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THE ROLE OF MESENCEPHALIC ASTROCYTE-DERIVED NEUROTROPHIC FACTOR (MANF) IN BIOMARKERS OF DIABETIC NEPHROPATHY

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Abstract

Objective: This study investigates the potential role of Mesencephalic Astrocyte-Derived Neurotrophic Factor (MANF) as a novel biomarker, evaluating its associations with established urinary biomarkers and glycemic control indicators in diabetes mellitus and diabetic nephropathy (DN).

Methods: A cross-sectional study was conducted involving 84 participants stratified by albuminuria levels into normoalbuminuria, microalbuminuria, and macroalbuminuria groups. MANF levels were quantified using enzyme-linked immunosorbent assays (ELISA), while urinary biomarkers and HbA1C were measured using standard clinical methods. Correlation analyses and multivariate regression models were employed to assess relationships between MANF and biomarkers.

Results: MANF levels demonstrated a significant upward trend across albuminuria categories, with the highest levels observed in the macroalbuminuria group (514.5±112.3 pg/mL, $p<0.01$). MANF positively correlated with HbA1C (R=0.78, $p<0.001$) and negatively with estimated glomerular filtration rate (eGFR) (R=0.72, $p<0.001$). These associations remained significant after adjusting for confounders such as age, sex, and body mass index (BMI).

Conclusion: MANF is a promising biomarker that reflects glycemic dysregulation and renal dysfunction, offering potential advantages over traditional markers. Its integration into clinical practice could enhance the early detection and personalized management of chronic kidney disease (CKD) and diabetes. Future longitudinal studies are warranted to validate its predictive value.

Keywords*: Kidney diseases, chronic, diabetic nephropathies, biological markers, blood glucose, neurotrophic factors.*

Introduction

Diabetic nephropathy (DN) is a leading cause of chronic kidney disease (CKD), often progressing silently until substantial renal damage occurs. Conventional biomarkers such as serum creatinine and albuminuria, though widely used, often lack the sensitivity and specificity required for early-stage detection, leading to diagnostic delays.[1]

Advancements in metabolomics and molecular diagnostics have enabled the identification of novel biomarkers, enhancing diagnostic precision and revealing underlying disease mechanisms.[2] Biomarkers like neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) underscore the value of integrating clinical and molecular data in nephrology.[3]

Mesencephalic astrocyte-derived neurotrophic factor (MANF) is a conserved protein implicated in endoplasmic reticulum (ER) stress, inflammation regulation, and cellular protection.[4, 8, 9] Initially characterized for its neuroprotective functions, MANF has since been detected in non-neuronal tissues, including the kidneys, where its expression increases in response to stress and injury. Its involvement in systemic metabolic pathways, such as those disrupted in diabetes, has also been documented.[10]

Urinary biomarkers like albumin-to-creatinine ratio (ACR) and HbA1C remain essential for evaluating renal and glycemic health.[5] Nevertheless, these conventional markers do not fully capture the complexity of underlying pathophysiological processes. Novel biomarkers such as MANF may offer additional diagnostic insight, particularly when integrated into current biomarker panels.[6, 7]

This study aims to investigate the associations between serum MANF levels and established biomarkers of renal function and glycemic control across varying stages of albuminuria.

Methods

Study Design

This study utilized a cross-sectional design to investigate the associations between MANF levels and established urinary biomarkers, including ACR and HbA1C. A cross-sectional approach is appropriate for identifying potential correlations and exploring the utility of MANF as a novel biomarker in clinical populations. By capturing a snapshot of data from diverse patients, this design allows for an in-depth evaluation of the relationships between MANF and markers of renal and metabolic health.[1]

Participant Selection

Participants were recruited based on specific inclusion and exclusion criteria to ensure the validity and reliability of the findings. Adults aged 18 to 75 years with a confirmed diagnosis of CDK or diabetes mellitus were included. All participants had available baseline clinical and laboratory data, including urinary albumin, serum HbA1C, and creatinine measurements. Exclusion criteria were established to minimize confounding variables and included patients with acute kidney injury, rapidly progressive glomerulonephritis, cancer, autoimmune diseases, or other inflammatory conditions unrelated to kidney function. These exclusions helped ensure that observed variations in MANF levels were primarily related to chronic conditions such as CKD and diabetes rather than acute or unrelated inflammatory responses.[5]

Data Collection

Spot urine samples were collected from participants in the morning to account for and minimize diurnal variations in biomarker levels. The standardization of collection protocols ensured consistency across samples and minimized pre-analytical variability. Serum MANF levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits, which provide high specificity and sensitivity for low-abundance proteins. This assay was complemented by automated clinical analyzers for urinary albumin and creatinine measurements, while blood HbA1C levels were determined through high-performance liquid chromatography (HPLC). This multi-pronged approach ensured that data collection was robust and aligned with clinical best practices.[8,9] For this study, venous blood samples were collected from the antecubital vein and transferred into standard biochemistry tubes as well as tubes containing K2EDTA. The biochemistry tubes were allowed to clot for 30 minutes before being centrifuged at 3000 rpm for 10 minutes. The obtained serum was then aliquoted and stored at -80°C until the serum MANF analysis was performed. Complete blood count (CBC), routine biochemistry, and urine parameters were analyzed immediately. CBC measurements were conducted using an automated analyzer (Sysmex XN-1000, Sysmex Company, Japan). Serum and urine biochemical parameters were determined with a standard biochemistry auto-analyzer (Cobas 8000, Roche Diagnostic Corp., Mannheim, Germany). Serum MANF levels were assessed using a commercially available ELISA kit (Elabscience, catalog number E-EL-H0504, lot number AGG9UZM2IC), with results expressed in pg/mL.

Statistical Analysis

Descriptive statistics, including means, medians, and standard deviations, were calculated to summarize patient demographics and baseline biomarker levels. Correlation analyses, using Pearson or Spearman methods depending on normality of the data distribution, were conducted to evaluate the relationships between MANF and urinary biomarkers such as ACR and HbA1C. Multivariate linear regression models were applied to adjust for potential confounding variables, including age, sex, body mass index (BMI), and baseline renal function. These adjustments ensured that the observed associations were not spurious and accounted for known influences on biomarker levels. Additionally, subgroup analyses were conducted to explore MANF's diagnostic utility across CKD stages and varying levels of glycemic control.[4,10] By employing rigorous methodologies and robust statistical analyses, this study aimed to provide reliable insights into the potential role of MANF as a biomarker in CDK and metabolic diseases.

Results

The study analyzed data from total of 84 participants, stratified into three groups based on albuminuria levels: normoalbuminuria, microalbuminuria, and macroalbuminuria. The average age of participants was 58.87±0.68 years, with a range from 33 to 83 years. Participants in the macroalbuminuria group exhibited higher mean HbA1C levels (9.82±2.41%) and lower estimated glomerular filtration rates (eGFR, approximated via spot urine albumin/creatinine mean of 100.11±185.69) compared to other groups, reflecting progressive metabolic and renal impairment. Baseline characterics of the study groups are given in Table 1.

Table 1. Baseline Characteristics

Parameter	**Normoalbuminuria** (n=36)	**Microalbuminuria** (n=30)	**Macroalbuminuria** (n=18)
Age (years) Mean±SD	55.6±9.2	59.8±10.1	62.5±11.3
Male (%)	50%	52%	58%
HbA1C (%) Mean±SD	6.2±0.8	8.1±1.4	9.8±2.4
MANF (pg/mL) Mean±SD	252.3±80.1	368.9±101.2	514.5±112.3
Systolic BP (mmHg) Mean±SD	125±15	142±18	160±21

SD: Standard deviation

The MANF levels demonstrated a significant upward trend across albuminuria categories. Participants in the macroalbuminuria group exhibited markedly elevated MANF levels (514.5±112.3 pg/mL), significantly higher than those in the normoalbuminuria group (252.3±80.1 pg/mL, $p<0.01$). This indicates a robust association between elevated MANF levels and the severity of albuminuria, potentially reflecting increased endoplasmic reticulum stress in advanced renal dysfunction.

Correlation analysis revealed statistically significant relationships between MANF levels and key clinical biomarkers. MANF positively correlated with HbA1C (R=0.78, $p<0.001$), suggesting an association with glycemic control. Conversely, a negative correlation was observed between MANF and eGFR approximated via spot urine albumin/creatinine (R=-0.72, $p<0.001$), indicating that higher MANF levels are linked to worsening renal function. These relationships remained significant after adjusting for confounding factors such as age, sex, and BMI.

The trends and relationships were further illustrated; in Figure 1, the scatterplot depicted a strong positive linear relationship between MANF levels and HbA1C, emphasizing the potential role of MANF in glycemic dysregulation.

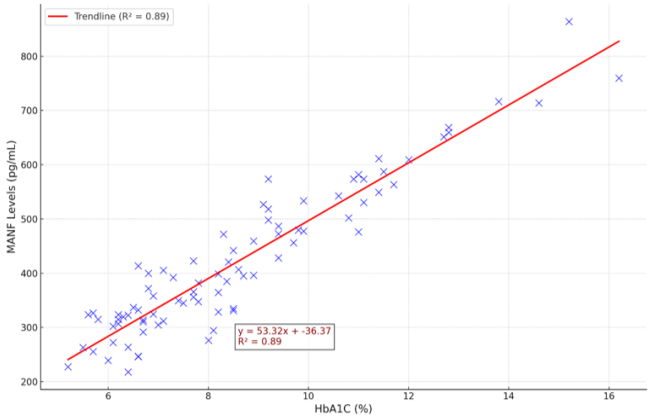

Figure 1. Scatterplot of MANF vs HbA1C

The boxplot given in Figure 2, displayed the distribution of MANF levels across albuminuria categories, highlighting the significant differences between groups.

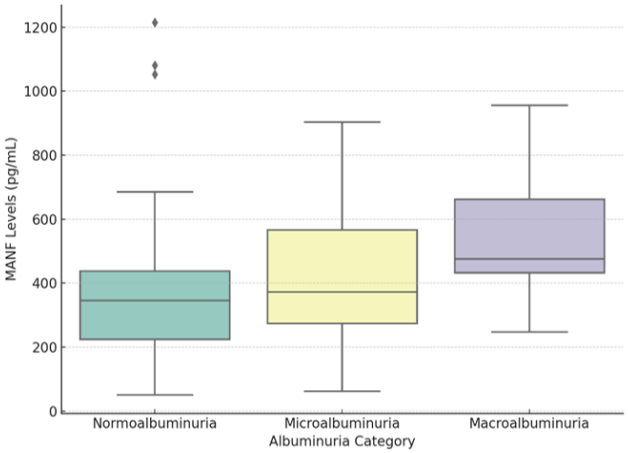

Figure 2. Boxplot of MANF Levels Across Albuminuria Categories

- **MANF Levels (Mean±SD):**
  - Normoalbuminuria: 252.3±80.1 pg/mL
  - Microalbuminuria: 368.9±101.2 pg/mL
  - Macroalbuminuria: 514.5±112.3 pg/mL
- **Correlation Coefficients:**
  - MANF vs HbA1C: R=0.89, $p<0.001$
  - MANF vs eGFR (spot urine albumin/creatinine): R=-0.72, $p<0.001$

In conclusion, the results indicate that MANF levels rise significantly with increasing albuminuria severity and correlate strongly with glycemic and renal biomarkers. These findings support the hypothesis that MANF serves as a novel biomarker for assessing renal dysfunction and metabolic regulation.

Discussion

The findings of this study underscore the significant correlations between MANF levels, glycemic control, and renal dysfunction, highlighting its potential as a biomarker for CDK and metabolic diseases. This section explores these associations, implications for clinical practice, and future research directions.

MANF's positive correlation with HbA1C reflects its association with glycemic dysregulation, as observed in diabetic populations where hyperglycemia accelerates kidney damage through oxidative stress and inflammatory pathways.[11,12] This finding aligns with existing research indicating that glycemic markers such as HbA1C and glycated albumin are critical in predicting renal and cardiovascular outcomes.[13,14] Additionally, MANF's role in mitigating endoplasmic reticulum stress and systemic inflammation provides a mechanistic basis for its association

with glycemic control.[10,15] Emerging biomarkers such as fructosamine and glycated albumin, though promising, often lack the comprehensive systemic insights offered by MANF.[16]

The elevated MANF levels in macroalbuminuria patients are consistent with studies identifying albuminuria as a key marker of CKD progression.[5,17] Albuminuria's role as a predictor of renal outcomes is well-established, but MANF's ability to reflect early endoplasmic reticulum stress and inflammation enhances its diagnostic value.[1,3] Recent advancements in biomarker research have emphasized the importance of combining systemic and local markers to improve diagnostic and prognostic accuracy, with MANF showing potential as a critical addition to this approach.[18,19]

The integration of MANF into clinical practice could revolutionize the management of CKD and diabetes by providing earlier detection and a better understanding of disease mechanisms. Current diagnostic tools, such as serum creatinine and eGFR, often fail to detect renal dysfunction at its earliest stages.[20] MANF offers a non-invasive, sensitive, and specific biomarker that reflects both renal and systemic health, bridging a critical gap in current diagnostic paradigms.[17] In clinical practice, monitoring MANF levels could allow for earlier interventions and personalized therapeutic strategies, potentially slowing CKD progression and improving outcomes in diabetic populations.[21]

Moreover, MANF's ability to correlate with multiple clinical parameters, including glycemic and renal biomarkers, underscores its utility as an integrative tool in precision medicine. By combining MANF with other novel biomarkers such as NGAL, KIM-1, and microRNAs, clinicians could achieve a more comprehensive understanding of disease progression and response to treatment.[22,23]

While the results are promising, this study's cross-sectional design limits causal inferences. Longitudinal studies are necessary to establish MANF's predictive value in CKD and DN. Additionally, exploring MANF's role in other systemic diseases could expand its clinical applications. Future research should focus on validating MANF in larger, diverse cohorts and integrating it into a multi-biomarker approach to improve diagnostic and prognostic accuracy.

This study highlights the promising role of MANF as a novel biomarker for renal and metabolic health. The significant correlations observed between MANF levels, glycemic markers, and renal dysfunction underscore its potential as an integrative tool for early detection and monitoring of CDK and DN. Unlike traditional markers, MANF reflects underlying pathophysiological processes such as endoplasmic reticulum stress and inflammation, offering a more comprehensive assessment of disease progression.

While this study provides foundational evidence supporting the utility of MANF as a biomarker for DN and glycemic dysregulation, several limitations must be acknowledged. First, the cross-sectional design restricts the ability to infer causality or temporal relationships between MANF levels and renal or metabolic biomarkers. Longitudinal studies are essential to determine whether changes in MANF levels precede or follow renal function decline and poor glycemic control. Second, the sample size, although adequate for preliminary correlations, may limit the generalizability of findings, particularly across diverse ethnicities, stages of CKD, or diabetes subtypes. Third, the study did not assess dynamic fluctuations in MANF over time or in response to therapeutic interventions, which could offer deeper insights into its clinical applicability. Additionally, only serum MANF levels were measured; incorporating urinary MANF levels in future studies may provide a more comprehensive evaluation of its role in renal pathology. Finally, while adjustments were made for confounders such as age, sex, and BMI, residual confounding due to unmeasured factors such as dietary habits, medication use, and comorbid conditions cannot be excluded. These limitations underscore the necessity of validating MANF's predictive value in larger, prospective, and multi-center cohorts, and of integrating it within a broader multi-biomarker framework to assess its robustness in clinical practice.

These findings pave the way for MANF's integration into routine clinical care, potentially transforming the management of CKD and diabetes to improve patient outcomes.

Conflict of Interest

No potential conflict of interest was reported by the author(s).

Compliance of Ethical Statement

Approval for the study was obtained from from the Kirsehir Ahi Evran University Clinical Research Ethics Committee (Decision No 2023-02/10), and written informed consent was obtained from all participating patients.

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Author's Contributions

K.T.: Study idea/Hypothesis, Design, Data Collection, Analysis, Literature Review, Writing, Critical Review; K.G.: Data Collection, Critical Review.

References

1. Fassett RG, Venuthurupalli SK, Gobe GC, Coombes JS, Cooper MA, Hoy WE. Biomarkers in chronic kidney disease: a review. *Kidney Int*. 2011;80(8):806-821. doi:10.1038/ki.2011.198
2. Zhao YY. Metabolomics in chronic kidney disease. *Clin Chim Acta*. 2013;422:59-69. doi:10.1016/j.cca.2013.03.033
3. Wasung ME, Chawla LS, Madero M. Biomarkers of renal function, which and when? *Clin Chim Acta*. 2015;438:350-357. doi:10.1016/j.cca.2014.08.039
4. Julkunen H. Metabolic blood biomarker profiling for chronic kidney disease prediction. *Nephrol Dial Transplant*. 2023;38. doi:10.1093/ndt/gfad063c_5650
5. Tesch GH. Review: Serum and urine biomarkers of kidney disease: a pathophysiological perspective. *Nephrology (Carlton)*. 2010;15(6):609-616. doi:10.1111/j.1440-1797.2010.01361.x
6. Zhang ZH, Chen H, Vaziri ND, et al. Metabolomic signatures of chronic kidney disease of diverse etiologies in the rats and humans. *J Proteome Res*. 2016;15(10):3802-3812. doi:10.1021/acs.jproteome.6b00583
7. Breit M, Weinberger KM. Metabolic biomarkers for chronic kidney disease. *Arch Biochem Biophys*. 2016;589:62-80. doi:10.1016/j.abb.2015.07.018
8. Kim Y, Lee H, Manson SR, et al. Mesencephalic astrocyte-derived neurotrophic factor as a urine biomarker for endoplasmic reticulum stress-related kidney diseases. *J Am Soc Nephrol*. 2016;27(10):2974-2982. doi:10.1681/ASN.2014100986
9. Liu H, Tang X, Gong L. Mesencephalic astrocyte-derived neurotrophic factor and cerebral dopamine neurotrophic factor: new endoplasmic reticulum stress response proteins. *Eur J Pharmacol*. 2015;750:118-122. doi:10.1016/j.ejphar.2015.01.016
10. Lindahl M, Danilova T, Palm E, et al. MANF is indispensable for the proliferation and survival of pancreatic β cells. *Cell Rep*. 2014;7(2):366-375. doi:10.1016/j.celrep.2014.03.023

11. Kim WJ, Park CY, Lee KB, et al. Serum 1,5-anhydroglucitol concentrations are a reliable index of glycemic control in type 2 diabetes with mild or moderate renal dysfunction. *Diabetes Care*. 2012;35(2):281-286. doi:10.2337/dc11-1462
12. Zhang H, Zuo JJ, Dong SS, et al. Identification of potential serum metabolic biomarkers of diabetic kidney disease: a widely targeted metabolomics study. *J Diabetes Res*. 2020;2020:3049098. doi:10.1155/2020/3049098
13. Inaba M, Okuno S, Kumeda Y, et al. Glycated albumin is a better glycemic indicator than glycated hemoglobin values in hemodialysis patients with diabetes: effect of anemia and erythropoietin injection. *J Am Soc Nephrol*. 2007;18(3):896-903. doi:10.1681/ASN.2006070772
14. Dozio E, Corradi V, Proglio M, et al. Usefulness of glycated albumin as a biomarker for glucose control and prognostic factor in chronic kidney disease patients on dialysis (CKD-G5D). *Diabetes Res Clin Pract*. 2018;140:9-17. doi:10.1016/j.diabres.2018.03.017
15. Looker HC, Colombo M, Hess S, et al. Biomarkers of rapid chronic kidney disease progression in type 2 diabetes. *Kidney Int*. 2015;88(4):888-896. doi:10.1038/ki.2015.199
16. Zelnick LR, Batacchi ZO, Ahmad I, et al. Continuous glucose monitoring and use of alternative markers to assess glycemia in chronic kidney disease. *Diabetes Care*. 2020;43(10):2379-2387. doi:10.2337/dc20-0915
17. Mizdrak M, Kumrić M, Kurir TT, Božić J. Emerging biomarkers for early detection of chronic kidney disease. *J Pers Med*. 2022;12(4):548. doi:10.3390/jpm12040548
18. Speeckaert M, Van Biesen W, Delanghe J, et al. Are there better alternatives than haemoglobin A1c to estimate glycaemic control in the chronic kidney disease population? *Nephrol Dial Transplant*. 2014;29(12):2167-2177. doi:10.1093/ndt/gfu006
19. Chaudhary K, Phadke G, Nistala R, Weidmeyer CE, McFarlane SI, Whaley-Connell A. The emerging role of biomarkers in diabetic and hypertensive chronic kidney disease. *Curr Diab Rep*. 2010;10(1):37-42. doi:10.1007/s11892-009-0080-z
20. Lousa I, Reis F, Beirão I, Alves R, Belo L, Santos-Silva A. New potential biomarkers for chronic kidney disease management—a review of the literature. *Int J Mol Sci*. 2020;22(1):43. doi:10.3390/ijms22010043
21. Galindo RJ, Beck RW, Scioscia MF, Umpierrez GE, Tuttle KR. Glycemic monitoring and management in advanced chronic kidney disease. *Endocr Rev*. 2020;41(5):756-774. doi:10.1210/endrev/bnaa017
22. Cardenas-Gonzalez M, Srivastava A, Pavkovic M, et al. Identification, confirmation, and replication of novel urinary microRNA biomarkers in lupus nephritis and diabetic nephropathy. *Clin Chem*. 2017;63(9):1515-1526. doi:10.1373/clinchem.2017.274175
23. Lecamwasam A, Ekinci EI, Saffery R, Dwyer KM. Potential for novel biomarkers in diabetes-associated chronic kidney disease: epigenome, metabolome, and gut microbiome. *Biomedicines*. 2020;8(9):341. doi:10.3390/biomedicines8090341