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Research Article

Role of Serum and Urinary Neutrophil Gelatinase Associated Lipocalin as A Biomarker in Patients with Diabetic Nephropathy

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Abstract

Diabetic nephropathy (DN) is a major complication of diabetes mellitus (DM). Early detection and intervention of DN can slow its progression and improve patient's outcomes. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of tubular damage might become a useful early biomarker for the evaluation of renal involvement in diabetic patients. We aimed to evaluate the serum and urine NGAL (sNGAL and uNGAL) in patients of diabetic nephropathy. The present study was conducted on 40 diagnosed patients of DN and 40 patients of diabetes mellitus without nephropathy. NGAL was analysed by enzyme linked immunosorbent assay (ELISA) and compared statistically between two groups. Serum and urinary NGAL levels were found significantly elevated in patients with DN as compared to DM ($p<0.05$). Therefore, NGAL might act as a potential biomarker in patients with diabetic nephropathy.

Key words: diabetes mellitus; diabetic nephropathy; sngal; ungal

1.Introduction

Diabetes mellitus (DM) is a state of metabolic dysregulation characterized by hyperglycemia which may emerge from defects in insulin secretion, insulin action, or both. Patients of DM may present with wide array of symptoms such as polyuria, polydipsia, weight loss, sometimes polyphagia, and blurred vision. It may be due to autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency or insulin resistance. [1] There are two main types of diabetes mellitus- type 1(T1DM) and type 2 (T2DM), most common being T2DM. T1DM has autoimmune pathology, is insulin dependent and mostly seen in children, whereas, T2DM is non-insulin dependent type and is commonly known as adult-onset diabetes. The associated chronic hyperglycemia in due course of time may lead to dysfunction or failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. It can lead to microvascular and macrovascular complications. Microvascular complications include nephropathy, neuropathy and retinopathy while macrovascular ones pertain mainly to cardiovascular system e.g., transient ischemic attack, angina pectoris, myocardial infarction, stroke and peripheral arterial disease. [2] Diabetic nephropathy (DN) is the leading cause of end-stage renal failure and death in diabetic patients. Hyperglycemia, increased blood pressure levels and genetic predisposition are several risk factors responsible for development of DN. [3] Increased urinary albumin excretion is an early indicator of DN which is mainly due to dysfunction of the glomerular barrier. It is categorized into two stages: microalbuminuria [with urinary albumin excretion (UAE) >20 μg/min and ≤199 μg/min] and macroalbuminuria (with UAE ≥200 μg/min). Early diagnosis of DN is crucial for its treatment. [3] Neutrophil gelatinase associated lipocalin (NGAL), also known as lipocalin -2 (LCN-2), is a member of lipocalin protein family and is encoded by LCN-2 gene on chromosome 9. It is one of the earlier proteins released from the renal tubules after ischemic or toxic damage. Tubular injury may lead to glomerular injury in diabetic patients and NGAL may be used as a biomarker to diagnose DN even earlier to development of microalbuminuria. [4] Therefore, to assess their association with DN, serum and urinary NGAL were analysed in diabetic patients with and without DN.

2. Materials and methods

For this study, 40 diagnosed patients of DN and 40 patients of diabetes mellitus were enrolled. Diagnosis was established with the help of detailed history and laboratory investigations. Staging was done according to joint committee on DN 2014. [5] All the subjects were divided into two groups: Group 1(cases)- patients with diabetic nephropathy (n=40) and group 2 (controls)- patients with diabetes mellitus without nephropathy (n=40).All the patients with diabetic nephropathy irrespective of age, sex and staging of the disease were included and the patients who were pregnant, lactating women, suffering from chronic diseases like tuberculosis, chronic liver disease, malabsorption syndromes, cardiovascular diseases, malignancies and taking vitamin supplements were excluded. Sample size was calculated keeping the power to 0.80. Blood samples and 24-hour urine samples were collected from all the subjects. Serum NGAL, urinary NGAL and albuminuria was analysed by enzyme linked immunosorbent assay (ELISA). [6-7] Routine biochemistry parameters were estimated on auto analyser (Randox Suzuka, United Kingdom) using standard kits provided by Randox laboratories and complete haemogram was done using an automated cell counter (Mindray, China, model no BC 5800). The data was compiled and analysed using Chi square test, Fisher's exact test. Quantitative data was analysed with student t-test (unpaired). Relationship between variables was analysed using Pearson's correlation coefficient. Demographic variables (i.e., age and sex) were investigated with the descriptive analysis. A probability of <0.05 was considered statistically significant.

3. Results

The mean age in cases was 55.22±10.54 years (30-70 years) and in controls was 55.2±14.90 years (24-86 years) (p>0.05). The sex ratio (male: female) was comparable as cases had sex ratio of 26:14 and controls had ratio of 27:13. The levels of serum urea, creatinine and uric acid were found statistically higher in cases as compared to controls (p <0.05) as shown in table 1. The levels of blood glucose and glycated haemoglobin (HbA1c) were not found statistically significant in the two groups (p >0.05) as shown in table 2. The difference in levels of sNGAL, uNGAL and albuminuria were found statistically highly significanty different (p<0.05) in two groups as shown in table 3. In cases, the correlation of serum HbA1c with sNGAL (r=0.16, p=0.32), HbA1c with uNGAL (r=0.01, p=0.95) and in controls the correlation of serum HbA1c with sNGAL (r=0.16, p=0.32) and HbA1c with uNGAL (r=-0.31, p=0.05) was not found statistically significant as shown in graphs 1 and 2.

		Cases	Controls	p value
Serum Urea (mg/dL)	Mean ± SD	112.75±66.04	29.80±14.6	0.00001
	Range	35-323	12-59	
Serum Creatinine (mg/dL)	Mean ± SD	4.68±2.51	0.8±0.2	0.00001
	Range	1.8-10.8	0.4-1.4	
Serum Uric acid (mg/dL)	Mean ± SD	6.09±2.99	3.9±1.57	0.00014
	Range	1.1-12.1	1.6-7.9	

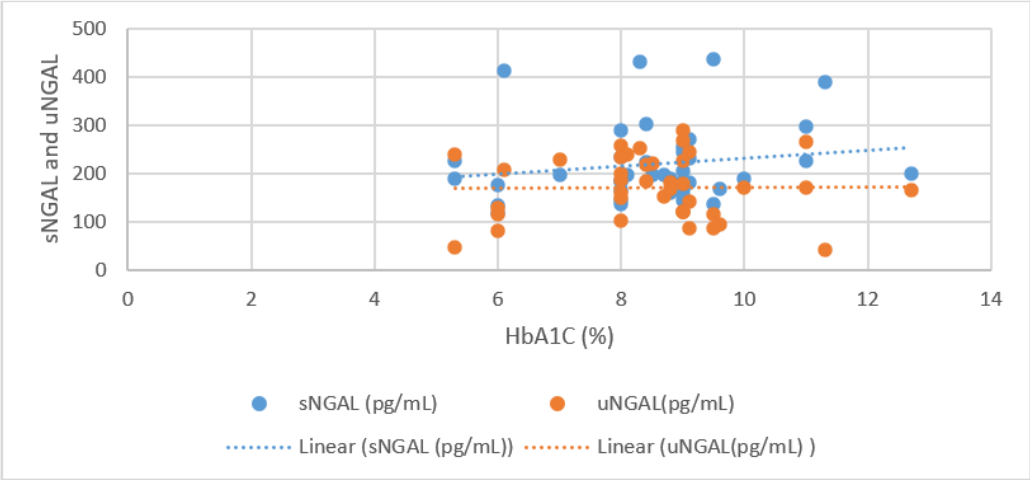
Table 1: Levels of serum urea, creatinine and uric acid in cases and controls

		Cases	Controls	p value
Fasting Glucose level (mg/dL)	Mean ± SD	178.55±59.90	160.47±51.91	0.153
	Range	110-331	110-315	
Post Prandial Glucose level (mg/dL)	Mean ± SD	265.88±88.02	253.30±93.49	0.537
	Range	135-461	150-473	
HbA1c (%)	Mean ± SD	8.51±1.56	8.03±2.23	0.270
	Range	5.3-12.7	5.4-11	

Table 2: Levels of fasting glucose, post prandial glucose and glycosylated haemoglobin (HbA1c) in cases and controls

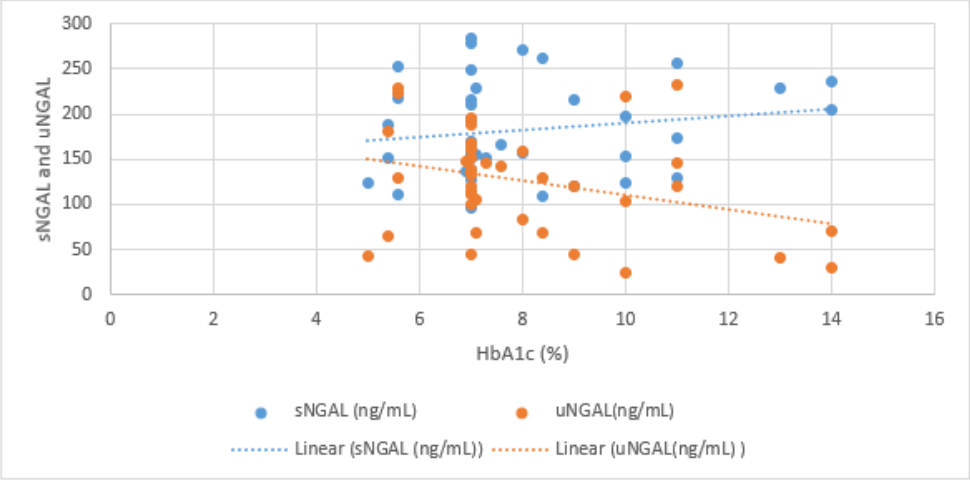
		Cases	Controls	p value
sNGAL (pg/mL)	Mean ± SD	218.91±80.70	182.21±52.35	0.0181
	Range	117.08-388.81	96.87-283.95	
uNGAL (pg/mL)	Mean ± SD	171.19±64.31	126.37±57.32	0.0015
	Range	45.76-288.46	24.30-232.76	
Urinart albumin (mg/day)	Mean ± SD	1052±288.58	137.2±54.08	0.00001
	Range	725-1580	49-267	

Table 3: Levels of sNGAL, uNGAL and urine albumin in cases and controls



Graph 1: Scattered diagram showing correlation between levels of serum HbA1c with sNGAL and uNGAL in cases

Correlation between levels of serum HbA1c with sNGAL and uNGAL in cases



Graph 2: Scattered diagram showing correlation between levels of serum HbA1c with sNGAL and uNGAL in controls Correlation between levels of serum HbA1c with sNGAL and uNGAL in controls

4. Discussion

DN is the most common and serious complication of diabetes affecting 20-40% of all the diabetic patients and it is the predominant cause of chronic kidney disease. [8] Serum levels of urea, creatinine and uric acid were found significantly higher in cases as compared to controls indicating the renal damage and reinforcing the diagnosis of DN in these patients. The serum urea is one of the established markers of glomerular damage. Several molecules like transform in growth factor beta (TGFβ), angiotensin convertase enzyme (ACE), angiotensin 1 (AT1), angiotensin 2(AT2), vascular endothelial growth factor (VEGF) and pro inflammatory cytokines are released due to alternating dilatation and relaxation of podocyte and mesangial cells. This process leads to structural and functional changes in the glomeruli, thereby, causing abnormal excretion of urea. [9] Secondly, hyperglycemia, over a span of few years, causes non-enzymatic attachment of glucose to proteins, lipids, nucleic acids resulting in the formation of advanced glycation end (AGE) products. These AGEs bind to receptors for AGE and activate the cell signaling molecules leading to increased oxidative stress and renal injury. Although, the serum creatinine is a more sensitive marker of DN as compared to serum urea levels, but its sensitivity is poor in the early stages of renal impairment because it does not rise until marked decrease in GFR occurs. In diabetic nephropathy, renal damage is to such an extent that cannot be early detected by raised serum creatinine levels due to inability of kidney to function properly and excrete creatinine. [10] The increased levels of serum uric acid in patients of diabetic nephropathy, though, incompletely understood but were found to be in accordance with the study done by Ali Momeni in diabetic patients. [11] It suggests that the increased levels of uric acid may be due to the glycosylation of inter renal proteins. Hyper glycaemia causes increase in glucose concentration within the mesangial cells leading to mesangial apoptosis and increased uric acid levels. Defective endothelial function and renin angiotensin system along with the insulin resistance are the contributing factors for increased levels of serum uric acid in patients with diabetic nephropathy. [12] Increased passage of albumin through the glomerular filtration barrier leads to microalbuminuria. Ultrastructural changes along with glomerular pressure and filtration rate are responsible for microalbuminuria. Though abnormal selective glomerular permeability can be confirmed in early DN but it does not correspond well with reported glomerular structural changes. The

systemic endothelial glycocalyx which is a protein-rich surface layer on the endothelium is severely damaged in diabetes which contributes to microalbuminuria. Microalbuminuria, systemic endothelial dysfunction and vascular disease are closely associated with one atheronals massively released in blood and urine from injured tubular cells. It is elevated even before the appearance of pathological albuminuria, possibly due to its small size. [13] Under normal circumstances, it is rapidly filtered and efficiently reabsorbed by proximal tubules, leaving only 0.1–0.2 % in the urine. [14] Source of NGAL can be renal or extra renal. Extra renal NGAL contributes to circulating NGAL, the elevated uNGAL levels could also be a consequence of higher circulating NGAL level. Therefore, both the sNGAL and uNGAL measurements contribute to determine if the increase in NGAL is from renal or extrarenal etiology. [15] NGAL is significantly released after renal tubular injury, much before the appearance of other molecules such as creatinine. There is a growing hypothesis that tubular phase of diabetic disease precedes classic glomerular lesions. For example, a tubular hypertrophy and a reduced organic ion transport activity were demonstrated to be already apparent before the onset of albuminuria, probably since the renal tubule is persistently exposed to a variety of metabolic and hemodynamic factors associated with diabetic disease. So, elevated NGAL may exhibit the degree of subclinical tubular impairment and represents an earlier measurable index of renal suffering. Therefore, it may be concluded that the serum and urinary levels of NGAL have the potential to act as biomarkers in patients with diabetic nephropathy and might possess a bearing in deciding or modifying the treatment modalities for these patients.

References:

1. Genuth S, Alberti KG, Bennett P. (2003). Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care*; 26:3160-3167.
2. Barrett EJ, Liu Z, Khamaisi M, King GL, Klein R, et al. (2017). Diabetic Microvascular Disease: An Endocrine Society Scientific Statement. *J ClinEndocrinolMetab*; 102:4343-4410.
3. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, et al. (2005). Diabetic nephropathy: diagnosis, *prevention, and treatment. Diabetes Care*; 28:164-176.
4. Kaul A, Behera MR, Rai MK, Mishra P, Bhaduaria DS, et al. (2018). Neutrophil gelatinase-associated lipocalin: as a predictor of early diabetic nephropathy in type 2 diabetes mellitus. *Indian J Nephrol*; 28:53.
5. (2020). kidney disease: Improving Global Outcomes (KDIGO) Diabetes Work Group. KDIGO Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. *Kidney* Int; 98:110-115.
6. Gan SD, Patel KR. (2013). Enzyme immunoassay and enzyme-linked immunosorbent assay. *J Invest Dermatol;* 12:133-134.
7. Ng LC, Teng LC, Ng ML, Sazali BS, Khalid BA. (2000). Microalbuminuria measurements by two in-house ELISA methods. *Malays J Pathol*; 22:73-78.
8. Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, et al. (2004). American Diabetes Association. *Nephropathy in diabetes.* Diabetes Care; 27:79-83.
9. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA. (1993). Expression of transforming growth factor beta is elevated in human and *experimental diabetic nephropathy*. Proc Natl AcadSci USA; 90:1814-1818.
10. Dabla PK. (2010). Renal function in diabetic nephropathy. *World J Diabetes;* 1:48-56.
11. Momeni A. (2012). Serum uric acid and diabetic nephropathy. *J Renal InjPrev*; 1:37-38.
12. Kanbay M, Yilmaz MI, Sonmez A, Turgut F, Saglam M, et al. (2011). Serum uric acid level and endothelial dysfunction in patients with nondiabetic chronic kidney disease. *Am J Nephrol;* 33:298-304.
13. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, et al. (2009). Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am SocNephrol* ;4: 337-344.
14. Kuwabara T, Mori K, Mukoyama M, Kasahara M, Yokoi H, SaitoY, et al. (2009). Urinary neutrophil gelatinase associated lipocalin levels reflect damage to glomeruli, proximal tubules and distal nephrons. *Kidney Int* ;75: 285-294.
15. Smertka M, Chudek J. (2011). Using NGAL as an Early Diagnostic Test of Acute Kidney Injury. Renal Failure; 34:130-133.

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