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Research Article

EVALUATION OF URINARY NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN AS AN EARLY MARKER OF NEPHROPATHY IN TYPE 1 DIABETIC ADOLESCENTS

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A R T I C L E I N F O

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A B S T R A C T

Background: Diabetic nephropathy (DN) is a major cause of morbidity and mortality among adolescents with type 1 diabetes. Clinical management and therapeutic intervention from early stage of DN is of major importance to prevent progression to end-stage renal disease. Renal tubulointerstitium plays an important role in the development and progression of diabetic nephropathy.
Aim: The aim of the present study was to assess and evaluate urinary Neutrophil Gelatinase-Associated lipocalin (uNGAL) as an early marker for diabetic nephropathy in type 1 diabetic adolescents.
Methodology: This is a case control study that included ninety adolescents. They were divided according to albumin/creatinine ratio (ACR) in urine into three groups: group I included thirty type 1 diabetic adolescents without microalbuminuria (<30 mcg/mg), group II included thirty type 1 diabetic adolescents with microalbuminuria (30–300 mcg/mg) and group III included thirty healthy controls. In addition to urinary NGAL, urine albumin/creatinine ratio, serum creatinine, estimated glomerular filtration rate (eGFR), fasting blood glucose (FBG), glycated hemoglobin (HbA1c) and other common biochemical parameters were measured according to standard methods and were assessed to correlate their values with urinary NGAL. Data analysis was done by using (SPSS) version 20.0. P value was set significant if < 0.05. All graphs were analyzed with graph pad version 17.0.
Results: Urinary NGAL was increased in diabetic patients compared to controls; interestingly, uNGAL was increased already in microalbuminuric patients (group II) and also in normoalbuminuric (group I) (p=0.000). It was increased proportionately to the severity of kidney function, poor glycemic control. Diabetic patients showed increased mean ACR values with respect to controls (p=0.000). Analysis of correlation revealed that urine NGAL was correlated with fasting blood glucose and blood pressure.
CONCLUSION: Urinary NGAL was significantly elevated in micro-albuminuric (group II) and some normo-albuminuric type 1 diabetes patients (GROUP I) compared with healthy controls (GROUP III) and it can predict diabetic nephropathy at early stage even before the development of microalbuminuria.

INTRODUCTION

Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control. Ongoing diabetes self-management education and support are critical to preventing acute complications and reducing the risk of long-term complications [1].Diabetic nephropathy (DN) is one of the most serious micro-vascular complications, which significantly impacts morbidity, mortality and quality of life. DN occurs in approximately one-third of all people with diabetes and is considered the leading cause of renal failure in developed and developing countries [2].

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DN is a micro-vascular complication of diabetes, and it is clinically characterized by persistent albuminuria, decline in glomerular filtration and function, and high risk of cardiovascular mortality and morbidity. DN, which is one of the micro-vascular complications of diabetes, is the largest cause of End Stage Renal Disease (ESRD) [3].

NGAL is a protein first identified in neutrophils but expressed at very low concentration in several tissues, including the lung, the gastrointestinal tract and the kidney upon inflammation and tissue injury. Circulating NGAL is filtered by the glomerulus and captured by the proximal tubule and only a minimal amount is excreted in urine. It's small molecular size and protease resistance could render it an excellent biomarker of renal injury [4, 5].

Interestingly, tubular biomarkers have shown that tubular dysfunction can be present early in DN, occasionally preceding glomerular injury. This observation underscores the fact that tubular biomarkers are early predictors of DN compared to microalbuminuria and other glomerular biomarkers. Examples of tubular biomarkers include NGAL, α-1-microglobulin, kidney injury molecule 1 (KIM-1), N-acetyl-β-D-glucosaminidase (NAG), cystatin C, and liver-type fatty acid-binding protein (L-FABP) [6].

MATERIAL AND METHODS

This is a case control study which involved 90 adolescents (44 males and 46 females) divided into three groups according to Albumin/Creatinine Ratio: group I included 30 type 1 diabetic adolescents without microalbuminuria (<30 mcg/mg) with mean age 13±2.7 years, group II included 30 type 1 diabetic adolescents with microalbuminuria (30–300 mcg/mg) with mean age 13.5±2.7 years and group III included 30 healthy controls with mean age 12.9±2.0 years. Urinary NGAL was measured using NGAL Enzyme-linked immunosorbent assay (ELISA) method. Urinary Albumin and Creatinine were estimated on fully automated chemistry analyzer. Albumin Creatinine Ratio (ACR) was calculated as mcg Albumin/mg Creatinine. Data analysis was done by using SPSS version 20.0. and graph pad. All T1DM adolescents are less than 3 years diabetes duration, T1DM adolescents with urinary tract infection, renal diseases and any inflammatory or chronic diseases were excluded from this study to avoid any interference in the NGAL measurement.

RESULTS

The demographic and disease-related characteristics of the patients which analysed by using ANOVA test are presented in Table 1. This study involved sixty patients with Type 1 diabetes mellitus adolescents and thirty healthy controls. They were categorized into (Group I) normoalbuminuria (N=30), (Group II) microalbuminuria (N=30), and (Group III) healthy controls (N=30), depending on their albumin/creatinine ratio in urine.

There was no significant difference between three groups for the gender, BMI, height, age and age on set between three groups but there was a significant longer duration in group II than Group I (p=0.003) which represent a risk factor for development op micro-albuminuria.

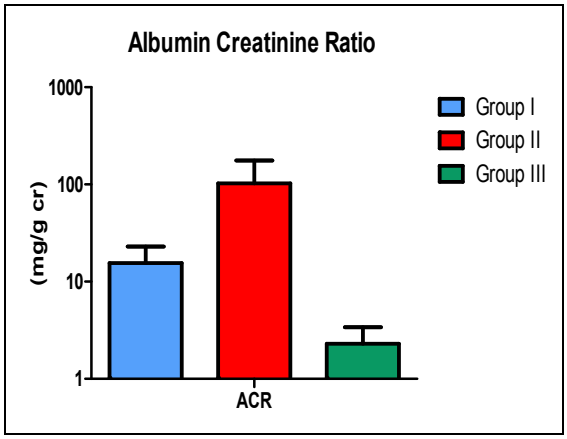

Figure 1 Estimated ACR in Group I, Group II and Group III (Controls)

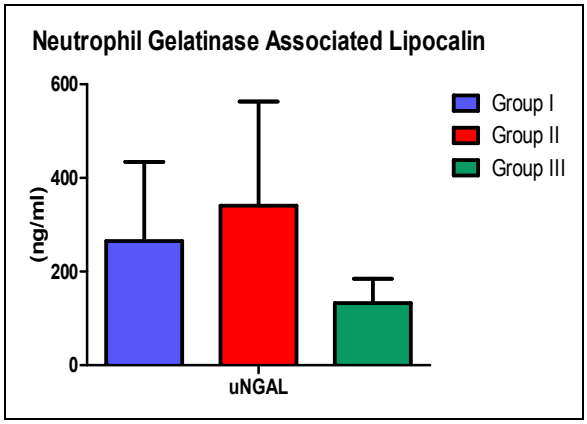

Figure 2 Estimated NGAL in Group I, Group II and Group III (Controls)

Table 1 Demographics and disease-related characteristics

Variables		(Group I) Normoalbuminuria T1DM	(Group II) Microalbuminuria T1DM	(Group III) Controls	ANOVA (P value)
Sex	Male %	19 (63.3%)	11 (36.7%)	14 (46.7%)	0.8
	Female %	11 (36.7%)	19 (63.3%)	16 (53.3%)	
Age (years) (mean±SD)		13±2.7	13.5±2.7	12.9±2.0	0.589
Age of onset of DM (years) (mean±SD)		7.9±2.4	7±3	-	0.2
Duration of diabetes (years) (mean±SD)		5.06±1.4	6.5±2.16	-	0.003
BMI (kg/m^2) (mean±SD)		20.6±4.4	22.06±6.2	19.3±2.4	.073
Height (cm) (mean±SD)		146±15	147.1±14.5	149.9±8.6	0.503
SBP (mmHg) (mean±SD)		119.5±19^{a**}	119±17^{b**}	103.3±13.2	0.000
DBP (mmHg) (mean±SD)		79.2±12.7^{a**}	79±10^{b**}	65±9.4	0.000
FBG (mg%) (mean±SD)		236±124^{a**}	269±117^{b**}	77.9±9.9	0.000
HbA1c(%)(mean±SD)		10.9±3^{a**}	12±2.6^{b**}	5.36±0.3	0.000
Insulin dose (u/kg/day) (mean±SD)		1.132±0.3	1.355±0.4	-	.031
AST U/L (mean±SD)		34±33.2	28.2±4.3	25.5±5.69	0.4
ALT U/L (mean±SD)		29.3±27.3	29.1±24.9	18.3±4.6	0.08
TC (mg/dl) (mean±SD)		176±30.3	194.7±55.9^{b**}	158.1±22.1	.002
TG (mg/dl) (mean±SD)		123.6±83.1^{a**}	116.1±62.9^{b*}	75.3±11	.006
HDL-C (mg/dl) (mean±SD)		43.6±10.5	50.6±11.9^{b*c**}	43.4±5.7	.007
LDL-C (mg/dl) (mean±SD)		110.1±24.5	125.8±46.4^{b*}	104±17.9	.029
Creatinine (mg%) (mean±SD)		0.8±0.2 ^{a**}	0.76±0.1	0.69±0.12	0.002
Urea (mg%) (mean±SD)		26.8±11.2	25.6±7.2	24.6±4.67	0.6
eGFR (ml/min/1.73m^2) (mean±SD)		111.1±25.6 ^{a**}	116.3±23.3 ^{b*}	132.3±25.5	0.004
ACR (mg/g cr) (mean±SD)		15.5±7.5^{a**}	102.7±73.5^{b**}	2.3±1.1	0.000
uNGAL (ng/ml) (mean±SD)		265.4±168.8	340.5±222.7^{b,c**}	132.8±52.1	0.000

* P-value < 0.05 is significant; **P-value < 0.005 is highly significant; a Significant between Group I and Group III; b Significant between Group II and Group III; c Significant between Group II and Group I; Appreviations: TC; Total Cholesterol; TG; Tri-Glycerides; HDL-C; High density lipoprotein cholesterol ; LDL-C; Low density lipoprotein cholesterol.

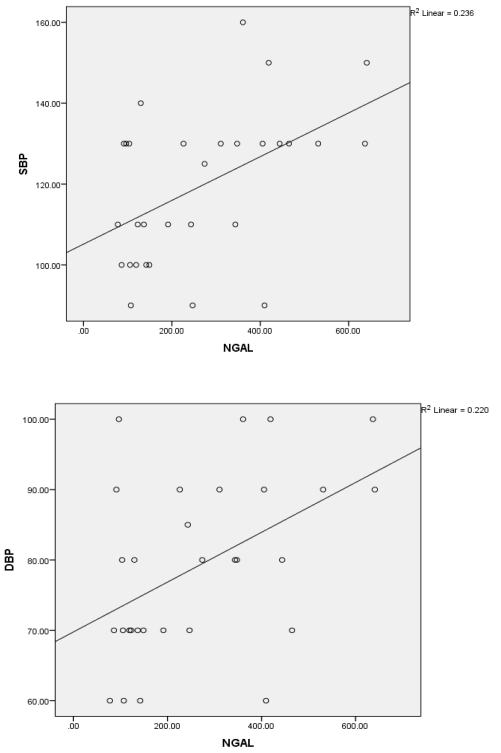

Figure 3 Correlation between Blood Pressure and NGAL in Group I

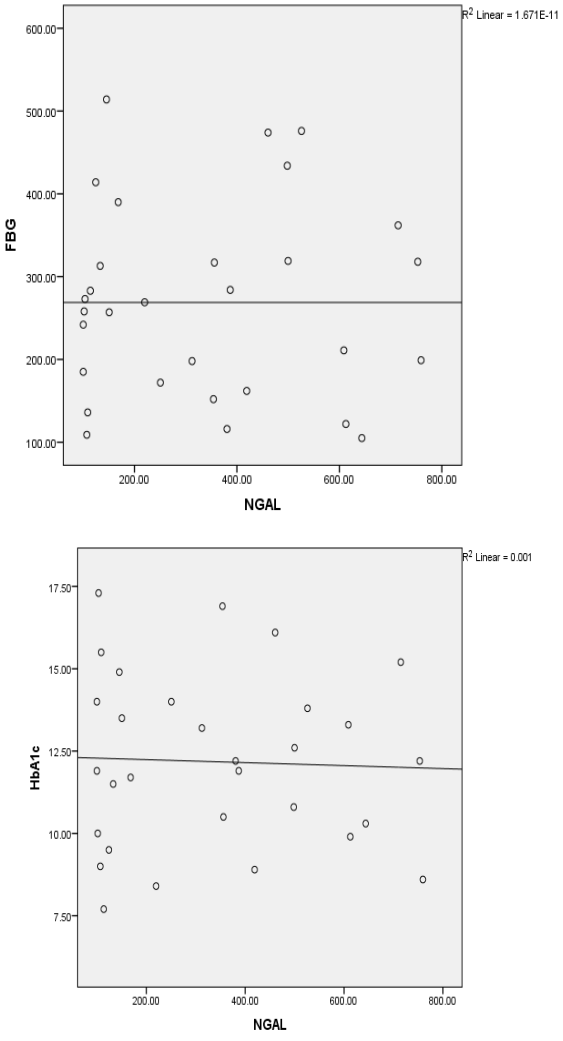

Figure 4 Correlation between Diabetes markers and NGAL in Group II

This study showed there was a highly statistically significant increase in the mean±SD of Albumin Creatinine Ratio (ACR) in (Group II) T1DM patients with nephropathy compared to (Group III) controls as shown in Fig.(1).

This study showed there was a highly statistically significant increase in the mean±SD of uNGAL in (Group II) T1DM patients with nephropathy patient groups than (Group III) control as shown in Fig. (2). This is an interesting finding and supports the hypothesis that uNGAL can be used as a marker for the early detection of diabetic nephropathy.

This study showed there was a highly statistically significant difference in the mean±SD of eGFR between T1DM patient Groups I, II compared to preserved renal function in controls (p 0.004) as shown in Table (1) which represent the impact of DM on renal function.

This study showed there is a highly significant difference between Group I and Group II for SBP and DBP (p=0.000) as shown in table (1), which means that elevated BP represents a risk factor for development of microalbuminuria. Also there is significant positive correlation between SBP, DBP and uNGAL in Group I T1DM patient without microalbuminuria as shown in Fig. (3), this may suggest an indirect predictive role of uNGAL as a cardiovascular morbidity marker. Undoubtedly, further studies investigating the endothelial dysfunction will further delineate the extent of microvascular damage in DN.

This study showed there is significant positive correlation between elevated FBG and uNGAL in Group II T1DM patient microalbuminuria, which support that uNGAL is strongly correlated with poor glycemic control which preceding DN as shown in Fig.(4).

This study showed there is statistical difference between the diabetic patient groups I or II and controls as regards glycosylated hemoglobin (HbA1c) and FBG as it was higher in group II (p=0.000). Also, there was a statistically significant difference in the mean±SD between the T1DM patients groups I and II for Insulin Dose u/kg/day (p=.031) which indicates that there are many risk factors for development of microalbuminuria in Type 1 diabetic patients as poor glycemic control, long duration of diabetes, high insulin dose and high blood pressure as shown in Table 1.

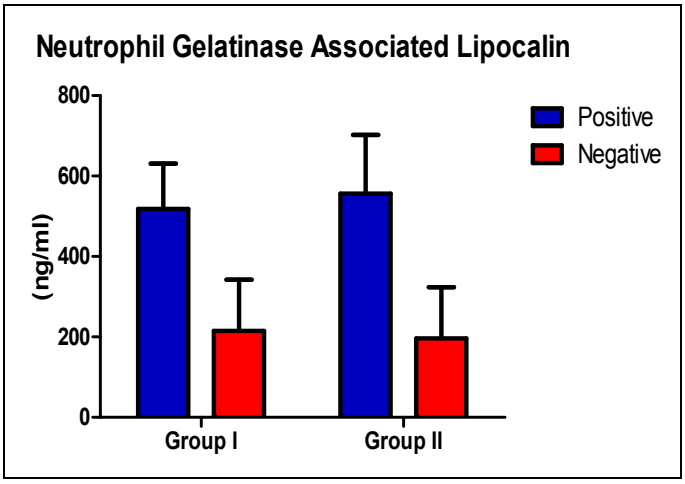

Figure 5 NGAL in relation to family history of Renal Disease in type 1 diabetics Group I and Group II

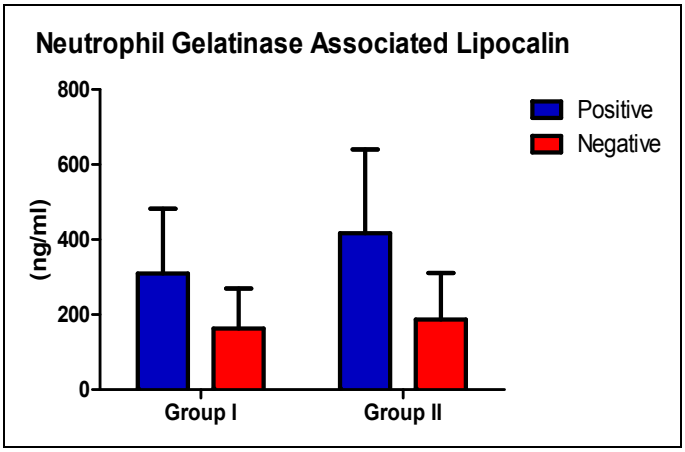

Figure 6 NGAL in relation to family history of diabetes mellitus in patients with type 1 diabetes mellitus Group I and Group II

The mean values of NGAL are higher in Group I and Group II diabetics with positive family history of Renal disease and DM compared with diabetics with negative family history for each disease in the same group as shown in fig. (5) and fig. (6) which supports the hypothesis that urine NGAL can be used as an early marker for early detection of diabetic nephropathy in presence of positive family history of renal disease or DM.

In diabetics with positive family history of diabetes mellitus than in Group I and Group II diabetics with negative family history of diabetes and the difference is statistically significant. The mean value of NGAL is higher in diabetics with positive family history of Renal disease than in Group I and Group II diabetics with negative family history of diabetes and the difference is statistically significant.

Liver enzymes have been tested for all groups to ensure that they have not liver disease. According to ALT and AST results, the patients with highly ALT and AST were excluded from this study so that there is no significant difference between patients Group I, Group II and Group III normal control.

DISCUSSION

Classically, albuminuria is regarded as the consequence of diabetes-induced glomerular damage. The onset of elevated levels of urinary albumin excretion is an early sign of diabetic nephropathy. Various studies have shown that in patients with diabetes, microalbuminuria predicts the occurrence of macroalbuminuria and renal function decline. As a result, high albuminuria has become an established marker of CKD in these patients [7].

More recently, it is increasingly appreciated that the renal tubule interstitium plays a role in the pathogenesis of diabetic nephropathy with prolonged exposure to a variety of metabolic and hemodynamic injuring factors that are associated with sustained hyperglycemia as contributing factors [8].

Markers of tubular damage are discovered and extensively investigated in predicting the occurrence of acute kidney injury after various nephrotoxic insults, such as ischemia during cardiac surgery, sepsis, and administration of contrast agents. Little research has been done about their role as early markers in patients with CKD [9].

The aim of this case control study was to assess urinary Neutrophil Gelatinase Associated lipocalin (uNGAL) and to evaluate as an early marker for diabetic nephropathy in type 1 diabetic adolescent. This study involved 60 patients with diabetes and 30 controls. It demonstrated that urine NGAL does not correlate with other diabetic indices; such as HbA1c and dermographic factors such as (age, gender, and Age of onset of DM. These findings are in accordance with reports from Bolignano *et al*. [10]. However, urine NGAL was noted to be correlating significantly with urine albumin/creatinine ratio and FBS. Few studies have reported the same findings in patients with chronic kidney disease [11–14], hence supporting idea that urine NGAL may be used as a marker for nephropathy.

In current study, there was no significant difference between three groups for BMI between all groups but there was a significant difference in SBP and DBP in Group I and Group II as it was higher as compared with controls (p=0.000).

Similarly, Alleyn *et al***.** reported no difference in BMI with respect to the occurrence of microalbuminuria [15].

This study showed the statistical difference between the diabetic patient groups I or II and the control group as regards glycosylated hemoglobin (HbA1c) and FBG as it was higher in group II (p=0.000). Also, there was a highly statistical significant difference in the mean±SD between the collective patient groups I and II for Insulin Dose u/kg/day (p=.031).

Similarly, in a study conducted by ***Tandon et al.,*** patients without any proteinuria showed HbA1c up to 7.0%. Amongst the microalbuminuric patients, they had HbA1c in the range of 8.1-10%. macroalbumin positive patients had HbA1c in the range of 10.1- 12%. A positive correlation was seen between degree of glycaemic control and development of proteinuria (p value <0.05) [16].

This study showed there was a highly statistical significant difference in the mean±SD of creatinine and eGFR between T1DM with nephropathy patient groups and control. On the other hand, there is no a significant difference between the diabetic patient groups and the healthy group in the mean±SD urea or between patients groups.

This was supported by *Sasso et al.,* study, showed that, In comparison with microalbuminuric patients, those with macroalbuminuria were characterized by a larger prevalence of greater renal impairment (57.8 ± 26.2 versus 68.2 ± 22.8 mL/min/1.73m 2 , P < 0.0001).HbA1c and haemoglobin values were influenced by both GFR and albuminuria [17].
This study showed there was a highly statistical significant increase in the mean±SD Albumin Creatinine Ratio (ACR) and NGAL in Group II T1DM with nephropathy patient groups than control.

This finding may support the hypothesis of a "tubular phase" of diabetic disease preceding overt diabetic nephropathy and hence the use of these markers, especially U.NGAL measurement for early evaluation of renal involvement even before appearance of microalbuminuria [18].
Similarly, *Siddiqi et al.,* results showed that U.NGAL levels were significantly high in cases as compare to controls [19].
Also, *Al-Rubeaan et al.* concluded that, NGAL had significantly increased in patients with microalbuminuria and marcoalbuminuria [20].

The association between the early tubular lesions in non-albuminuric patients with type 1 diabetis and NGAL was further supported by published studies [21, 22, 23].

This study showed, the mean value of ACR is higher in diabetics with positive family history of renal disease than in Group I and Group II diabetics with negative family history of diabetes and the difference is statistically significant.

Similarly, *Arar et al.,* Fifty-three percent of diabetic relatives presented with a high urinary albumin/creatinine ratio (≥0.03) and were unaware of having DN ($P \leq 0.05$). Although an overwhelming majority of participants perceived diabetes as running in their family, only 36% of respondents felt that DN ran in their family or believed that a family history of diabetes or having a sibling with DN were important risk factors for developing DN [24].

This study showed, there are a significant positive correlation of SBP, DBP, and NGAL. On the other hands, there was non-significant positive correlation between Urea and NGAL and negative non-significant correlation between Liver Function, eGFR and NGAL.

The *Mahfouz et al.,* study showed a significant correlation between the urinary levels of NGAL with the presence of high SBP [25].

CONCLUSION

Urinary NGAL is rapidly emerging as a very promising predictive tool in a variety of renal and non-renal conditions. The horizons for its applications are rapidly expanding. It can be used early to predict many disorders, especially diabetic nephropathy in future. The present study supports these findings by showing an association between higher urinary NGAL levels and greater progress in diabetic nephropathy.

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