

Evaluation of Neutrophil Gelatinase Associated Lipocalin, As A Biomarker of Renal Injury in Type 2 Diabetic Patients

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ABSTRACT

Aim: To evaluate urinary excretion of Neutrophil Gelatinase Associated Lipocalin (NGAL) in type 2 diabetic patients with different stages of nephropathy.

Methods: It was a cross-sectional study conducted at Shaikh Zayed Hospital, Lahore from Oct 2009 – Oct 2010. Ninety five subjects both males and females were divided into two groups. 75 type 2 diabetic patients (33 males and 42 females) were included in the Diabetic group and 20 healthy individuals were taken as Controls. Urinary Neutrophil Gelatinase Associated Lipocalin (uNGAL) was measured using NGAL Elisa method. Urinary Albumin and Creatinine were estimated on fully automated chemistry analyzer. Albumin Creatinine Ratio (ACR) was calculated as mg Albumin/g Creatinine. On the basis of ACR, diabetic patients were divided into 3 groups. Normoalbuminuric (<30mg Alb/g Cr), Microalbuminuric (30-300mg Alb/g Cr) and Macroalbuminuric (>300 mg Alb/g Cr).

Results: The diabetic group showed highly increased levels of uNGAL (male diabetics mean=212.8±45.3 ng/ml, male controls mean=5.36±0.55 ng/ml and female diabetics mean=158.2±28.7 ng/ml, female controls mean=7.85±1.45 ng/ml). These levels increased in parallel with the severity of the renal disease. The ACR of female diabetics (279.62±106.17 mg Alb/g Cr) was significantly higher than the ACR of female controls (3.76±1.50 mg Alb/g Cr) and ACR of male diabetics (796.88±571.88 mg Alb/g Cr) was significantly higher than the ACR of male controls (1.44±0.78 mg Alb/g Cr). ACR of all the 3 diabetic groups showed a positive correlation with uNGAL except normoalbuminuric females.

Conclusion: uNGAL relates closely with renal function markers. It may prove to be a sensitive and non-invasive biomarker for the early detection of diabetic nephropathy as well as renal injuries caused by reasons other than diabetes.

Keywords: NGAL, Diabetic Nephropathy, Microalbuminuria.

INTRODUCTION

Human Neutrophil Gelatinase Associated Lipocalin (NGAL) also known as Lipocalin-2 was isolated in 1993¹. It was originally identified as a 25 kda glycosylated protein covalently bound to Gelatinase from human neutrophils, where it represents one of the neutrophil secondary granule proteins². Later, it was also found to be located in bone marrow cells as well as lung, bronchi, stomach, small intestine, colon, pancreas, prostate, thymus and kidneys.³ Human NGAL consists of a single polypeptide chain of 179 amino acid residues⁴. It possesses diverse physiological functions such as transporting iron, inducing apoptosis and regulating immune response⁵. Although it is expressed at low levels in several human tissues, it is markedly induced in injured epithelial cells including the kidney⁶.

Diabetic nephropathy is one of the most common microvascular complication of diabetes mellitus⁵. It develops in approximately 40% of all type 2 diabetic patients, characterized by persistent albuminuria, elevated blood pressure and a progressive decline in kidney function leading towards end stage renal disease (ESRD)⁷. In another prospective study, it was shown that one-fifth of the type 2 diabetics with nephropathy developed ESRD during the 5 year observation period⁸. Early detection of renal injury leads to an early referral and consequently improved outcome. For quite a long time, the impaired renal function of patients with diabetic nephropathy is mainly reflected by laboratory detection of serum creatinine, and blood urea nitrogen, both of which are not sensitive enough to detect early change of renal function⁹.

Creatinine clearance correlates better with GFR than serum creatinine but it is not useful in determining the exact level of renal function. It can only signify whether renal function is normal, moderately or severely reduced¹⁰. Other researchers have also reported that relation between Body Mass

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Index (BMI) and serum creatinine or creatinine clearance is inconsistent.¹¹ In agreement with the stated fact Wu and Parikh conclude that serum creatinine and creatinine clearance are late biomarkers of diabetic nephropathy¹².

Microalbuminuria, the screening test for the detection of nephropathy, refers to an abnormally increased excretory rate of albumin in the urine in the range of 30-300mg/g Cr. It is the earliest manifestation of renal disease in type 1 and type 2 diabetes¹³. However, it occurs due to diffuse diabetes induced glomerular damage¹⁴. Whereas, more recent studies have revealed that in diabetic patients renal function is better related to the degree of renal tubulointerstitial impairment than to the severity of glomerular lesions¹⁵. In the same perspective, Valmadrid et al have concluded that the onset of microalbuminuria may be a late finding in type 2 diabetics given its strong association with cardiovascular disease and increased risk of mortality¹⁶.

In recent years, NGAL has emerged as one of the most promising biomarker in the diagnostic field of acute and chronic renal diseases. It is massively released from renal tubular cells after various injuring stimuli.¹⁷ It is highly accumulated in the cortical tubules, blood and urine after nephrotoxic and ischemic injury due to its sustained production by the inflamed but vital tubular cells¹⁸. Certain studies have stated the greater sensitivity and specificity of urinary NGAL over serum NGAL. Kuwabara and colleagues emphasized that uNGAL is a very early and sensitive biomarker of kidney injury¹⁹. Another study postulated that because of its small molecular size (25kda) and resistance to degradation, NGAL is readily excreted and detected in the urine²⁰. Most importantly, NGAL derived from the systemic sources does not affect uNGAL measurements since any filtered NGAL is rapidly and efficiently reabsorbed by the proximal tubule²¹. Thus, any urinary excretion of NGAL is likely only when there is concomitant proximal tubular injury or increased de novo NGAL synthesis¹⁹.

Currently, microalbuminuria is the only most sensitive marker used for the early detection of renal injury. The present study is aimed at evaluating urinary excretion of NGAL in type 2 diabetics with different stages of nephropathy as depicted by their albumin creatinine ratios. This is being done to assess the potential relationship between this tubular biomarker and the severity of renal involvement in these patients.

MATERIALS AND METHODS

It was a cross sectional study conducted at Shaikh Zayed Hospital, Lahore from Oct 2009 to Oct 2010.

Ninety five (95) subjects both male and female were divided into two groups. Seventy five (75) type 2 diabetics (33 males and 42 females) were included in the Diabetic group and twenty (20) healthy individuals (8 males and 12 females) were taken as Controls. The criteria for inclusion in the diabetic group was diagnosed type 2 Diabetes Mellitus for more than 10 years. Exclusion criteria was patient having diagnosed acute or chronic renal disease, history of renal transplant, pregnancy or any other systemic disease or malignancy. Both patients and controls participated with prior consent. The study was approved by the Ethical Committee of Federal Post Graduate Medical Institute, Sh. Zayed Hospital, Lahore.

uNGAL was measured using Bioporto's Rapid Elisa Kit 037 (Denmark). Urinary Albumin and Creatinine were estimated on fully automated Roche 902 chemistry analyzer. Albumin to Creatinine ratio (ACR) was calculated as mg Alb/ g Cr by spot urinary creatinine. On the basis of ACR, type 2 diabetics were divided into 3 groups: Normoalbuminuric (<30 mg Alb/ g Cr), Microalbuminuric (30-300 mgAlb/g Cr) and Macroalbuminuric (>300 mgAlb/g Cr).

Statistical analysis was done on SPSS version 15.0. Results are expressed as mean±SD. Students 't' test was used for comparison between two groups and ANOVA was used for comparison between more than two groups. The association between uNGAL and other variables was observed by calculating correlation coefficient 'r'. A 'p' value <0.05 was considered significant.

RESULTS

There was no significant difference between the mean ages, weight and height of the diabetic and control groups. BMI of female diabetics was significantly higher ($p<0.001$) than BMI of female controls. Mean fasting serum glucose of male and female diabetics was significantly higher ($p<0.001$) than fasting serum glucose of controls (Table 1).

Mean serum creatinine of both male and female diabetics was significantly higher ($p<0.001$) as compared to their respective controls. The mean creatinine clearance of male diabetics was found to be 70.44 ± 4.82 ml / min which was significantly lower ($p<0.01$) as compared to the mean creatinine clearance of male controls which was 123.77 ± 14.58 ml/min. Similarly, the mean creatinine clearance of female diabetics was 75.71 ± 3.63 ml/min which was significantly lower ($p<0.001$) as compared to female controls which was 124.85 ± 9.43 ml/min (Table 1).

The mean ACR of male diabetics was found to be 796.88 ± 571.88 mg/g which was significantly higher ($p<0.001$) as compared to the mean ACR of

male controls which was 1.44 ± 0.78 mg/g, whereas, in the female diabetics this ratio was 279.62 ± 106.17 mg / g which was significantly higher ($p < 0.05$) than the female controls who had the mean ACR of 3.76 ± 1.50 mg/g. The mean uNGAL of male diabetics was calculated to be 212.77 ± 45.29 ng/ml which was significantly higher ($p < 0.001$) as compared to male controls with mean uNGAL of 5.36 ± 0.55 ng/ml. Similarly, mean uNGAL of diabetic females was found to be 158.14 ± 28.73 ng/ml which was significantly higher ($p < 0.001$) in comparison to female

controls who had mean uNGAL of 7.85 ± 1.45 ng/ml. (Table 1)

After dividing the type 2 diabetics into three groups i.e., normoalbuminuric, microalbuminuric and macroalbuminuric, uNGAL was correlated with ACR of these groups. All the three diabetic groups both males and females showed a significant positive correlation of uNGAL with ACR except normo-albuminuric females (Table 2)

Table 1: Age, Weight, Height, BMI, Systolic BP, Diastolic BP, FBG, uNGAL, Creatinine Clearance, ACR and Hb A1c in Male and Female groups. Mean \pm SEM is given. Figure in parenthesis indicates number of cases in each group

Group	Male Control (08)	Male Diabetics (33)	Female Controls(12)	Female Diabetics(42)
Age (yr)	52.90 \pm 1.97	55.91 \pm 1.13	51.40 \pm 3.07	52.36 \pm 1.22
Weight (Kg)	68.20 \pm 2.70	71.15 \pm 1.82	61.60 \pm 3.54	67.26 \pm 1.74
Height (m)	1.64 \pm 0.02	1.66 \pm 0.02	1.57 \pm 0.04	1.56 \pm 0.01
BMI (kg/m ²)	25.30 \pm 1.17	25.52 \pm 0.50	25.00 \pm 1.37	27.52 \pm 0.62***
Systolic BP (mm Hg)	121.00 \pm 2.21	123.33 \pm 3.25	116.00 \pm 2.21	121.90 \pm 2.96
Diastolic BP(mm Hg)	84.90 \pm 1.91	77.58 \pm 1.57**	78.10 \pm 2.40	77.14 \pm 1.50
FBG (mg/dl)	83.30 \pm 3.72	144.18 \pm 11.53***	79.90 \pm 3.42	179.12 \pm 11.77***
Urinary NGAL(ng/ml)	5.36 \pm 0.55	212.77 \pm 45.29***	7.85 \pm 1.45	158.14 \pm 28.73***
Creatinine clearance (ml/min)	123.77 \pm 14.58	70.44 \pm 4.82***	124.85 \pm 9.43	75.71 \pm 3.63***
ACR (mg/g)	1.44 \pm 0.78	796.88 \pm 571.88***	3.76 \pm 1.50	279.62 \pm 106.17***
Hb A1c	5.48 \pm 0.12	9.30 \pm 0.39***	5.51 \pm 0.14	9.08 \pm 0.20***
Serum Creatinine	0.65 \pm 0.06	1.49 \pm 0.23***	0.56 \pm 0.05	1.02 \pm 0.06***

*p < 0.05 significantly lower or higher as compared to control

**p < 0.01 highly significantly lower or higher as compared to control

***p < 0.001 highly significantly lower or higher as compared to control

Table 2: Correlation of uNGAL with Albumin Creatinine ratio. Coefficient of correlation (r) is given. Figure in parenthesis indicates number of cases in each group.

Group Compared	Control		Normo albuminuric		Micro albuminuric		Macro albuminuric	
	Male (08)	Female (12)	Male (10)	Female (20)	Male (12)	Female (16)	Male (6)	Female (6)
uNGAL with ACR	0.354	0.603	0.690**	0.596	0.710**	0.886**	0.955**	0.990**

DISCUSSION

The current study revealed highly raised uNGAL levels and urinary albumin creatinine ratios (ACR) of all type 2 diabetics when compared with a well matched control group. A characteristic trend of increased uNGAL was seen in parallel with the severity of the renal involvement as reflected by their albuminuria and creatinine clearance. In essence, both male and female diabetic patients show a significantly higher uNGAL and ACR and a decrease in serum creatinine clearance which is consistent with the severity of nephropathy and defines no significant difference in gender.

This study has also depicted a highly significant positive correlation between uNGAL and ACR, reflecting a close association of this biomarker with renal injury. These results were in close agreement with the studies performed by Yang and Bolignano in 2009.^{5,14}

It was previously thought that ACR is uniformly associated with a decrease in GFR in type 2 diabetics.^{22,23} However, more recent studies have shown that it is not true for all type 2 diabetics but only in those who exhibit diabetic glomerulopathy.²⁴ In another study, type 2 diabetics were identified with a progressive decrease of creatinine clearance despite of no increase in albuminuria.²⁵ Similarly, in this study, uNGAL was also found to be elevated in diabetics with no evidence of pathological albuminuria. This revealed that uNGAL precedes pathological albuminuria, which supports the growing hypothesis of a 'tubular phase' of diabetes that precedes the manifestation of classic glomerular lesions.¹⁵ Thus, any increase in uNGAL might express the degree of subclinical tubular impairment which may contribute to timely diagnosis leading to better prognosis. As it is depicted, uNGAL may represent an earlier, non-invasive index of renal involvement in the progression of diabetes.

In further studies, uNGAL measurements ideally should be correlated with other potential biomarkers of renal injury like Cystatin C, KIM-1(Kidney Injury Molecule 1) and Beta – 2 microglobulin etc. to ascertain its degree of sensitivity and specificity as a marker of renal injury.

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