

Serum adiponectin among different stages of type 2 diabetes mellitus patients

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Background: Type 2 diabetes mellitus is a chronic metabolic disorder that leads to micro and macrovascular complications. Nephropathy is the most common microvascular complication. For diagnosis of nephropathy in T2DM patients by using urinary albumin, this is not a sensitive and specific biomarker because it is elevated in other conditions. we aimed to evaluate the serum adiponectin for early detection of nephropathy in patients with type 2 diabetes mellitus. **Methods:** A total of 60 subjects were included in the present study and further subdivided into 30 patients T2DM with normoalbuminuria, 30 patients with microalbuminuria. All the patients were included after obtaining institutional ethical permission and consent forms. Blood and urine samples were collected from all the subjects and proceed with further analysis. Appropriate statistical analyses were used for different types of data analysis. **Results:** Increased levels of FBS, PPBS, HbA1C and serum adiponectin were observed in two groups of T2DM patients. Statistically elevated levels of serum Urea, Creatinine and Uric acid levels were observed in patients T2DM with microalbuminuria when compared to T2DM with normoalbuminuria. The serum adiponectin was positively correlated with FBS, PPBS, HbA1C, Urea, Creatinine, Uric Acid and Urinary Albumin in patients with two groups of T2DM. **Conclusion:** This study concluded that measuring the serum adiponectin levels may be useful for the early detection of nephropathy in patients with T2DM.

Keywords: Type 2 Diabetes Mellitus, HbA1C, Adiponectin and Urine Albumin

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Introduction

Type 2 diabetes mellitus is a chronic metabolic disease due to both genetic and environmental factors play an important role in insulin resistance. In this condition, β -cell function in the pancreas declines gradually over some time before the onset of clinical hyperglycemia. Several mechanisms have been proposed for insulin resistance, including increased non-esterified fatty acids, inflammatory cytokines, adipokines, and mitochondrial dysfunction, whereas glucotoxicity, lipotoxicity and amyloid formation have been attributed to be responsible for β -cell dysfunction [1-2]. Diabetic nephropathy (DN) is one of the major and most common microvascular complications in patients with T2DM and develops as progressive kidney disease as a result of angiopathy of capillaries in the renal glomeruli and causes nephrotic syndrome and diffuse glomerulosclerosis [2-4]. DN is presently the single commonest cause of end-stage kidney failure worldwide and is acknowledged as an independent risk factor for cardiovascular disease [5]. Microalbuminuria is defined as albumin excretion of 30-229 mg/24 hours and proteinuria as protein excretion of > 500 mg in 24 hours in the setting of diabetes. Screening for diabetic nephropathy may be accomplished by either a 24-hour urine collection or a spot urine measurement of microalbumin [6-7]. Poor glycemic control is identified as a critical etiological factor for nephropathy in diabetes patients. Studies have shown that high HbA1c levels which are reflective of poor metabolic control are associated with an increased incidence of nephropathy [8]. In addition, hyperglycemia associated metabolic complications such as the formation of advanced glycation end products (AGEs) also contributes to renal disease in diabetes [9]. AGEs are formed as a result of the irreversible attachment of reducing sugars to the amino acids of proteins and are proposed to be involved in the pathophysiological mechanisms underlying diabetic nephropathy through the generation of oxidative stress, increased production of cytokines and growth factors and also by interacting with the renin-angiotensin-aldosterone system [10-11].

Adiponectin is an adipocytokine and it has anti-inflammatory, antiatherogenic, and antidiabetic properties. It is also normally present on the endothelium and in the smooth muscle cells of intrarenal arteries and on the endothelium of glomerular and peritubular capillaries.

Circulating adiponectin exists mainly in three isoforms which include a low molecular weight trimeric form (LMW), a hexameric middle molecular form (MMW) and a high molecular weight form (HMW) consisting of 12-36 monomers; globular form and full-length form are the minor circulating fractions of adiponectin [12-13]. The mean plasma concentration of adiponectin was reported to be higher compared to other adipocytokines such as leptin and IL-6, ranging from 5-30 mg/L or 5-30 μ g/mL, accounting for about 0.01% of all plasma proteins in humans [14]. The plasma half-life of adiponectin is seventy-five minutes and the protein is mainly cleared by the liver and also kidneys. The high molecular weight form has the slowest clearance rate and relatively constant serum levels. Adiponectin acts through three cellular receptors namely, Adiponectin receptor 1 (AdipoR1), Adiponectin receptor 2 (AdipoR2), and T-cadherin. Adiponectin plays an important role in metabolic regulation and energy homeostasis, mainly through its effects on carbohydrate and lipid metabolism [15]. Adiponectin inhibits hepatic gluconeogenesis by inhibiting genes involved in glucose production thereby decreasing glucose levels.

The glucose-lowering effect appears to be independent of insulin. Through its local action in key metabolic tissues, adiponectin promotes insulin sensitization and therefore improves whole-body energy homeostasis. On lipids, adiponectin increases β -oxidation of fatty acids in skeletal muscle and suppresses lipid accumulation in the liver. Adiponectin also exerts strong protection against several pathological events in various cells by suppressing cell death, inhibiting inflammation, and enhancing cell survival [16]. Though mainly synthesized and secreted from adipocytes, plasma adiponectin levels were found to be decreased with increasing visceral obesity and correlated with insulin resistance and the development of type 2 diabetes. The adiponectin receptor AdipoR1 was found to be expressed on endothelial cells, podocytes, mesangial cells, Bowman's capsular epithelial cells of the glomerulus, and also by the proximal tubular cells [17]. AdipoR1 has a greater affinity for the globular form of adiponectin and can cause activation of the AMPK pathway. AMPK is considered to be required for the maintenance of renal physiology. Activation of AMPK results in the activation of energy-generating processes while inhibiting energy-consuming processes and the generation of free radicals [18].

These beneficial effects of adiponectin on podocytes reduce the permeability of podocytes to albumin and thus protect against the development of albuminuria. The present study is to determine the serum adiponectin among different stages of type 2 diabetes mellitus patients.

Materials and Methods

Settings: This study was conducted in the Department of Pathology at the Institute of Medical Science, Karnataka.

Duration and Type of The study: This is a cross-sectional observational study conducted from April 2017- Feb 2020.

Sampling Methods: A total of 60 patients were diagnosed with type 2 diabetes mellitus as per American Diabetes Association (ADA) criteria. 30 Normoalbuminuria is defined as urinary albumin to creatinine ratio (ACR) <30 mg/g creatinine; 30 microalbuminuria is defined as ACR in the range of 30-299 mg/g creatinine.

Sample Size: 60 Cases

Inclusion Criteria: All the subjects Age >30 years, Patients diagnosed with type 2 Diabetes mellitus based on ADA criteria in different stages of nephropathy.

Exclusion Criteria: Type 1 Diabetes Mellitus, Non-Diabetic Renal Disease, Urinary Tract Infections, Individuals on thiazolidinediones, anti-inflammatory and immunosuppressive drugs, Thyroid and liver disease, Macrovascular complications such as cardiovascular, cerebrovascular and peripheral vascular diseases, Active inflammatory disease excluded from this study.

Data collection and Procedures: Four (4) mL of fasting venous blood sample was collected from all the subjects into two tubes: 1 mL into a tube containing (anti glycolytic and anticoagulant), and 3 mL into a plain tube. Plasma samples were separated immediately and plain samples were allowed to clot and separated by centrifugation at 3000 rpm for 15 min.

The separated samples were transferred into appropriately labelled aliquots and stored at -80 0C until biochemical analysis was done. Spot urine sample was collected along with the blood sample and was processed immediately for albumin and creatinine.

The FBS, PPBS, Urea, Creatinine, Uric acid, HbA1C and Urinary Albumin were analysed by using laboratory standard methods, serum adiponectin analysed ELISA Method.

Ethical considerations and Permission: All the subjects were recruited in the study after informed consent. The study was approved by the Institutional ethics committee.

Statistical Analysis: The data was checked for the difference in the adiponectin levels between patients and controls was analysed using unpaired two-tailed Student’s t-test or Mann Whitney U test as appropriate. The association between the variables was studied using Pearson or Spearman correlation analysis. Statistical analysis was performed using Microsoft Excel spreadsheets and SPSS for windows version 16.0. A p-value <0.05 was considered statistically significant.

Results

Table 1 shows the data distribution among the two groups of type 2 diabetes mellitus significant difference was observed across the four groups for fasting blood sugar, postprandial blood sugar, glycated haemoglobin, serum adiponectin and urine albumin creatinine ratio. All the two groups of patients had significantly higher fasting blood sugar, post prandial blood sugar, blood urea, creatinine, uric acid, HbA1C, Urinary Albumin and serum adiponectin levels in patients T2DM with microalbuminuria when compared to T2DM with Normoalbuminuria (p<0.0001**).

Table-1: Demographic characteristics and biochemical parameters studied in two groups of type 2 diabetes mellitus patients

Parameter	T2DM with Normoalbuminuria	T2DM with Microalbuminuria
Age (years)	47.93 ± 5.34	52.80 ± 5.99
FBS (mg/dL)	88.93 ± 11.52	185.67 ± 74.92
PPBS (mg/dL)	124.69 ± 44.14	238.27 ± 96.69
Serum urea (mg/dL)	33.87 ± 12.37	78.07 ± 13.56
Serum creatinine (mg/dL)	1.01 ± 0.19	4.79 ± 0.88
Serum Uric Acid (mg/dL)	4.23 ± 2.27	11.46 ± 9.82
HbA1C (%)	3.18 ± 9.82	10.32 ± 8.70
Urinary Albuminuria (mg/dL)	15.70 ± 5.47	82.90 ± 8.70
Serum Adiponectin (ng/mL)	12.79 ± 0.88	17.01 ± 12.98

Table 2: Show the Pearson correlation between the two groups of T2DM patients. The serum adiponectin was positively correlated with plasma fasting blood sugar, postprandial blood sugar, serum urea, creatinine, uric acid and Urinary Albuminuria levels were in two groups of T2DM Patients.

Table 2: Shows the Pearson correlation analysis in between serum Adiponectin with Other biochemical parameters among two groups of T2DM Patients

Parameter	Name of The Parameters	r value	P-value
	Fasting Blood Sugar (mg/dL)	0.164	0.079
	Post Prandial Blood Sugar (mg/dL)	0.079	0.854
	Serum Urea (mg/dL)	0.687	0.0001**
	Serum Creatinine (mg/dL)	0.549	0.0001**
	Serum Uric Acid (mg/dL)	0.637	0.0001**
	HbA1C (%)	0.289	0.0001**
	Urinary Albuminuria (mg/dL)	0.401	0.0001**

Discussion

Diabetic nephropathy, also known as diabetic kidney disease is one of the microvascular complications of diabetes mellitus that is characterized by the occurrence of persistent microalbuminuria, decreased renal function, hypertension and an increased risk of cardiovascular morbidity and mortality and is associated with type 1 or type 2 diabetes mellitus [19]. Diabetic nephropathy is the most common cause of end-stage renal disease and renal replacement therapy. Although the incidence of diabetic nephropathy is higher in type 1 diabetes, due to the continuing increase in the prevalence rates of type 2 diabetes along with decreased mortality rates from cardiovascular disease, patients with type 2 diabetes account for about one-third of all patients requiring renal replacement therapy. Further, it is predicted that the number of type 2 diabetes patients requiring renal replacement therapy will increase. The increased circulating adiponectin levels could be due to an increased adiponectin synthesis in adipose tissue and its secretion into the blood in an attempt to overcome the microvascular damage in the advanced stage of diabetic nephropathy [20-21]. Since recent times, the relationship between adiponectin and kidney function is gaining increasing recognition. However, the majority of studies exploring the relationship of adiponectin with renal function were conducted on diseased individuals. Monomers and dimers of adiponectin that are small enough to cross the glomerular filtration barrier were found to be excreted in the urine of healthy individuals [22].

Proximal and distal tubular cells and were shown to synthesize and excrete adiponectin. Moreover, adiponectin is also normally present on the endothelium and in the smooth muscle cells of intrarenal arteries and on the endothelium of glomerular and peritubular capillaries. Adiponectin inhibited mesangial cell proliferation and expression of type IV collagen, laminin, and fibronectin thus suggesting its renoprotective effects thereby suppressing the development and progression of DN [23]. Sharma et al; have shown that adiponectin knockout mice had increased albuminuria that could be reversed by the administration of adiponectin, however, the same beneficial effect could not be extrapolated to the entire spectrum of diabetic nephropathy since the relationship between serum adiponectin levels and albuminuria was found to exhibit a biphasic pattern [24]. In patients with normoalbuminuria, adiponectin was found to have a negative association with albuminuria, on the other hand, in patients with more advanced albuminuria serum adiponectin levels correlated positively with albuminuria [25]. Accordingly, earlier studies have reported significantly higher adiponectin levels in the presence of advanced diabetic nephropathy. A recent study showed that plasma adiponectin levels increased significantly with the progression of diabetic nephropathy [26].

Similarly, another study reported that although serum adiponectin levels in the diabetic group did not show any significant difference as compared with the control group, the highest concentrations of adiponectin were observed in patients with macroalbuminuria when compared to those with normoalbuminuria and microalbuminuria, or the control group [27]. The serum total and HMW adiponectin levels correlated positively with the severity of diabetic nephropathy and retinopathy [28]. One more study found that diabetic patients with overt nephropathy had elevated serum levels of adiponectin that appear to be increased in proportion to the degree of renal tubular injury and tubulointerstitial inflammation [29]. According to them, the increase in circulating adiponectin in overt diabetic nephropathy patients might be a physiological response to mitigate renal tubular injury and to prevent the further progression of diabetic nephropathy through its anti-inflammation and anti-atherogenic effects [30]. They observed that both serum and urinary adiponectin levels were significantly increased in patients with overt diabetic nephropathy.

Moreover, they have also observed a positive correlation between serum adiponectin and urine adiponectin levels in all patients of type 2 diabetes irrespective of the degree of diabetic nephropathy [31]. Thus, according to them, the increase in the serum adiponectin levels are caused by the increased synthesis in adipose tissue and secretion of the marker rather than reduced clearance due to impaired renal function [32]. They have also concluded that the renal protective role of adiponectin is mainly brought about by its effects on podocytes and is mediated by the AMPK pathway and also observed that overexpression of adiponectin in streptozotocin induced diabetics resulted in reduced proteinuria. Thus, these findings indicate that adiponectin exhibits renoprotective effects mainly through its action on podocytes [33]. Thus, findings of the present study indicate that serum adiponectin levels are increased in type 2 diabetes mellitus patients with Normo and microalbuminuria. Although adiponectin is known to protect against the development of proteinuria, the exact relationship between adiponectin and albuminuria appears to be complex and needs to be further evaluated. Moreover, adiponectin was also found to be involved in the beneficial effects observed with the medical treatment of albuminuria using angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers (ARBs), Thiazolidinediones [34]. Previously one more study has shown that adiponectin reduces the degree of microalbuminuria and exhibits renal protective effects by improving endothelial dysfunction and uncoupling of the glomerular vascular endothelial growth factor (VEGF)-nitric oxide (NO) axis in streptozotocin-induced type 2 diabetic rats with early diabetic nephropathy [35]. Hence, understanding the pathophysiological relationship between adiponectin and albuminuria might help in elucidating the exact role played by this adipocytokine in the development of albuminuria in various clinical conditions such as type 2 diabetes mellitus. This has clinical implications in that besides being a marker of nephropathy, albuminuria is considered an important risk factor for cardiovascular disease in patients with diabetes mellitus.

Conclusion

The present study evaluated serum adiponectin concentrations in individuals with type 2 diabetes mellitus with normoalbuminuria, microalbuminuria.

The elevated levels of serum adiponectin in patients with type 2 diabetes with normoalbuminuria, before excretion of urinary microalbuminuria. Serum adiponectin concentration in the circulation is increased in patients with advanced diabetic nephropathy, thus suggesting its potential role in the pathophysiological process underlying the development of nephropathy which is characterized by albuminuria.

What new this study adds to existing knowledge

For diagnosis of nephropathy in T2DM Patients by using Urinary Microalbuminuria, but is not an accurate, sensitive and specific biomarker for early detection of nephropathy, by using serum adiponectin monitoring were useful for early detection, sensitive and specific biomarker for nephropathy in T2DM patients because it is elevating before excretion of urinary Microalbumin.

Authors Contribution

Dr. Rashmi GS Basavaraj, Data collection, Literature review, Manuscript preparation, Manuscript editing, Final approval. **Dr. Ravikumar Malladad**, Patients Selection, Collection of Samples, Data Analysis and Statistical analysis.

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