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Role of Angiopoietin-Like Protein 4 (ANGPTL4) as a Predictive Marker for Diabetic Nephropathy in Type 2 Diabetes

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ABSTRACT

Background: Angiopoietin-like protein 4 (ANGPTL4) is a glycoprotein that regulates LPL activity during fasting and thereby affects triglyceride metabolism. In the nephrotic syndrome, ANGPTL4 has been proposed as a relationship between hypertriglyceridemia and albuminuria. In this study, the levels of circulating ANGPTL4 in individuals having diabetic nephropathy (DN) were investigated, as well as their relationship with confirmed DN.

Aim of the work: In this study, we wanted to explore a potential role of ANGPTL4 under conditions of T2D alone and DN, where a changes in ANGPTL4 levels would be permissive for the development of a kidney condition as a complication of diabetes. This would promote the use of ANGPTL4 as a biochemical marker for the detection of nephropathy in patients with diabetes

Patients and Methods: This study included 60 patients. 20 patients who had T2D but no DN (T2D group I) 20 patients who had T2D and DN (DN group II) 20 individuals without T2D and no nephropathy (control group). We assessed serum ANGPTL4 levels in all groups via an enzyme-linked immunosorbent assay (ELISA).

Result: In our study, patients with T2D with DN had significantly greater ANGPTL4 levels than patients with T2D without DN and control subjects (healthy group). HBA1c showed a positive correlation.

Conclusion: Use of ANGPTL4 as a Predictive Marker for DN in T2D.

Keywords: T2DM with DN; Angiopoietin-like protein 4 (ANGPTL4); HBA1c.

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INTRODUCTION

Diabetes type 2 is a complicated condition characterized by insulin resistance and abnormal pancreatic activity (IR). In numerous studies, IR has been linked to moderate to severe chronic kidney failure (CRF). It was recently discovered in diabetic nephropathy patients in the early stages of mild kidney illness.¹

The estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (ACR) are the primary tools for measuring glomerular damage and changes in kidney function in clinical practice today. Nevertheless, a number of factors decrease the precision and dependability of these indicators, making them less accurate for detecting diabetic kidney disease early on.²

Adipocytes, hepatocytes, myocytes, macrophages, endothelium, as well as intestinal cells release ANGPTL4, a glycoprotein having a molecular weight of 45–65 kDa.³

ANGPTL4 has been intensively studied since its discovery, with reports indicating that it has a function in a variety of physiologic and pathologic situations that involve energy homeostasis, angiogenesis, cancer, and healing of wounds.⁴

ANGPTL4 has been extensively studied because of its involvement in lipid metabolism, particularly as a strong inhibitor of LPL, which regulates LPL activity to limit TG removal from circulation. In this approach, ANGPTL4 overexpression lowers TG levels in circulation while ANGPTL4 deficiency raises circulating TG levels. By means of this regulatory mechanism, by reducing extracellular TG hydrolysis, ANGPTL4 would protect cells from lipotoxicity and subsequent FA absorption.⁵

Defects in LPL activity, on the other hand, would result in hypertriglyceridemia, a common nephrotic syndrome symptom.⁶

ANGPTL4 has been identified as the missing link in the nephrotic syndrome between hypertriglyceridemia and albuminuria.⁷

ANGPTL4 levels in the blood are strongly linked to the severity of DN. In T2DM patients, blocking ANGPTL4 in the kidney may delay the onset and development of DN.

PATIENTS AND METHODS

The research was conducted in the department of internal medicine (at Al Hussein Hospital) and an outpatient clinic. Ethical committee regulations will be followed, and the patient will be asked to sign a written informed consent form that includes an explanation of the procedures' possible hazards.

The research comprised 60 people who were split into 3 groups depending on their BMI, age, and gender: 20 people who had T2D but no DN (T2D group I), 20 individuals who had T2D and DN (DN group II), and 20 individuals without T2D and no nephropathy (DN group III) (control group).

Exclusion criteria: Patients with clinically significant renal, liver, neurological, endocrinal, cardiovascular, autoimmune, infection, any acute inflammation or other major systemic diseases, including malignancy. Patients who got antibiotics and nonsteroidal anti-inflammatory medications, corticosteroids, or cytotoxic drugs before taking blood for the study. Patients who smoke and/or drink are at risk.

All patients underwent a thorough medical history, general and local examinations were done with special attention to measurements of BMI and BP, and laboratory tests such as fasting blood glucose, kidney and liver function tests, and HBA1c levels, TC (total cholesterol), LDL-C (low-density lipoprotein-C), HDL-C (high-density lipoprotein-C), and triglycerides (high-density lipoprotein C), as well as CRP, were all measured.

The albumin and creatinine levels in urine specimens were determined. The Modification of Diet in Renal

Disease research equation was used to calculate the eGFR. ANGPTL4 will be measured using an ELISA.

Fresh, mid-stream urine was collected from all patients and refrigerated at -20 C to determine the albumin/creatinine ratio. Microalbuminuria is diagnosed with Bayer CLINITEK Microalbumin Reagent Strips, a semi-quantitative approach. The CLINITEK Analyzer was used for the analysis. According to the manufacturer, the Bayer Microalbustix test offers a sensitivity of 90% and a specificity of 88% for the albumin/creatinine ratio.

ANGPTL4 Enzyme Linked Immunosorbent Assay (ELISA): ANGPTL4 protein levels in plasma have been determined with a Magnetic Luminex Assay kit (R & D Systems Europe, Ltd., Abingdon, UK) according to the instructions of the manufacturer.

Plasma samples were thawed on ice to prevent repeating freeze-thaw cycles. There was no evidence of cross-reactivity with other proteins.

Statistical Analysis

The data was analyzed using the computer software program Statistical Package for the Social Sciences (SPSS) version 20, Chicago.

The descriptive characteristics of the study patients were presented as mean SD (standard deviation) and evaluated using an ANOVA test.

At P 0.05, the probability (P-value) has been considered significant.

To define the degree of relationship between two sets of variables, Pearson's correlation coefficient (R) test was used.

RESULTS

		Groups						Stat. test	P-value
		Group: I (n = 20)		Group: II (n = 20)		Group: III (n = 20)			
Sex	Male	11	55%	11	55%	12	60%	X ² = 0.13	0.934 NS
	Female	9	45%	9	45%	8	40%		
Age (years)	Mean	48.5		44.7		45.2		F = 2.2	0.120 NS
	±SD	5.9		6.3		6.7			
BMI (kg/m ²)	Mean	29.9		34.1		26.8		F = 18.2	< 0.001 HS
	±SD	2.7		5.0		3.5			

Table 1: comparisons between studied groups as regard demographic data

This table shows:

Highly statistically significant (**p-value < 0.001**) increased BMI in group II (34.1 ± 5 kg/m²) when compared with group I (29.9 ± 2.7 kg/m²) and group III (26.8 ± 3.5 kg/m²).

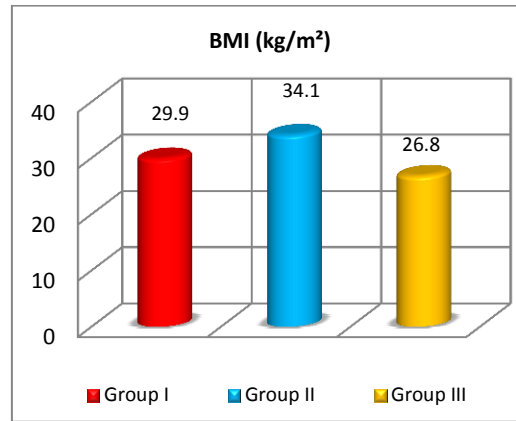


Fig. 1: comparisons between studied groups as regard BMI.

		Groups			F	P-value
		Group I (n = 20)	Group II (n = 20)	Group III (n = 20)		
Creatine (mg/dl)	Mean	0.7	2.7	0.7	66.4	< 0.001 HS
	±SD	0.2	1.1	0.2		
Urea (mg/dl)	Mean	24.5	26.1	24.1	0.77	0.465 NS
	±SD	5.9	4.3	6.2		
ALB/Creat (mg ALB/g Creat)	Mean	14.05	1695.0	8.3	237.8	< 0.001 HS
	±SD	4.5	488.3	4.7		

Table 2: comparisons between studied groups as regard kidney functions

This table shows:

- Highly statistically significant (**p-value < 0.001**) increased Creat in group II (2.7 ± 1.1 mg/dl) when compared with group I (0.7 ± 0.2 mg/dl) and group III (0.7 ± 0.2 mg/dl).
- Highly statistically significant (**p-value < 0.001**) increased ALB/Creat ratio in group II (1695 ± 488.3 mg ALB/g Creat) when compared with group I (14.05 ± 4.5 mg ALB/g Creat) and group III (8.3 ± 4.7 mg ALB/g Creat).

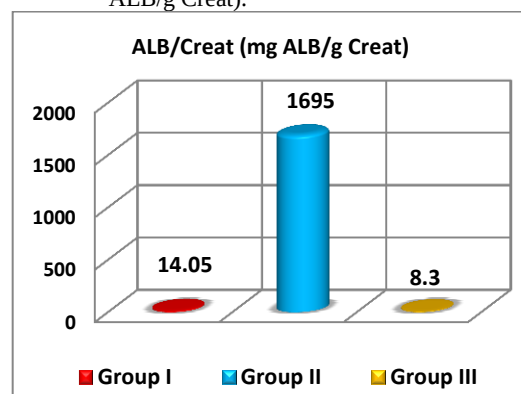


Fig. 2: comparisons between studied groups as regard ALB/Creat ratio.

		I vs II	I vs III	II vs III
Creat	LSD	2.02	0.045	2.07
	p-value	< 0.001 HS	0.827 NS	< 0.001 HS
ALB/Creat ratio	LSD	1498	5.75	1454
	p-value	< 0.001 HS	0.949 NS	< 0.001 HS

Table 3: Post-Hoc test for multiple comparisons between studied groups as regard Creat and ALB/Creat ratio.

As regard Creat, there was:

- Highly statistically significant difference (**p-value < 0.001**) between groups I & II.
- Highly statistically significant difference (**p-value < 0.001**) between groups II & III.

As regard ALB/Creat ratio, there was:

- Highly statistically significant difference (**p-value < 0.001**) between groups I & II.
- Highly statistically significant difference (**p-value < 0.001**) between groups II & III.

		Groups			F	P-value
		Group: I (n = 20)	Group: II (n = 20)	Group: III (n = 20)		
FBS (mg/dl)	Mean	108.8	198.0	77.0	104.3	< 0.001 HS
	±SD	7.0	46.6	6.6		
PPBS (mg/dl)	Mean	154.7	260.9	103.3	173.4	< 0.001 HS
	±SD	14.0	43.8	10.8		
HbA1C (%)	Mean	9.2	9.8	4.6	103.3	< 0.001 HS
	±SD	1.4	1.5	0.3		

Table 4: comparisons between studied groups as regard blood glucose assessment

This table shows:

- Highly statistically significant (**p-value < 0.001**) increased FBS in group II (198 ± 46.6 mg/dl) when compared with group I (108.8 ± 7 mg/dl) and group III (77 ± 6.6 mg/dl).
- Highly statistically significant (**p-value < 0.001**) increased PPBS in group II (260.9 ± 43.8 mg/dl) when compared with group I (154.7 ± 14 mg/dl) and group III (103.3 ± 10.8 mg/dl).
- Highly statistically significant (**p-value < 0.001**) increased HbA1C in group II (9.8 ± 1.5 %) when compared with group I (9.2 ± 1.5 %) and group III (4.6 ± 0.3 %).

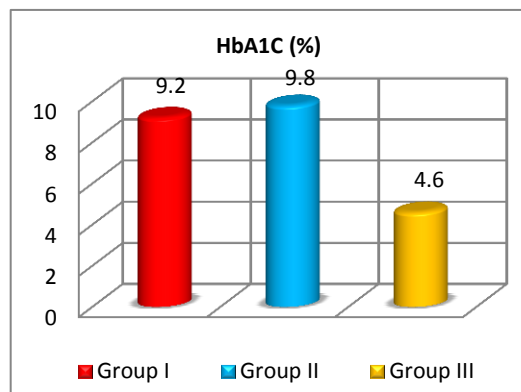


Fig. 3: comparisons between studied groups as regard HbA1C.

		Groups			F	P-value
		Group: I (n = 20)	Group: II (n = 20)	Group: III (n = 20)		
ANGPTL4 (ug/ml)	Mean	174.0	230.3	179.3	71.8	< 0.001 HS
	±SD	14.7	18.7	15.5		

Table 5: comparisons between studied groups as regard ANGPTL4

This table shows: highly statistically significant (**p-value < 0.001**) increased ANGPTL4 in group II (230.3 ± 18.7 ug/ml) when compared with group I

(174 ± 14.7 ug/ml) and group III (179.3 ± 15.5 ug/ml).

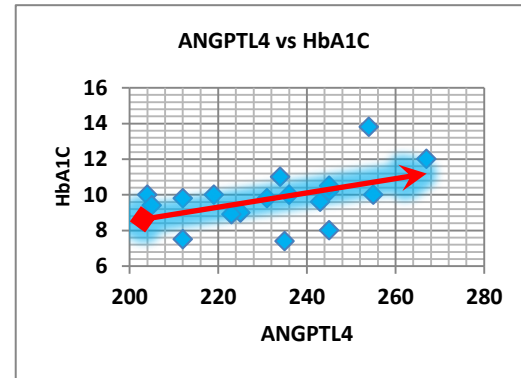


Fig. 4: Positive correlation between ANGPTL4 and HbA1C in group II.

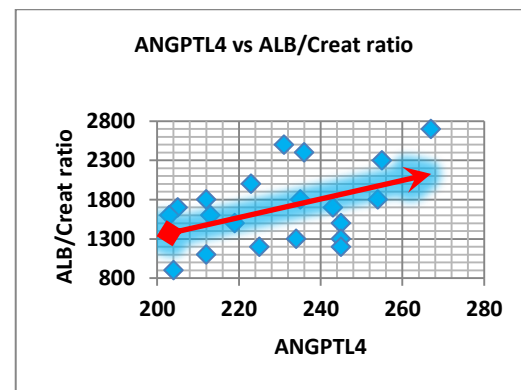


Fig. 5: Positive correlation between ANGPTL4 and ALB/Creat ratio in group II.

DISCUSSION

Diabetes-related kidney disease (DKD) represents a major cause of end-stage renal disease (ESRD), with a 44% rise in the global frequency expected by 2030.⁸ It is distinguished by higher urine albumin excretion, proteinuria, and a decrease in estimated glomerular filtration rate (eGFR) in the absence of other renal disorders. The risk of kidney damage is reduced by half when DKD is detected and managed early.⁹

Angiopoietins are vascular growth factors that help blood vessels develop and repair. In both physiology and pathology, angiopoietins play a critical function in glomerular capillaries. Glomerular capillaries are distinguished by a fenestrated endothelium that lies on a basal membrane as well as specialized epithelial cells (podocytes) that coat the glomerular filter's exterior layers with their interdigitating foot processes.¹⁰

Angiopoietin-like proteins are released proteins involved in energy metabolism. Adipocytes, hepatocytes, (cardio) myocytes, endothelial cells, and macrophages are among the cell types that express ANGPTL4.¹¹

As a result, ANGPTLs shield triglyceride-rich lipoproteins (TRLs) from LPL-mediated lipid hydrolysis, or lipolysis, in the bloodstream. In the opposite direction, LPL becomes resistant to ANGPTL inhibition after binding the endothelial membrane protein glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1).¹²

The goal of this research was to investigate the ANGPTL4 role in diabetic nephropathy as well as assess the risk factors associated with diabetic nephropathy.

In this study, 40 T2 diabetic patients and 20 healthy volunteers participated (normoglycemic individuals with normal renal function).

The participants have been allocated into 3 groups: Group 1 is made up of 20 diabetics who have normoalbuminuria (30 mg/g). Group 2 is made up of 20 diabetics with albuminuria (more than 30 mg/g). Group 3 is made up of 20 healthy, non-diabetic people.

According to the current study's findings, poor glycemic control is linked to a significantly higher likelihood of diabetic nephropathy.

When compared to groups I (108.8 mg/dl) and III (77.6 mg/dl), FBS levels in group II (198.46.6 mg/dl) increased statistically significantly (p-value 0.001).

Furthermore, when comparing groups I (9.2 percent) and III (9.2 percent), a statistically significant (p value 0.001) increase in HbA1C was seen in group II (9.8 percent). 4.6 percent.

AlRubeaan performed a randomized cross-sectional observational study in which 54,670 T2D patients above the age of 25 were recruited from the Saudi National Diabetes Registry and assessed for DN.

Diabetic nephropathy affected 10.8 percent of the population, with microalbuminuria accounting for 1.2 percent, macroalbuminuria accounting for 8.1 percent, and ESRD accounting for 1.5 percent.

Poor glycemic control was found to be significantly greater in nephropathic individuals, microalbuminuria patients, macroalbuminuria patients, and ESRD patients, with 60.6 percent, 61.1 percent, 59.5 percent, and 63.6 percent, respectively, as measured by the percentage of patients with a HbA1c of more than 8%.¹³

Nephropathic patients had significantly higher HbA1c levels (p value =0.0001) than non-nephropathic individuals.

The Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) researchers discovered that patients who received an intensive glycemic

strategy showed a significantly lower risk of poor renal function than those who received a conventional glycemic control strategy.¹⁴ In another study, strict blood glucose control (target HbA1c of 7.1%) delayed microalbuminuria when compared to standard therapy (target HbA1c of < 9.1 percent).¹⁵

The current study's findings revealed a highly statistically significant (p-value 0.001) increase in ANGPTL4 in group II (239.3 18.7 ug/ml) when compared to groups I (174 14.7 ug/ml) and III (179.3 15.5 ug/ml). This means that in T2D patients, ANGPTL4 acts as a biochemical indicator for diabetic nephropathy.

In their study, Al Shawaf and her colleagues discovered a significant rise in plasma ANGPTL4 levels in DN cases when compared to those with T2D and healthy people.

The study included 36 healthy people and 86 people with T2D, 37 of whom had a normal renal function and 49 of whom had DN.¹⁶

ANGPTL4 levels were higher in DN patients (241:56, 14:1 g/ml) than in the control group (178:43, 24:09 g/ml). Surprisingly, the levels of ANGPTL4 in T2D patients and healthy people were comparable.

Yu examined 1207 T2DM patients in a cross-sectional study. Patients having UACRs of 30 mg/g, 30–300 mg/g, or > 300 mg/g have been classified as having normal to mild, moderate, or severe albuminuria. According to the researchers, increased ANGPTL4 levels have been linked to a greater risk of CKD and albuminuria in T2DM patients.¹⁷

The findings of this study matched those of Ma et al., who studied diabetics with normoalbuminuria (n = 40), microalbuminuria (n = 40), and macroalbuminuria (n = 40). For example, urine ACRs of 30 lg/mg, 30-299 lg/mg, and C300 lg/mg, for example, were utilized to differentiate between normoalbuminuria, microalbuminuria, and macroalbuminuria.¹⁸

The researchers discovered that diabetics with microalbuminuria and macroalbuminuria had significantly higher Angptl4 levels than cases with normal-albuminuria and healthy people.¹⁸

Data from the National Health and Nutrition Examination Survey (NHANES) in the United States, which included a large sample of diabetics, supported these findings. Diabetes affected 2,894 adults (13.6 percent) of the 21,205 study participants. Diabetics, on average, were 59 years old, had a FBG of 1552 mg/dl, and a HbA1c level of 7.2 percent; 80.3 % were overweight (BMI 25), and 49.1 % were obese (BMI 30). Diabetes was found to be more prevalent in adults as weight classes increased, rising from 8% in normal-weight people to 43% in obese class 3 people.¹⁹

According to the findings of a recent study, obesity has a deleterious effect on kidney function in diabetes patients. In terms of BMI, we discovered statistically significant (p -value 0.001) differences among groups I and II (29.9 $\pm$ 2.7 kg/m² versus 34.1 $\pm$ 5.5 kg/m²)

Chen¹ did research in China that comprised 264 people with T2D and biopsy confirmed DN, which was similar to ours. The mean age of DN patients was 53–19.06 years (range 31–80). Individuals have been allocated into 3 groups based on their BMI: obese (28.0 kg/m² (n = 59), overweight (25–30 kg/m² (n = 83), and lean (18–25 kg/m² (n = 122). The three groups had the same mean ages and sex ratios, as well as comparable FBG, HbA1c, average blood pressure, and previous treatment. Obesity has been linked to an increased risk of proteinuria.²⁰

Another recent study found a total of 1077 sufferers with T2D participated in the observational prospective longitudinal follow-up research. Patients with T2D were 58.3 years old on average and had diabetes for a mean of 11 years. Nephropathy was discovered to affect 90.7 percent of the population in a multiple logistic regression study. The development of diabetic nephropathy was found to be strongly linked to BMI.²¹

CONCLUSION

Our findings demonstrated the levels of circulating ANGPTL4 in individuals having DN were found to be significantly higher.

We propose ANGPTL4 as a novel biomarker for predicting the risk of nephrotic syndrome. Increased ANGPTL4 levels in T2DM patients indicate the severity of nephropathy. There this positive correlation between A1c and nephropathy in type 2 DM.

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