Association of serum adiponectin levels with albuminuria among type 2 diabetes mellitus patients

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Abstract

Introduction: Adiponectin is an adipocytokine produced by adipose tissue. It has insulin sensitizing effects, potential anti-inflammatory and anti-atherogenic properties. Adiponectin is known to play a protective role on kidneys by preventing albuminuria. A lower concentration of adiponectin is noted, in conditions of resistance to insulin, diabetes mellitus, and CKD. But among the CKD micro and macro albuminuric daibetic nephropathy patients have increased adiponectin levels. Hence there is an ambiguity concerning albuminuria in diabetes mellitus and increased levels of adiponectin. In this background, the present study was taken to investigate correlation of serum adiponectin levels with degrees of albuminuria in type 2 diabetic patients.
Materials and Methods: Study included 60 diabetic patients and they were classified into three groups based on the degree of albuminuria. The levels of serum adiponectin and albuminuria was analyzed among the groups and healthy controls
Results: The levels of serum adiponectin and albuminuria was analysed among the groups and healthy controls. A statistically significant difference was found between type 2 diabetic patients as compared to healthy controls ($p < 0.001$). And it was significantly higher in type 2 diabetes patients with macroalbuminuria when compared to healthy controls ($p < 0.001$, ANOVA).
Conclusion: It concluded that among the diabetic nephropathy patients, serum levels of adiponectin are increasing with the progression of renal failure as well as the levels of albuminuria. Since, hypoadiponectinemia is associated with inflammation, atherogenic properties and insulin resistance, adiponectin is secreted more likely so as to alleviate their detrimental effects in diabetic nephropathy patients.

Keywords: Adiponectin, Albuminuria, Chronic kidney disease, Diabetic nephropathy.

Introduction

Diabetes mellitus (DM) is a systemic disorder resulting from hyperglycemia, caused either due to absolute or relative deficiency of insulin, decreased glucose utilization and increased gluconeogenesis. It is a major global health problem, affecting almost all age groups. Diabetes' prevalence is predicted to exponentially increase globally from 171 million to 366 million in over three decades, with its maximum impact in India.[1] Probably, by 2030, it may even affect nearly 79.4 million individuals in India. Diabetes is the most common cause of end stage renal disease. Poor glycemic control leading to chronic hyperglycemia is identified as a critical etiological factor for nephropathy in diabetes patients.[2] Diabetic nephropathy develops as a result of complex interaction between various pathways including metabolic, hemodynamic, inflammatory and other pathways. The key pathological event in diabetic nephropathy is damage to the glomerular basement membrane. This results in microalbuminuria, a condition characterized by the appearance of low but abnormal levels (≥ 30 to 300mg/day or 20µg/min) of albumin in urine and this is one of the earliest clinical evidences of nephropathy.[3] Although the occurrence of diabetic nephropathy is more frequently associated with type 1 diabetes, due to the continuing increase in the prevalence of type 2 diabetes along with decreased mortality rates due to cardiovascular disease, those with type 2 diabetes account for nearly 1/3rd of all patients requiring renal replacement.[4] Further, it is found that type 2 diabetes patients requiring renal replacement therapy will multiply in the coming years unless the condition is diagnosed early and treatment initiated promptly. Hence there is a need for in-depth research on clinical course of diabetic nephropathy, various factors contributing to its pathophysiology.

Until recently, adipose tissue was considered as a lipid storing organ. However, it is now considered as an active endocrine organ that participates in several important physiological functions through actively secreting a number of cytokines known as adipocytokines. Adiponectin is a 30kDa adipocytokine exert multiple biological effects and contribute to the regulation of homeostasis significantly.Since recent times, adiponectin has attracted great attention due to its anti-inflammatory, antiatherogenic, and antidiabetic properties.[5] And now a days, the relationship between adiponectin and kidney function is gaining increasing recognition. Adiponectin is known to play a protective role on kidneys through the activation of AMP activated protein kinase (AMPK).[6] The development of albuminuria in patients with diabetes was found to be

influenced by adiponectin. Various studies have also shown that adiponectin plays an important pathophysiological role in insulin resistance, diabetes and inflammation.[7] Although adiponectin is secreted by mature adipose tissue cells, serum adiponectin levels bear an inverse relation with adipose tissue mass and thus, low adiponectin levels were observed in obesity related diseases such as type 2 Diabetes mellitus.[8] Although elevated serum adiponectin levels have been found among diabetic nephropathy patients with micro and macro albuminuria, however, there is an ambiguity concerning albuminuria in diabetes mellitus and increased levels of adiponectin.[9] 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.

In this background, the present study was taken up to explore serum adiponectin levels among type 2 diabetic patients in comparison with controls and to evaluate the correlation of serum adiponectin levels with degrees of albuminuria in type 2 diabetic patients

Materials and Methods

60 type 2 diabetes mellitus patients, diagnosed as per American Diabetes Association (ADA)[10] criteria attending Basaveshwara Medical College Hospital & Research Centre, Chitradurga were included in the present study. This cross-sectional comparative study was conducted in the year 2017. The diabetic patients were classified into three groups based on the degree of albuminuria (albumin creatinine ratio). Normoalbuminuria is defined as urinary albumin to creatinine ratio (ACR) <30 mg/g creatinine; microalbuminuria is defined as ACR in the range of 30-299 mg/g creatinine and macroalbuminuria is diagnosed when the ACR was ≥300 mg/g creatinine.[11] 60 healthy individuals were included as controls. All the subjects were recruited in the study after obtaining their informed consent. The study was approved by Institutional ethics committee. Type 2 Diabetes mellitus patients, diagnosed based on ADA criteria, presenting in different stages of nephropathy were taken as cases. Patients with 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 were excluded from the study.

Sample collection

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 labeled aliquots and biochemical analysis was done. Spot urine sample was collected along with the blood sample and was processed immediately for urinary creatinine and albumin. According to ADA guidelines, albuminuria can be screened by measuring albumin-creatinine ratio in spot urine sample collected randomly.[10] Serum adiponectin levels were analyzed by ELISA method. Urinary Albuminuria is analyzed by estimating the ratio of albumin and creatinine in random urine sample by urine ACR analyzer (Immunoturbidimetric method). Fasting blood sugar, blood urea and serum creatinine were measured by laboratory standard methods.

Statistical Analysis

The data was checked for normality of distribution using Kolmogorov Smirnov test. All the characteristics are summarized descriptively. For continuous variables, the summary statistics of N, mean, standard deviation about the arithmetic mean were used. For categorical data, the number and percentage were used in the data summarized.

Variations in the levels of adiponectin between cases and controls was analysed using unpaired Student's t-test (2-tailed). The difference in adiponectin levels among patients in various stages of nephropathy and controls was assessed using Analysis of variance (ANOVA).The association between the variables was studied using Pearson correlation analysis. Data was compiled in Microsoft excel spread sheets and analyzed using SPSS for windows version 16.0. A p value <0.05 was considered statistically significant.

Results

Patient's characteristics are shown in Table 1. The mean serum Adiponectin levels of 60 diabetic patients studied was 12.99 (± 10.86, SD) and the median inter quartile range of urinary albuminuria were 82.90(15.35-416.20). A statistically significant difference was found in the serum adiponectin levels between type 2 diabetic patients as compared to healthy controls (p value < 0.001, independent student t test).

Table 1: Patients characteristics and biochemical parameters studied in controls and type 2 diabetes mellitus patients

Parameter	Controls (n=60) (Mean ± SD)	T2DM (n=60) (Mean ± SD)
Age (years)	49.13 ± 8.20	50.27 ± 8.66
BMI (kg/m2)	24.22 ± 2.94	25.14 ± 3.45
FBS (mg/dL)	98.93 ± 11.52	221.13 ± 74.59
Serum urea (mg/dL)	22.07 ± 7.72	38.27 ± 23.76
Serum creatinine (mg/dL)	1.03 ± 0.19	1.58 ± 1.06
Serum adiponectin (µg/mL)	4.23 ± 2.27	12.99 ± 10.86 *
Urinary ACR* (mg/g creatinine)	9.09(7.25-10.99) **	82.90(15.35-416.20) **

BMI: Body Mass Index; FBS: Fasting Blood Sugar; ACR: Albumin Creatinine Ratio
*p value < 0.001, independent student t test applied.
** Median (Inter quartile range)

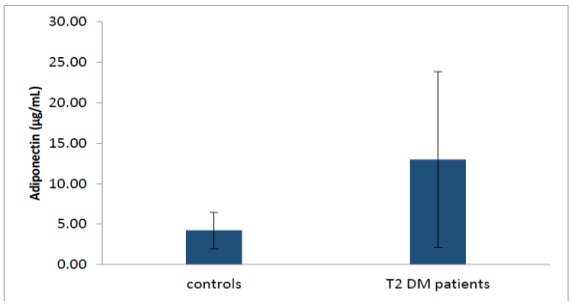

Fig. 1: Graphical representation of serum adiponectin levels in controls and cases (Type 2 diabetes mellitus patients)

Fig. 1 shows the serum adiponectin levels in type 2 diabetes patients and controls.

Table 2: Patients characteristics and biochemical parameters studied in controls and three groups of type 2 diabetes mellitus patients

Parameters	Group 1 (n=60) (Mean ± SD)	Group 2 (n=19) (Mean ± SD)	Group 3 (n=23) (Mean ± SD)	Group 4 (n=18) (Mean ± SD)	p-value
Age (years)	49.13 ± 8.20	46.93 ± 7.34	50.80 ± 5.99	53.07 ± 11.20	0.226
BMI (kg/m2)	24.22 ± 2.94	25.52 ± 3.65	25.68 ± 3.81	24.23 ± 2.88	0.366
FBS (mg/dL)	98.93 ± 11.52	214.67 ± 74.92	238.27 ± 96.69	210.47 ± 45.20	<0.001
Serum urea (mg/dL)	22.07 ± 7.72	23.87 ± 12.37	22.87 ± 3.07	68.07 ± 13.56	<0.001
Serum creatinine (mg/dL)	1.03 ± 0.19	1.01 ± 0.59	0.94 ± 0.20	2.79 ± 0.88	<0.001
Serum adiponectin (µg/mL)	4.23 ± 2.27	10.32 ± 8.70	11.46 ± 9.82	17.01 ± 12.98	<0.001
Urinary ACR * (mg/g creatinine)	9.09 (7.25-10.99)	12.00 (7.50-15.70)	82.90 (44.10-136.40)	857.70 (414.70-1598.04)	<0.001

Data was expressed as Mean ± SD;
* refers to Median / Inter quartile range (IQR)

Grouping of subjects: Group 1 included controls; Group 2 involved type 2 diabetes patients with normoalbuminuria; Group 3 consisted of type 2 diabetes patients with microalbuminuria and Group 4, of type 2 diabetes patients with macroalbuminuria

BMI refers Body Mass Index; FBS refers to Fasting Blood Sugar and ACR refers to Albumin Creatinine Ratio.

ANOVA test is applied to test the significant difference in mean among the different groups.

Table 2 shows the mean values and SD of various biochemical parameters studied in controls and three groups of type 2 diabetic patients. Significant difference was observed across all four groups for fasting blood sugar, serum urea, serum creatinine, serum adiponectin and urinary ACR. All three groups of patients had significantly higher levels of FBS, in comparison with controls ($p<0.001$). Further, serum adiponectin levels were significantly higher in type 2 diabetes patients with macroalbuminuria than those with healthy controls ($p<0.001$).

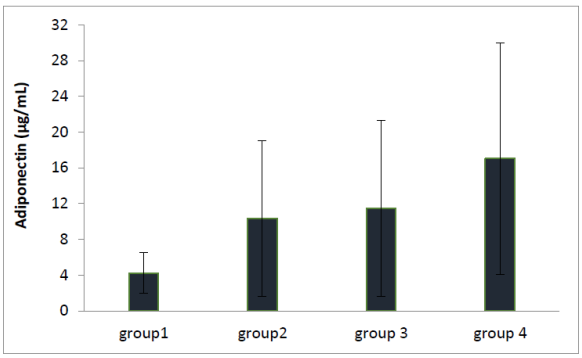

Fig. 2: Graphical representation of serum adiponectin levels among controls and cases (three groups of type 2 diabetes mellitus patients)

Group 1 → controls; Group 2 → type 2 diabetes patients with normoalbuminuria; Group 3 → type 2 diabetes patients with microalbuminuria and Group 4 → type 2 diabetes patients with macroalbuminuria

Fig. 2 graphically shows serum adiponectin levels in controls and various groups of patients with type 2 diabetes mellitus

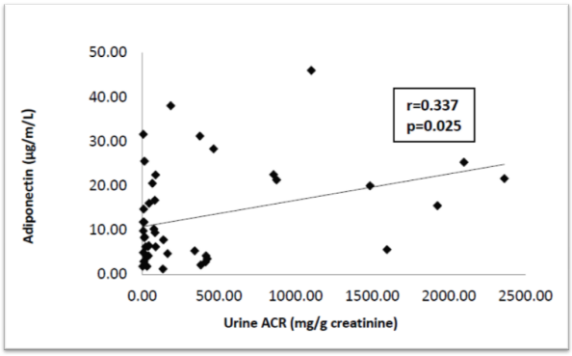

Fig. 3: Association of serum adiponectin levels with urine albumin creatinine ratio in type 2 diabetes mellitus patients

Fig. 3 shows that there is significant and positive correlation between serum adiponectin levels and urinary albumin creatinine ratio (r=0.337, p=0.025)

Table 3: Levels of serum adiponectin in relation to albuminuria

Albuminuria stages (Median (Q1, Q3)	N	Mean ± SD
Healthy controls 9.09 (7.25-10.99)	60	4.23 ± 2.27
Normoalbuminuria 12.00 (7.50-15.70)	19	10.32 ± 8.70
Microalbuminuria 82.90 (44.10-136.40)	23	11.46 ± 9.82
Macroalbuminuria 857.70 (414.70-1598.04)	18	17.01 ± 12.98

p<0.001 between groups, ANOVA test is applied.

Analysis of variance (ANOVA) showed significant difference in adiponectin levels across the four study groups (p<0.0001) (table-3). Further analysis using post hoc tests showed that patients with macroalbuminuria had significantly higher adiponectin levels when compared to controls (17.01±12.98 and 4.23±2.27 for macroalbuminuria and controls, respectively; p=0.011). However, adiponectin concentration was not significantly different between other groups studied.

Discussion

Until recently, adipose tissue was considered as an inert storage organ. However, it is now well recognized that the adipocytes synthesize and secrete a number of proteins that are actively involved in energy homeostasis, glucose and lipid metabolism, neuroendocrine and cardiovascular function, and various other physiological functions. Besides its role in energy metabolism, adiponectin which is a newly discovered protein exerts pleiotropic beneficial effects including insulin sensitizing, anti-inflammatory, anti-oxidant, anti-apoptotic and anti atherogenic effects.[12] Though adiponectin is mainly obtained from adipose tissue, its circulating levels were found to be decreased in obesity and its related disorders such as insulin resistance and type 2 diabetes mellitus. Low levels of serum adiponectin were also found to predict the incidence of type 2 diabetes.[13] Adiponectin was shown to play a protective role on kidneys through several mechanisms and protects against the development of albuminuria. The development of albuminuria, which is a marker of diabetic renal disease, was further found to be influenced by adiponectin.[14]

In the present study, serum adiponectin levels were measured in type 2 diabetes mellitus patients, classified based on amount of protein excretion into three groups (Table 2) and the levels were compared with healthy controls. In this study, significant difference in serum adiponectin levels across the four study groups (p<0.0001) was found, with macroalbuminuric patients, having significantly higher adiponectin levels when compared to controls. (Table 3). Similar results of elevated levels of adiponectin in patients with end stage kidney failure were also previously reported in type 2 diabetic patients.[15]

Studies conducted by Koshimura et al. Galovicova et al and Kim et al have shown similar findings of elevated adiponectin levels in diabetic nephropathy with macro-albuminuria. This increased levels of adiponectin may be due to either 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, or a decreased clearance of adiponectin in the setting of impaired renal function, or a combination of both.[16-18] Fujita et al, in their study, have documented that 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, whereas, Pradeepa et al., could not observe any significant difference in serum levels of adiponectin in diabetic patients with and without nephropathy.[19] In contrast, Jung et al., reported decreased levels of serum adiponectin in diabetic patients with nephropathy when compared to those without nephropathy.[20]

When the relationship between adiponectin and albuminuria measured as albumin creatinine ratio (ACR) was analysed using correlation analysis in the present study, it was found that there was significant and positive association between serum adiponectin and urinary ACR (p=0.025) (figure-3). Similarly, Galovicova et al., and Fujita et al., also observed a positive association between these two parameters.[17,21]

Conclusion

Among type 2 diabetic patients, plasma levels of adiponectin are increasing with the progression of renal failure as well as the levels of albuminuria. Since, hypoadiponectinemia is associated with inflammation, atherogenic properties and insulin resistance, adiponectin is secreted more likely so as to alleviate their detrimental effects in diabetic patients.

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