Serum Visfatin as a Novel Marker of Endothelial Dysfunction in Patients with Type 2 Diabetes

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ABSTRACT

Introduction: Atherosclerotic vascular complications are the main cause of premature morbidity and mortality of patients with CKD. Endothelial dysfunction is an early predictor of atherosclerosis. Visfatin levels negatively correlated with glomerular filtration rate (GFR) and endothelial function.

Aim: The aim of this study is to evaluate serum visfatin level in patients with type 2 diabetic nephropathy patients and to find its association with microalbuminuria.

Material and methods: Sixty type 2 diabetic subjects (T2DM) with more than 5 year diabetic duration in the age group of 35 to 60 years and thirty age and sex matched healthy controls were selected for the study. Serum visfatin, insulin levels and urine microalbumin were assessed.

Results: There was significant increase in the level of plasma visfatin, urinary visfatin in microalbuminuric diabetic patients compared to normoalbuminuric diabetic patients.

Conclusion: it is presumed that Plasma visfatin can be a novel surrogate marker for the clinical assessment of endothelial dysfunction and CVD risk in type 2 diabetes.

Keywords: Nicotinamide Phosphoribosyltransferase (NAmPRTase), Masked Hypertension (MHT), Vascular Cell Adhesion Molecule-1 (VCAM-1), Intercellular Cell Adhesion Molecule-1 (ICAM-1) and Melanoma Cells Adhesion Molecule-1 (MCAM-1)

INTRODUCTION

Diabetic nephropathy is the leading cause of chronic kidney diseases and affects ~40% of type 1 and type 2 diabetic patients. Vascular complications can be observed even in early nephropathy of diabetic and also non-diabetic subjects.[1,2] Many studies have investigated the association between microalbuminuria and atherothrombosis.[3] Endothelial dysfunction has long been proposed as one of the earliest signs of diabetic microangiopathy.[4] Recent data indicate that the association between microalbuminuria and atherothrombotic disease cannot be explained entirely by markers of endothelial dysfunction and chronic inflammation.[5] Endothelial dysfunction associated with insulin resistance appears to precede the development of overt hyperglycemia in patients with Type 2 diabetes mellitus (T2DM).[6] Therefore, endothelial dysfunction may be a critical early target for the prevention of atherosclerosis and CVD in patients with diabetes mellitus.[7]

Visfatin is an adipokine that was identified in 2004, and its name suggests that it is produced predominantly and secreted in visceral fat.[8] Visfatin gene encodes 491 amino acids with molecular weight of 52 kDa and it is identical to pre-B cell colony-enhancing factor, which was described in 1994 as a cytokine that is produced by lymphocytes that acts on the maturation of lymphocytes as a regulator of inflammatory responses. Visfatin recently recognized as the formerly described nicotinamide phosphoribosyltransferase (NAmPRTase), which is the limiting enzyme in nicotinamide adenine dinucleotide biosynthesis that is involved in the generation of reactive oxygen species (ROS).[9] Study of Mu J et al., 2011 reported that visfatin levels correlated with high sensitive C-reactive protein (hs- CRP) levels and carotid IMT, suggesting that visfatin may play an important role in uremia-related atherosclerosis.[10] In patients with CKD, visfatin levels positively correlate with soluble markers of endothelial dysfunction such as vascular (VCAM-1), intercellular (ICAM-1) and melanoma cells adhesion molecule-1 (MCAM-1).[11] The relation between visfatin levels and endothelial function in CKD is not merely descriptive, but may also have a functional impact since visfatin levels negatively correlated with glomerular filtration rate (GFR) and endothelial function estimated as flow-mediated dilation (FMD) in brachial artery.[12] Indeed, the improvement of endothelial function after kidney transplantation correlates with a reduction in circulating visfatin levels.[13] So the aim of this study is to evaluate serum visfatin level in patients with type 2 diabetic nephropathy patients and to find its association with microalbuminuria.

MATERIAL AND METHODS

Sixty Type 2 diabetic patients of both sexes with more than 5 yr diabetic duration aged between 35-60 years on oral hypoglycemic drugs, were selected for our study. The informed consent was taken from all the study subjects and the study was approved by Institutional Human Ethics Committee (IHEC). Experiments were performed in accord-

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ance with Helsinki declaration of 1975. The diabetic patients were divided into two groups according to albumin-to-creatinine ratio (ACR): Group I- Thirty patients with normoalbuminuria (<30 mg/g creatinine).Group II - thirty patients with microalbuminuria (30–299 mg/g creatinine). We excluded the patients on insulin, Smokers, Alcoholics, Tobacco chewers, urinary tract infection, history of other renal diseases and acute or chronic infection or inflammatory disorders, neoplastic disorders, liver dysfunction, thyroid disorders history of acute myocardial infarction, stroke, and peripheral vascular disease. Thirty healthy age, sex matched subjects were selected as control.

Biochemical analysis: 5 ml of venous blood samples were withdrawn from the anticubital vein from all subjects after overnight fast under aseptic conditions. Routine laboratory investigations were performed immediately using autoanalyser and aliquots were stored at −80 °C for further estimation of serum levels of insulin and visfatin.

Post prandial blood sample was collected for plasma glucose (PPG) analysis. First morning urine samples were collected in sterile container and used for microalbumin and creatinine estimation. Urine samples were centrifuged at 3000 rpm, 10 minutes to remove particulate matter and stored at −80°C for the analysis.

Routine laboratory investigations included fasting glucose, creatinine, urea,uric acid, sodium, potassium, chloride, haematological parameters,lipid profile. HbA1C is estimated by ion exchange resin method and fasting insulin (Diametra, Spello, Italy) and visfatin (Sincere Biotech; Ltd, Beijing, China) by ELISA.

Homeostasis model assessment for insulin resistance (HOMA-IR) HOMA- IR was calculated from the fasting glucose and insulin values using the formula;

HOMA – IR = fasting insulin X fasting glucose (mM/L)/22.5[14]

RESULTS

Table -1. Body mass index, systolic BP and diastolic BP were significantly elevated in diabetic patients compared to control group.

Table 2. There was significant increase in the level of FPG, PPG, HbA1c, insulin, HOMA-IR (14) and ACR in diabetic patients compared to control subjects. There was a significant increase in the level of PPG, serum insulin, HbA1C, insulin, HOMA-IR, urine microalbumin, and ACR in microalbuminuria T2DM compared to normoalbuminuria T2DM.

Table 3. Serum cholesterol, triglycerides, LDL - cholesterol were within normal limits in diabetic patients with

Parameters	Controls (n=30)	Normoalbuminuria T 2DM (n=30)	Microalbuminuria T 2 DM(n=30)
Age	46.7±4.0	47.1±6.7	49.4±4.6
Body mass index (BMI)	24.5±1.4	26.8±3.8 ^{a*}	27.5±3.4 $^{a\#}$
Waist/Hip ratio	0.93±0.04	0.94±0.06	0.93±0.05
Systolic BP(mmHg)	112.9±8.1	126.5±15.5$^{a\#}$	128.8±13.5 ^{a*}
Diastolic BP(mm Hg)	75.1±4.4	79.7±8.5 $^{a\#}$	80.5±6.9 $^{a\#}$
Duration DM (years)	-	8.4±2.1	9.4±3.3

a - Controls vs Normoalbuminuria T 2DM, Microalbuminuria T 2 DM, b -Normoalbuminuria T 2DM vs Microalbuminuria T 2 DM, * p value <0.001, # p value <0.05

Table-1: Baseline parameters in controls and study groups

Parameters	Controls (n=30)	Normoalbuminuria T 2DM (n=30)	Microalbuminuria T 2 DM(n=30)
FPG (mg/dl)	83.2±5.8	126.0±22.8^{a*}	138.3±34.4^{a*}
PPG(mg/dl)	109.8±9.5	177±20.6^{a*}	210.4±44.4^{a*,b*}
HbA1C	5.4±0.5	7.3±0.8^{a*}	8.7±0.8^{a*,b*}
Insulin (µIU/mL)	6.7±0.6	10.5±2.7^{a*}	15.2±4.4^{a*,b*}
HOMA-IR	1.43±0.19	3.2±0.8^{a*}	4.8±1.6^{a*,b*}
Urine Microalbumin (mg/L)	17.3±2.1	17.9±2.8	78.9±13.2 ^{a*,b*}
Urine creatinine (mg/dl)	87.3±5.8	79.7±11.1^{a*}	69.5±19.9 $^{a*,b\#}$
Urine albumin Creatinine ratio (ACR)(mg/gm. of creatinine)	19.6±2.9	24.8±3.2 ^{a*}	133.3±42.9 ^{a*,b*}

Table-2: Plasma glucose, serum insulin, HbA1c, HOMA-IR, urine microalbumin, urine creatinine and ACR in controls and study groups.

Parameters	Controls (n=30)	Normoalbuminuria T 2DM (n=30)	Microalbuminuria T 2 DM(n=30)
Serum cholesterol (mg/dl)	168.9±8.8	185.3±19.4^{a*}	194.9±22.7^{a*}
Serum Triglycerides (mg/dl)	95.9±7.1	130.1±35.9^{a*}	140.2±35.0^{a*}
HDL cholesterol (mg/dl)	44.0±2.4	39.2±2.9^{a*}	38.5±2.4^{a*}
LDL cholesterol (mg/dl)	105.7±8.8	120.0±16.0^{a*}	128.3±22.2^{a*}

Table-3: Lipid profile parameters in controls and study groups.

Parameters	Controls (n=30)	Normoalbuminuria T 2DM (n=30)	Microalbuminuria T 2 DM(n=30)
Serum urea(mg/dl)	24.5±4.3	27.8±4.2 [a#]	31.4±5.0 [a*,b#]
Serum creatinine(mg/dl)	0.6 ±0.1	0.7±0.2	0.9±0.3 [a*,b#]
Plasma Visfatin (pg/ml)	46.5±6.1	58.3±9.1 [a*]	104.0±12.8 [a*,b*]
Urine Visfatin (pg/mg of Creatinine)	21.0±3.7	41.3±7.6 [a*]	68.9±30.5 [a*,b*]

Table-4: Plasma and urinary visfatin, Renal profile parameters in controls and study groups.

Parameters	Plasma Visfatin Correlation Coefficient (r)	Urinary Visfatin Correlation Coefficient (r
Albumin Creatinine Ratio	0.689**	0.582**
FPG	0.234	0.113
PPG	0.354*	0.298*
HbA1C	0.387**	0.279*
HOMA-IR	0.421**	0.321**
Cholesterol	0.245	0.115
TGL	0.337**	0.345**
HDL	-0.297*	-0.282*
LDL	0.153	0.185

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed)

Table-5: Correlation between plasma visfatin and urinary visfatin with albumin creatinine ratio, FPG, PPG, HbA1C, HOMA-IR, serum triglycerides and HDL cholesterol

normoalbuminuria and microalbuminuria even there is a significant difference compared to controls. HDL - cholesterol significantly decreased in diabetic patients but within the normal range.

Table 4. There was significant increase in the level of plasma visfatin, urinary visfatin in diabetic patients compared to control subjects. The levels of plasma visfatin, urinary visfatin in microalbuminuric diabetic patients was significantly increased compared to normoalbuminuric diabetic patients. Serum urea, creatinine were within normal limits in diabetic patients even there is a significant difference between control and study groups.

Correlation analysis demonstrated a positive association between plasma and urinary visfatin with albumin creatinine ratio, PPG, HbA1C, HOMA-IR, serum triglycerides and negative association with HDL cholesterol.

STATISTICAL ANALYSIS

Statistical analyses were carried out with SPSS 20.0. Values were expressed as mean ± standard deviation, p value < 0.05 was considered statistically significant. Normally distributed data were explored by using one-way ANOVA

DISCUSSION

Diabetes is a major cause of both microvascular and macrovascular complications. In our study diabetic patients had increased BMI, waist to hip ratio and higher systolic blood pressure compared with age- and sex-matched healthy controls (Table 1). The development and the complications of T2DM is due to the complex interaction between genetic and environmental factors, mainly by lifestyle and dietary habits which can either accelerate or slow down disease progression.[15] Takeno et al., 2012[16] reported that masked hypertension (MHT) and vascular damage in patients with type 2 diabetes, could be also at increased risk of cardiovascular disease. Improvement in endothelial function, inflammatory activity and urinary albumin excretion by antihypertensive therapy has been reported in hypertensive type II diabetic individuals, regardless of the type of antihypertensive therapy used.[17]

In our study we observed significantly high serum cholesterol, LDL and TGL in T2DM compared with healthy controls. Hyperglycemia causes alterations in lipid metabolism which bring about a series of adverse effects, including enhanced HDL clearance, decreased apoA-1 transcription and accelerated HDL glycation leads to atherosclerosis.[18,19]

We have observed that HOMA-IR was significantly increased in microalbuminuric diabetics compared with normoalbuminuric diabetics. Insulin resistance may be linked to endothelial dysfunction by a number of mechanisms, including disturbances of sub cellular signaling pathways common to both insulin action and nitric oxide (NO) production. The presence of microalbuminuria reflecting a state of generalized endothelial dysfunction, is a risk factor for cardiovascular disease and mortality and it is used as a predictor of cardiovascular end organ damage especially diabetic nephropathy.[20]

The present study demonstrated that plasma and urinary visfatin levels were significantly increased in T2DM patients compared with healthy individuals. We found a positive association between proteinuria and the intracellular protein NAMPT/visfatin as reported earlier studies.[21] Several studies reports that visfatin could be a surrogate marker of systemic inflammation in type 2 diabetic patients.[22] Visfatin has been implicated in the pathogenesis of various metabolic disorders, such as metabolic syndrome, type 2 diabetes mellitus and obesity.[23,24] Visfatin induces the cellular expression of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), IL-8, IL-6 and MMP-9 by activating the p38-MAPK signaling pathway which may help perpetuating vascular inflammation.[25] Visfatin is associated with endothelial adhesion molecules and FMD%, suggesting that visfatin is an important biomarker for prediction of endothelial dysfunction and future coronary heart diseases in chronic kidney disease patients.[26] A positive association between plasma and urinary visfatin with albumin creatinine ratio, PPG, HbA1C,HOMA-IR,serum triglycerides and negative association with HDL cholesterol were observed(table5). Visfatin is to be involved in the

promotion of proatherogenic activities through changes in lipid metabolism and endothelial smooth muscle function.[27]

CONCLUSION

Hence monitoring of Serum visfatin levels could be a novel surrogate marker of endothelial dysfunction and coronary artery disease risk in type 2 diabetic patients. Further prospective studies are required to prove this hypothesis.

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