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Comparison of Urinary NGAL With Conventional Renal Function Markers in Type 2 Diabetic Patients

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ABSTRACT

Objective: To compare and correlate urinary neutrophil gelatinase-associated lipocalin (NGAL) with serum creatinine and creatinine clearance, as biomarkers of renal injury in type 2 diabetic patients. **Materials and Methods:** It was a cross-sectional study conducted at Shaikh Zayed Hospital, Lahore in the Department of Biochemistry from October 2009 to October 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'. uNGAL was measured using Bioporto's NGAL Rapid Elisa Kit 037. Serum creatinine was estimated on fully automated chemistry analyzer. Creatinine clearance was calculated by using the Cockcroft and Gault formula. uNGAL levels were compared and correlated with the sCreatinine and creatinine clearance of the Diabetic and the Control group. **Results:** The diabetic group showed elevated sCreatinine and significantly low creatinine clearance along with highly increased levels of uNGAL, when compared with the controls. When correlated, uNGAL had a significant positive correlation with sCreatinine and a highly significant negative correlation with creatinine clearance of the female diabetics only. **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

Diabetic nephropathy is one of the most common micro vascular complications of diabetes mellitus, which greatly affects the life quality and survival of the patients. It develops in more than 40% of all type 2 diabetic patients.[1] For quite a long time, the impaired renal function of these patients is assessed by serum creatinine, and blood urea nitrogen, both of which are insensitive to early changes in renal function.[2]

Serum creatinine has been used to screen for kidney diseases for almost 80 years.[3] Wu and Parikh state the limitations of serum creatinine being influenced by muscle mass, gender, age, race, medications and its delayed elevation till the injury becomes well established.[4] Recently, it has been postulated, that a substantial number of patients have evidence of tubular injury without significant elevation in their serum creatinine.[5] Creatinine clearance correlates better with GFR than creatinine but it can only signify whether renal function is normal, moderately or severely reduced.[6] Heymsfield and colleagues have reported that relation between body mass index and serum creatinine or creatinine clearance is also inconsistent.[7] In agreement with the stated fact, Wu and Parikh conclude that serum creatinine and creatinine clearance are late biomarkers of diabetic nephropathy.[4]

In recent years, NGAL has emerged as one of the most promising biomarkers in the diagnostic field of acute and chronic renal diseases. Human NGAL was discovered in 1993.[8] It was originally identified as a 25 Kda, glycosylated protein covalently bound to Gelatinase from human neutrophils.[9,10] Later, it was also found to be located in bone marrow, lung, bronchi, stomach, small

intestine, colon, pancreas, prostate, thymus and kidneys.[11,12] It is revealed that NGAL is massively released from renal tubular cells after various injuring stimuli preceding the rise in serum creatinine.[13] It becomes highly accumulated in the cortical tubules, blood and urine after nephrotoxic and ischemic injury as shown by Mori and Nakao. They proposed that increase in NGAL is the consequence of its sustained production by inflamed but vital tubular cells, whereas the rise in serum creatinine and the decline in GFR are the mere outcome of a general loss of nephrons.[14] In patients undergoing treatments, such as contrast medium administration and cardiac surgery, the rise in NGAL level predicts the onset of acute kidney injury, anticipating the following rise in serum creatinine.[15,16]

Certain studies have stated the greater sensitivity and specificity of urinary NGAL over serum NGAL. Owing to its small molecular size, and resistance to degradation, NGAL is readily excreted and detected in the urine.[17] Importantly, NGAL derived from systemic sources does not effect urinary NGAL measurements, since any filtered NGAL is rapidly and efficiently reabsorbed by the proximal tubule.[18] Therefore, increased urinary NGAL excretion most likely results from a combination of an early intrinsic tubular cell response to injury and a later component reflecting the inability of the damaged tubule cell to completely reabsorb filtered NGAL.[19]

MATERIALS AND METHODS

Ninety five subjects, both males and females were divided into two groups. Seventy five patients above 30 years of age were included in the 'diabetic group' and 20 healthy subjects (10 males and 10 females) of the similar age were included in the 'control group'. The criteria for inclusion in the diabetic group was diagnosed type 2 diabetes mellitus for more than 10 years. Exclusion criteria was patient having acute or chronic renal disease, history of renal transplant, pregnancy or any other systemic disease or malignancy.

Serum creatinine estimation was performed on the Dimension AR Clinical Chemistry Auto analyzer. The kit was provided by Siemens Healthcare Diagnostics UK. Creatinine clearance was calculated by CG formula (Cockcroft and Gault), proposed in 1976.[20]

$$\text{Creatinine clearance (ml/min)}^{21} = \frac{(140 - \text{age}) \times \text{weight (kg)}}{72 \times \text{serum Cr (mg/dl)}}$$

Bioporto's NGAL Rapid Elisa Kit 037, made in Denmark was used to measure the amount of human NGAL in urine. The normal range is 2.1–9.6ng/ml as reported by Bolignano.[13]

Statistical analysis was done on SPSS version 15.0. Results of uNGAL, serum creatinine and creatinine clearance were expressed as mean± SEM. Student's t test was used for comparison between two groups. The association between uNGAL and other variables was observed by calculating correlation coefficient 'r'. A 'p' value of less than 0.05 was considered statistically significant.

RESULTS

There was no significant difference between the mean age, weight, height and BMI of the diabetic and control groups (Table 1) .The mean serum creatinine of male diabetics was not significantly different from that of male controls. Whereas, mean serum creatinine of female diabetics was found to be 1.06±0.06 mg/dl which was significantly higher ($p<0.001$) as compared to the mean serum creatinine of female controls which was 0.55±0.05 mg/dl. The mean creatinine clearance of male diabetics was found to be 70.44±4.82ml/min which was significantly lower ($p<0.01$) as compared to the mean creatinine clearance of male controls which was 123.77±14.58ml/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. The mean urinary NGAL 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±1.45ng/ml (Table 1).

Table 1: Age, weight, height, BMI, systolic BP, diastolic BP, FBG, uNGAL, serum creatinine and creatinine clearance in male and female groups. Mean± SEM is given. figure in parenthesis indicates number of cases in each group.

Group	Male		Female	
	Control (n=10)	Diabetics (n=33)	Controls (n=10)	Diabetics (n=42)
Age (yr)	52.90±1.97	55.91±1.13	51.40±3.07	52.36±1.22
Weight (Kg)	68.20±2.70	71.15±1.82	61.60±3.54	67.26±1.74
Height (m)	1.64±0.02	1.66±0.02	1.57±0.04	1.56±0.01
BMI (kg/m^2)	25.30±1.17	25.52±0.50	25.00±1.37	27.52±0.62
Systolic BP (mm Hg)	121.00±2.21	123.33±3.25	116.00±2.21	121.90±2.96
Diastolic BP(mm Hg)	84.90±1.91	77.58±1.57**	78.10±2.40	77.14±1.50
FBG (mg/dl)	83.30±3.72	144.18±11.53***	79.90±3.42	179.12±11.77***
Urinary NGAL(ng/ml)	5.36 ± 0.55	212.77±45.29***	7.85 ± 1.45	158.14±28.73***
Serum Creatinine(mg/dl)	0.75 ± 0.10	1.66 ± 0.31	0.55 ± 0.05	1.06 ± 0.06
Creatinine Clearance (ml/min)	123.77±14.58	70.44 ± 4.82**	124.85 ± 9.43	75.71 ± 3.63***

* $p<0.05$ significantly higher as compared to control; ** $p<0.01$ significantly higher as compared to control; *** $p<0.001$ significantly lower as compared to control

Table 2: Correlation of uNGAL with Serum Creatinine and Creatinine Clearance (CG formula). Coefficient of correlation (r) is given.

Group Compared	Control		Diabetic	
	Male	Female	Male	Female
uNGAL with serum Creatinine	0.127	0.182	0.314	0.313*
uNGAL with creatinine clearance	-0.038	0.404	-0.301	-0.446**

* $p<0.05$ significantly higher as compared to control; ** $p<0.01$ significantly higher as compared to control

uNGAL when correlated with serum creatinine of all the groups showed significant ($p<0.05$) positive correlation ($r = 0.31$) with serum creatinine of female diabetics only. Similarly, when correlated with creatinine clearance, only female diabetics showed a highly significant ($p<0.01$) negative correlation ($r = 0.45$) with uNGAL (Table 2).

DISCUSSION

uNGAL was evaluated in type 2 diabetics and was correlated with current renal function markers i.e. serum creatinine, and creatinine clearance, to assess its role as a biomarker of renal injury.

The results showed a significant rise in the serum creatinine of female diabetics and a significant decline in the creatinine clearance of both male and female diabetics. This revealed that the renal function of the male diabetics had also been compromised but was not manifested by their normal creatinine levels. This was in accordance with the study that had previously reported that many patients show evidence of tubular injury without a significant rise in their serum creatinine.[5]

The current study clearly showed that all type 2 diabetic patients had elevated uNGAL levels when compared to a well matched control group. A characteristic trend of increased uNGAL values was seen in accordance with the severity of the renal involvement. It reached higher levels in patients with manifested diabetic nephropathy as reflected by the decline in their creatinine clearance. Moreover, male diabetics, who didn't show any significant increase in their serum creatinine values, had nephropathy, which was reflected by a significant decrease in their creatinine clearance in proportion to increased uNGAL levels.

These results are in agreement with those reported in the study performed in 2009, in which type 2 diabetic patients with similar duration of diabetes, have also shown significantly higher values of uNGAL.[22]

Serum creatinine had significant positive

correlation with uNGAL in female diabetics just as is reported by Bolignano and Damman.[23,24] Creatinine clearance when correlated with uNGAL, showed a significant negative correlation in the female diabetics. Bachorzewska's work also depicted the same correlation between NGAL and creatinine clearance.[25] Hence, it is depicted that, both serum creatinine (direct correlation) and creatinine clearance (inverse correlation), the main clinical indices of renal function correlate well with uNGAL.

Diabetic nephropathy doesn't develop within the initial years of diabetes and timely diagnosis can lead to effective treatment and better prognosis. uNGAL, as seen, might become a sensitive and non – invasive tool for the evaluation of renal involvement in the progression of diabetes.

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