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ENDOCRINOLOGY, NUTRITION & METABOLISM | RESEARCH ARTICLE

Study the association of adiponectin with inflammation and hypercoagulability in case of type 2 diabetic subjects with renal dysfunction

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Abstract: Adiponectin is a 30-kDa polypeptide chiefly secreted by adipose tissue and plays a pivotal role in cardiovascular complication and kidney disease. The aim of this study was to find if there was any association of adiponectin with inflammation and hypercoagulability in case of type 2 diabetic subjects with renal dysfunction. From May 2012 to January 2014, 205 outpatients with normo, micro, and macroalbuminuria having type 2 diabetes (T2DM) were included in this study. We found a U-shaped association of adiponectin with albumin excretion. Macroalbuminuria showed a positive correlation with adiponectin ($p < 0.0001$). Plasma F1+2 was strongly associated with degree of renal failure ($p < 0.001$). CRP, IL6, TNFα, and pentosidine status showed a linear increase from normoalbuminuria and microalbuminuria to macroalbuminuria ($p < 0.001$, $p < 0.0001$). Monitoring adiponectin, pasma F1+2, inflammatory and oxidative stress markers provide a predictive value for presence of macrovascular complications in patients with type 2 diabetes having renal dysfunction.

Subjects: Biochemistry; Biology; Endocrinology

Keywords: T2DM; Adionectin; F1+2; IL6; CRP; Pentosidine

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PUBLIC INTEREST STATEMENT

Adiponectin is a 30-kDa polypeptide chiefly secreted by adipose tissue and plays a pivotal role in cardiovascular complication and kidney disease. The aim of this study was to find if there was any association of adiponectin with inflammation and hypercoagulability in case of type 2 diabetic subjects with renal dysfunction. Monitoring adiponectin, pasma F1+2, inflammatory and oxidative stress markers provide a predictive value for presence of macrovascular complications in patients with type 2 diabetes having renal dysfunction.

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1. Introduction

Diabetic nephropathy (DN) is an important long-term complication of diabetes (Adler et al., 2003). It is characterized by the development of proteinuria with a subsequent decline in glomerular filtration rate (Pathania, Rathaur, Yadav, Jayara, & Chaturvedi, 2013). Traditional risk factors including albuminuria, eGFR, hypertension, elevated level of triglyceride, cholesterol, LDL partly explain the excess risk of developing predominant complication in DN. Adiponectin, the dominant secretory product of adipocytes, is a marker and perhaps a mediator of metabolic and cardiovascular disease in T2DM (Sook Lee et al., 2013). It, also known as Acrp30, AdipoQ, gelatin-binding protein of 28 kDa, is an adipocyte-specific protein that enhances insulin sensitivity and promotes lipid metabolism (Díaz-Soto et al., 2014). It circulates in plasma in three forms: a trimer (low-molecular weight [LMW]), a hexamer (trimer-dimer) of medium-molecular weight (MMW), and a larger multimeric high-molecular weight (HMW) form (Scherer, Williams, Fogliano, Baldini, & Lodish, 1995). Adiponectin is elevated in kidney disease including nephrotic syndrome, and particularly in end-stage kidney disease, with levels up to three times higher compared to the normal population in case of type 1 diabetes (Jorsal et al., 2013). There has not been sufficient work on the adiponectin level especially on the patients with type 2 diabetes in different stages of chronic kidney disease (CKD). Adiponectin has systemic anti-inflammatory effects which may affect global insulin sensitivity (Fantuzzi, 2013). DN is a manifestation of an ongoing acute-phase response that is primarily characterized by alterations of the so-called acute-phase proteins, such as C-reactive protein (CRP). Elevated level of IL-6, which is the main stimulator of the production of the most acute-phase protein, increases the risk of DN. In addition to IL-6, other cytokines, like TNFα is a central mediator of inflammatory reactions (Barzilay et al., 2001; Festa, D'Agostino, Tracy, & Haffner, 2002; Pickup, Mattock, Chusney, & Burt, 1997; Pickup & Crook, 1998; Pradhan, Manson, Rifai, Buring, & Ridker, 2001; Schmidt et al., 1999; Vozarova et al., 2002). Cardiovascular disease in DN subject is very frequent. Several traditional factors such as dyslipidemia, albuminuria, and hypertension cannot fully explain the progression of this kind of pathogenesis. Thus, several putative nontraditional risk factors for accelerated atherosclerosis, such as advanced glycation end product (AGE), advanced oxidation protein product, have recently attracted increased interest. Hyperglycemia induced oxidative stress damage to the endothelial glycocalyx and prothrombotic shift in coagulation and fibrinolysis. It has been shown that endothelial damage precedes atherosclerotic changes of the vascular wall. Prothrombin F1+2, a good marker of thrombin generation in plasma, can be used as a biomarker to assess the risk of developing cardiovascular disease in DN subjects (Aso et al., 2004; Catena, Zingaro, Casaccio, & Sechi, 2000; Shlipak et al., 2003; Tufano et al., 2001).

In this study, we investigated the correlation of serum adiponectin, IL6, TNFα, eGFR (Estimated Glomerular filtration rate—Cockcroft–Gault equation), hypercoagulative factor in a large urban population suffering fromT2DM and T2DM-associated renal dysfunction. Results have been analyzed with reference to healthy control subjects without any previous history of diabetes. The measurement of adiponectin and plasma F1+2 may therefore carry an additional prognostic value for diabetes and its associated complications beyond the currently recognized set of risk factors.

2. Material and methods

2.1. Study design

Blood sample was collected from voluntary donors with history of T2DM ($n = 143$, male:92, female:51) under treatment in IPGMER/SSKM hospital, Kolkata. The control (Group A) blood samples ($n = 62$, male:34, female:28) were collected from healthy voluntary donors of the Department of Endocrinology, SSKM hospital, Kolkata. T2DM subjects were divided into three groups based on their urinary albumin excretion (UAE). Normoalbuminuria (Group B) was defined as UAE less than 30 mg/24 h, microalbuminuria (Group C) as UAE 30–299 mg/24 h, and macroalbuminuria (Group D) as UAE more than 300 mg/24 h. The postprandial blood sugar level for diabetic patients was > 200 mg/dl while it was < 140 mg/dl for healthy subjects (without any family history of DM). The glycated hemoglobin (HbA1c) was considered to be normal at < 6% and that of diabetic patient with poor glycemic control was > 6%. Subjects with history of cardiac diseases, smoking, alcohol intake, pregnancy, malignancy or any inflammatory disorders were excluded from the study group. Study was approved by Institutional ethical committee.

2.2. Isolation of plasma and serum
Blood samples were collected from the subjects after 12 h of fasting in a heparin vial simultaneously with another vial in absence of any anticoagulant. Blood serum and plasma were separated by centrifugation of the blood and was stored at −80°C for further analysis.

2.3. Estimation of adiponectin
Adiponectin level in blood serum was determined by using sandwich ELISA technique (Demeditec kit, Germany). The antisera have little or no cross reactivity. The analytical sensitivity of the ELISA yields < 0.6 ng/ml.

2.4. Assay of CRP
CRP level in blood plasma was determined by using sandwich ELISA technique (Ray Biotech Inc.). Absorbance was measured with an ELISA reader at 450 nm.

2.5. Estimation of IL6
IL6 level in blood serum was determined by using sandwich ELISA technique (Krishgen Biosystem kit).

2.6. Estimation of TNFα
TNFα level in blood serum was determined by using sandwich ELISA technique (Krishgen Biosystem kit).

2.7. Assay of plasma pentosidine
Plasma pentosidine level in blood serum was determined by using sandwich ELISA technique (My biosource).

2.8. Investigation of AOPP (advanced oxidation protein product) level
Assay of AOPP was carried out using plasma from different groups of patients, according to the method of Witko-Sarsat et al. Plasma was diluted 5 times with PBS (pH 7.4), chloramin-T (1–100 mmol) (LOBA-CHEMIE) was used as a calibrator. The reaction was initiated by the addition of 50 ml of 1.16 mol/l potassium iodide(KI), after 2 min 200 ml of glacial acetic acid was added into it. The absorbance of the reaction mixture was monitored immediately at 340 nm. The level of AOPP was expressed in mmol of chloramin-T equivalent per liter of plasma (mmol/l).

2.9. Plasma prothombin F1+2
Plasma prothombin F1+2 level in blood was determined by using sandwich ELISA technique (Cloud clone Corp, Uscn Lifescience Inc).

2.10. Statistical analysis
The results have been expressed as mean ± standard error. Differences between the groups were considered significant at $p < 0.05$. Student's *t*-test was used for comparing biochemical variables. Data were interpreted using the analysis of variance (ANOVA) followed by Schaeffer's method of multiple (Scheffe, 1959) comparisons. Statistical evaluation was performed by Statistica 6.0 and SPSS 10.0 software. Pearson correlation was applied to linear regression analysis between variables.

3. Results
Demographic and hematological characteristics like level of fasting blood glucose, total cholesterol, LDL, triglycerides, eGFR, creatinine clearance of control and patients have been presented in Table 1. This result has revealed a significant disparity in case of BMI, Fasting, PP, HbA$_1$C, total cholesterol, and ACR. Glycemic control has been found to be the worst in patients with macroalbuminuria.

Plasma adiponectin level was lower in diabetic subjects in comparison to control subjects (14.2 ± 3.5 μg/ml, 19.19 ± 4.7 μg/ml, $p < 0.05$). In control group as well as in diabetic subjects, men had lower adiponectin level compared to women (12.4 ± 2.7 μg/ml, 8.9 ± 2.5 μg/ml and 18.08 ± 3.5 μg/ml,

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Table 1. Demographic characteristics in various subgroups based on urinary albumin excretion (UAE)				
	Group A (Control)	**Group B (T2DM)**	**T2DM with nephropathy**	
			Group C (Microalbuminuria)	**Group D (Macroalbuminuria)**
N	74	69	62	
			42	20
BMI (kg/m^2)	23 ± 4.5	27 ± 2.4	27.2+3.5	28.4+1.9
PP (mg/dl)	105 ± 14	190 ± 20	230 ± 15	355 ± 17
HbA1c (%)	5.8 ± 0.79	8+	9.5+	10.8+
BP (mm Hg)	127 ± 5.9/80 ± 4.0	140 ± 5.8/89 ± 2.6	150 ± 5.6/95 ± 5	175 ± 5.8/100 ± 6.2
Creatinine clearance (ml/min)	99.2 ± 15.8	87.0 ± 25.1	78.5 ± 22.6	37.1 ± 16.5
eGFR	≥90	80–60	45–60	<45
LDL (mg/dl)	101 ± 9.2	124 ± 13	130 ± 8.5	142 ± 10.2
HDL (mg/dl)	45 ± 7.67	39 ± 5.33	35 ± 4.4	28 ± 4.1
Tryglyceride (mg/dl)	160 ± 14.67	210 ± 20.85	274 ± 15.32	369 ± 22.28
Cholesterol (mg/dl)	150 ± 17.23	202 ± 29.49	225 ± 23.72	245 ± 18.54

Notes: Group A: Control; Group B: UAE < 30 mg/24 h; Group C: UAE 30–299 mg/24 h; Group D: UAE > 300 mg/24 h.

15.4 ± 1.2 μg/ml). In obese patients, plasma adiponectin level has been found to be lower when compared to lean patients. Plasma adiponectin level in type 2 diabetics was also negatively correlated with early features of nephropathy (microalbuminuria). However, in patients with established CKD (macroalbuminuria), adiponectin level was elevated and positively predicts the progression of disease. We found a U-shaped association of adiponectin with albumin excretion (Figure 1).

Our data indicate that diabetes as a whole was strongly associated with elevated levels of CRP, IL-6, and TNFα (Table 2). The associations remained statistically significant even after controlling for BMI and age ($p = 0.001$).

AOPP and pentosidine an AGE (oxidative stress marker) were found to be significantly higher in case of T2DM ($p < 0.01$) and DN ($p < 0.001$) group in comparison to control. Pentosidine level were gradually higher from microalbuminuria to macroalbuminuria group and reflect a positive correlation ($r = 0.57$, $p < 0.01$) in subjects with DN (Figure 1).

Figure 1. Correlation between adiponectin (μgm/ml) and UAE (mg/24 h).

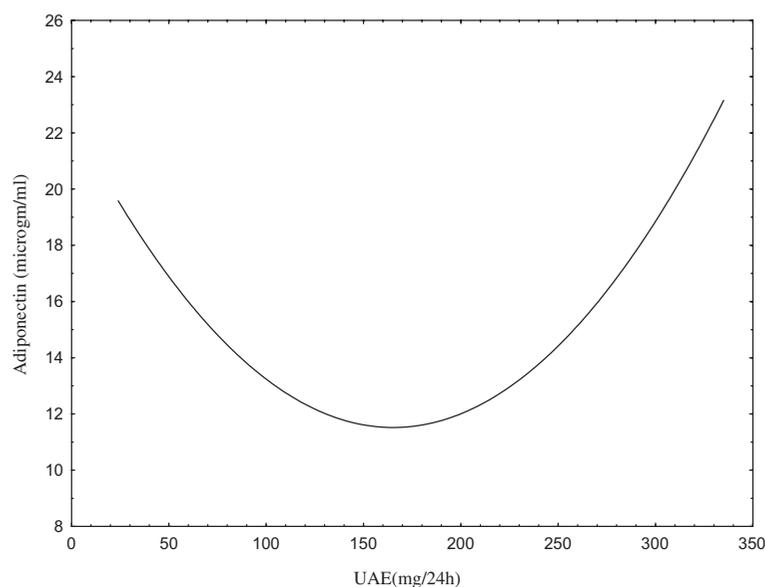Notes: U-shaped association was noticed between adiponectin and urinary albumin excretion.

Table 2. Data are expressed as the mean ± SD				
	Group A (Control)	**Group B (T2DM)**	**Group C (Microalbuminuria)**	**Group D (Macroalbuminuria)**
Adiponectin (μg/ml)	19.19 ± 4.7	14.2 ± 3.5	13.8 ± 4.1	20.4 ± 3.2*
AOPP (μmol/l)	84 ± 10.1	147 ± 9.8	183 ± 11.2	225 ± 8.7**
Pentosidine (pmol/ml)	78 ± 7.9	109 ± 9.1	128 ± 9.7	158 ± 10.3**,#
CRP (mg/l)	7.6 ± 1.6	14.45 ± 1.7	17.75 ± 1.76	26.7 ± 2.1$
TNFα (pg/ml)	2.7 ± 0.71	3.9 ± 0.46	4.9 ± 0.55	6.1 ± 0.68
IL6 (pg/ml)	1.4 ± 0.57	2.90 ± 0.94	3.3 ± 0.96	5.26 ± 1.1
F1+2 (ng/ml)	3.43 ± 0.8	4.92 ± 0.87	5.52 ± 0.78	7.15 ± 0.83@

Notes: Group A: Control; Group B: UAE < 30 mg/24 h; Group C: UAE 30–299 mg/24 h; Group D: UAE > 300 mg/24 h.

*$p < 0.05$ versus Group C.

**$p < 0.01$ versus Group C.

#$p < 0.001$ versus Group A.

$$p < 0.001$ versus Group A.

@$p < 0.01$ versus Group B.

Plasma concentration of F1+2 was significantly higher in Group D (macroalbuminuria) than that in diabetes subject without nephropathy (Group B) ($p < 0.01$) as well as in Group C (microalbuminuria) ($p < 0.05$). Correlation of metabolic parameters like PP, HbA1c, cholesterol, and triglyceride with the endothelium dependent factor (F1+2) reflected a positive correlation ($r = 0.66$, $p < 0.001$; $r = 0.54$, $p < 0.01$; $r = 0.45$, $p < 0.01$; $r = 0.41$, $p < 0.01$, respectively). Simple linear regression analysis detected a negative correlation between HDL and Plasma F1+2 ($r = -0.06$, $p < 0.02$).

Simple linear regression analysis between adiponectin and F1+2 reflect a paradoxical nature. Pooled value of adiponetic of control, T2DM and DN (microalbuminuria) subjects showed a negative correlation but in the subjects having macroalbuminuria, it seemed a positive correlation.

4. Discussion

Increases in number of cases of DN in several classes of populations have drawn attention worldwide. This study has focuses on plasma adiponectin level along with hypercoagulative factor F1+2, inflammatory marker and oxidative stress marker in T2DM, T2DM with renal dysfunction, and control subjects. It is already established that hypoadiponectinemia is correlated with early features of nephropathy (microalbuminuria) (von Eynatten et al., 2009). Tsioufis et al. (2005) evaluated the level of adiponectin in nondiabetic hypertensive men related to microalbuminuria. They found that the microalbuminuria was associated with lower adiponectin levels. Yano et al. (2007) examined the association between adiponectin and low-grade albuminuria in obese and lean nondiabetic patients and found that the urine albumin excretion was significantly higher in obese patients with low adiponectin levels compared to obese patients with high adiponectin levels. Our study has shown the same pattern of association between adiponectin and microalbuminuria in case of T2DM subjects. This remained same even after adjusting for age, systolic blood pressure, and fasting glucose level. But paradoxically this scenario was changed in case of macroalbuminuria. We noticed that patients with macroalbuminuria on an average had a higher level of adiponectin as compared to the patients with normoalbuminuria and microalbuminuria. A possible reason for this kind of finding may be the mechanism of action of adiponectin in the kidney appears to be related to AMPK activation and NADPH oxidase (Georgios & Dimitrios, 2014; Sweiss & Sharma, 2014). Several studies are needed to clarify this pathway and examine the role of probable targets of adiponectin-AMPK-Nox pathway for nephropathy. Till date the exact role of the kidney in the biodegradation and excretion of adiponectin is unclear. Recently Georgios et al. have enlightened this pathway (Georgios & Dimitrios, 2014). Adiponectin is also known to play an important role in the development of hypercoagulative factors, mediator of atherosclerosis, but its physiologic role is yet to be fully addressed. In our study, we found that plasma F1+2 was positively correlated with adiponectin in macroalbuminuria group having T2DM. Since endothelial damage plays a pivotal role in the pathogenesis of

cardiovascular disease, it can be assumed from this study that T2DM subjects having macroalbuminuria and hyperadiponectinemia have more tendency to develop CVD in comparison with age-matched healthy control subjects. Turbulence in glucose metabolism enhances the process of inflammation and oxidative stress. In recent past, important research works in the area of T2DM related complication, have established that inflammation and oxidative stress related parameters could be crucial factors in disease pathogenesis. CRP, TNFα, and IL6 are exquisitely sensitive systemic markers of inflammatory condition. Pentosidine and AOPP are oxidative stress markers and they play significant role in the progression of CVD and CKD (Chakraborty et al., 2011). This study has shown that the inflammatory and oxidative stress markers exhibit a linear increase from normoalbuminuria and microalbuminuria to macroalbuminuria.

Our study concludes that adiponectin is a novel risk marker of CVD in patients having various stages of kidney disease. Monitoring adiponectin, plasma F1+2, inflammatory and oxidative stress markers, provide a predictive value for presence of macrovascular complications in patients with type 2 diabetes having renal dysfunction.

Abbreviations

T2DM;   Type 2 diabetes mellitus
CVD;   Cardiovascular disease
DN;   Diabetic nephropathy

Competing interest
The authors declare that there is no competing interest associated with this manuscript.

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