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The Association between Serum Adropin Level and Metabolic Complications of Type 2 Diabetes Mellitus

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ABSTRACT

Background: Several recent reports indicated presence of low levels of serum adropin among patients who had type 2 diabetes mellitus (T2DM) when compared with non-diabetic cases. However, more investigations are needed to determine the clinical importance of this adropin reduction as an early marker for diabetic nephropathy (DN).

Objective: Evaluation of the performance of serum adropin levels as an early biomarker for DN in T2D patients.

Patients and Methods: In a cross-sectional study, sixty cases with T2DM were enrolled in addition to 20 healthy nondiabetic subjects. The diabetic patients were further subdivided according to albuminuria level into, non albuminuric group (20 patients), microalbuminuric group (20 patients) and macroalbuminuric group (20 patients). We used enzyme-linked immunosorbent assay for determination of serum adropin levels among all participants.

Results: Serum adropin levels were much lower among macroalbuminuric diabetic cases compared with non albuminuric, microalbuminuric cases as well as healthy controls ($p < 0.001$). Negative correlations were revealed between serum adropin levels and all of the following: age, estimated glomerular filtration rate (eGFR), serum creatinine (SCr), blood urea nitrogen (BUN), random blood sugar (RBS), HbA1c, low-density lipoprotein (LDL), cholesterol, triglycerides (TG), and urine albumin to creatinine (ACR), meanwhile there was a positive correlation with hemoglobin (HB)%, total protein, high density lipoprotein (HDL) and albumin.

Conclusions: DN is associated with reduced adropin levels, and serum adropin may be a useful diagnostic marker for early identification of DN among type 2 diabetic cases.

Keywords: Adropin, T2DM, Diabetic nephropathy.

INTRODUCTION

Diabetes mellitus is the highest prevalent endocrinal disorder worldwide and represents a growing public health concern as about 425 million people are already diagnosed as diabetic according to 2017 global statistics of diabetes mellitus, by 2045 calculations suggest that number will increase to 629 million [(1)]. In Egypt, it is estimated that 15.6% of all persons aged 20-79 have type 2 diabetes [(2)]. In addition to being the major etiology of chronic kidney disease as well as end-stage renal disease (ESRD), which impact thirty to forty percent of patients who had diabetes, and the most common complication, which contribute to high morbidity and mortality among these cases is diabetic nephropathy (DN) [(3,4)].

Encoded by the Enho gene, the new peptide hormone adropin was detected for the first time in the year of 2008 [(5)]. Adropin helps maintain a steady level of energy in the body. Animal researches evaluating the various effects of adropin on the metabolic process revealed its effect on improving insulin resistance and glucose metabolism by decreasing endogenous hepatic glucose production, and enhancing glucose utilization, via activation of the insulin signaling pathways as Akt phosphorylation and glucose transporter 4 receptor. Adropin decreases levels of low-density lipoprotein cholesterol, total cholesterol, and serum triglycerides while raising levels of HDL cholesterol [(6-8)].

DN is the most common cause of CKD and ESRD in diabetic patients affecting more than one-third of patients. DN is characterized by both structural and functional abnormalities. Renal injury in diabetes is caused by several interrelated pathophysiological mechanisms, including hyperglycemia, hyperlipidemia, hypertension, chronic low-grade inflammation, oxidative stress and aldosterone-renin-angiotensin system activation, which leads to glomerulosclerosis and tubulointerstitial fibrosis and expression of profibrotic mediators such as PAI-1 and transforming growth factor β1 [(4)]. Currently, there is no curative treatment for DN except to delay its progression to ESRD. As a result, developing cutting-edge treatment strategies for people at high risk and identifying innovative biomarkers for early identification of DN are crucial [(3, 4)].

Administering adropin to streptozotocin-induced type 2 diabetes in rats reduced blood glucose levels and improved insulin resistance, although serum adropin levels were lower and adropin expression was higher in these rats' kidney tissue. These findings provide more evidence that adropin may have a part in the etiology of type 2 diabetes and its complications, particularly regarding DN [(9, 10)].

We aimed at this work to examine the correlations between serum adropin levels and diabetic metabolic and renal complications.

PATIENTS AND METHOD

In a cross-sectional study, sixty patients with T2DM were enrolled in addition to 20 healthy non diabetic subjects as control group. The American

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Diabetes Association (ADA) criteria for determining type 2 diabetes were used to make the diagnosis. The ratio of urine albumin to creatinine (ACR) was utilized to divide type 2 diabetics into three groups: twenty normoalbuminuria cases group (ACR level was less than 30 mg/g), twenty microalbuminuria cases group (ACR between 30 and 300 mg/g), and twenty macroalbuminuria cases group (ACR to be higher than 300 mg/g).

Exclusion criteria: if they had other endocrinal disorders, malignancy, active infection, or cardiovascular diseases. The control group included 20 healthy subjects. None of them received medication or dietary or had a history of diabetes.

A complete clinical examination was done on all participants and fasting venous blood samples were collected.

Morning venous blood samples (10 mL total) were collected from each patient. After an 8-hour fast, two blood samples (3 ml each) were collected on a plain vacutainer tube (red-topped). One was for serum separation that was used to test for liver, and kidney function tests, and fasting blood sugar. Analysis was performed using dedicated reagents on Cobas 8000 (Roche Diagnostics, Germany). While the second tube was utilized to analyze serum adropin levels, CVs for intra-assay of less than 9% were achieved utilizing an enzyme-linked immunosorbent test kit purchased from Biotechnology co, CV >11% between tests. Detection Limit Range: 0.01 - 100 pg/mL (Sensitivity: 4.735 pg/mL).

Two blood samples (2 ml each) were collected on EDTA vacutainer tubes (lavender-topped). One was used for complete blood count (CBC) on Sysmex XS autoanalyzer (Sysmex Corporation, Japan). The other was used for HbA1C analysis on Cobas 6000 (Roche Diagnostics, Germany).

Another vacutainer sample was taken after an additional 4 hours of fasting, and it was analyzed on the Cobas 8000 with specific reagents for the lipid profile (Roche Diagnostics, Germany). The serum was separated using a centrifuge at 1200x g for 10 minutes after 30 minutes at room temperature had passed and the plain tubes had been retrieved and allowed to clot.

A random morning urine sample was used for the measurement of ACR on Cobas 6000 (Roche Diagnostics, Germany).

Ethical approval:

Zagazig Medical Ethics Committee of the Zagazig Faculty of Medicine gave its approval to this study. All participants gave written consent after receiving all information. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

Under the assumptions of = 0.05 (two-tailed) and power = 90%, the sample size was calculated using preliminary data from our lab. Serum adropin concentrations could be differentiated between the three T2DM subgroups and the control group with a minimum of 11 individuals. Means and standard deviations were used to summarize the quantitative data, which were compared by one-way ANOVA test. Qualitative data were presented as frequency and percentage and were compared using chi-square tests. Using a ROC curve, the optimum threshold was determined. Pearson's technique was used to examine the associations between serum adropin and other variables. P<0.05 was considered significant.

RESULTS

As shown in **Table (1),** Age differed significantly between the studied groups, where diabetic microalbuminuric group showed higher mean age followed by diabetic macroalbuminuric group, diabetic non albuminuric group than the control group.

Body mass index (BMI) differed significantly between the studied groups as the diabetic non albuminuric cases revealed the highest BMI and the lowest BMI was found among the control group. On the other hand, a non-statistically significant difference was found as regards sex ensuring matching of studied groups.

Table (1): The study groups demographics

Variable		Control Group (n=20)	Diabetic non albuminuric group (n=20)	Diabetic microalbuminuric group (n=20)	Diabetic macro albuminuric group (n=20)	P
Age (years) Mean±SD		36.5±11.55	60±7.5[a]	63.5±3.32[a]	63.15±3.41[a]	**<0.001***
BMI (Kg/m2) Mean±SD		26.52±0.89	28.3±1.2[a]	27.99±1.27[a]	27.59±0.88[a]	**<0.001***
Variable		**No (%)**	**No (%)**	**No (%)**	**No (%)**	**P**
Sex	**Female**	10 (50)	15 (75)	14 (70)	12 (60)	0.363
	Male	10 (50)	5 (25)	6 (30)	8 (40)	

a: The significant difference in comparison to control group, *: Significant

As shown in **Table (2),** with the exception of WBCs, all the laboratory parameters differed significantly between the studied groups.

Table (2): Laboratory investigations of the studied groups:

Variable	Control Group (n=20)	Diabetic non albuminuric group (n=20)	Diabetic microalbuminuric group (n=20)	Diabetic macro albuminuric group (n=20)	P
WBCs (x10^3/µL)	6.74±1.54	7.46±1.19	7.31±1.06	7.11±1.92	0.753
Hb (g/dl)	12.16±2.32	12±1.56	9.74±1.18[a,b]	9.07±0.85[a,b]	**<0.001***
Platelets (x10^3/µL)	321.6±76.69	278.1±9.87	371.3±54.96[b]	304.5±19.24[c]	**0.008***
Creatinine (mg/dL)	0.83±0.09	0.83±0.08	0.97±0.18[a,b]	1.65±0.24[a,b,c]	**<0.001***
BUN (mg/dL)	15.53±2.58	11.98±2.54	23.6±7.94[a,b]	33.35±1.71[a,b,c]	**<0.001***
Albumin (g/dL)	4.27±0.31	4.45±0.32[a]	3.54±0.22[a,b]	2.82±0.23[a,b,c]	**<0.001***
Total Protein (g/dL)	7.24±0.56	7.33±0.47	5.99±0.44[a,b]	4.52±0.42[a,b,c]	**<0.001***
ACR (mg/g)	11.75±2.61	16.3±6.88	136.3±6.36[a,b]	364.7±10.95[a,b,c]	**<0.001***
HbA1c %	5.01±0.22	8.29±0.84[a]	8.66±0.37[a,b]	8.58±0.47[a]	**<0.001***
RBS (mg/dL)	87.65±12.48	233.6±51.92[a]	261.65±49.87[a]	332.25±73.47[a,b,c]	**<0.001***
Cholesterol (mg/dL)	151.8±24.04	158.35±38.02	230.75±19.12[a,b]	251.9±5.87[a,b,c]	**<0.001***
HDL-C (mg/dL)	61.9±9.56	64.3±11.71	44.05±2.19[a,b]	33.05±4.67[a,b,c]	**<0.001***
LDL-C (mg/dL)	69.35±5.49	76.95±3.05	144.6±9.21[a,b]	172.3±13.15[a,b,c]	**<0.001***
TG (mg/dL)	89.05±7.01	76.25±6.81	257.55±27.89[a,b]	383.15±12.27[a,b,c]	**<0.001***

(a, b, and c for the significant difference when a: compared to control group, b: compared to diabetic non albuminuric group, c: compared to diabetic microalbuminuric group)
*: Significant

As shown in **Table (3),** the mean eGFR levels differed significantly between the studied groups as diabetic patients with macro albuminuria showed lower mean values compared to other groups, also serum adropin levels differed significantly between the studied groups as diabetic patients with macro albuminuria showed lower mean values compared to other groups

Table (3): Mean values of eGFR, and serum adropin among the studied groups

Variable	Control Group (n=20)	Diabetic non albuminuric group (n=20)	Diabetic microalbuminuric group (n=20)	Diabetic macro albuminuric group (n=20)	P
eGFR (ml/min/1.73m) Mean±SD	94.9±8.08	98.12±13.28	96.28±11.01	120.03±14.59[a,b,c]	**<0.001***
Serum adropin (pg/ml) Mean±SD	6.87±1.42	4.44±0.4[a]	3.44±0.32[a,b]	2.25±0.49[a,b,c]	**<0.001***

(a, b, and c for the significant difference when a: compared to control group, b: compared to diabetic non albuminuric group, c: compared to diabetic microalbuminuric group), *: Significant

In this study, we correlated serum adropin concentrations with several demographic and clinical variables (**Table 4**). Adropin showed significant positive correlations with each of (HB%, Albumin, total protein, and HDL). While it showed significant negative correlations with each of (age, creatinine, BUN, HbA1c, RBS, ACR, cholesterol, LDL, TG, eGFR).

Table (4): Correlation between adropin and different parameters (n=80):

variables	Adropin (pg/ml)	
	R	P
Age	**-0.337**	**0.008***
BMI	0.220	0.091
WBC	0.072	0.584
HB%	**0.583**	**< 0.001***
PLT	-0.014	0.915
Creatinine	**-0.802**	**<0.001***
BUN	**-0.683**	**<0.001***
Albumin	**0.874**	**<0.001***
Total Protein	**0.876**	**<0.001***
Hba1c %	**-0.304**	**0.018***
RBS	**-0.574**	**<0.001***
ACR	**-0.858**	**<0.001***
Cholesterol	**-0.739**	**<0.001***
HDL	**0.811**	**<0.001***
LDL	**-0.778**	**<0.001***
TG	**-0.761**	**<0.001***
eGFR	**-0.552**	**<0.001***

r= Pearson correlation coefficient, *: Significant

Adropin's potential as a new biomarker for recognizing diabetic nephropathy from people without nephropathy and healthy controls was analysed using ROC curve analysis. **Figure 1** displays the ROC analysis results. The ROC curve in the current investigation suggested that a cutoff value of 3.75 (pg/ml) for adropin was optimum for distinguishing between diabetic and nondiabetic nephropathy (**Table 5)**.

Table (5): validity of adropin (pg/ml) in predicting diabetic neuropathy

Variables	AUC	95% CI	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
S. adropin	0.894	0.802-0.987	3.75	82.5%	90%	94.3%	72%	85%

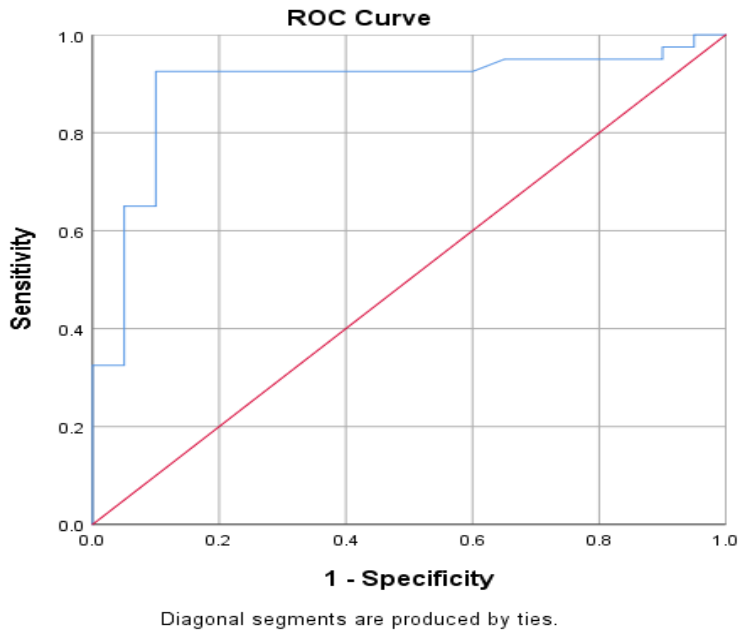

Figure (1): ROC curve of serum adropin (pg/ml) in predicting DN

DISCUSSION

Among its many roles, adropin's regulation of insulin release plays a role in regulating glucose, lipid, and protein metabolisms; it was detected in the year of 2008 by **Lovren *et al.* [11].** Several experimental animal studies had linked adropin with development of T2D as the study by **Gao *et al.* [12]**, where adropin knockout mice had suffered severe insulin resistance and its metabolic consequences as dyslipidemia and adiposity. Serum adropin levels may be linked to type 2 diabetes and its metabolic consequences, however this association has only been studied in a small number of humans. The purpose of this research was to investigate the association between serum adropin levels and metabolic disorders such as DN and dyslipidemia.

As regards the serum adropin levels correlation with the degree of microalbuminuria (as early predictor of DN), we found that diabetic cases with macroalbuminuria had significantly lower serum adropin levels when compared to cases with no albuminuria, microalbuminuria, and healthy controls ($p < 0.001$). Microalbuminuric and macroalbuminuric patients with T2D had lower adropin concentrations compared to non albuminuric patients, with macroalbuminuric patients having the lowest serum adropin levels. Our findings agreed with those of **Hu and Chen [13],** who found an inverse relationship between serum adropin and albuminuria in type 2 diabetes.

Meanwhile, we revealed statistically significant negative correlation between serum adropin and eGFR, the same results were reported by other investigators as **Hu and Chen [13], Es-haghi *et al.* [14] and Berezina *et al.* [15].**

Regarding parameters of glucolipid hemostasis (cholesterol, HDL-C, TG, RPG and HbA_1c) our results were in line with other studies [14, 16], we found that serum adropin levels was negatively correlated with HbA1c, as an indicator for glycemic control. These findings were supported by animal studies where adropin knockout rats had severe insulin resistant that improved after adropin injection [12].

According to literature review, adropin can ameliorate insulin resistance via decreasing endogenous hepatic glucose production, enhancing glucose utilization through sensitizing insulin signaling pathways as Akt phosphorylation, beside activating glucose transporter 4 receptor [5,6].

Our results revealed a strong negative correlation between serum adropin concentrations and cholesterol, TG and LDL and a positive correlation with HDL. These finding were in concordance with the results of other studies [14-18]. This association between serum adropin and dyslipidemic profile encouraged other investigators to assess the correlation between adropin levels and metabolic dysfunction associated with NAFLD, where **Zhang *et al.* [19]** and **Li *et al.*[20]** found strong correlation between low adropin concentrations with development of metabolic dysfunction-associated fatty liver disease (MAFLD) indicating that adropin could serve as potential biomarker to predict the development of metabolic complications in T2D patients [17,18].

A meta-analysis by **Soltani *et al.* [21],** involving 5 studies and 643 participants, found that serum adropin concentrations were significantly lower in overweight and obese individuals compared to normal-weight individuals. These results corroborated our observation that there is an inverse correlation between serum adropin levels and body mass index. The results were replicated in both in vivo and in vitro investigations [20-23].

Our work has several limitations, the first being that, being a cross-sectional study, we cannot infer a causal association between adropin and the data we examined. Second, the relatively small sample size of this study, so obtained results cannot be generalized on larger populations. Nevertheless, our current study added to the emerging literature about the significant role of adropin in development of DN as well as other diabetic metabolic complications.

CONCLUSION

DN is associated with reduced adropin levels, and serum adropin may be a useful diagnostic marker for early identification of DN among type 2 diabetic cases.

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