World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Correlation of spot urine protein creatinine ratio with twenty four urine protein and serum lipid profile in type 2 diabetic nephropathy patients

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Received: 11-10-2014 / Revised: 21-11-2014 / Accepted: 26-11-2014

ABSTRACT

The present study aimed to analyze the association between proteinuria as measured by 24 urine protein and urine protein creatinine ratio, and lipid variables in diabetic patients with or without nephropathy. The study subjects were sixty four diabetic patients with nephropathy and fourty seven diabetic patients without complications. Levels of triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol were estimated in serum samples. In 24 hour urine samples, total protein was estimated, and in spot urine samples, levels of creatinine and protein were estimated. Diabetic nephropathic patients showed significantly higher serum levels of triglycerides and significantly lower serum level of HDL cholesterol, in comparison to diabetic patients without complications. The total cholesterol and LDL cholesterol levels did not differ between the two groups. Urinay protein excretion (24 hours) and urine protein creatinine ratio were significantly higher in patients with diabetic nephropathy than the diabetic without complications. Urinary protein: creatinine ratio showed significant positive correlation with 24 hr urine protein and serum triglycerides, and negative correlation with serum HDL cholesterol, in diabetic nephropathic patients. Urine protein creatinine ratio had sensitivity of 100%, specificity of 90.90%, and positive prediction rate of 91.67% to diagnose diabetic nephropathy. Future studies with larger sample size correlating urine protein: creatinine ratio with clinical staging of nephropathy, and analyzing its value in prognosis and follow up of treatment, are required.

Keywords: HDL level, LDL level and Nephropathy

INTRODUCTION

Diabetic nephropathy and proteinuria*:* Diabetic nephropathy is characterized by persistent proteinuria, arterial hypertension, declining glomerular filtration rate and plasma lipid abnormalities (Selby et al., 1990). It is the leading cause of chronic kidney disease in patients starting renal replacement therapy. Diabetic nephropathy has been classsifically defined by the presence of proteinuria>0.5 g/24 hours (Gross et al., 2005). This stage has been referred to as overt nephropathy, macroalbuminuria or proteinuria.

The mechanism by chronic hyperglycemia leads to end stage renal disease is incompletely defined. However, it involves interaction of soluble factors such as growth factors, angiotensin II etc, hemodynamic alteration in the renal microcirculation and structural changes in glomerulus. Proteinuria is the hallmark of diabetic nephropathy. Often, a phase of microalbuminuria precedes the phase of clinically evident proteinuria. In microalbuminuria, the albumin concentration is too low to be detected by dipstick method but can be measured by radioimmunoassay or immuno-turbidimetric method (Inomato et al., 1989). Proteinuria is the most important early predictor of renal injury by current standards. 24-h urine collections to evaluate total protein excretion have been replaced in pediatric practice with random urine total protein: creatinine and urine albumin: creatinine ratios (Hogg et al., 2000; Leung et al., 2007; Xin et al., 2004). Multiple studies in adults and children have validated the use of randomly voided samples to estimate the total protein excretion in individuals (Hogg et al., 2000; Abitbol et al., 2006).

Diabetic nephropathy and cardiovascular risk*:* There is accumulating evidence suggesting that the risk for developing diabetic nephropathy and cardiovascular disease starts when urinary excretion rate values are still within the normal range (Gross et al., 2005). Cardiovascular disease

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is a common complication both in type 1 and type 2 diabetes mellitus, especially for patients with diabetic nephropathy. The endothelial cell adhesion molecules, intercellular adhesion molecule-1or vascular cell adhesion molecule-1, play a crucial role in the initiation of atherosclerosis. Levels of both cell adhesion molecules are raised by the diabetic and kidney disease states (Wu, et al., 2005). Dyslipidemia contributes to the development of microvascular diseases in diabetes (Thomas et al., 2006). Studies have attempted to establish the association of microalbuminuria and serum lipids in diabetic nephropathy, and observed that the relationship depended on the stage of the disease and wide variations due to genetic factors (Thomas, 2006).

Objectives of the Present Study: In the present study we have made an attempt to analyze the association between proteinuria as measured by 24 urine protein and urine protein creatinine ratio, and lipid variables in diabetic patients with or without nephropathy.

MATERIALS AND METHODS

***Study subjects*:** This hospital based study was done at GEMS Medical College and Hospital. The study duration was from May 2014 to September 2014. Diabetic patients visiting the outpatient department and diabetic nephropathic patients admitted in nephrology ward for treatment were the subjects of this study. Group-1 comprised of type 2 diabetic nephropathic patients (n=65) and Group-2 comprised of Diabetic patients without any complications (n=47). The diagnosis of diabetic nephropathy was based on clinical examination, urine microalbumin and glycated hemoglobin. The age group of the patients was 35 to 60 years, and both males and females were included in the study. Patients with co morbid hypertension, ischemic heart disease, or other significant medical illness were excluded.

Sample collection and assays done: Blood samples were collected taking aseptic precautions, and sera were separated. Both random and 24 hour's urine samples were collected. In the serum samples, levels of triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol were estimated by standard, enzymatic spectrophotometric methods (Burtis et al., 2012; Tietz, 2006). In 24 urine samples, total protein level was estimated by turbidometrtic methods (Burtis et al., 2012; Tietz, 2006). In random urine samples, total protein was estimated by turbidometric method and creatinine was estimated by Jaffe's kinetic method (Burtis et al., 2012). The assays were done Cobas 6000 automated analyzer using reagent kits of Roche Diagnostics. The procedures were as per manufacturer's kit inserts. Internal and external quality control programmes were employed to ensure precise and accurate results.

Data analysis: The obtained data were expressed as mean ± S.D. and significance of results was evaluated by Student's "t" test. Karl Pearson's Correlation analysis was done to evaluate correlation among biochemical parameters.

RESULTS

The results of this study are presented in table 1. Diabetic nephropathic patients showed significantly higher serum levels of triglycerides and significantly lower serum level of HDL cholesterol, in comparison to diabetic patients without complications. The total cholesterol and LDL cholesterol levels did not differ between the two groups. Urinay protein excretion (24 hours) and urine protein creatinine ratio were significantly higher in patients with diabetic nephropathy than the diabetic without complications. There was a significant positive correlation ($r=0.84$, $p<0.001$) between urinary protein and urinary protein-creatinine ratio among patients with diabetic nephropathy. Diabetic nephropathicpatients also showed a significant negative correlation of 24 hr urine protein with serum HDL cholesterol ($r= -0.62$, $p <0.001$). Urine protein-creatinine ratio showed significant positive correlation with serum triglycerides ($r = 0.765$, $p<0.001$), and negative correlation with serum HDL cholesterol ($r= -0.678$, $p<0.001$). Using the optimal spot urine protein-creatinine ratio cut off point of 0.20, all the 33 patients with more than 300 mg/24 h of protein excretion could be identified, giving a sensitivity of 100%, specificity of 90.90%, and positive prediction rate of 91.67%. The spot urine protein-creatinine ratios less than 0.19 yielded a sensitivity of 100% for exclusion of significant proteinuria.

Table 1: Biochemical Parameters in Serum and Urine of Diabetic Patients (values are mean and SD of number of samples indicated)

Parameters	Group-1 : Type 2 DM With Nephropathy (n=65)	Group-2 : Type 2 DM Without Complications (n =47)
Serum Total Cholesterol (mg/dl)	211 ± 23.2	201 ± 17
Serum LDL Cholesterol (mg/dl)	112 ± 21	105 ± 18
Serum HDL Cholesterol (mg/dl)	31 ± 9 *	43 ± 5.5
Serum Triglycerides (mg/dl)	179 ± 36 *	119 ± 21
24 hr urinary protein (mg/day)	326 ± 57 *	38 ± 11
Urinary protein-creatinine ratio, spot urine	0.37 ± 0.16 *	0.07 ± 0.01

*Statistically significant difference when compared to group-2; $p<0.001$.

DISCUSSION

The present study made an attempt to assay the levels of 24 hour urine proteins, urine protein to creatinine ratio, and serum levels of lipids in diabetic patients with and without nephropathy. This study observed significant changes in serum triglycerides, serum HDL cholesterol, urine protein protein to creatinine ratio and 24 hours urine protein in patients with diabetic nephropathy when compared to diabetic patients without complications. There was significant correlation of urine protein to creatinine ratio with 24 hours urine protein, serum triglycerides and serum HDL cholesterol. We observed increased levels of triglycerides and decreased levels of HDL-cholesterol in the serum in patients with diabetic nephropathy. Previous studies have shown that lipid abnormalities in patients with diabetes mellitus is a major problem and associated with increased risk of cardiovascular disease (Onuigbo et al., 2011). The most common pattern of dyslipidemia in such patients consists of elevated levels of serum triglyceride (TG) and low levels of HDL-cholesterol. In the present study, we observed that urine protein creatinine ratio showed positive correlation with serum triglycerides and negative correlation with serum HDL cholesterol in diabetic nephropathy patients. The mechanisms for dyslipidemia in diabetic nephropathy are multifactorial and complex.

Long-term hyperglycemia is proposed to cause generalized vascular endothelial damage, which reduces functional lipoprotein lipase, leading to increased triglyceride (TG) levels and decreased high-density lipoprotein cholesterol (HDL-C). In overt-diabetic nephropathy, hypoproteinemia markedly increases low-density lipoprotein cholesterol (LDL-C), and renal failure specifically increases remnant lipoproteins and decreases HDL-C and LDL-C (Hirano, 2013). For quantification of proteinuria, timed urine collections (usually performed over 24 hours) were considered as gold standard, but have major limitations like time consumption and error in sample collection. Estimation of total protein in spot urine sample, avoids the 24-hour urine collection, but is largely influenced by the body hydration (Biradar et al., 2011). This variation can be eliminated by factoring the spot urine total protein concentration by urine creatinine concentration, i.e. the urine protein to creatinine ratio (Chitalia et al., 2001; Torng et al., 2001; Xin et al., 2004). In the present study, we observed significant positive correlation of urine protein creatinine ratio with 24-hour protein excretion. Assaay of urine protein creatinine ratio requires a random urine sample unlike the inconvenience of collecting 24 hour urine sample especially in outpatients for assessing proteinuria in diabetic nephropathy. This study has shown that urine protein creatinine ratio had a sensitivity of 100%, specificity of 90.9% and positive prediction rate of 91.67% in identifying patients with 24 urine protein excretions of > 300 mg/24 hours. These findings are in concordance with earlier which observed high sensitivity and specificity of urine protein creatinine ratio in diagnosis of diabetic nephropathy (Biradar et al., 2011; Chitalia et al., 2001; Torng et al., 2001).

This study made an attempt to analyze the clinical utility of urine protein creatinine ratio in spot urine sample as an alternative to 24 hours urine protein estimation. It has also correlated the urine protein creatinine ratio with serum lipid profile. Thus, the clinical significance of spot urine protein creatinine ratio as a rapid diagnostic test for diabetic nephropathy, and as a predictor of cardiovascular risk by its correlation of dyslipidemia, is evident from this study.

Conclusions

Urine protein creatinine ratio is a sensitive and specific marker of clinical use in diagnosis and prognosis in diabetic nephropathy. There was a significant correlation of proteinuria with dyslipidemia in diabetic nephropathy.

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