Adiponectin level in diabetic kidney disease the relationship with glycemic control and microvascular complications; a mystery unresolved

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Abstract

Background: Defining new predictive biomarkers in diabetic kidney disease (DKD) would provide a window of opportunity for preventive and/or therapeutic interventions to prevent or delay the onset of irreversible long-term micro and or macro vascular complications. Adiponectin (ADPN) has been variously associated with diabetic microvascular complications; however, no comprehensive clinical data exist examining the association between adipocytokines and the presence of these complications.

Aim of study: we aimed to measure the plasma levels of adiponectin in patients with type 2 diabetes mellitus, to assess whether these levels vary with the different stages of DKD according to their e GFR and to evaluate its relation to their microvascular complications and glycemic control.

Methods: This is a prospective observational study including 100 T2DM classified into two groups according to their albuminuria levels and estimated GFR. Participants subjected to thorough history taking and clinical examination. Serum level of ADPN was assessed in all patients.

Results: Serum ADPN levels were significantly lower in T2DM patients with nephropathy (P = 0.001), while their levels were non-significantly higher in patients with non-proliferative retinopathy or neuropathy. Their levels were lowered with more advanced stages of DKD with nephropathy and the decrement was dependent on their severity (P<0.001). Levels of ADPN with cutoff value of < 22600 (μg/mL) had ability to diagnose microvascular complications in our diabetic patients with sensitivity (81%) and specificity (27%). Multivariate logistic regression analysis showed that the odds ratio for the presence of nephropathy in the lowest tertile of ADPN was 1.09 (95% CI; 11.45- 13.08, P= 0.06), therefore, ADPN was not an independent risk factor for diabetic nephropathy. However, its higher level was independently associated with increased odds for the presence of neuropathy in particular. Conclusions: ADPN plays a role in the pathogenesis of microvasculopathy in diabetic patients and help to identify high-risk patients and modulate the therapeutic potential in the prevention of DKD.

Keywords: *T2DM; ADPN: Adiponectin; DKD Diabetic Kidney Disease; ESRD: End Stage Renal Disease.*

1. Introduction

Diabetic kidney disease is the most serious microvascular complication of diabetes and the largest single cause of ESRD in many developed countries [1]. DKD is also associated with an increased cardiovascular mortality. It occurs as a result of interaction between both genetic and environmental factors. Hyperglycemia, hypertension, and genetic predisposition are the major risk factors. However, the exact mechanisms of DKD are unclear [2]. Inflammation may be a key factor which is activated by the metabolic, biochemical, and hemodynamic derangements known to exist in the diabetic kidney [4]. Hemodynamic-mediated vascular injury was identified as one mechanism in the pathogenesis of diabetic nephropathy [4]. Sustained increase in glomerular capillary pressure driven by increase in plasma flow had been observed, might be damaging to glomerular endothelial, epithelial, and mesangial cells, thereby initiating and contributing to the progression of nephropathy [4]. Alt-

hough numerous mediators of diabetic hyperfiltration had been proposed, the exact mechanism remained unclear [3&4]. Progressive microvascular vaso-degeneration is the major factor in progression of diabetic complications [5]. Adipocytokines are adipose tissue-derived molecules with hormone-like actions that are produced mainly by adipocytes. They are established metabolic regulators with functions in several other systems including inflammation [6]. Adiponectin (ADPN) is one of the most abundant adipocytokines and it is an anti-inflammatory and cardioprotective protein. [7] While adiponectin is generally measured with high-throughput assays as total circulating levels. ADPN has been shown to protect against cardiovascular disease for the general population with problematic metabolic syndrome. Interestingly, Adiponectin levels are inversely related to the degree of adiposity [8]. Therefore, its concentrations are reduced in obesity, in conditions with resistance to insulin, in diabetes mellitus (DM). A physiological role for ADPN has not been fully established. ADPN is thought to inhibit the endothelial expression of adhesion molecules VCAM -1and

ICAM -1, which is triggered by inflammatory cytokines, insulin resistance [9]. We aimed to measure the plasma levels of adiponectin in patients with T2DM, to assess whether these levels vary with the different stages of DKD according to their eGFR and to evaluate its relation to their microvascular complications and glycemic control.

2. Materials and methods

The present study was conducted on 100 T2DM patients with DKD. The study was approved by the ethical committee of Faculty of Medicine, Assiut University and a written informed consent was obtained from each participant. The type 2 diabetic patients (diagnosed according to ADA2010) were classified according to their albuminuria levels (Group A) and to their estimated GFR (Group B) into two groups. Group A subdivided into group A1 patients with normoalbuminuria (defined as < 30 mg/24hour), group A2 patients with microalbuminuria (defined as 30-299 mg/24hour) and group A3 patients with macroalbuminuria (defined as >300 mg/24hour). Group B sub classified into 5 subgroups according to eGFR stages; G1 (≥ 90 ml/min/1.73 m²), G2 (60–89 ml/min/1.73 m²), G3 (3059ml/min/1.73m²), G4 (1529ml/min/1.73m²) and G5 (≤15ml/min/1.73m²). Patients with albuminuria (rather than due to diabetes mellitus) had past history of cardiovascular events (heart failure or history of coronary artery disease), infections, apparent autoimmune disease, liver cell failure, respiratory failure and acute diabetic complications, hypertension and diabetic patients on regular hemodialysis were excluded. The estimated glomerular filtration rate (eGFR) was calculated by the modification of diet in renal disease (MDRD) equation [39]. Patients groups were matched for sex and age. All patients were submitted to full history taking, thorough clinical examination, and anthropometric measures to determine the body mass index (BMI), The abdominal ultrasound were done to exclude patients with obstructive uropathy. Electrocardiogram (ECG) and Trans-thoracic Echocardiography (TTE) were performed to all participants. Fundus examination was performed by an ophthalmologist after maximum pupillary dilatation using ophthalmoscope to identify diabetic retinopathy. After centrifugation to yield platelet-poor plasma from samples on anticoagulant (3.8% sodium citrate) and serum from clotted blood samples, serum and plasma samples was stored in aliquots at _20 °C until assay. The following routine laboratory investigations were done in all patients groups: Peripheral hemogram was performed on whole blood samples on EDTA using Beckman Coulter Hmx, USA, and HbA1c% will be measured by high-performance liquid chromatography. Liver function tests, kidney function tests and Lipid profile were measured by standard laboratory methods using Hitachi 911 autoanalyzer. 24 hours urinary albumin excretion was measured by the Immulite analyzer. Urinary protein by dip stick method was performed and the quantitative urine albumin/creatinine ratio in morning spot urine samples was used for standard microalbuminuria determination. Serum levels of Adiponectin was assessed by ELISA technique using (EIA-3418) KIT, DRG international inc., USA). Urine creatinine and albumin was measured with spectrophotometric analysis. Urinary protein by dip stick method was performed and the quantitative urine albumin/creatinine ratio in morning spot urine samples was used for standard microalbuminuria determination.
Statistical analysis: Statistical analyses will be carried out using the Statistical Package for Social Sciences (SPSS). Descriptive statistics for each variable will be determined. Normally distributed data will be expressed as mean + standard deviation. Median and minimum-maximum values will be used for variables without a normal distribution. data with a normal distribution will be compared by Student t test and ANOVA test. Comparisons of continuous variables with an asymmetric distribution were will be made by using the Mann-Whitney U test and Kruskal-Wallis test. Associations between the variables will be explored using the Pearson correlation and Spearman's rho (for data that will not normally distributed). Binary logistic regression analysis will be performed to define variables associated with albuminuria. Receiver-operating characteristic

(ROC) analyses will be used to compare the performance and prognostic power of the Adrenomedullin, Leptin and Adiponectin for albuminuria and diabetic microvascular complications. the predictive validities will be quantified as the area under the ROC curves (c statistics), and the comparisons of c statistics will be performed by MedCalc statistic software. A P value less than 0.05 will be considered significant.

3. Results

The demographic data of all studied our T2DM patients; 64 (64%) patients were males. Their age ranged 32- 48 years with mean ± SD of 40.55 ± 7.33 years. The mean ± SD of disease duration was 7.61 ± 2.86 years. The mean ± SD of MAP 52.55±5.89. Most of patients (72%) were overweight; their body mass index (BMI) ranged 21-33 (kg/m²) with mean ± SD of 27.09 ± 5.55 (kg/m²).

Table 1: The Clinical Main Vascular Complications in the All Studied Groups

Complications	Frequency (%)
Microvascular complications	100 (100%)
• Retinopathy	
• Non-proliferative	70 (70%)
• Proliferative	50 (71.4%)
• Nephropathy	20 (28.6%)
• Yes	
• No	63 (63%)
• Neuropathy	37 (37 %)
• Yes	
• Motor	82 (82%)
• Sensory	13 (15.9%)
• Sensorimotor	41 (50 %)
• Autonomic	38 (46.3%)
• No neuropathy	28 (34.1%)
	18 (18%)

Data was expressed in form of frequency (percentage).

Half of the patients (50%) received oral hypoglycemic agents and 92% were on conservative renal replacement therapy while only 8 patients (8%) were on hemodialysis on demand (secondary to acute kidney injury happened, whatever the cause, during the study). The frequency of microvascular complications were present in 100 % of our cases manifested as follow: Retinopathy in 70% of patients; 28.6% of them had proliferative changes while 71.4% of them had non-proliferative changes, 82% of our patients had neuropathy; motor in 15.9%, sensory in 50 %, sensorimotor in 46.3% and autonomic features in 34.1%. Most of our studied patients had DKD with nephropathy as shown in Table 1 .

Table 2: Classification of Studied DKD Patients According to their Egfr and the Degree of Albuminuria

Parameters	Frequency (%)
Stage of DKD	
i) (≥ 90 ml/min/1.73 m²)	33 (33%)
ii) (60-89 ml/min/1.73 m²)	16 (16%)
iii) (30-59 ml/min/1.73 m²)	47 (47%)
iv) (15-29 ml/min/1.73 m²)	4 (4%)
v) (< 15 ml/min/1.73 m²)	0 (0%)
Degree of albuminuria	
A1: Normoalbuminuria (< 30mg/24hr)	18 (18%)
A2: Microalbuminuria (30-300 mg/24hr)	31 (31%)
A3: Macroalbuminuria (> 300 mg/24hr)	51 (51%)

Stage of DKD (diabetic kidney disease) according to e GFR (ml/min/1.73 m²); Data was expressed in form of frequency (percentage).

Table 3: The Relationship between Different Stages of DKD and the Degree of Albuminuria

Stage of DN (ml/min)	Degree Albuminuria (mg/24 hour)	Total number patients	P - value

	Norm albuminuria (A1: < 30)	Microalbuminuria (A2: 30-300)	Macroalbuminuria (A3: > 300)		
I (> 90)	13 (13%)	16 (16%)	4 (4%)	33 (33%)	0.001
II (60-89)	3 (3%)	7 (7%)	6 (6%)	16 (16%)	0.09
III (30-59)	2 (2%)	8 (8%)	37 (37%)	47 (47%)	0.02
IV (15-29)	0	0	4 (4%)	4 (4%)	-
V (<15)	0	0	0	0	-
Total	18 (18%)	31 (31%)	51 (51%)	100 (100%)	-

DKD (diabetic kidney disease). Data was expressed in from of frequency (%) respecting to total patients count. P value was considered significant if < 0.05.

Table 2 showed that our studied DKD patients were classified according to estimated glomerular filtration rate (eGFR) (ml/min/1.73 m^2); into five stages from I-V where the most of them (47%) had stage III while none of patients had stage V. Our patients were classified according to albuminuria as follows: eighteen patients (18%), had normoalbuminria and thirty-one of them (31%) had microalbuminuria while fifty-one patients (51%) had macroalbuminuria. Stage I DKD was manifested in 33% of our patients with variable degree of albuminuria mainly microalbuminuria (16%), these differences were of highly statistically significance with P value $\leq$ 0.001. Stage III DKD was manifested in 47% of our patients, 37% of them had an overt nephropathy. These differences were of statistically significance with P value $\leq$ 0.02 However, no statistical significant difference among our patients with stage II and VI. Moreover, none of our Patients was in stage V as shown in Table 3. There were highly statistically significant differences between different grades of albuminuria and the basic characteristics of our studied patient groups; BMI, disease duration, HbA1c (24.09 ± 4.09, 4.44 ± 3.69

and 7.34 ± 1.23 in A1 versus 26.32 ± 5.01, 5.61± 3.33 and 9.91 ± 1.0301 in A2 versus 26.91 ± 6.78, 8.15 ± 3.55 and 10.78 ± 2.2111 in A3 with P value $\leq$ 0.001 for each respectively. Male gender was a characteristic feature in our T2DM patients had increasing degree of albuminuria with highly statistically significant difference between its different grades (11% in A1 versus 23% in A2 versus 36% in A3 with P value $\leq$ 0.001 for each respectively). Moreover, There were highly statistically significant differences between different grades of albuminuria and the mean serum levels of Adiponectin in our patients (55.72 ± 19.03, 135.39 ± 46.01, 183.01 ± 30.11; P $\leq$ 0.001 respectively). Patients with Macroalbuminuria had more disease duration, HbA1c, BMI, serum levels of adiponectin compared with those with Microalbuminuria and Normoalbuminuria with significant P values (P< 0.05). Regarding the age of patients, the increased age, the more advanced grade of albuminuria but with no significant statistical difference between different grades of albuminuria. It was noted that patients with Microalbuminuria had values in between other two groups (normal and macroalbuminuria) regarding the significant parameters as shown in Table 4.

Table 4: The Clinical and Laboratory Basics of Studied Patients According To Degree of Albuminuria

Variables	Degree Albuminuria (mg/g cr)			P*-value	P1-value	P2-value	P3-value
	Normoalbuminuria (A1: < 30) (No=18)	Microalbuminuria (A2: 30-300) (No=31)	Macroalbuminuria (A3: > 300) (No=51)				
Age (years)	37.45 ± 3.24	39.61 ± 2.13	38.54 ± 3.45	0.71	0.09	0.34	0.47
Gender (%) Male Female	11 (61.1%) 7 (38.9%)	23 (25.8%) 8 (74.2%)	36 (70.6%) 15 (29.4%)	0.001	0.03	0.01	0.02

BMI (kg/m²)	24.09 ± 4.09	26.32 ± 5.01	26.91 ± 6.78	0.04	0.03	0.001	0.44
Duration (years)	4.44 ± 3.69	5.61± 3.33	8.15 ± 3.55	0.001	0.56	0.01	0.001
HbA1c (%)	7.34 ± 1.23	9.91 ± 1.03	10.78 ± 2.21	0.001	0.001	0.01	0.02
Adiponectin (µg/mL)	22988 ± 4109.35	16907.64 ±3436.23	12939.35 ± 2705.45	0.04	0.001	0.01	0.001

Nominal date was expressed in form of frequency (%) and compared with Chi² test while continuous one was expressed in form of mean ± SD and compared with one-way ANOVA test. P value considered of statistical significant if < 0.05. HbA1c; glycosylated hemoglobin, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure *result of ANOVA test ; P1 compared patients with normoalbuminuria and those with microalbuminuria, P2 compared patients with normoalbuminuria and those with macroalbuminuria, P3 compared patients with macroalbuminuria and those with microbuminuria.

Table 5: The Relation of the Levels of Adiponectin among Our Studied Patients with Different Stages of Diabetic Kidney Disease

Stage of DKD (No= 100) Mean ± SD					P -value					
Stages	Stage I (No =3)	Stage II (No = 16)	Stage III (No =33)	Stage IV (No =33)	P1-value	P2-value	P3-value	P4-value	P5-value	P6-value
ADPN (µg/mL)	18908.3 3 ± 1235.98	15503.3 2 ± 2009.11	13782.9 8 ±1983.0 3	6171.4 5 ± 983.24	0.09	0.04	0.02	0.78	0.14	0.03

DKD: diabetic kidney disease, ADPN: adiponectin, Data was expressed in form of mean ± SD and compared with one-way ANOVA test. P value considered of statistical significant if < 0.05 P* indicated to ANOVA test; P* value 0.001for each, p1 indicated comparison between grades I and II, P2 indicated comparison between grades I and III P3 indicated comparison between grades I and IV, P4 indicated comparison between grades II and III, P5 indicated comparison between grade II and IV, P6 indicated comparison between grade III and IV.

Table 5 showed that patients with more advanced stages of DKD, the lower serum levels of Adiponectin (18908.33 ± 1235.98, 15503.32 ± 2009.11, 13782.98 ± 1983.03 and 6171.45 ± 983.24 respectively) with variable significance values.

Table 6 showed that 50 patients had non-proliferative retinopathy while proliferative retinopathy presented in 20 patients. The mean serum levels of adiponectin in patients with non-proliferative retinopathy were statistically non-significantly higher than those levels with patients with proliferative retinopathy (16518.23± 6553.13 versus 15232.86 ± 7262.09 with P ≥ 0.35).

Moreover, eighty-two (82%) patients had different types of diabetic neuropathic affection while 18 (18%) patients had no neuropathy. Those patients with diabetic neuropathy had statistically non-significantly higher mean levels of adiponectin compared with those patients without neuropathy (16791.11 ±4213.09 versus 15811.65± 3513.22 with P ≥ 0.35). Regarding to proteinuria, 63 patients had proteinuria while no proteinuria presented in 37 patients. The mean serum levels of adiponectin in patients without proteinuria were statistically significantly higher than those levels with patients with proteinuria (18322.59 ± 2100.13 versus 14601.69 ± 2221.49 with P ≤ 0.001). Statistically non-significant negative correlations of the mean levels of adiponectin with age of patients, BMI, duration of the disease, HbG, blood glucose, HbA1c, blood urea and serum creatinine (r = - 0.17, r = -0.02, r = -0.21, r = -0.14, r = -0.11, r = -0.04, r = -0.41 and r =- 0.11, with P ≥ 0.05 respectively) with a highly statistically significant negative correlation with albuminuria ((r =

- 0.44 ; P ≤ 0.001) were found. However, a highly statistically significant positive correlation of the mean level of Adiponectin with creatinine clearance (r = 0.3; P ≤ 0.001) was found. Regarding the lipid profile, Adiponectin had statistically significant negative correlations with LDL and TG (r= -0.55 and r= -0.61 with P ≤ 0.04 and 0.01 respectively). Nonetheless, there were statistically non-significant correlations between cholesterol and HDL levels with Adiponectin (r= 0.21, P ≥ 0.05 for each) as shown in Table 7 and Figures1a, b and 2a, b.

Table 6: The Relation between of Microvascular Complications with Adiponectin Levels in Studied Diabetic Patients

Microvascular complications	No	Adiponectin (µg/mL) Mean± SD	P-value
• Retinopathy • Non-proliferative retinopathy • Proliferative retinopathy	50 20	16518.23± 6553.13 15232.86 ± 7262.09	0.35
• Neuropathy • Yes • No	82 18	15811.65± 3513.22 16791.11 ± 4213.09	0.35
Nephropathy • With proteinuria • Without proteinuria	63 37	14601.69 ± 2221.49 18322.59 ± 2100.13	0.001

Data was expressed in from mean ± SD. Student t test was used to compare between both groups and
P value was considered statistically significant if < 0.05.

Table 7: The Correlations of Adiponectin with Different Parameters in the Studied Patients

Parameters	Adiponectin	
	r =	P-value
Age	-0.17	0.52
BMI	-0.02	0.45
Duration of DM	-0.21	0.11
HbG Level	-0.14	0.16
Blood glucose level	-0.11	0.21
HbA1c level	-0.04	0.65
Serum creatinine	-0.11	0.33
Creatinine clearance	0.31	0.001
Albuminuria	-0.44	0.001
LDL	-0.55	0.04
TG	-0.61	0.01
HDL	0.21	0.32
TC	0.21	0.55

TC: Cholesterol TG: triglycerides. P value indicated the significance of correlation where correlation was significant if P value <0.05 while r value indicated to strength of correlation.
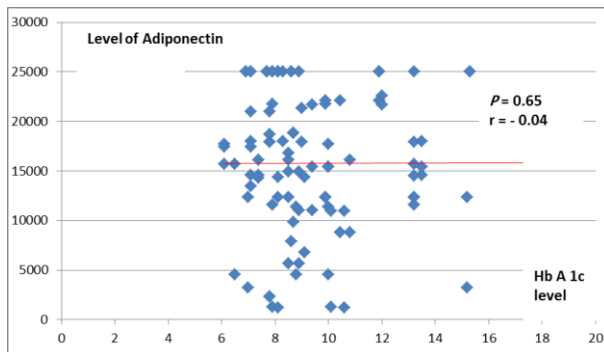Figure 1a: Correlation between level of Adiponectin and HbA1c in the studied patients

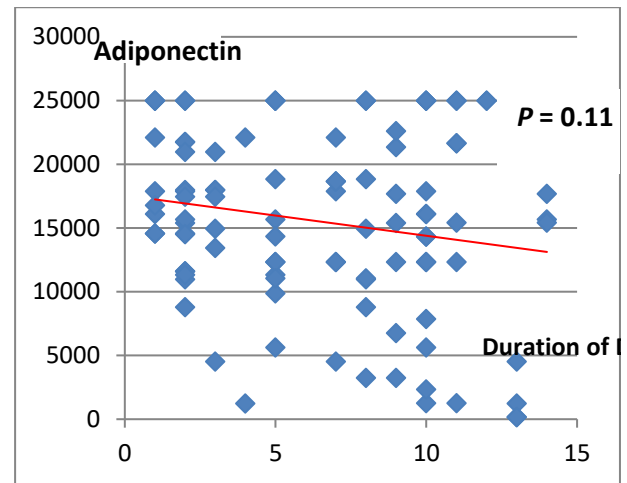**Fig. 1:** A) Correlation between Adiponectin and Duration of DM.

Fig. 2: A) Correlation between Adiponectin and Creatinine Clearance.
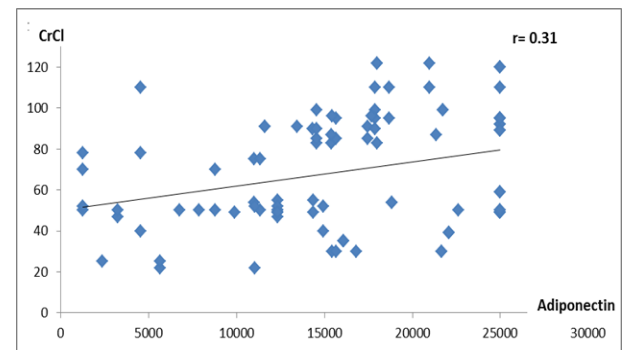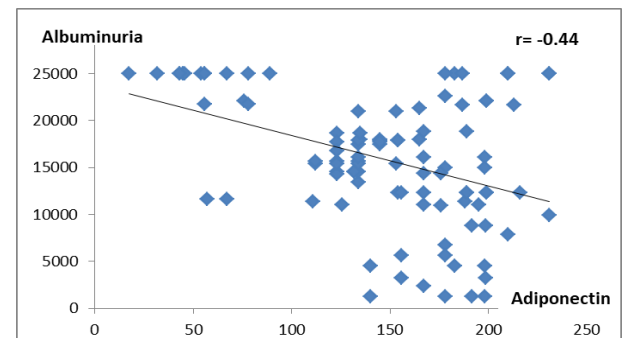

**Fig. 2:** b) Correlation between Adiponectin and Albuminuria.

Multivariate logistic regression analysis for prediction of diabetic nephropathy showed that the odds ratio for the presence of nephropathy in the lowest tertile of adiponectin was 1.09 (95% confidence interval 11.45- 13.08, P= 0.06) therefore, adiponectin was
not an independent risk factor for diabetic nephropathy, while duration of DM (OR= 2.33, 95%CI= 2.45- 4.78, P= 0.04) was considered as an independent risk factor for diabetic nephropathy as shown in Table 8. In the current study, the Level of Adiponectin with cutoff value of < 22600 (µg/mL) was able of to detect the diagnosis and prognosis of microvascular complications in our patients with diabetic nephropathy with sensitivity (81%) and specificity (27%) with 86% Positive predictive value (PPV) and 20 % negative predictive value (NPV) as shown in Table 9 and Figure 3.

Table 8: Multivariate Regression Analysis for Prediction of DKD with Nephropathy

Variables	Odd ratio (OR)	95% CI	P value
Age	0.98	1.09- 2.33	0.07
Sex	0.34	2.34- 3.07	0.11
Duration of DM	2.33	2.45- 4.78	0.04
Body mass index	1.4	6.78- 7.68	0.45
Adiponectin	1.09	11.45-13.08	0.06

Table 9: Receiver-Operating Characteristic (ROC) Analyses for Prediction of Microvascular Complications in Our Studied Patients

ROC curve	Adiponectin
Area under the curve	0.61
Cut off point	< 22600
P value	0.01
Sensitivity	81%
Specificity	27%
Positive predictive value	86%
Negative predictive value	20%
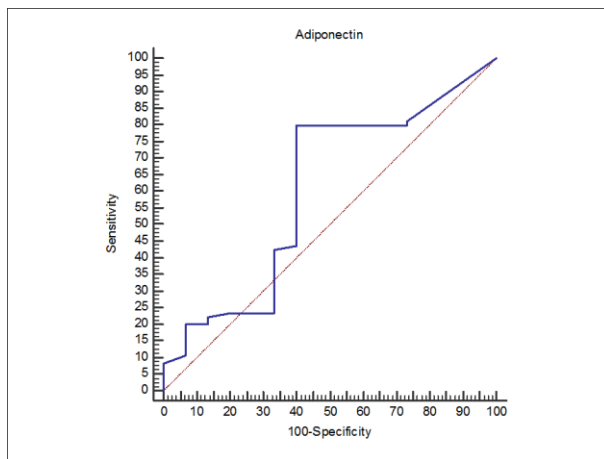
Fig. 3: Prediction of Microvascular Complications in Our Studied Patients.

4. Discussion

Type 2 diabetes (T2DM) has been postulated to be a generalized inflammatory condition resulting from obesity-induced dysregulation of adipocytes, which produce an excess of inflammatory cytokines [10]. Persistent inflammatory state further contributes to the development of the extensive vascular disease characteristic of diabetes and implicate in the in the progression of chronic kidney disease [11] [12]. The disturbance of diabetic complications is based on microangiopathy. Vascular bed damage may be originated by chronically stimulated high glucose and advanced glycosylated end product, which might be mediated through cytokines, adipokines, and oxidized stress like oxidized low density lipoprotein [4]. However, there is still limited information on the relationship between microangiopathies and adipocytokines, such as adiponectin and leptin in patients with T2DM [6]. The present study aimed to assess plasma level of adiponectin in patients with DN and assess its correlation with glycemic control and other diabetic microvascular complications.

In the current study, most of our diabetic patients had macroalbuminuria were associated with more advanced stages of diabetic nephropathy. Our results were in agreement with Baghel et al., 2014 [13] who stated that degree of albuminuria is associated with elevated serum creatinine and decrease creatinine clearance reflecting kidney function impairment. However, Kramer et al., 2003 [14] stated that CKD with creatinine clearance (<60 ml/min/1.73 m2) may occur in the absence of increased urine microalbumin excretion in a substantial proportion of adults with T2DM. Although albuminuria is considered a key aspect of the pathogenesis of progressive kidney dysfunction, the progressive reduction in GFR could be described in patients with T2DM in the absence of proteinuria indicating that the associations with albuminuria and reduced eGFR are strong and independent across the range of observed values in T2DM patients. Also Caramori 2003, Toshiharu et al., 2009 and MacIsaac et al., 2006 [15 – 17] reported that this phenomenon of reduced GFR in the absence of significant albuminuria was previously described in several epidemiologic data of patients with T2DM, although the clinical squeals in such patients has not been previously determined.

In the current study, Most of our diabetic patients had microvascular complications. The diabetic microvascular complications are principally driven by vascular inflammation. The potential role of adipocytokines in vascular disease is intriguing; in particular, diminishing the inflammatory state of diabetes may be central in modifying risk for progressive endothelial dysfunction and renal disease [23]. Therefore, the role of adiponectin had been addressed in our patients. The statistically significant lowest levels of adiponectin were found in our T2DM patients especially those patients with diabetic nephropathy; neuropathy and those had proliferative retinopathy. In spite of our patients with sensory affection had statistically significant higher levels of adiponectin, the patients with no neuropathic affection had the statistically significant highest mean levels suggesting that adiponectin is generally considered to be an anti-inflammatory adipocytokine, and has extensive vasculo-protective actions. In addition, multiple logistic regression analysis showed that higher serum adiponectin levels were independently associated with increased odds for the presence of neuropathy. This finding was in agreement with Kato et al., 2008 [19] who stated that serum adiponectin was not correlated with neuropathy with the diagnosis of peripheral neuropathy based on typical symptoms and current perception threshold or nerve conduction study. Notably, the prevalence of diabetic retinopathy in the present study population was lower, and the severity of retinopathy was milder. Our result of the present study was in consistent with Kato et al., 2008 and Jung et al., 2014 [19] [20] who found that adiponectin level is increased in the more advanced stages of diabetic retinopathy of T2DM.

Also our results were in agreement with Yilmaz et al., 2008 [21] who reported that plasma adiponectin concentrations are lower in patients with diabetic retinopathy than those without it. It remains to be determined whether serum adiponectin level is causally associated with the development and progression of retinopathy or increased as a compensatory response. Zhang et al., 2017 [5] have shown that diabetic retinopathy has features of chronic and subclinical inflammation. Therefore, in response to inflammation promoting diabetic retinopathy, serum adiponectin might be increased as a compensatory process. Conversely, another possible explanation is that adiponectin could worsen diabetic retinopathy, although this appears to be contrary to the general concept of adiponectin as a beneficial hormone.

In our study, there were highly statistically significant differences between different stages of diabetic nephropathy and level of adiponectin. The more advanced stage of diabetic nephropathy, the more lower the level of adiponectin. These findings were in agreement with Sharma et al., 2008 and Doumatey et al., 2012 [22] [23] who found that serum adiponectin and albuminuria levels were inversely correlated. Moreover, we found low levels of plasma adiponectin in patients with Macroalbuminuria, compared to those without Macroalbuminuria. The one explanation is that kidney plays a substantial role in ADPN removal from the plasma by absorbing and degrading the peptide. Ljubic et al., 2015 [25] were attributed the decreases of adiponectin levels in T2DM patients to obesity. Notably, Jung et al., [20] suggested that renal ADPN degradation is already impaired in patients with mild to moderate renal insufficiency. Our results were in disagreement with Galovicova et al., 2012 [26] who reported higher plasma adiponectin levels in those with Macroalbuminuria, compared to those who had normoalbuminuria as well as nicroalbuminuria concluding that diabetic nephropathy potentially plays a very important role in increasing the synthesis and secretion of adiponectin.

Interestingly, in our study, an inverse association was reported between adiponectin and renal dysfunction, consistent with that study by LeveyAS et al., 2007 [26]. Furthermore, whether lesser degrees of renal impairment influence adiponectin concentrations is unclear. In the present study, the mean eGFR and serum creatinine between patients with nephropathy and those without nephropathy were not significantly different, indicating mild renal impairment. Therefore, the present data suggest that serum adiponectin level might be negatively associated with renal dysfunction in mild nephropathy. Although the reason why the serum adiponectin level is higher in chronic kidney disease (CKD) patients than in subjects without CKD remains unclear, cystatin C could be one of the suggested causes.

Moreover, our patients who had diabetic macrovascular complications had the statistically significant highest levels of adiponectin. This finding was in agreement with Wang et al., 2015 and Yazici et al., 2012 [27] [28] who reported that patients with these macrovascular complications display low concentrations of plasma adiponectin levels. It could be hypothesized that increased hyperlipidemia, which is ultimately involved in macrovascular complications, might play a role in decreasing circulating adiponectin. Interestingly, low plasma adiponectin favors progression of atherosclerosis and vascular plaque formation [28]. Also, in the present study, the multiple links of adiponectin to several metabolic risk factors, such as triglycerides, cholesterol, HDL-C, LDL –C had been addressed. Our study found that the level of adiponectin was significantly inversely related with the LDL, and triglycerides, these results were in agreement with Alitinova et al., 2007 [29] who explain these results to inflammation, hyperglycemia and insulin resistance. Furthermore, Izadi et al., 2013 [30] reported that dyslipidemia which is characterized by low concentrations of apolipoprotein AI and high concentrations of TG-rich lipoproteins have an inverse relationship with serum adiponectin concentrations. Some authors also had shown that plasma adiponectin regulates TG rich lipoprotein metabolism and lipid metabolism regulatory enzymes suggesting that adiponectin has been effective on pathology of lipid metabolism and influenced by BMI. However, we did not found any correlation between adiponectin and HDL. Our results were in contrast to Maruyama et al., 2009 [31] who reported that ADPN is involved in the regulation of lipid metabolism where a decreased ADPN is linked to hyperlipidemia, low HDL concentration and the appearance of small dense LDL particles in young men in our study, we found the lowest levels of adiponectin among male diabetic patients. This result suggested that adiponectin as a cardiovascular protector has diminished more in male diabetic patients. The lower level of adiponectin in male diabetic patients may be due to a number of factors but a higher concentration of serum testosterone is likely one of them. Our finding was in agreement with Ding et al., 2006 [32] who stated that testosterone also plays a role in the pathogenesis of type 2 diabetes. They reported that serum testosterone had reduced in male patients with T2DM, whereas it was increased in the female patients supposing that testosterone reduces the risk of type 2 diabetes in men but increases the risk in women. Given the anti-diabetic and anti-atherogenic actions of adiponectin and the potential anti-diabetic actions of testosterone in men, reduced adiponectin and testosterone concentrations in males may at least in part account for the higher incidence of cardiovascular disease in men due to a diminished protective effect of these endogenous hormones. In our study, we found non -significant inverse associations of adiponectin with duration of disease, BMI, poor glycaemia and renal dysfunction. These results came in agreement with Kato et al., 2008 [19] who reported that plasma adiponectin levels were correlated negatively with BMI, insulin resistance, and poor glycaemia. Since hypo-adiponectinemia is associated with inflammation, atherogenic properties and insulin resistance, adiponectin is secreted more likely so as to alleviate their detrimental effects in diabetic ESRD patients. It may also explain the decreased plasma levels of adiponectin in obese patients, as the degree of the obesity is deteriorating. However, the relationship between adiponectin and kidney function is complex., a in our study showed a significant low adiponectin level is associated with T2D patients but adiponectin levels seem to increase with increasing duration of T2D and in diabetic patients with nephropathy. These observations were in agreement with Looker et al 2004, Isobe et al., 2005 and Kadowki and Yamauchi 2005 [33 - 35] who suggested that circulating levels of adiponectin are heavily influenced by the pathophysiologic state of individuals as a result of multiple mechanisms, including reduced clearance by the kidney (and degree of sensitivity or resistance to adiponectin).

In agreement with Rao et al [36], our result stated that, even, with the cutoff value of Adiponectin < 22600 (µg/mL) of high sensitivity (81%) and low specificity (27%) which has 86% Positive predictive value ; PPV, ADPN could not be considered as independent factor for prediction the Microvascular complications in our patients with diabetic nephropathy.

5. Conclusion

Based on these results, adiponectin is supposed to play a role in the pathogenesis of microvasculopathy in T2DM patients. So, it can be used to identify high-risk patients and modulating its action would have therapeutic potential in the prevention of diabetic nephropathy. In particular, higher serum adiponectin was independently associated with increased odds for the presence of neuropathy. Future prospective studies with larger numbers of patients are required to establish a direct relationship between plasma adipocytokines concentrations and the development or severity of diabetic microangiopathies.

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