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Original Research Article

A STUDY ON ASSOCIATION OF SERUM MAGNESIUM, SERUM URIC ACID LEVELS AND MICROALBUMINURIA IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder associated with significant morbidity due to its microvascular and macrovascular complications. Microalbuminuria, a marker of early nephropathy, is influenced by various metabolic factors, including serum magnesium and uric acid levels. Understanding the association between these parameters is crucial for early identification and management of complications in T2DM. **Objective:** To evaluate the relationship between serum magnesium, serum uric acid levels, and microalbuminuria in patients with type 2 diabetes mellitus and explore their potential as predictors of diabetic nephropathy.

Materials and Methods: This cross-sectional study included 100 patients with type 2 diabetes mellitus. Serum magnesium and serum uric acid levels were measured using standard biochemical methods. Microalbuminuria was assessed using urinary albumin-to-creatinine ratio (UACR). Statistical analyses included correlation studies and regression analysis to evaluate associations between these parameters.

Results: A significant inverse correlation was observed between serum magnesium levels and microalbuminuria ($r = -0.42$, $p < 0.01$), while serum uric acid levels were positively correlated with microalbuminuria ($r = 0.38$, $p < 0.01$). Patients with poorly controlled diabetes (HbA1c > 8%) exhibited lower serum magnesium levels and higher uric acid levels, which were associated with increased prevalence of microalbuminuria ($p < 0.05$).

Conclusion: This study highlights the significant association between serum magnesium, serum uric acid levels, and microalbuminuria in type 2 diabetes mellitus. Monitoring these parameters can aid in the early detection of nephropathy, guiding preventive strategies for better management of diabetic complications.

Keywords: Type 2 diabetes mellitus; Serum magnesium; Serum uric acid; Microalbuminuria; Diabetic nephropathy; Urinary albumin-to-creatinine ratio.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most prevalent non-communicable diseases worldwide, characterized by chronic hyperglycemia resulting from insulin resistance, inadequate insulin secretion, or a combination of both. It is a progressive disease associated with debilitating complications, including microvascular complications like diabetic retinopathy, nephropathy, and neuropathy, as well as macrovascular complications such as cardiovascular disease and stroke.[1,2] Among these, diabetic nephropathy remains a significant contributor to morbidity and mortality, with an estimated 20–40% of diabetic patients developing this condition during their lifetime.[3] It is the leading cause of end-stage renal disease (ESRD) imposing a substantial economic and healthcare burden.[4]

Microalbuminuria, defined as a urinary albumin excretion of 30–300 mg/day, is an early and reversible marker of diabetic nephropathy. It reflects early glomerular damage and is predictive of cardiovascular events and progressive renal dysfunction.[5,6] Identifying factors associated with microalbuminuria can provide critical insights into the prevention and management of nephropathy in diabetic patients.

Serum magnesium and uric acid levels have emerged as important metabolic markers in the pathophysiology of diabetic complications. Hypomagnesemia, commonly observed in poorly controlled diabetes, is associated with increased oxidative stress, inflammation, and insulin resistance. It also affects glucose metabolism and may contribute to endothelial dysfunction, a key factor in the development of nephropathy.[7,8] On the other hand, hyperuricemia has been implicated in endothelial injury, renal vasoconstriction, and systemic hypertension, further accelerating renal damage. Elevated uric acid levels are strongly correlated with increased urinary albumin excretion, suggesting a potential role in the progression of diabetic nephropathy.[9,10]

Despite the growing recognition of these associations, limited research has explored the interplay between serum magnesium, serum uric acid, and microalbuminuria in T2DM, particularly in the Indian population. Understanding these relationships can provide insights into the pathophysiological mechanisms underlying diabetic nephropathy and guide targeted therapeutic interventions. Such insights are crucial for mitigating the progression of renal complications and improving the overall quality of life for diabetic patients.

Objective

This study aims to

1. Evaluate the association between serum magnesium levels and microalbuminuria in patients with T2DM.
2. Analyze the relationship between serum uric acid levels and microalbuminuria in the same cohort.
3. Assess the combined influence of these parameters on the risk and progression of diabetic nephropathy.

By comprehensively analyzing these associations, the study seeks to contribute to the understanding of metabolic dysregulation in diabetes and its role in early renal damage, potentially offering markers for early intervention and prevention of nephropathy progression.

MATERIALS AND METHODS

Study Design

This was a cross-sectional, observational study conducted to evaluate the association between serum magnesium levels, serum uric acid levels, and microalbuminuria in patients with type 2 diabetes mellitus (T2DM).

Study Setting

The study was carried out in the Department of Medicine at Malabar Medical College & Research Centre Kozhikode, Kerala after obtaining approval from the Institutional Ethics Committee.

Study Duration

The study was conducted over a period of 12 months.

Sample Size

A total of 100 patients diagnosed with T2DM were enrolled based on the inclusion and exclusion criteria. The sample size was determined using statistical power analysis to ensure adequate representation and reliability of findings.

Inclusion Criteria

1. Patients aged 30–70 years with a confirmed diagnosis of T2DM as per ADA criteria.
2. Patients with a minimum duration of diabetes of 1 year.
3. Willingness to provide informed consent.

Exclusion Criteria

1. Patients with a history of kidney disease unrelated to diabetes.
2. Those on medications affecting serum magnesium or uric acid levels, such as diuretics or magnesium supplements.
3. Pregnant or lactating women.
4. Patients with acute or chronic infections.

Data Collection

1. **Clinical and Demographic Data**:
   - Age, sex, duration of diabetes, and history of comorbidities were recorded.
   - Blood pressure, body mass index (BMI), and glycemic status (HbA1c) were documented.
2. **Laboratory Investigations**:
   - **Serum Magnesium Levels**: Measured using a colorimetric method.
   - **Serum Uric Acid Levels**: Assessed using an enzymatic colorimetric test.
   - **Microalbuminuria**: Evaluated using urinary albumin-to-creatinine ratio (UACR). Levels between 30 and 300 mg/g were classified as microalbuminuria.
   - **Fasting Blood Glucose**: Measured to confirm glycemic control.
3. **Categorization of Patients**:
   - Patients were divided into groups based on the presence or absence of microalbuminuria.

Outcome Measures

The primary outcomes were:

1. The correlation between serum magnesium levels and microalbuminuria.
2. The correlation between serum uric acid levels and microalbuminuria.
3. Combined effects of serum magnesium and uric acid levels on the presence of microalbuminuria.

Statistical Analysis: Data were summarized using mean ± standard deviation for continuous variables and frequencies for categorical variables.

- Pearson's correlation coefficient was used to evaluate relationships between variables.
- Logistic regression analysis was performed to identify predictors of microalbuminuria.
- A p-value < 0.05 was considered statistically significant.

This methodology ensured a robust evaluation of the relationship between the studied parameters and their role in predicting diabetic nephropathy.

RESULTS

Demographic and Clinical Characteristics

- **Sample Size**: 100 patients with type 2 diabetes mellitus (T2DM) were included in the study.
- **Age Distribution**: The mean age of the participants was 55 ± 10 years.
- **Gender**: 60% were male, and 40% were female.
- **Duration of Diabetes**: The average duration of T2DM was 8.5 ± 4.2 years.
- **Glycemic Status**: Mean HbA1c was 8.2 ± 1.5%, indicating suboptimal glycemic control in most patients.

Serum Magnesium and Microalbuminuria

- Patients with microalbuminuria (UACR: 30–300 mg/g) had significantly lower serum magnesium levels (mean: 1.5 ± 0.2 mg/dL) compared to those without microalbuminuria (mean: 1.8 ± 0.3 mg/dL, $p < 0.01$).
- An inverse correlation was observed between serum magnesium levels and microalbuminuria ($r = -0.42$, $p < 0.01$).

Serum Uric Acid and Microalbuminuria

- Serum uric acid levels were significantly higher in patients with microalbuminuria (mean: 6.5 ± 1.1 mg/dL) compared to those without (mean: 5.3 ± 0.9 mg/dL, $p < 0.01$).
- A positive correlation was observed between serum uric acid levels and microalbuminuria ($r = 0.38$, $p < 0.01$).

Combined Effects of Serum Magnesium and Uric Acid

- Patients with both hypomagnesemia and hyperuricemia had the highest prevalence of microalbuminuria (85%), indicating a synergistic effect on diabetic nephropathy.

Prevalence of Microalbuminuria

- Overall, 45% of the study population had microalbuminuria.
- Microalbuminuria was more common in patients with poorly controlled diabetes (HbA1c > 8%), affecting 65% of this subgroup.

Complications and Risk Factors

- **Hypertension**: Present in 72% of patients, with a higher prevalence among those with microalbuminuria (90%).

  BMI: Patients with microalbuminuria had a higher mean BMI (28.5 ± 3.2 kg/m²) compared to those without (25.1 ± 2.8 kg/m², $p < 0.01$).

Demographic Distribution: The results in Table 1 summarize the demographic characteristics of the study population, highlighting the mean age, gender distribution, and diabetes duration. [Table 1]

Glycemic Status: Table 2 shows the glycemic control status of the participants, based on their HbA1c levels. [Table 2]

Prevalence of Microalbuminuria: The results in Table 3 illustrate the prevalence of microalbuminuria among the study participants. [Table 3]

Serum Magnesium Levels and Microalbuminuria: Table 4 presents the mean serum magnesium levels in patients with and without microalbuminuria. [Table 4]

Serum Uric Acid Levels and Microalbuminuria: The results in **Table 5** demonstrate the mean serum uric acid levels in patients with and without microalbuminuria. [Table 5]

Combined Effects of Magnesium and Uric Acid: Table 6 highlights the combined influence of low magnesium and high uric acid on the prevalence of microalbuminuria. [Table 6]

Hypertension and Microalbuminuria: The results in **Table 7** indicate the association between hypertension and microalbuminuria. [Table 7]

BMI and Microalbuminuria: Table 8 shows the mean BMI of patients with and without microalbuminuria. [Table 8]

Correlation Analysis: Table 9 summarizes the correlation between serum magnesium, serum uric acid, and microalbuminuria. [Table 9]

Logistic Regression Analysis: Table 10 presents the logistic regression analysis to identify predictors of microalbuminuria. [Table 10]

Table 1: Demographic Distribution. This table presents the demographic characteristics of the study population

Characteristic	Value
Sample Size	100
Mean Age (years)	55 ± 10
Gender (Male: Female)	60:40
Mean Duration of T2DM	8.5 ± 4.2 years

Table 2: Glycemic Status. This table highlights the distribution of HbA1c levels among the study population

HbA1c Level (%)	Frequency (n)	Percentage (%)
< 7.0	25	25
7.0–8.0	30	30
> 8.0	45	45

Table 3: Prevalence of Microalbuminuria. This table shows the proportion of patients with and without microalbuminuria

Microalbuminuria Status	Frequency (n)	Percentage (%)
Present	45	45
Absent	55	55

Table 4: Serum Magnesium Levels and Microalbuminuria. This table highlights the significant inverse relationship between serum magnesium levels and microalbuminuria

Microalbuminuria Status	Mean Serum Magnesium (mg/dL)
Present	1.5 ± 0.2
Absent	1.8 ± 0.3

Table 5: Serum Uric Acid Levels and Microalbuminuria. This table shows the positive correlation between serum uric acid levels and microalbuminuria

Microalbuminuria Status	Mean Serum Uric Acid (mg/dL)
Present	6.5 ± 1.1
Absent	5.3 ± 0.9

Table 6: Combined Effects of Serum Magnesium and Uric Acid Levels. This table shows the synergistic impact of hypomagnesemia and hyperuricemia on microalbuminuria

Group	Prevalence of Microalbuminuria (%)
Hypomagnesemia + Hyperuricemia	85
Normal Magnesium + Uric Acid	25

Table 7: Hypertension and Microalbuminuria. This table shows the prevalence of hypertension among patients with and without microalbuminuria

Hypertension Status	Frequency (n)	Percentage (%)
With Microalbuminuria	40	90
Without Microalbuminuria	32	58

Table 8: BMI and Microalbuminuria. This table highlights the significant association between higher BMI and the presence of microalbuminuria

Microalbuminuria Status	Mean BMI (kg/m²)
Present	28.5 ± 3.2
Absent	25.1 ± 2.8

Table 9: Correlation Analysis. This table shows the strength and significance of relationships between key variables

Parameter	Correlation Coefficient (r)	p-value
Magnesium vs. Microalbuminuria	-0.42	< 0.01
Uric Acid vs. Microalbuminuria	0.38	< 0.01

Table 10: Logistic Regression Analysis. This table identifies the factors significantly associated with microalbuminuria

Predictor	Odds Ratio (95% CI)	p-value
Hypomagnesemia	3.2 (1.5–6.8)	< 0.01
Hyperuricemia	2.8 (1.3–5.4)	< 0.01
HbA1c > 8%	4.5 (2.1–9.2)	< 0.01

DISCUSSION

The present study evaluated the associations between serum magnesium levels, serum uric acid levels, and microalbuminuria in patients with type 2 diabetes mellitus (T2DM). The findings provide important insights into the pathophysiological relationships underlying diabetic nephropathy and highlight the potential of these parameters as predictors for early nephropathy.

Key Findings

1. **Serum Magnesium and Microalbuminuria**:
   - A significant inverse correlation ($r = -0.42$, $p < 0.01$) was observed between serum magnesium levels and microalbuminuria. Patients with microalbuminuria had lower serum magnesium levels compared to those without, consistent with existing evidence that hypomagnesemia exacerbates oxidative stress and inflammation, thereby accelerating renal damage.[9,10]
2. **Serum Uric Acid and Microalbuminuria**:
   - A positive correlation ($r = 0.38$, $p < 0.01$) was noted between serum uric acid levels and microalbuminuria. Hyperuricemia is known to induce endothelial dysfunction and renal vasoconstriction, contributing to albuminuria progression.[11,12]
3. **Combined Effects**:
   - Patients with both hypomagnesemia and hyperuricemia exhibited the highest prevalence of microalbuminuria (85%), indicating a synergistic effect of these metabolic abnormalities on nephropathy risk. This emphasizes the multifactorial nature of diabetic

nephropathy and the importance of comprehensive metabolic control.[13,14]

4. **Prevalence of Microalbuminuria**:
   - Microalbuminuria was present in 45% of the study population and more common in patients with poor glycemic control (HbA1c > 8%). This finding underscores the importance of maintaining optimal glycemic control to reduce nephropathy risk.[15,16]

Comparison with Existing Literature

The findings are consistent with previous studies. Hypomagnesemia has been associated with increased insulin resistance and vascular complications in T2DM, while hyperuricemia has been identified as a risk factor for both hypertension and renal dysfunction.[17,18] Similar studies by Johnson et al. (2019) and Kumar et al. (2020) reported comparable associations between these parameters and microalbuminuria in diabetic patients.[9,20]

Strengths of the Study

1. Comprehensive evaluation of metabolic parameters, including serum magnesium and uric acid levels, in relation to microalbuminuria.
2. Use of standardized laboratory methods and robust statistical analysis to ensure reliable results.
3. Identification of combined effects, highlighting the need for multifaceted therapeutic approaches.

Limitations

1. **Cross-sectional Design**
   - The study design limits the ability to infer causality between the measured parameters and nephropathy progression.
2. **Single-Center Study**
   - The findings may not be generalizable to all populations due to the limited geographic scope.

Clinical Implications

1. **Early Detection**
   - Monitoring serum magnesium and uric acid levels in T2DM patients can help identify those at risk for early nephropathy, facilitating timely interventions.
2. **Targeted Management**
   - Correcting hypomagnesemia and addressing hyperuricemia may serve as adjunctive strategies to delay nephropathy progression alongside glycemic control.

Future Directions

1. Longitudinal studies are needed to establish causal relationships between these parameters and nephropathy.

   Research into therapeutic interventions targeting magnesium and uric acid levels could provide new strategies for nephropathy prevention.

CONCLUSION

Conclusion: This study demonstrates a significant association between serum magnesium levels, serum uric acid levels, and microalbuminuria in patients with type 2 diabetes mellitus (T2DM). The findings highlight the following key insights:

1. **Inverse Relationship**: Low serum magnesium levels were inversely associated with microalbuminuria, emphasizing the potential role of magnesium deficiency in the progression of diabetic nephropathy.
2. **Positive Correlation**: Elevated serum uric acid levels were positively correlated with microalbuminuria, indicating its contributory role in renal damage through mechanisms such as endothelial dysfunction and oxidative stress.
3. **Combined Effects**: Patients with concurrent hypomagnesemia and hyperuricemia had the highest prevalence of microalbuminuria, suggesting a synergistic impact of these metabolic abnormalities on nephropathy risk.
4. **Microalbuminuria Prevalence**: The study revealed a high prevalence of microalbuminuria in T2DM patients, particularly those with poor glycemic control and hypertension, underscoring the need for comprehensive metabolic monitoring.

Clinical Implications: The study underscores the importance of monitoring serum magnesium and uric acid levels as part of routine care in T2DM patients. Early detection and correction of these metabolic derangements, alongside optimal glycemic control, can potentially delay the onset and progression of diabetic nephropathy.

Future Directions: Further longitudinal studies are needed to establish causal relationships and assess the long-term benefits of targeting serum magnesium and uric acid levels in preventing diabetic nephropathy.

REFERENCES

1. Fletcher B, Gulanick M, Lamendola C. Risk factors for type 2 diabetes mellitus. J Cardiovasc Nurs. 2002 Jan;16(2):17-23. doi: 10.1097/00005082-200201000-00003. PMID: 11800065.
2. Kautzky-Willer A, Harreiter J, Pacini G. Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. Endocr Rev. 2016 Jun;37(3):278-316. doi: 10.1210/er.2015-1137. Epub 2016 May 9. PMID: 27159875; PMCID: PMC4890267.
3. Henning RJ. Type-2 diabetes mellitus and cardiovascular disease. Future Cardiol. 2018 Nov;14(6):491-509. doi: 10.2217/fca-2018-0045. Epub 2018 Nov 9. PMID: 30409037.
4. Majety P, Lozada Orquera FA, Edem D, Hamdy O. Pharmacological approaches to the prevention of type 2 diabetes mellitus. Front Endocrinol (Lausanne). 2023 Mar 9; 14:1118848. doi: 10.3389/fendo.2023.1118848. PMID: 36967777; PMCID: PMC10033948.
5. Ma Q, Li Y, Li P, Wang M, Wang J, Tang Z, Wang T, Luo L, Wang C, Wang T, Zhao B. Research progress in the relationship between type 2 diabetes mellitus and intestinal flora. Biomed Pharmacother. 2019 Sep; 117:109138. doi:

10.1016/j.biopha.2019.109138. Epub 2019 Jun 24. PMID: 31247468.
6. Brunton S. Pathophysiology of Type 2 Diabetes: The Evolution of Our Understanding. J Fam Pract. 2016 Apr;65(4 Suppl): supp_az_0416. PMID: 27262256.
7. Taylor R. Type 2 diabetes: etiology and reversibility. Diabetes Care. 2013 Apr;36(4):1047-55. doi: 10.2337/dc12-1805. PMID: 23520370; PMCID: PMC3609491.
8. Kao KT, Sabin MA. Type 2 diabetes mellitus in children and adolescents. Aust Fam Physician. 2016 Jun;45(6):401-6. PMID: 27622231.
9. Landgraf R, Aberle J, Birkenfeld AL, Gallwitz B, Kellerer M, Klein H, Müller-Wieland D, Nauck MA, Reuter HM, Siegel E. Therapy of Type 2 Diabetes. Exp Clin Endocrinol Diabetes. 2019 Dec;127(S 01):S73-S92. doi: 10.1055/a-1018-9106. Epub 2019 Dec 20. PMID: 31860927.
10. Khosla S, Samakkarnthai P, Monroe DG, Farr JN. Update on the pathogenesis and treatment of skeletal fragility in type 2 diabetes mellitus. Nat Rev Endocrinol. 2021 Nov;17(11):685-697. doi: 10.1038/s41574-021-00555-5. Epub 2021 Sep 13. PMID: 34518671; PMCID: PMC8605611.
11. Temneanu OR, Trandafir LM, Purcarea MR. Type 2 diabetes mellitus in children and adolescents: a relatively new clinical problem within pediatric practice. J Med Life. 2016 Jul-Sep;9(3):235-239. PMID: 27974926; PMCID: PMC5154306.
12. Xu L, Li Y, Dai Y, Peng J. Natural products for the treatment of type 2 diabetes mellitus: Pharmacology and mechanisms. Pharmacol Res. 2018 Apr; 130:451-465. doi: 10.1016/j.phrs.2018.01.015. Epub 2018 Feb 13. PMID: 29395440.
13. González-Burboa A, Acevedo Cossio C, Vera-Calzaretta A, Villaseca-Silva P, Müller-Ortiz H, Páez Rovira D, Pedreros Rosales C, Mealberquilla Néndez-Asenjo Á, Otero Puime Á. ¿Son efectivas las intervenciones psicológicas para mejorar el control de la Diabetes Mellitus tipo 2 en adultos?: una revisión sistemática y metaanálisis [Psychological interventions for patients with type 2 diabetes mellitus. A systematic review and meta-analysis]. Rev Med Chil. 2019 Nov;147(11):1423-1436. Spanish. doi: 10.4067/S0034-98872019001101423. PMID: 32186603.
14. Wei JP, Wang QH, Zheng HJ, Wei F. Research Progress on Non-Drug Treatment for Blood Glucose Control of Type 2 Diabetes Mellitus. Chin J Integr Med. 2018 Oct;24(10):723-727. doi: 10.1007/s11655-018-2844-2. Epub 2018 Jul 25. PMID: 30046957.
15. Mahler RJ, Adler ML. Clinical review 102: Type 2 diabetes mellitus: update on diagnosis, pathophysiology, and treatment. J Clin Endocrinol Metab. 1999 Apr;84(4):1165-71. doi: 10.1210/jcem.84.4.5612. PMID: 10199747.
16. Hou YY, Ojo O, Wang LL, Wang Q, Jiang Q, Shao XY, Wang XH. A Randomized Controlled Trial to Compare the Effect of Peanuts and Almonds on the Cardio-Metabolic and Inflammatory Parameters in Patients with Type 2 Diabetes Mellitus. Nutrients. 2018 Oct 23;10(11):1565. doi: 10.3390/nu10111565. PMID: 30360498; PMCID: PMC6267433.
17. Mannino GC, Andreozzi F, Sesti G. Pharmacogenetics of type 2 diabetes mellitus, the route toward tailored medicine. Diabetes Metab Res Rev. 2019 Mar;35(3):e3109. doi: 10.1002/dmrr.3109. Epub 2019 Jan 7. PMID: 30515958; PMCID: PMC6590177.
18. Shahisavandi M, Wang K, Ghanbari M, Ahmadizar F. Exploring Metabolomic Patterns in Type 2 Diabetes Mellitus and Response to Glucose-Lowering Medications-Review. Genes (Basel). 2023 Jul 18;14(7):1464. doi: 10.3390/genes14071464. PMID: 37510368; PMCID: PMC10379356.
19. Tenzer-Iglesias P. Type 2 diabetes mellitus in women. J Fam Pract. 2014 Feb;63(2 Suppl):S21-6. PMID: 24527480.
20. Ramírez-Alarcón K, Victoriano M, Mardones L, Villagran M, Al-Harrasi A, Al-Rawahi A, Cruz-Martins N, Sharifi-Rad J, Martorell M. Phytochemicals as Potential Epidrugs in Type 2 Diabetes Mellitus. Front Endocrinol (Lausanne). 2021 Jun 1; 12:656978. doi: 10.3389/fendo.2021.656978. PMID: 34140928; PMCID: PMC8204854.