly higher mean levels of fasting blood sugar (FBS), HbA1c, serum creatinine, and microalbuminuria than the control group, with all differences being statistically significant ($p < 0.00001$ for all parameters).

Similarly, the study by Kare et al. (2021) emphasizes the significant role of diabetes duration and glycemic control in the development of microalbuminuria. Their findings showed a linear correlation between microalbuminuria, glycemic control, and diabetes duration, with most patients having an average diabetes duration of 8 years.

The study by Kiconco et al. (2019) reported a high prevalence of microalbuminuria among diabetic patients, with serum creatinine and glucose levels significantly associated with microalbuminuria. Similarly, our study demonstrated a strong positive correlation between HbA1c and microalbuminuria (Rs = 0.7608, p = 0.001), emphasizing the link between poor glycemic control and kidney damage. Additionally, a moderate positive correlation was observed between serum creatinine and microalbuminuria levels (Rs = 0.5024, p = 0.001), supporting the role of creatinine as a reliable marker of kidney dysfunction in diabetes. Furthermore, the positive correlation between diabetes duration and serum creatinine levels (Rs = 0.5557, p = 0.001) aligns with Kiconco et al.'s findings that extended diabetes duration may exacerbate kidney damage. However, unlike Kiconco et al.., our study did not find a significant correlation between FBS and microalbuminuria (Rs = -0.271; p = 0.10), potentially due to variations in glucose regulation among participants. These results underscore the importance of early detection of microalbuminuria, alongside monitoring serum creatinine and HbA1c levels, to assess and manage the risk of diabetic nephropathy.

As highlighted in the study by Ghanta & Belli (2020), the incidence of microalbuminuria increases with the duration of diabetes mellitus and higher HbA1c levels. Microalbuminuria was also significantly associated with elevated serum cholesterol levels, while a mild negative correlation was observed between triglycerides, very low-density lipoprotein cholesterol (VLDL), and microalbuminuria. These findings align with and support the present study while also emphasizing the potential value of including additional biomarkers to provide a more comprehensive understanding of diabetic nephropathy.

The findings in this study will be a valuable addition to the ongoing research efforts in diabetes and its related complications. Future longitudinal studies are needed to further explore the progression of microalbuminuria and its impact on kidney function in diabetic patients. Information in this study will help future studies to derive the regional incidence and prevalence of T2DM and its complications.

Conclusion

There is a strong relationship between poor glycemic control and the prevalence of microalbuminuria in diabetic patients. Microalbuminuria was more prevalent in patients with uncontrolled diabetes (HbA1c ≥ 7%) and increased with the duration of diabetes. Regular monitoring of HbA1c and microalbuminuria is essential for preventing late complications, including end-stage renal disease

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Conflict of interest

Nil

Reference

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Abbreviations:

1. **T2DM -** Type 2 Diabetes Mellitus
2. **ESRD -** End-Stage Renal Disease
3. **HbA1c -** Glycated Haemoglobin
4. **ACR -** Albumin to Creatinine Ratio
5. **DKD -** Diabetic Kidney Disease
6. **FBS -** Fasting Blood Sugar
7. **DM -** Diabetes Mellitus
8. **ANOVA -** Analysis of Variance
9. **EDTA -** Ethylenediaminetetraacetic Acid
10. **R_S -** Spearman's Rank Correlation Coefficient
11. **BioRender -** A software tool for creating scientific diagrams
12. **ACR -** Albumin-Creatinine Ratio