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Serum Midkine as an Early Predictor of Diabetic Nephropathy in Type 1 Diabetes Mellitus

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Abstract:

Introduction: Diabetic nephropathy (DN) is a prevalent and severe microvascular consequence of DM that is linked to higher rates of illness and death in individuals with diabetes. Currently, microalbuminuria remains the most reliable method for diagnosing the condition. But, there is an urgent need for new biomarkers to allow earlier diagnosis and management for better outcomes.

Aim of study: To evaluate the significance of serum Midkine (MK) as a biomarker for early identification of DN in children diagnosed with type 1 diabetes mellitus (T1DM) before the onset of microalbuminuria.

Subjects and Methods: This study comprised a sample size of 100 children, of which 50 had T1DM (25 macroalbuminuric cases (group 1), 25 normoalbuminuria cases (group 2), and 50 healthy participants served as the control group (group 3). In each of the three groups, a comprehensive medical history, clinical examination, and laboratory evaluation of hemoglobin A1c% (HbA1c%), lipid profile, urinary microalbuminuria (MA), and serum MK were conducted.

Results: Comparing the two diabetic groups to the control group, our research demonstrated a significant rise in serum MK. Moreover, serum MK concentrations were greater among those with microalbuminuria compared to those with normoalbuminuria. According to the analysis of the receiver operating characteristic (ROC) curve, microalbuminuria could be predicted with a sensitivity of 96% and specificity of 90 % using an MK cutoff value of 260 pg/ml.

Conclusions: Serum MK is a practicable, novel, and beneficial indicator for assessing renal involvement in children with T1DM, particularly in normoalbuminuric children.

Keywords: Type 1 DM; Diabetic nephropathy; Microalbuminuria; Midkine.

1. Introduction

Diabetic nephropathy (DN) is a prevalent and severe microvascular consequence of DM that is related to higher rates of illness and death in cases with diabetes [1].

DN is responsible for thirty to 50% of cases of end-stage renal disease (ESRD). It is expected that approximately 40% of cases with DN will need renal replacement therapy [2].

The Normoalbuminuric screening is now the most widely used and reliable sign in medical practice for predicting and detecting diabetic kidney involvement in pediatric diabetes [3].

The clinical practice consensus recommendations of the International Society for Pediatric and Adolescent Diabetes serve as the foundation for detecting albuminuria and related abnormalities. Normoalbuminuric is identified when the quantity of albumin in a 24-hour urine sample falls within the range of 30-300 mg, or when the amount of albumin in at least two out of three initial morning urine samples is between 30-300 mg/L. Normoalbuminuria is a state in which the amount of albumin in the urine is equal to or less than 30 mg/L in all urine samples collected in the early morning. The level of Normoalbuminuric is assessed using the urinary albumin creatinine ratio (UACR), which should be between 2.5 and 25 mg/mmol for males and 3.5 and 25 mg/mmol for females. This measurement is based on at least two out of three urine samples collected in the morning. Macroalbuminuria is characterized by the occurrence of albumin levels over 300 mg/L in a minimum of 2 morning urine samples [4].

While albuminuria can serve as a biomarker for predicting and detecting diabetic kidney disease (DKD), its utility is limited in that not all diabetic children exhibiting micro or macroalbuminuria will demonstrate a decline in kidney function. Additionally, UACR, eGFR (estimated glomerular filtration rate), infection, diet, fever, hydration status, hemodynamics, tension, physical activity, periods, and hyperglycemia are all variables that can affect albuminuria levels. A significant number of Normoalbuminuric cases (up to 40%) may also return to normoalbuminuria if their blood pressure (BP) and glycemic levels are strictly managed. Consequently, Normoalbuminuric may be transient [3].

Similarly, when significant proteinuria is present, the glomerulus sustains severe injury; certain diabetic cases with normoproteinuria develop progressive renal insufficiency; this condition is referred to as normoproteinuric diabetes [5].

Thus, urine albumin is inadequate and imprecise as an early biomarker of DN. The utilization of the Schwartz method for estimating GFR using serum creatinine helps to circumvent these difficulties, although it tends to underestimate the actual renal function [3].

Hence, there is a need for novel biomarkers that can more accurately evaluate the renal condition of those with DN, while being less affected by factors like age and sex. Additionally, novel biomarkers can accurately describe the impact of pharmacological treatment over time, enabling the identification of the most effective dosage and kind of medication [6].

Several indicators of damage to the proximal tubular cells may be identified in the urine of diabetic patients in the early stages of the disease, even when there is no apparent harm to the glomeruli. This suggests that proximal tubular injury is an early occurrence and not only a result of glomerular injury [7].

Midkine (MK), a cytokine that binds to heparin, has a role in the development of kidney disorders by increasing the movement and activation of white blood cells. Animal models and small case-control investigations have shown that MK may serve as a pathological biomarker in CKD. However, this hypothesis has not yet been verified in prospective human research [8].

To the best of our understanding, there is currently no accessible evidence on the connection between serum MK and DN in children who have T1DM. The present investigation aimed to evaluate the diagnostic utility of serum MK as a new biomarker in predicting Normoalbuminuric, hence enabling early detection of diabetic nephropathy in children with T1DM.

2. Subjects and Methods

2.1. Subjects

This case-control research involved 100 children; 50 cases with type 1 Diabetes Mellitus who have been getting treatment at Fayoum University Hospital pediatric endocrine clinic for a minimum of 2 years

and the other 50 children were healthy children of the same age and gender considered as controls.

Inclusion criteria

Patients from both genders aged between 5 and 16 years with type 1 DM for at least two years.

Exclusion criteria

We excluded patients with any chronic infections, liver diseases, immunosuppressive states, autoimmune or connective tissue diseases, or any other condition that may cause albuminuria as nephrotic syndrome and glomerulonephritis.

Excluded were cases who were taking antihypertensive, lipid-lowering, or antiplatelet medications.

2.2. Methods

- Demographic information, including patients' ages and sexes was gathered.
- Anthropometric measurements including height, weight and BMI together with systolic and diastolic blood pressure were also obtained.
- Detailed medical history was taken from all diabetic patients including age of onset of DM, duration of the disease and insulin types and doses.
- All diabetic patients were tested for urinary Normoalbuminuric and accordingly classified into two groups:

  Group A: cases with urinary Normoalbuminuric.

  Group B: cases without urinary Normoalbuminuric.

- All the study populations were tested for serum midkine levels.

Serum MK was detected by the ELISA technique utilizing the Human Midkine ELISA kit provided by (BT LAB, China) and using ChroMate (Awareness Technology INC, USA) as a microplate reader.

2.3. Statistical Methods

The data were encoded utilizing SPSS software version 22 on Windows 7 to facilitate its loading into Microsoft Access for analysis. The application was created by SPSS Inc., based in Chicago, USA. Simple descriptive analysis of quantitative parametric data in the form of % and numbers for qualitative data, and arithmetic means for measuring central tendency and

standard deviations for assessing dispersion. The inclusion of quantitative data in the research was preceded by a one-sample Kolmogorov-Smirnov test to assess normality for each group, followed by the selection of inferential statistics. Parametric quantitative data: Self-contained samples to compare quantitative measures of 2 independent groups, the t-test was applied. Utilize the one-way ANOVA test to compare quantitative measures across multiple independent groups of quantitative data, while the Benferroni post-hoc test is employed to determine the significance level among any 2 groups. The chi-square test is employed to compare two or more qualitative groups when qualitative data is involved. Pearson bivariate correlation test for determining the relationship among variables Evaluation of the sensitivity and specificity of a novel test utilizing the "Receiver Operating Characteristic" (ROC) curve. A P-value below 0.05 was deemed to indicate statistical significance.

3. Results

This case-control research was performed on 100 subjects, divided into three groups: 25 cases of diabetes mellitus with Normoalbuminuric (Group I), 25 cases of diabetes mellitus without Normoalbuminuric (Group II) and 50 controls (Group III).

The study population comprised 37 females and 63 males, with a mean age of 13.8 ±2.5 for group (I), 13.6 ±3 years for group (II) and 10.8 ±3.1 for group (III). no statistically significant variations were noted in the basic demographics of the two study groups (**Table 1).**

Table 1. Comparisons of demographic characters in variant examined groups.

Variables		**Group I**	**Group II**	**Group III**	***P*-value**
Sex	Male	7 (28%)	12 (48%)	18 (36%)	0.33
	Female	18 (72%)	13 (52%)	32 (64%)	
Age (years)		13.8 ±2.5	13.6 ±3	10.8 ±3.1	0.9[a] 0.2 [b,c]

a: significance between GI and GII, b: significance between GI and GIII, c: significance between GII and GIII.

All the study populations were tested for serum midkine and they were compared to each other. A statistically significant variance was noted with (p <0.05) among study groups regarding serum MK level with a greater level in group I than in group II and the lowest level in group III. In addition, a statistically significant variance was noted (p <0.05) among study groups regarding serum MK levels (**Table 2)**.

Table 2. Comparisons of Serum MK level in different study groups.

Variables	Group I	Group II	Group III	*P*-value
Age (years)	408.6±146.5	323±82.4	203.9±44.2	0.003^{a}* $<0.001^{b,c}$*

*a: significance between GI and GII, b: significance between GI and GIII, c: significance between GII and GIII. * significant.*

A correlation between serum Midkine level and level of Normoalbuminuric was performed among diabetic patients. we found that a statistically significant positive relationship was noted (p <0.05) between serum MK level and Normoalbuminuric (**Table 3).**

Table 3: Relationship among Serum MK with the level of Normoalbuminuric among diabetic patients.

Variables	R	*P*-value
Normoalbuminuric	0.64	<0.001*

** significant.*

Then serum Midkine sensitivity and specificity in the diagnosis of diabetic nephropathy was observed. we noted that there was statistically significant sensitivity (p <0.05) to serum MK level to differentiate cases of diabetes with Normoalbuminuric from controls with a Sensitivity and specificity test of (96%, and 90%) at the cutoff of 259.5. To differentiate cases of diabetes without Normoalbuminuric from

controls the sensitivity and specificity test of (62%, and 88%) at the cut-off value of 244.5. Finally, to differentiate cases of diabetes with, and without Normoalbuminuric the sensitivity and specificity test of (72%, and 52%) at the cut-off values of 313 (**Table 4)**.

Table 4: Sensitivity and specificity of serum MK level in diagnosis of cases with Normoalbuminuric, and diabetic cases without Normoalbuminuric.

Variable	Sensitivity	Specificity	AUC	Cut off point	*P*-value
DM with Normoalbuminuric from controls	96%	90%	96%	259.5	<0.001*
DM without Normoalbuminuric from controls	62%	88%	93.9%	244.5	<0.001*
DM with/without Normoalbuminuric	72%	52%	68.6%	313	0.02*

** significant.*

4. Discussion

Currently, there is a rising interest in discovering novel biomarkers that might potentially substitute GFR and ACR, or enhance their predictive capability, for the early detection of DKD and the prognosis of its advancement [9].

Diabetic children with Normoalbuminuric had significantly elevated levels of MK contrasted to those with normoalbuminuria and the control group in this research. Significantly greater amounts of MK were seen in normoalbuminuric children compared to controls, which is in line with the outcomes of Metwally et al. (2021) [10].

This indicates that there is a correlation between serum MK levels and subclinical tubular impairment; therefore, these levels may serve as a measurable indicator of renal involvement before the development of Normoalbuminuric. In addition, urinary Normoalbuminuric is substantially and positively correlated with

MK, which is in line with the outcomes of Metwally et al. (2021) [10]. This indicates that MK has an impact on the extent of renal involvement and could potentially serve as a metric for dividing DM into distinct stages [10].

We agree with Kosugi et al.'s (2006) findings that kidney biopsy samples from 8 adult cases had DN exhibited pronounced tubular atrophy, interstitial fibrosis, and interstitial cell infiltration. MK induction was observed in the glomeruli, tubules, and interstitium of these DN specimens. Furthermore, the expression of MK was found in all studied patients, which exhibited different phases of DN. The data aligned with the MK expression pattern in a mouse model induced by Streptozotocin [11].

Our study also showed a statistically significant sensitivity of the serum MK level to differentiate cases of diabetes with Normoalbuminuric from controls with a Sensitivity and specificity test of (96%, and 90%) at cutoff (259.5). to differentiate cases of diabetes without Normoalbuminuric from controls the sensitivity and specificity test of (62%, and 88%) at the cut-off value of 244.5. finally, to differentiate cases of diabetes with and without Normoalbuminuric the sensitivity and specificity tests of (72%, and 52%) at the cut-off value of 313.

5. Conclusion

MK is higher in Normoalbuminuric patients than non-Normoalbuminuric and controls and is higher in non-Normoalbuminuric than controls. It’s also correlated with the degree of MA. Particularly in normoalbuminuric children, serum MK is a practical, novel, and beneficial indicator for assessing renal involvement in children with T1DM.

Ethical approval and consent to participate: The Committee of Ethics in Fayoum University Hospital and Faculty of Medicine approved this study and numbered in its session number (99) on 9th of October 2022, all the participants were informed about the details of the study with documented written informed consent.

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