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Research Article

Role of Angiopoietin like protein-8 as a predictive marker of diabetic nephropathy in type 2 diabetes mellitus

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Abstract

Background: Diabetic nephropathy is a critical complication of T2DM. Its prevalence is 30-40% of individuals with long-standing T2DM. DN can cause damage to the small blood vessels of the kidneys impairing their functions in filtering waste and excess fluids from the blood that may ultimately lead to end-stage kidney disease (ESKD). Although several markers are available for diagnosis of DN, it is important to find better markers for better clinical management. Angiopoietin like protein-8 (ANGPTL8) is produced in the liver and adipose tissue. It has role in regulation in lipid metabolism and regulation of insulin sensitivity. **Aim:** To evaluate serum level of ANGPTL8 in patients diagnosed with DN and to investigate the relation between ANGPTL8 and severity of DN. **Methods:** The study was conducted on 80 subjects divided into 60 patients diagnosed with T2DM with DN and 20 patients diagnosed with T2DM without DN as a control group, All patients undergo Complete medical examination, radiographic imaging (including renal ultrasonography) and laboratory testing including FBG, HbA1c,urea, creatinine,eGFR, AST, ALT, albumin,lipid profile, CRP, serum ferritin, A/C ratio and serum ANGPTL8. **Results:** Serum ANGPTL8 levels showed statistically significant increase in patients with diabetic nephropathy compared to those without, and were independently associated with markers of proteinuria (ACR) and hypoalbuminemia. Although no significant correlation was observed with glycemic control markers or renal filtration parameters (eGFR, serum creatinine). **Conclusion:** Serum level of ANGPTL8 was higher among patients with DN which highlights its value in DN progression and were independently associated with markers of proteinuria (ACR) and hypoalbuminemia. it could be an early predictor for DN.

Key words: ANGPTL8, Diabetic nephropathy, Risk factors**.**

Introduction

Diabetic nephropathy is one of the most chronic serious microvascular complications of T2DM. It is characterized by a persistent increase in proteinuria. It is considered a primary contributor to end-stage renal failure that affects approximately 15% or more of T2DM [(1)]. Several risk factors contribute to the development of DN including genetics, ethnicity, obesity, dyslipidemia, age & smoking[(1)]. The mechanism by which DN occurs is a combination of hemodynamic abnormalities, metabolic disturbance & hormonal imbalance that can end by ESKD [(2)].

If DN progresses to ESKD, the only effective treatment is dialysis and kidney trans-plantation. Therefore, more and more

researches are needed for novel markers that predict diabetic nephropathy among type II diabetic patients. ANGPTL8 is a secretory protein & atypical member of angiopoietin-like family. It is a 22-kDa protein with 198 amino acids [3]. ANGPTL8 main role is to regulate lipoprotein lipase (LPL) activity. It has role in triglyceride metabolism through its interaction with ANGPTL3 and ANGPTL4[4]. ANGPTL8 has been investigated as a potential biomarker for various metabolic diseases, including obesity and diabetes, due to its association with lipid metabolism[5].

ANGPTL8 has role in the regulation of several inflammatory signaling pathways as it can modulate the activity of inflammatory cytokines which play central role in the body's immune response[6]. The level of circulating ANGPTL8 has been associated with atherogenic lipid profiles in high risk cohorts with T2D or cardiovascular disease. Elevated plasma ANGPTL8 levels were associated with higher all-cause mortality risk in Chinese population with T2D [7]. In addition to this, Yang et al suggested a potential role of ANGPTL8 in the pathogenesis of albuminuriain T2D due to the strong link between ANGPTL8 and albuminuria [8]. Previous studies showed altered angiopoietin like protein-8 circulating level in type 2 diabetes mellitus [9]. Whether or not the alteration in ANGPTL8 level can be a predictive marker for increased diabetic nephropathy remains unclear, so more studies are required to indicate the role of ANGPTL8 in diabetic nephropathy.

Aim of the work

To evaluate serum levels of ANGPTL8 in patients diagnosed with DN and to investigate the relation between ANGPTL8 and severity of DN.

Subjects and methods

Study design

This prospective case-control study included a total of 80 participants; 60 were patients with T2DM with DN. The remaining 20 participants were selected as a control group subjects who are T2DM without DN. The participants were chosen from the internal medicine department and clinic at Minia University hospital - Faculty of Medicine between April and October of 2024. The hospital's ethics committee approved the study, and each participant provided a written consent. The date of approval February 13, 2024, and the approval number is 1055: 02/2023. Patients with type 1 diabetes mellitus, chronic kidney disease, malignancy, acute infection and blood loss, or transfusion were excluded from the study. All patients undergo comprehensive medical history taking, physical examination, radiographic imaging (including renal ultrasonography) and laboratory testing including Fasting blood glucose, HbA1c, urea, s. creatinine, eGFR, AST, ALT, albumin, lipid profile, CRP, serum ferritin, A/C ratio and serum ANGPTL8. Our patients were divided into 2 groups **Group I (patients' group):** It included 60 patients diagnosed with diabetic nephropathy. Diagnosis of DN was based on persistent albuminuria (>300 mg/day) that is confirmed on at least 2 occasions taken at least 3-6 months apart and progressive decline in the glomerular filtration rate and **Group II (Control group):** It included 20 subjects collected from patients presented with T2DM with normal kidney function.

Blood sampling protocol:

About 8 ml of fasting venous blood sample was withdrawn from each subject and was divided as follow: 2 ml in ethylene diamine tetra acetic acid (EDTA) containing tube for determination of HbA1c, 6 ml were let to be clotted in a plain tube for thirty minutes then centrifuged for fifteen minutes at about 3000 rpm. Resulted serum was used for determination of Fasting blood glucose, urea, creatinine, AST, ALT, albumin, lipid profile, CRP and ferritin. The remaining part was stored at -20 ^{o}C for determination of ANGPTL8 later by ELISA.

Urine sampling protocol: 30-60 ml of clean catch first morning urine sample was collected for measuring albumin/creatinine ratio.

Laboratory methods:

Fasting blood glucose, urea, creatinine, AST, ALT, albumin, triglyceride, cholesterol & HDL cholesterol were measured by auto-analyzer SELECTRA PRO XL, ELITech Group, clinical chemistry automation systems, Netherland. The level of (LDL-c) was determined by Friedwald's equation. A/C ratio was determined by (auto-analyzer SELECTRA PRO XL, ELITech Group, clinical chemistry automation systems, Netherland), CRP and HbA1c were determined by (auto-analyzer Mindray SPL 1000, Shenzhen Mindray Bio-Medical Electronics CO., LTD, China), serum Ferritin was determined by electrochemiluminescence immunoassay (ECLIA) using cobas e411, Roche Diagnostics GmbH, Hitachi High-Technologies Corpo-ration, Tokyo, Japan. eGFR was calculated using serum creatinine with the 2021 chronic kidney disease epidemiology collaboration (CKD-EPI) creatinine equation. The CKD-EPI equation for estimating the glomerular filtration rate (GFR) is expressed as follows: eGFR = 142 × min (SCr/κ, 1) α × max (SCr/κ, 1)−1.200 × 0.9938Age × 1.012 [if female] Where: SCr is the serum creatinine level. κ is a constant (0.7 for females and 0.9 for males).α is -0.329 for females and -0.411 for males Age is the age of the individual in years. Serum ANGPTL8 was measured by ELISA, the kit was supplied by Bioassay Technology Laboratory, China for quantitative detection of human ANGPTL8 in serum.

Statistical analysis:

Statistical package of the social sciences (SPSS) version 27 was used for statistical analysis. We used the Kolmogorov-Smirnov rest to make sure the data was normal. When it came to non-parametric quantitative data, the median (IQR) was used, whereas number and percentage were used for qualitative data. Two groups were compared using One-way ANOVA (Analysis of Variance) for parametric data. The Mann-Whitney U test was employed to compare two groups for non-parametric data. For data that was not parametric, Spearman's rank correlation was used. When the p-value was less than 0.001, it was deemed very significant, and when it was less than 0.05, it was deemed significant.

Results

Comparison between 2 studied groups as regard demographic data:

There was statistically significant difference between group I and group II in terms of age (P <0.001) but there was no statistically significant difference regarding gender distribution (P = 0.594) (**table 1).**

Table (1): Comparison between the two studied groups as regards demographic data:

Variables	Group I N=60	Group II N=20	P- value
Age *Mean ± SD* *Range*	56 ±10.5 (38-83)	40.9 ±5.5 (33-50)	**<0.001***
Sex *Male (%)* *Female (%)*	38 (63.3%) 22 (36.7)	14 (70%) 6 (30%)	0.594

*: Significant level at p value< 0.05

Comparison between 2 studied groups as regard laboratory investigations

There was high statistically significant increase in FBG, HbA1c, Serum creatinine and blood urea levels in group I when compared to group II (P < 0.001 for both parameters)., Group I showed highly statistically significant decrease in eGFR compared to Group II eGFR (P < 0.001), moreover, A/C ratio showed highly significantly elevation in Group I compared to Group II (P < 0.001).

Regarding liver enzymes, there were no statistically significant differences observed in the levels of AST (P = 0.095) or ALT (P = 0.152) between the two studied groups however, serum albumin showed highly statistically significant decrease in group I in comparison with Group II (P < 0.001).

The results of lipid profile reveal that cholesterol & HDL cholesterol showed highly statistically increase in group I compared to group II (P = 0.035 & 0.007, respectively) while LDL levels did not differ significantly (P = 0.165). Triglyceride levels were significantly higher in Group I compared to Group II (P = 0.003). There was no statistically significant difference in the TG:HDL ratio between Group I and Group II groups (P = 0.563). The LDL:HDL ratio was significantly lower in Group I compared to Group II (P = 0.005) **(table 2).**

Table (2): Comparison between the two studied groups as regards laboratory investigations

Variables	Group I N=60	Group II N=20	P- value
Fasting serum glucose(mg/dl)			
Median	175.5	130	**<0.001***
IQR	(155.5-200)	(130-140)	
HbA1c(%)			
Median	9.8	7.1	**<0.001***
IQR	(8.4-11.5)	(6.8-7.1)	
Urea(mg/dl)			
Median	145.5	30	**<0.001***
IQR	(118-183)	(29-35.3)	
Creatinine(mg/dl)			
Median	4.9	0.8	**<0.001***
IQR	(3.8-6.9)	(0.8-0.9)	
eGFR			
Median	12.5	126	**<0.001***
IQR	(8-17)	(109-132)	
A/C ratio(mg/gm)			
Median	2107	16.3	**<0.001***
IQR	(1336.1-2905.8)	(11.8-19.2)	
AST(U/L)			
Median	18	13.5	0.095
IQR	(14-28.5)	(11-20)	
ALT(U/L)			
Median	20	17.5	0.152
IQR	(16-25.5)	(12-29)	
Albumin(g/dl)			
Median	2.9	3.6	**<0.001***
IQR	(2.8-3)	(3.5-4.2)	
Cholesterol(mg/dl)			
Median	244	175.5	**0.035**
IQR	(184-311)	(164-243)	
HDL cholesterol(mg/dl)			
Median	64	39.5	**0.007**
IQR	(38-78)	(21-49)	
LDL cholesterol(mg/dl)			
Median	134.5	101	0.165
IQR	(97-215)	(96-176)	
Triglyceride(mg/dl)			
Median	138	91	**0.003**
IQR	(93.3-205)	(86-124.3)	
TG: HDL Ratio			
Median	2.2	2.6	0.563
IQR	(1.4-3.8)	(2-3.1)	
LDL: HDL Ratio			
Median	2.4	3.4	**0.005**
IQR	(1.3-4.1)	(2-8.6)	

Comparison between the two studied groups as regards Inflammatory markers and serum level of ANGPTL8:

The results in **table (3)** reveal that highly statistically significant increase in CRP and ferritin levels were found in group I compared to group II (P < 0.001 for both) **.** Moreover, ANGPTL8 levels were significantly higher in Group I compared to Group II. These differences were highly statistically significant (P < 0.001).

Table (3): Comparison between the two studied groups as regards Inflammatory Markers and serum level of ANGPTL8:

Variables	Group I N=60	Group II N=20	P- value
CRP(mg/l) *Median* *IQR*	56.5 (32-103.5)	4 (3-6)	**<0.001***
Ferritin(ng/ml) *Median* *IQR*	700.5 (532-902.3)	505.5 (420.8-520.3)	**<0.001***
ANGPTL8(ng/l) *Median* *IQR*	400.5 (331.8-560)	253 (190.8-292)	**<0.001***

*: Significant level at p value< 0.05

Diagnostic performance of A/C ratio, serum creatinine and ANGPTL8 in predicting diabetic nephropathy:

The area under the A/C ratio ROC curve AUC was 1.00 with sensitivity and specificity of 100% for both, when the cut-off value was ≥261.8 (**p<0.001***). The area under the creatinine ROC curve AUC was 1.00 with sensitivity and specificity of 100% for both, when the cut-off value was ≥1.5 (**p<0.001***), The area under the ANGPTL8 ROC curve was 0.907 with sensitivity and specificity of 86.7% and 90% respectively, when the cut-off value was ≥307.5 (**p<0.001***). **(fig 1),(fig2),(fig3).**

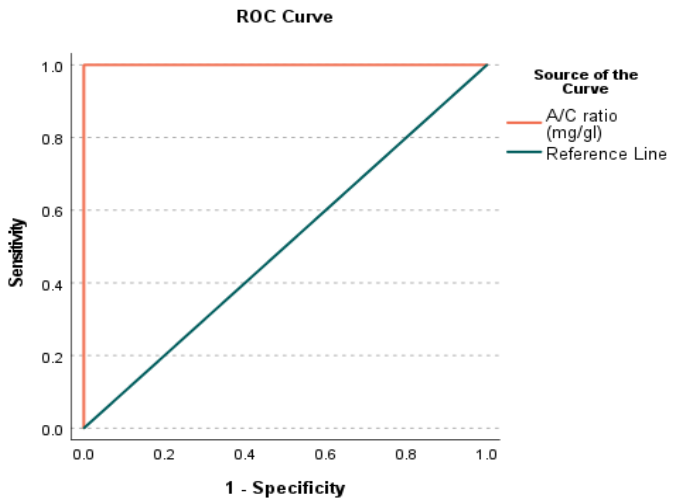

Figure (1): ROC curve analysis of A/C ratio (mg/gm) in predicting DN.

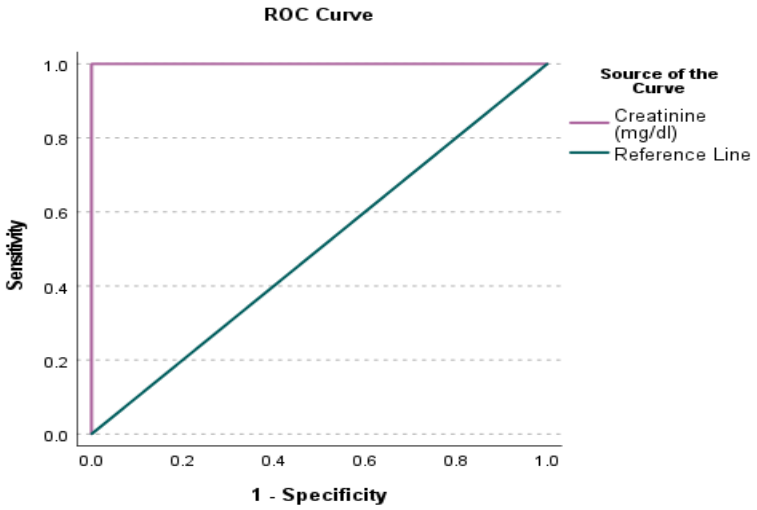

Figure (2): ROC curve analysis of serum creatinine in predicting DN.

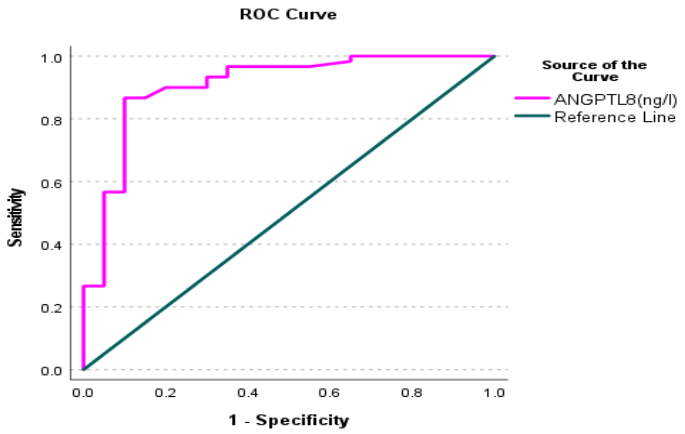

Figure (3): ROC curve analysis of serum ANGPTL8 in predicting DN.

Correlations between ANGPTL8 with other laboratory investigations:

There was statistically significant positive moderate correlation between ANGPTL8 & A/C ratio (r = 0.411, p = 0.001). however, there was statistically significant negative moderate correlation between ANGPTL8 & serum albumin (r = -0.462, p < 0.001). Moreover, ANGPTL8 showed statistically significant positive weak correlation with ferritin (r=0.305, p=0.018). In addition, There were positive weak correlations for ANGPTL8 with cholesterol (r=0.338, p=0.008), LDL cholesterol (r=0.354, p= 0.006) & LDL:HDL ratio (r=0.274, p=0.034) however, there was no significant correlation with TG, HBA1c,serum creatinine and eGFR(Figures 4,5,6,7,8).

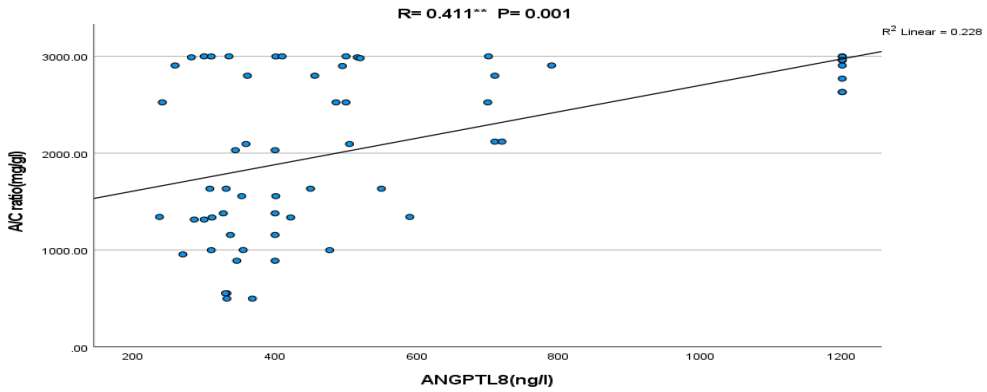

Figure (4) Correlation of ANGPTL8 (ng/l) vs A/C ratio (mg/gm) among Group I

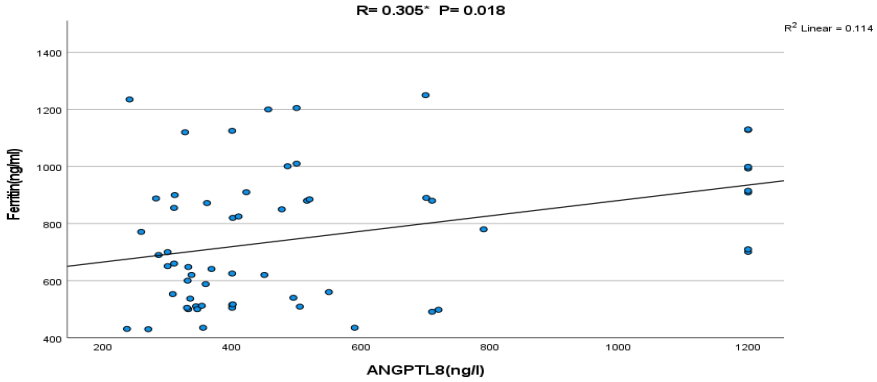

Figure (5): Correlation of ANGPTL8 (ng/l) vs ferritin among Group I

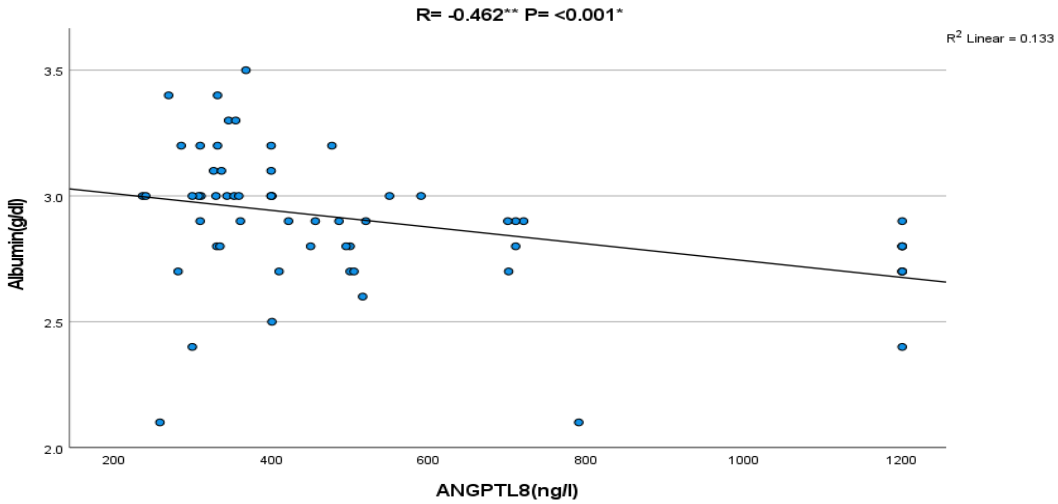

Figure (6): Correlation of ANGPTL8 (ng/l) vs serum albumin among Group I

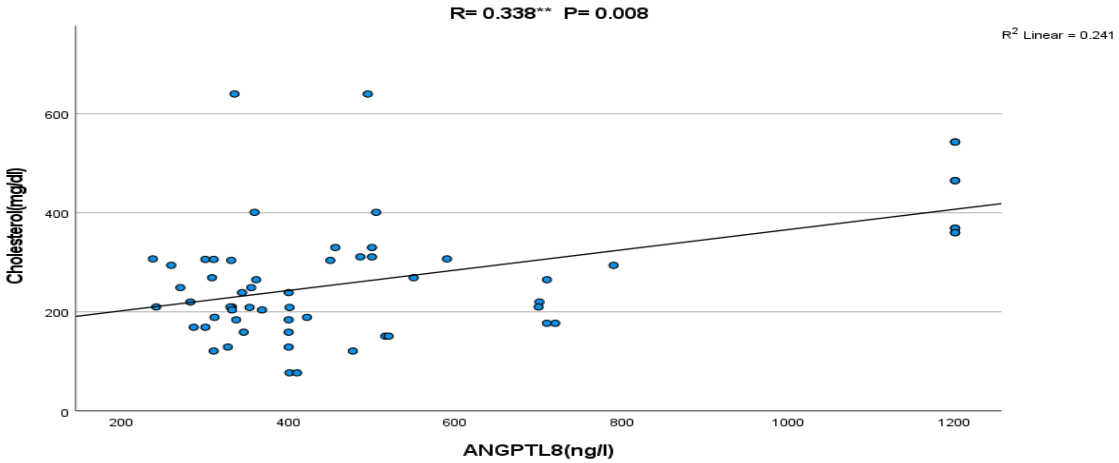

Figure (7): Correlation of ANGPTL8 (ng/l) vs cholesterol (mg/dl) among Group I

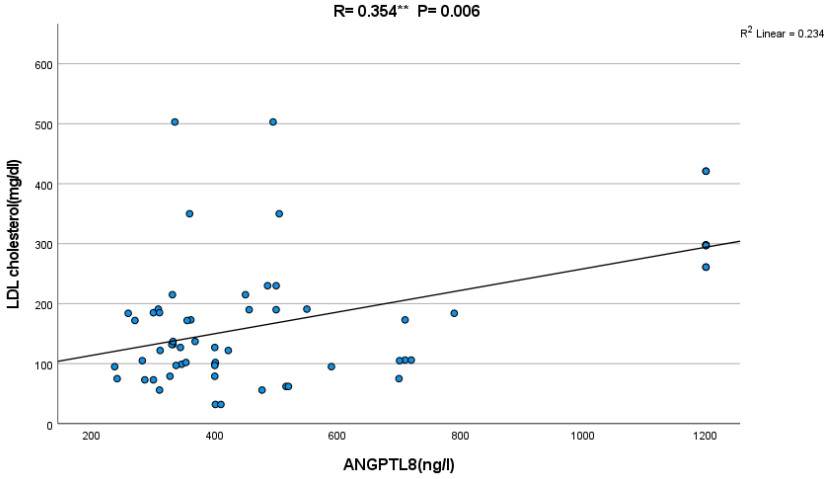

Figure (8): Correlation of ANGPTL8 (ng/l) vs LDL cholesterol (mg/dl) among Group I

Table (4) multiple linear regression analysis for predictors of ANGPTL8 among studied cases by enter method

Dependent variable is ANGPTL8							
Model		Unstandardized Coefficients		Standardized Coefficients	P value	95.0% Confidence Interval for B	
		B	Std. Error	Beta		Lower Bound	Upper Bound
1	(Constant)	904.155	695.038		0.20	-500.56	2308.88
	Age	-4.270	3.387	-0.138	0.21	-11.11	2.57
	Fasting serum glucose	2.873	1.022	0.325	**0.008***	.80	4.93
	HbA1c(%)	50.103	38.103	0.286	0.19	-26.90	127.11
	Urea(mg/dl)	-.938	.800	-0.179	0.24	-2.55	0.67
	Creatinine(mg/dl)	8.215	29.894	0.071	0.78	-52.2	68.63
	AST(U/L)	1.422	4.532	0.069	0.75	-7.73	10.58
	ALT(U/L)	-12.437	5.546	-0.493	0.03	-23.64	-1.22
	Albumin(g/dl)	-315.633	173.618	-0.288	0.07	-666.52	35.26
	eGFR	-.770	5.140	-0.030	0.88	-11.15	9.61
	Albumin in urine(mg/dl)	1.024	.760	0.769	0.18	-.51	2.56
	Creatinine in urine (g/dl)	-1402.584	1919.898	-0.360	0.46	-5282.84	2477.67
	A/C ratio(mg/gl)	-.153	.098	-0.438	0.02	-0.60	-0.04
	CRP (mg/l)	-1.850	1.118	-0.210	0.10	-4.11	0.41
	Ferritin(ng/ml)	.257	.169	0.206	0.13	-.08	0.59
	HDL cholesterol(mg/dl)	-1.146	3.599	-0.117	0.75	-8.42	6.12
	LDL cholesterol(mg/dl)	2.001	.745	0.745	**0.01***	.4	3.50
	Triglyceride(mg/dl)	.270	.813	0.074	0.74	-1.37	1.91
	TG:HDL ratio	11.865	39.212	0.088	0.76	-67.38	91.11
	LDL:HDL ratio	-26.466	36.355	-0.187	0.47	-99.94	47.01

Table (4) presents a multiple linear regression analysis using the enter method to identify predictors of ANGPTL8 levels.

The model includes a range of variables, such as metabolic markers (e.g., fasting serum glucose, HbA1c, cholesterol), renal function indicators (e.g., urea, creatinine, albumin in urine), liver enzymes (e.g., AST, ALT), and inflammatory markers (e.g., CRP, ferritin). The standardized coefficients (Beta values) indicate the relative strength and direction of each predictor's association with ANGPTL8 when all other variables are held constant. Notably, fasting serum glucose (Beta = 0.325, p = 0.009) and cholesterol (Beta = 0.745, p = 0.01) show significant positive associations, suggesting that higher levels of these markers correlate with increased ANGPTL8. In contrast, ALT (Beta = -0.493, p = 0.03) exhibits a strong negative association, implying that elevated ALT levels are linked to lower ANGPTL8. The A/C ratio (Beta = -0.438, p = 0.02) also shows a significant negative relationship, indicating its potential role in modulating ANGPTL8. However, many variables, such as age (Beta = -0.128, p = 0.21), HbA1c (Beta = 0.286, p = 0.19), and CRP (Beta = -0.210, p = 0.10), did not reach statistical significance, highlighting their limited predictive power in this model.

Discussion

Diabetic nephropathy is one of the most serious complications of T2DM. It is considered a primary contributor to end-stage renal failure that affects approximately 15% or more of T2DM[10].It is a microvascular disease that is characterized by gradual progression of renal insufficiency by thickening of glomerular and tubular membranes, augmentation of mesangial matrix that end by glomerulosclerosis & tubulointerstitial fibrosis[11].The clinician and research community focus on the increasing global prevalence of T2DM and subsequently DN. Early diagnosis and prevention of DN is important to suppress the morbidity and mortality progression of DN in patients with T2DM. Therefore, exploring potential markers that have a significant role in early diagnosis & prevention of DN is very important [12].

ANGPTL8 is a secreted protein that is mainly expressed in the liver and adipose tissue. It is a new regulator of lipid metabolism as ANGPTL8 has role in clearance of triglycerides by suppressing lipoprotein lipase activity [13]. ANGPTL8 has been investigated as a potential biomarker for various metabolic diseases, including obesity and diabetes, due to its association with lipid metabolism [13]. The correlation between changes in ANGPTL8 levels and DN and its underlying mechanisms is still unremarkable. Therefore, more clinical and experimental studies are needed, targeting its role in mitigating kidney damage in DN. As a result, we conducted this study to assess the level of ANGPTL8 in T2DM patients with DN (group I) in comparison with T2DM patients without DN (group II) and the correlation between ANGPTL8 & markers of DN.

In the current study, DN patients were older than diabetic patients without nephropathy and this was in agreement with Morton JI et al., Study who found that the incidence of ESKD in T2DM increased with long duration of diabetes as well as age. Incidence rates were higher in old categories of age of onset in the first 10-15 years of diabetes [14].

Results of the present study showed that most patients of DN are males, this is similar to Ren X et al., study that reported that DN is highly common in males with a female to male ratio 1:1.7. This sex difference is related to hormonal influence as estrogen has protective effect on kidneys, genetic factors, socioeconomic and lifestyle factors [15].

As regard laboratory investigations in the current study, fasting serum glucose level and HbA1c were significantly increased among DN patients compared to diabetic patients only, DN develpement is associated with bad glycemic control. Similarly, Matsukuma Y et al., found that increased high-normal levels of HbA1c are considered to be risk factors for arteriolar wall thickening of the kidney which affects kidney function on long run duration & has role in DN progression [16]. Arnold F et al., found that lowest HbA1c levels were seen with the lowest decline of kidney function [17]. In contrary, Al-Dabet MM et al., found that there was persistence of albuminuria in spite of marked improvement in blood glucose levels. This was explained by nearly half of the changes that occur under the influence of hyperglycemia in gene expression persisted in spite of blood glucose reduction reflecting hyperglycemic memory [18]

Our study showed a statistically significant increase in serum urea and creatinine levels in patients with diabetic nephropathy (DN) compared to diabetic patients without nephropathy, along with a significant decrease in estimated glomerular filtration rate (eGFR) in DN patients. Similarly, Julián MT et al. reported a decline in eGFR, with a slope of 2.05 ml/min/1.73m² per year in DN patients, reflecting disease progression[19]. Consistently, Bramlage P et al. found that renal impairment is highly prevalent in patients with T2DM, with an annual incidence rate of 6.6%, and over three years, 30.9% of patients experienced an eGFR decline of 12 ml/min/1.73m² or more [20].

Moreover, this study showed that there was highly statistically significant elevation in A/C

ratio in DN patients compared to Diabetic patients without nephropathy. Similarly, Shi Y et al. found that a higher urinary A/C ratio is significantly associated with an increased prevalence of DN as higher urinary A/C ratio occurs due to glomerular damage under the effect of high blood glucose level, increased albumin excretion as kidneys' filtering capacity decline[21].

Current study showed highly statistically significant decrease in serum albumin. Similarly, Cheng T et al. found that a negative association between serum albumin and renal function as the decline in serum albumin was associated with poor kidney function. This occurs due to increase leakage of albumin in urine due to poor filtration function of kidneys, decreased synthesis of albumin by liver as DN alters protein synthesis in liver & increase catabolism of albumin that occur with DN [22].

Regarding inflammatory markers among studied groups Our study showed that CRP and ferritin were significantly higher in DN patients compared to non-diabetic nephropathy. Similarly, Lin CC et al., found that 1-unit increase in log-transformed CRP was found to be associated with a 15% increase in DN risk that can occur as a consequences of disease associated inflammation as high glucose level can induce high CRP level by activation of NF-κB and TGF-β/Smad3 signaling that lead to renal inflammation and fibrosis so a direct relation between increased CRP level & DN risk was found in this study supporting a causal role of CRP in the inflammatory process that occurs in DN [23].

Similarly, Dubey K et al., found that serum ferritin was higher in patients with microalbuminuria as against patients having normal A/C ratio (p-value- 0.04). Patients with poor glyemic control had higher ferritin level (p-value- 0.024) so ferrtin can be used as a marker for DN [24]. Similarly, Wu YH et al., found that a 10 ng/ml increase in ferrtin level was associated with a 1.12-fold higher adjusted risk for the incidence of chronic kidney disease. This increase in serum ferritin may be due to chronic inflammation that occurs in DN as ferritin is acute phase reactant & oxidative stress that occurs in DN can upregulate ferritin synthesis [25].

Our study demonstrated that cholesterol and triglyceride levels were significantly higher in DN patients compared to non-DN patients. Similarly, Zhao Y et al. found that elevated remnant cholesterol was associated with an increased risk of progression to ESKD (end-stage kidney disease) in DN patients, with a one-SD increase correlating with a higher risk. This dyslipidemia may be attributed to insulin resistance and hyperglycemia, which promote lipid overproduction, inflammation, oxidative stress, and disruption of lipid metabolism characteristic of diabetic nephropathy [26].
In line with our findings, Zaidi IA et al., reported that triglyceride levels were significantly higher in T2DM patients with DN compared to those without ($p < 0.05$), highlighting the important role of hypertriglyceridemia in the onset and progression of DN. The underlying mechanisms likely involve insulin resistance and hyperglycemia leading to increased lipid synthesis, disturbed lipid metabolism, and enhanced inflammatory and oxidative processes in diabetic nephropathy [27].

Moving on to the primary biomarker of interest in this study, ANGPTL8 exhibited notable alterations that may be critically relevant to the progression of diabetic nephropathy (DN). Our findings revealed that ANGPTL8 levels were significantly higher in patients with DN compared to those without, consistent with previous studies by AlMajed et al.,[11] and Li et al., which also reported elevated ANGPTL8 levels in T2DM patients with DN compared to those without nephropathy [28]. Additionally, Emara et al., in a study involving 80 individuals (60 T2DM patients with DN and 20 apparently healthy controls), demonstrated that ANGPTL8 levels were significantly elevated in the macro-albuminuria group compared to healthy controls and other diabetic groups (normoalbuminuria and microalbuminuria) ($p <0.05$) [5].

The elevated serum levels of ANGPTL8 in DN patients may be attributed to its role in inflammatory processes, which are known contributors to the pathogenesis of DN. Furthermore, our data demonstrated that ANGPTL8 levels were positively correlated with the albumin-to-creatinine ratio (ACR) and negatively correlated with serum albumin levels, findings consistent with several previous studies. For example, Alshawaf et al., reported a positive correlation between ANGPTL8 and urinary albumin [29], while Emara et al. [5] observed a positive correlation between ANGPTL8 and ACR, and a negative correlation with serum albumin.

These observations may be explained by the presence of albuminuria in DN and by the upregulation of ANGPTL8 under conditions of inflammation and stress, wherein albumin acts as a negative acute-phase reactant. Moreover, in multiple linear regression analysis, ANGPTL8 remained independently associated with both ACR and serum albumin levels, suggesting a potential role for ANGPTL8 in the progression of diabetic nephropathy through its association with proteinuria and hypoalbuminemia.

In addition to its relationship with proteinuria markers, we further explored the association between ANGPTL8 and metabolic parameters such as glucose metabolism, insulin resistance, and lipid profile to better understand its broader role in disease progression. Several studies have explored the relationship between ANGPTL8 and glucose metabolism and insulin resistance.

For example, Hu et al. found that in newly diagnosed type 2 diabetic patients, serum ANGPTL8 levels were positively correlated with fasting plasma glucose and 2-hour post-OGTT glucose levels [30]. Similarly, ISSA Y.A et al. reported a positive correlation between ANGPTL8 and HbA1c [31].
However, in our study, no significant correlation was observed between ANGPTL8 levels and either fasting plasma glucose or HbA1c. This finding aligns with Li M et al., who also reported no significant association between ANGPTL8 and HbA1c [28] Although no simple bivariate correlation was found between fasting glucose and ANGPTL8 levels, fasting glucose emerged as an independent predictor of ANGPTL8 levels in the multiple linear regression analysis ($p = 0.008$).

This suggests that fasting glucose may play a contributory role in modulating ANGPTL8 levels when adjusted for other confounding factors such as lipid profile and renal function parameters, highlighting the complex interplay between glucose metabolism and ANGPTL8 regulation in the context of diabetic nephronpathy.

Regarding the lipid profile, previous studies have reported a positive association between ANGPTL8 levels and triglycerides (TG) as well as other lipid parameters. However, in the present study, although ANGPTL8 demonstrated a positive correlation with total cholesterol and LDL cholesterol, no significant association was observed with TG levels among diabetic nephropathy patients.
Notably, LDL cholesterol was found to be an independent predictor of serum ANGPTL8 levels ($p = 0.01$).

This finding is consistent with reports by Stefanska et al.,[32] and Emara et al.,[5], who demonstrated that elevated LDL or total cholesterol levels were significantly associated with higher ANGPTL8 concentrations.
The underlying mechanism may involve the inhibitory effect of ANGPTL8 on lipoprotein lipase (LPL) activity, leading to impaired triglyceride metabolism and indirect accumulation of LDL particles.

Despite these findings, conflicting results have been reported in the literature; for example, Emara et al., [5] reported a positive correlation between ANGPTL8 and triglyceride levels. Such differences may be due to variations in sample size, age groups, ethnic backgrounds, or disease stages. Further studies are warranted to clarify these inconsistencies and better define the relation-ship between ANGPTL8 and lipid metabolism in diabetic nephropathy.

Interestingly, no significant correlation was observed between serum ANGPTL8 levels and either estimated glomerular filtration rate (eGFR) or serum creatinine levels in our diabetic nephropathy patients. This finding contrasts with AlMajed et al. [11], who reported a negative correlation between ANGPTL8 and eGFR. Our findings may suggest that ANGPTL8 is more closely associated with markers of proteinuria and inflammation rather than direct measures of renal filtration function. Further studies are needed to explore the dynamics of ANGPTL8 levels across different stages of diabetic kidney disease progression.

To assess the diagnostic potential of ANGPTL8 in discriminating diabetic nephropathy, ROC curve analysis was conducted, comparing its performance with established markers such as A/C ratio and serum creatinine.
In our study, all enrolled patients exhibited gross proteinuria and reduced eGFR, which may explain the exceptionally high diagnostic accuracy observed with the ACR, reaching an area under the curve (AUC) of 1.00.

The cut-off value for ANGPTL8 was determined to be ≥307.5 ng/mL, with an AUC of 0.907, sensitivity of 86.7%, and specificity of 90%. more studies are required to validate this cut off.

Our study also has several limitations including small sample size, the absence of a microalbuminuria group, and the lack of a healthy control subjects, further studies involving larger and more diverse populations are necessary to validate these findings and include patients across different stages of diabetic nephropathy (normoalbuminuria, microalbuminuria, macroalbuminuria) to better understand the dynamics of ANGPTL8 changes across disease progression also to correlate serum ANGPTL8 levels with renal biopsy findings to further validate its role in kidney pathology in addition , Investigate the molecular mechanisms linking ANGPTL8 to inflammation, proteinuria, and renal injury in diabetic nephropathy and finally to evaluate the potential utility of ANGPTL8 as a prognostic biomarker for disease progression and response to therapeutic interventions to establish the potential role of ANGPTL8 as a diagnostic biomarker for diabetic nephropathy.

In conclusion, our study highlights the potential significance of ANGPTL8 as a biomarker in diabetic nephropathy. We found that serum ANGPTL8 levels were significantly elevated in patients with diabetic nephropathy compared to those without, and were independently associated with markers of proteinuria (ACR) and hypoalbuminemia. Although no significant correlation was observed with glycemic control markers or renal filtration parameters (eGFR, serum creatinine), ANGPTL8 demonstrated a promising diagnostic performance in distinguishing diabetic nephropathy, with high sensitivity and specificity. The role of ANGPTL8 appears to be more closely related to inflammatory processes and proteinuria.

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