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Correlation of Beta ig-h3 levels with Albumin Creatinine Ratio in Type 2 Diabetic Patients

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Abstract

Background: *Diabetic nephropathy may be a consequence of the actions of certain cytokines and growth factors. Prominent among these is transforming growth factor beta (TGF-beta) because it promotes renal cell hypertrophy and stimulates extracellular matrix accumulation.*

Aim: *The aim of this study was to evaluate the plasma and urinary βig-h3 levels in type 2 diabetic patients and examined the association with albumin creatinine ratio.*

Materials and methods: *Sixty type 2 diabetic patients with more than 5 year diabetic duration in the age group of 35 to 60 years were selected for this study and 30 age matched healthy individuals were selected as control group. Plasma and urinary beta ig-h3 levels were assessed by ELISA method and microalbumin by turbilatex method.*

Results: *The plasma and urinary beta ig-h3 levels were significantly increased in type 2 diabetic patients compared with healthy control group, and significantly increased in microalbuminuric diabetic patients compared to normoalbuminuric diabetic patients. The plasma and urinary beta ig-h3levels were positively correlated with the albumin creatinine ratio (ACR), glycated hemoglobin (HbA1C) and insulin resistance and negatively correlated with HDL cholesterol.*

Conclusion: *Plasma and urinary β ig-h3 could be useful to identify the early stage of diabetic nephropathy.*

Key Words: *Diabetic nephropathy, Beta ig-h3, Albumin creatinine ratio*

I. Introduction

Diabetic nephropathy (DN) is a major complication of type 2 diabetes mellitus. DN is the most common cause of end-stage renal disease, and it markedly enhances the risk of cardiovascular events [1]. The progressive increase in urinary albumin excretion coupled with elevated blood pressure leading to declined glomerular filtration are characteristics of DN [2]. Consequently, it is necessary to identify the factors responsible for these abnormalities and that facilitate the progression of renal complications [3, 4]. Of these, the cytokine transforming growth factor-β (TGF- β) is a pro-sclerotic growth factor implicated in the pathogenesis of diabetic nephropathy. Beta ig-h3 (Transforming growth factor-beta-induced protein TGFBI) is an extracellular matrix protein which is induced in many cells by TGF- β and key factor in the development of renal hypertrophy and the accumulation of extracellular matrix [5-7]. Beta ig-h3 forms a fibrillar structure and interacts with several other extracellular matrix proteins, including fibronectin and type I collagen [8]. Beta ig-h3 is also known to affect cell growth and differentiation [9-11]. Since beta ig-h3 is an extracellular matrix protein that plays a role in a wide range of physiological and pathological conditions, we explored the importance of plasma and urinary βig-h3 levels in type 2 diabetic patients and examined the association with albumin creatinine ratio (ACR).

II. Materials And Methods

A total of 60 type 2 diabetic patients of both sexes with more than 5 year diabetic duration , aged between 35-60 years on oral hypoglycemic drugs, attending diabetic out-patient department of Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamil Nadu, India, were selected for our study . The included diabetic patients were categorized into two groups based on albumin creatinine ratio (ACR). Groups were divided as follows: 30 patients with normoalbuminuria (<30 mg/g creatinine), 30 patients with microalbuminuria (30–299 mg/g creatinine).We excluded the patients based on the following criteria: Patients on insulin, smokers, alcoholics, tobacco chewers, abnormal urinary sediment, urinary tract infection,

history of other renal disease and active or chronic persistent infection or inflammatory disorders, neoplastic disorders, thyroid disorders, liver dysfunction, history of acute myocardial infarction, stroke, and occlusive peripheral vascular disease. Thirty healthy age and sex matched subjects were selected as controls. The informed consent was obtained from all the study subjects and the study was approved by the Institutional Human Ethics Committee (IHEC). Experiments were done in accordance with Helsinki declaration of 1975.

Biochemical analysis

A fasting blood and urine samples were obtained from the subjects immediately after enrolment. Blood samples were centrifuged at 2000×g for 10 min. Samples were analyzed for routine investigations plasma glucose (FPG), lipid profile (Total Cholesterol, HDL, and Triglycerides), glycosylated hemoglobin (HbA1C) and urine microalbumin, urinary creatinine. Plasma and urinary beta ig-h3, insulin assessed by ELISA and the 2 hour post prandial venous plasma glucose (PPG) estimation was also done.

Statistical analysis

Statistical analysis 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 analyzed by using one-way ANOVA. The Pearson correlation test was used for correlation analysis.

III. Results

Table 1: Baseline parameters in control and type 2 diabetic patients

Parameters	Controls (n=30)	Normoalbuminuria T2DM (n=30)	MicroalbuminuriaT2DM (n=30)
Age	47.7±3.9	48.1±6.7	49.1±4.4
Body mass index (BMI)	24.3±1.3	26.9±3.6 [a*]	26.5±3.5 [a#]
Waist/Hip ratio	0.91±0.03	0.92±0.06	0.92±0.04
Systolic BP(mmHg)	113.9±7.1	123.5±15.5[a#]	126.6±12.5 [a*]
Diastolic BP (mm Hg)	74.1±3.4	78.7±7.5 [a#]	78.5±6.6 [a#]
Duration DM (years)	-	8.1±2.0	9.2±3.0

Data are expressed as mean ±SD, *p<0.001, #p<0.05 was considered statistically significant.
a - Controls vs Normoalbuminuria T 2DM, Microalbuminuria T 2 DM
b -Normoalbuminuria T 2DM vs Microalbuminuria T 2 DM

Table 2: ACR, FPG, PPG, HbA1C, HOMA-IR, Lipid profile, Urea, Creatinine and Beta ig-h3 levels in control and type 2 diabetic patients

Parameters	Controls(n=30)	Normoalbuminuria T2DM (n=30)	MicroalbuminuriaT2DM (n=30)
Urine albumin Creatinine ratio (ACR) (mg/gm. of creatinine)	18.6±2.7	22.8±3.2 [a*]	127.3±40.9 [a*,b*]
FPG (mg/dl)	82.2±5.7	123.0±22.6 [a*]	135.3±33.4 [a*]
PPG(mg/dl)	107.8±9.2	170±20.7 [a*]	203.4±42.4 [a*,b*]
HbA1C	5.5±0.4	7.1±0.8 [a*]	8.2±0.9 [a*,b*]
HOMA-IR	1.3±0.18	3.1±0.9 [a*]	4.7±1.6 [a*,b*]
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*]
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 β ig- h3 (ng/ml)	2.4±0.7	5.3±1.4 [a*]	12.0±2.9 [a*,b*]
Urinary βig -h3 (ng/mg of Creatinine)	1.2±0.5	4.5±1.7 [a*]	7.9±3.8 [a*,b*]

Data are expressed as mean ±SD, *p<0.001, #p<0.05 was considered statistically significant.
a - Controls vs Normoalbuminuria T 2DM, Microalbuminuria T 2 DM
b -Normoalbuminuria T 2DM vs Microalbuminuria T 2 DM

Table 3: Correlation between plasma beta ig-h3 &measured parameters in type 2 diabetic patients

Parameters	**Correlation Coefficient(r)**
Albumin Creatinine Ratio	0.934**
Urinary beta ig-h3	0.787**
FBS	0.274*
PPBS	0.374**
HbA1C	0.503**
HOMA-IR	0.481**
Cholesterol	0.386**
TGL	0.252
HDL	-0.310*
LDL	0.178

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

Table 4: Correlation between urinary beta ig-h3 &measured parameters in type 2 diabetic patients

Parameters	Correlation Coefficient(r)
Albumin Creatinine Ratio	0.771***
FBS	0.054
PPBS	0.060
HbA1C	0.381**
HOMA-IR	0.422**
Cholesterol	0.297*
TGL	0.178
HDL	-0.284*
LDL	0.126

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

IV. Discussion

Diabetic nephropathy is characterized by hypertrophy of the glomerular, tubuloepithelial structures and thickening of glomerular, tubular basement membranes and progressive accumulation of extracellular matrix proteins (fibronectin, collagens and laminin) in the mesangium and the interstitium [12]. The manifestations of DN may be a consequence of the actions of certain cytokines and growth factors such as transforming growth factor beta (TGF-beta) because it promotes renal cell hypertrophy and stimulates extracellular matrix accumulation [13].

In the present study, we observed that plasma and urinary beta ig-h3 levels were significantly increased in type 2 diabetic patients compared with healthy controls and there was also significant difference observed in microalbminuric diabetic patients compared with normoalbuminuric diabetic patients. High glucose as well as glycated albumin and advanced glycation end products (AGE) induce TGF-β over expression in mesangial cell culture, but not in podocytes [14-16]. Furthermore, high glucose and exogenous TGF-β1coordinately increased α3 (IV) collagen and vascular endothelial growth factor (VEGF) expression. Thus, high glucose seems to enhance the effects of ambient TGF-β1 on α3 (IV) collagen and VEGF production by increasing the expression of TGF-β type II receptor [17]. Song et al., [18] reported that rat mesangial cells grown in high glucose concentration expressed decreased collagenase activity, and this action was mediated by autocrine TGF-β.

In this study, plasma and urinary beta ig-h3 levels showed strong positive correlation with ACR, HbA1C and HOMA-IR, and negative correlation with HDL cholesterol. The chronic hyperglycemia, increased non-enzymatic glycation of proteins, de novo synthesis of diacylglycerol and subsequent activation of protein kinase C, increased intracellular glucosamine production, and enhanced renal production of vasoactive agents (angiotensin II, endothelins, thromboxane) have all been shown to increase the expression of TGF-β in cultured renal cells and animal models of diabetic nephropathy [19-21]. So, the development of glomeular hypertrophy and glomerulosclerosis is likely caused by high activity of the TGF-β system [22-27].

The incipient diabetic kidney disease are characterized by an increase in kidney size, glomerular volume, enhanced glomerular filtration rate (GFR) and later by the development of mesangial cell proliferation, accumulation of glomerular extracellular matrix, increased urinary albumin excretion, glomerular sclerosis, and tubular fibrosis. The subsequent overt diabetic nephropathy is clinically characterized by proteinuria, hypertension, and progressive renal insufficiency [28, 29]. Although microalbuminuria is usually considered the first clinical expression of DN, some normoalbuminuric T2DM patients have significantly more advanced diabetic glomerulopathy lesions with normal GFR [30, 31]. Recent studies have suggested that TGF-β might initiate the early diabetic renal changes and might be critically involved in the development of diabetic kidney disease [32, 33].

Hence, we conclude that plasma and urinary β ig-h3 could be useful to identify the early stage of diabetic nephropathy.

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