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ORIGINAL ARTICLE

Neutrophil Gelatinase-Associated Lipocalin as Early Sign of Diabetic Kidney Injury in Children

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ABSTRACT

Background: Type 1 diabetes mellitus (T1DM), the most common childhood endocrine disorder, leads to numerous complications of the macro and microvascular system. Diabetic nephropathy (DN) is one of the major complications of T1DM with end-stage renal disease (ESRD). Aim of the work: This research would determine whether the rates of urinary NGAL (uNGAL) and Doppler ultrasonography can predict early stages of diabetic kidney injury in childhood. **Methods**: A case control study was conducted in pediatrics outpatient clinics at Zagazig University Hospitals on 32 participants, 16 ones of them were a diabetic children at outpatients clinic and matched with 16 healthy subjects**. Results:** The results of the study revealed that, at cutoff point of 56.4 ng/ml, Urinary NGAL was 100% sensitive, 81.2% specific and 90.6% accurate, that there was a statistical significant difference between the studied groups in Urinary NGAL and Urinary NGAL/Cr ratio. Cases group had significantly higher Urinary NGAL and Urinary NGAL/Cr ratio than control group. **Conclusion**: NGAL is a rising and promising marker for early detection of DN. It can detect the tubular phase of DN even before the onset of Microalbuminuria.

Keywords: Urinary NGAL, T1DM, Diabetic Nephropathy, Childhood.

INTRODUCTION

Type 1 diabetes mellitus (T1DM), the most common childhood endocrine disorder, results in multiple complications of the macro and microvascular system**[1]**. Diabetic nephropathy (DN) is one of the major complications of T1DM with end-stage renal disease (ESRD) **[2]**. Microalbuminuria is now known to be the first indication of DN. It has been known, however, that some DN-related histopathological changes occur before microalbuminuria starts **[3]**. Therefore, multiple studies have investigated non-invasive methods for diagnosing DN in earlier stages **[4]**. The main focus of these studies was on neutrophil gelatinase-associated lipocalin (NGAL), the most promising biomarker for early detection of acute kidney injury (AKI) **[5]**. NGAL, a member of the superfamily of lipocalin protein, is released from the renal tubular cells in response to various acute and chronic kidney insults such as contrast nephropathy, IgA nephropathy, and lupus nephritis **[6]**. Tubulointerstitial damage as well as glomerular changes in DN **[7]** have been shown to be associated with renal functional degradation. It was therefore considered that NGAL can predict diabetic renal damage as a tubular marker **[8]**.

Although DN is rarely manifested in childhood, it is known that changes that lead to kidney function deterioration begin in childhood. Increased intrarenal resistive index (RI) was recently shown as a role of creatinine clearance in adults with diabetic nephropathy. Nevertheless, there is still a shortage of RI evidence in children with diabetes, and the connection between increased intrarenal RI and altered renal hemodynamics remains unclear**[9]**.

The aim of this study is to assess whether urinary NGAL (uNGAL) levels and

Doppler ultrasonography can predict the early phases of diabetic kidney injury in childhood.

Methods:

A case control study was conducted in pediatrics endocrinology outpatient clinics at Zagazig University Hospitals in period of 6 months duration. 32 participants, 16 were a diabetic children at outpatients clinic and matched with 16 healthy subjects. **Inclusion criteria:** Type 1 diabetes mellitus (T1DM), less than 18 years and Both genders were included **Exclusion criteria:** Diabetic patients had clinical signs or laboratory evidence of kidney disease, diabetic patients with microalbuminuria, patients with other autoimmune diseases or medicines other than insulin, and patients with urinary tract infection at urinary sample time.

All patients included in this studied subject to the following: age, gender, residency, dietary history and family history of DM or CKD. Details about disease onset, duration, regimen of insulin therapy, number of hospital admissions and frequency of DKA or hypoglycemia were alsocollected. Special focus was done on DM complications such asretinopathy and neuropathy.

Complete physical examination: was done for all patients. Anthropometric measurements are accurately reported, including weight, height and body mass index. Percentiles were determined using Egyptian children and adolescents reference data **[10].**

Blood pressure was measured using the auscultatory method and Percentiles were estimated based on the American Academy of Pediatrics 2017 guidelines**[11].**

Laboratory investigations: Investigations included HbAlC, serum creatinine, urinary albuminexcretion, serum and urinary nrutrophil gelatinase-associated lipocalin (NGAL). The first morning urine was collected and centrifuged, the Supernatant was removed and the specimens held at -40°C until they were checked. The lipocalin associated with neutrophil gelatinase (NGAL) was assessed. Serum and urine with human lipocalin-associated neutrophil gelatinase (NGAL) ELISA package. The Sensitivity of this kit is 10.5113ng/ml with assay range of 12ng/ml to 3000ng/ml. It is highly specific for NGAL detection. No cross-reactivity between NGAL and other substances was detected. Normal urine NGAL level is estimated to be around 20ng/ml. Filtration rate glomerular (ml / min/1,73 m^2) was calculated based on serum creatinine (mg/dl) and height (cm) , using the following bedside

Twenty four hours urine collection was done to measure urinary. Excretion of the albumin. The definition of microalbuminuria is urinary albumin. 30-300 mg/24 hours of excretion, measured on a minimum of 2 of 3 measurements over a period of 2 to 3 months. Evaluation of the Doppler ultrasound.

Ethical Clearance: Written Informed consent was taken from the patient parents to participate in the study. Approval for performing the study was obtained from Pediatrics and Clinical Pathology Departments, Zagazig University Hospitals after taking Institutional Review Board (IRB) approval. The work has been carried out in accordance with the code of ethics of the world medical association (Decleration of Helsinki) for studies involving humans.

Statistical Analysis

Data collected, coded, entered and analyzed using Microsoft Excel software, throughout history, basic clinical examination, laboratory investigations and outcome measures. Data were then imported into the Social Sciences Statistical Package (SPSS version 20.0) (Social Sciences Statistical Package) for analysis software. Depending on the type of qualitative data represented by number and percentage, quantitative continuous group represented by mean ± SD, the following tests were used to test differences for meaning;., correlation by Pearson's correlation or Spearman's. For significant results, the P value was set at < 0.05 & < 0.001 for high significant results.

RESULTS

Our study found that no statistical significant difference between the studied groups in all demographic characteristics except the age as case group had significantly had higher age than control group (Table 1).

Our study found that a statistical significant difference between the studied

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groups in diabetic ketoacidosis and polyphagia, polydipsia and polyuria (Table 2).

Our study found that a statistical significant difference between the studied groups in Urinary NGAL and Urinary NGAL/Cr ratio. Cases group had significantly higher Urinary NGAL and Urinary NGAL/Cr ratio than control group (Table 3).

Our study found that no statistical significant association between Urinary NGAL and demographic characteristics of the studied cases. (Table 4).

Our study found that at cutoff point of 56.4 ng/ml, Urinary NGAL was 100% sensitive, 81.2% specific and 90.6% accurate. Also, at cutoff point of 77.9 ng/ml, Urinary NGAL/Cr ratio was 100% sensitive, 87.5% specific and 93.8% accurate. (Figure 1)

Table (1): Demographic characteristics of the studied groups

Variables	Case group (n=16)	Control group (n=16)	Test of sig.	P
Age (years): Mean ± SD	11.8 ± 2.2	9.4 ± 2.1	T 3.2	**0.003** **S**
Sex, n (%): Male Female	5 (31.3%) 11 (68.8%)	8 (50.0%) 8 (50.0%)	χ^2 1.2	0.2
Residence. n (%): Urban Rural	6 (37.5%) 10 (62.5%)	7 (43.8%) 9 (56.3%)	χ^2 0.1	0.7
Family history of DKA, n (%): Positive Negative	10 (62.5%) 6 (37.5%)	6 (37.5%) 10 (62.5%)	χ^2 2.0	0.1
Family history of renal disease, n (%): Positive Negative	4 (25.0%) 12 (75.0%)	3 (18.8%) 13 (81.3%)	χ^2 0.2	0.6

Table (2): Clinical characteristics of the studied groups:

Variables	Case group (n=16)	Control group (n=16)	Test of sig.	P
BMI (kg/m^2): Mean ± SD	19.8 ± 3.6	19.7 ± 4.0	T 0.1	0.9
SBP (hgmm^2): Mean ± SD	100.6 ± 10.8	98.8 ± 8.7	T 0.09	0.9
DBP (hgmm^2): Mean ± SD	55.6 ± 5.1	59.1 ± 4.6	T 0.5	0.5
DKA, n (%):	4 (25.0%)	0 (0.0%)	fisher	**0.01 (S)**
Vulvo-vaginitis, n (%):	3 (18.8%)	0 (0.0%)	fisher	0.1
Polys (polyphagia, polydipsia and polyuria), n (%):	9 (56.3%)	0 (0.0%)	fisher	**<0.001(HS)**

Table (3): Laboratory characteristics of the studied groups:

Variables	Case group (n=16)	Control group (n=16)	Test of sig.	P
Micro-albuminuria(mg/24 hours): Mean ± SD	19.1 ± 5.8	18.0 ± 6.4	T 0.5	0.6
Serum creatinine(mg /dl): Mean ± SD	0.61 ± 0.2	0.6 ± 0.12	T 0.07	0.9
HgA1c (%): Mean ± SD	8.8 ± 1.6	3.1 ± 1.4	T 10.8	**<0.001** **HS**
GFR (ml/min/1.73 m^2):			T	

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Variables	Case group (n=16)	Control group (n=16)	Test of sig.	P
Mean ± SD	97.0 ± 6.6	94.6 ± 6.2	1.1	0.3
Urinary NGAL(ng/ml): Mean ± SD	76.7 ± 7.3	30.0 ± 10.1	T 15.0	**<0.001** **HS**
Urinary NGAL/Cr (ng/ml): Mean ± SD	134.6 ± 44.0	52.0 ± 21.8	MW 4.5	**<0.001** **HS**

Table (4): Association between Urinary NGAL and demographic characteristics of the studied cases:

Variables	Urinary NGAL (ng/ml) Mean ± SD	T	P
Sex: Male Female	 72.7 ± 8.0 78.6 ± 6.5	1.6	0.1
Residence: Urban Rural	 78.4 ± 9.5 75.7 ± 6.0	0.7	0.4
Family history of DKA: Positive Negative	 76.1 ± 7.9 77.9 ± 6.7	0.4	0.6
Family history of renal disease: Positive Negative	 76.7 ± 8.1 76.7 ± 7.4	0.004	0.9
Type of feeding during 1st 6 months: Exclusive breast feeding Artificial feeding Mixed feeding	 77.0 ± 4.4 77.0 ± 6.1 76.2 ± 11.4	 KW 0.2	 0.9
Age of weaning: ≤ 12 months > 12 months	 77.7 ± 6.3 76.2 ± 8.1	 T 0.4	 0.7

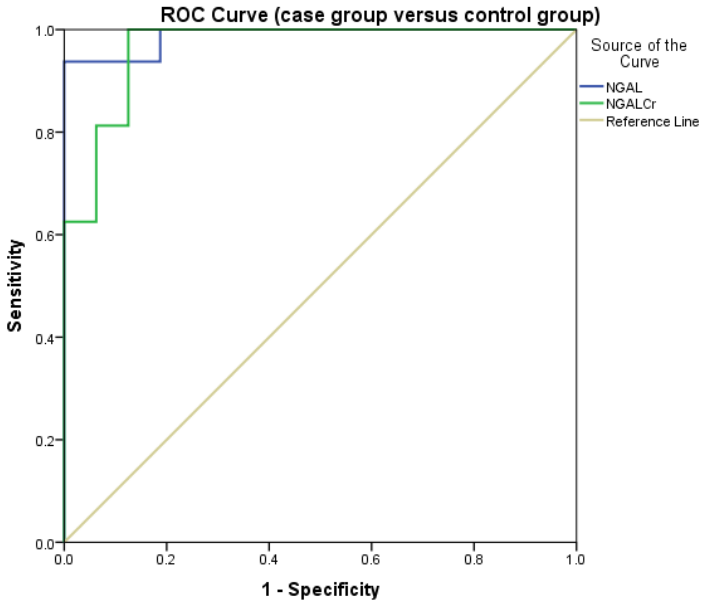

Figure (1): Receiver operating characteristics curve of Urinary NGAL and Urinary NGAL/Cr ratio

DISCUSSION

Type 1 diabetes is a chronic autoimmune disease characterized by the

selective destruction of beta-cells in pancreatic islet cells, together with the formation of antibodies against beta-cell components. Type 1 diabetes is most commonly diagnosed with symptomatic hyperglycemia in children and adolescents, and imparts the immediate need for exogenous insulin replacement. Two peaks of type 1 diabetes appear to occur in childhood and puberty **[12] .**

Diabetic nephropathy is a condition characterized by the production of urinary albumin in abnormal amounts. In this analysis, they analyzed the demographic characteristics of the studied groups and found that the mean ± SD age of the case group was 11.8 ± 2.2 and 9.4 ± 2.1 for the control group, and found that there was no statistically significant difference in all demographic characteristics between the studied groups except the age group as the case group had significantly higher age than the control group.

Compared to our sample, **Yuruk et al., [13]** consisted of 76 children (36 males and 40 females) and 35 children (18 males and 17 females) in the control group. The mean age of the patient and control groups was 12.43±3.87 years and 11.14±3.77 years.

T1DM's classic children's presentations are polyuria, polydipsia, and weight loss. A significant number of patients with diabetic ketoacidosis (DKA) may have a significant mortality risk for these children. **[13]**

The hand study evaluated the clinical characteristics of the participants and found that there was a statistically significant difference between the studied groups in DKA and polys, whereas there was no statistically significant difference between them with respect to BMI, BP and Vulvo-vaginitis.

In line with our research, **Yuruk et al.,[13]** found that there were no significant differences in sex and BMI between the patient and control groups (p>0.05).

The present study revealed a statistically significant difference between the groups studied in urinary NGAL and the ratio of urinary NGAL/Cr. The group of cases had a significantly higher urinary NGAL and NGAL / Cr ratio than the group of controls.

In agreement with our study, **Yuruk et al.[13]** found that mean uNGAL levels in the patient group were significantly higher than in the control group (100.16±108.28 ng / mL vs 21.46±18.59 ng / mL; p=0.0001), and mean uNGAL / Cr values were also significantly higher in the patient group than in the control group (118.93±117.97ng/mg vs. 32.1±51.48 ng / mg; p=0.0001).

UNGAL levels in diabetic adults and children have only been tested in a few studies**[15]** .

Bolignano et al. [16] Among adults with type 2 DM, found higher uNGAL levels and fractional NGAL excretion rates than in controls. In T1DM patients, uNGAL / Cr were stated to be higher than in the adult control group by **Nielsen et al. [8]** and in normoalbuminuric adolescents by **Demir et al. [15].**

Zachwieja et al. [17] For non-microalbuminuric children with T1DM, higher levels of uNGAL were also identified than in controls. The most relevant question at this point is whether high uNGAL or uNGAL/Cr levels suggest diabetic kidney injury before microalbuminuria occurs.

The present study showed a statistically significant difference in HgA1c among the studied groups.

In another **Yuruk et al. [13]** in which patients are classified into three subgroups such as those with better glycemic control (HbA1c: 6.5-7.5%; n=20), medium glycemic control (HbA1c: 7.5-9%; n=31), and weak glycemic control (HbA1c > 9%; n=25). There were no significant differences between these groups with respect to uNGAL (p=0.423) and uNGAL / Cr (p=0.371), this is in line with our results where there was no statistically significant association between urinary NGAL and glycemic control of the cases studied and between the ratio of urinary NGAL / Cr and glycemic control of the cases studied.

The on - the-hand study found that there was no statistically significant association between urinary NGAL and demographic features of the cases studied, similar to our

study, Nektaria et al. study**[18].** It was found that NGAL values were not influenced by age, T1D age and puberty, suggesting that the various physiological mechanisms that occur during puberty were independent. While in the cases examined, there was a statistically significant correlation between the urinary NGAL / Cr ratio and renal history. Cases with positive history of renal disease had significantly higher Urinary NGAL/Cr ratio.

The current study also found that there was no statistical significant association between Urinary NGAL and either feeding history or clinical characterstics of the studied cases, and there was no statistical significant association between Urinary NGAL/Cr ratio and either feeding history or clinical characteristics of the studied cases

CONCLUSION

NGAL is a rising and promising marker for early detection of DN. It can detect the tubular phase of DN even before the onset of Microalbuminuria. While our study highlights the potential rule of NGAL as a marker of DN.

LIMITATION OF STUDY

The conclusions of this study were limited by a small sample size in one center only. Further follow up of these patients is critical to identify and detect the trends in NGAL levels with disease progression.

RECOMMENDATION

Various locations and centers and higher number of cases with Different age groups should be implemented in future studies to Facilitate generalization of the results. This will help in expanding our knowledge about this novel biomarker and provide us with basis for implementation of its use in risk assessments and follow up of TIDM patients.

Declaration of interest

The authors report no conflicts of interest. The authors along are responsible for the content and writing of the paper.

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