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Evaluation of Serum Tumor Necrosis Factor Alpha (TNFα) in Type 1 Diabetic Children and Adolescents as Predictor for Diabetic Nephropathy in Correlation to Proteinuria

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ABSTRACT

Background: Diabetic nephropathy (DN) is a major complication of type 1 diabetes mellitus (T1DM) and a leading cause of chronic kidney disease. Tumor necrosis factor alpha (TNFα), an inflammatory cytokine, may serve as a biomarker for early detection of DN.

Objective: To evaluate serum TNFα levels in children and adolescents with T1DM and assess its potential as a predictor for DN in correlation with proteinuria and clinical variables.

Methods: This cross-sectional study included 50 children with T1DM for over 5 years, subdivided into two groups based on urinary albumin-creatinine ratio (ACR): Group I (normoalbuminuria, n=25) and Group II (microalbuminuria, n=25). A control group (n=30) of age- and sex-matched healthy children was included. Serum TNFα levels were measured using ELISA. Clinical and laboratory parameters were analyzed for correlations.

Results: Serum TNFα levels were significantly higher in diabetic patients compared to controls (p=0.036) but not significantly different between normoalbuminuric and microalbuminuric groups (p=0.401). ROC analysis identified a TNFα cutoff of 5.1 µg/ml with 76% sensitivity and 63.6% specificity for distinguishing T1DM patients from controls. No significant correlations were found between TNFα and age, diabetes duration, glycemic control, or BMI, except for a weak association with height in the microalbuminuric group (p=0.048).

Conclusion: Serum TNFα may serve as a differentiating marker between diabetic and non-diabetic children but lacks predictive value between normoalbuminuria and microalbuminuria groups. Its role in early DN detection requires further validation.

KEYWORDS: Type 1 diabetes, TNFα, diabetic nephropathy, proteinuria.

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INTRODUCTION

Diabetic nephropathy (DN), recently also named as diabetic kidney disease (DKD), is one of the most common diabetic microvascular complications and has become the leading cause of chronic kidney diseases in the world (1).

DN is defined as persistent proteinuria >500 mg/24 hours or albuminuria >300 mg/24 hours or >200 µg/min and is usually associated with hypertension and a diminishing glomerular filtration rate (GFR) (2).

The term 'biomarker' is commonly used, but a precise definition has not been agreed upon. One definition has been provided by the World Health Organization (WHO), which has defined biomarkers as 'any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease (3).

TNF-α is an important cytokine produced under high glucose conditions by macrophages, renal tubular cells, and glomerular mesangial cells. Apart from being a major participant in promoting inflammation, TNF-α is known to induce apoptosis and accumulation of ECM in glomerular and tubular regions leading to alteration of glomerular filtration, tubular permeability, and reabsorption. Moreover, aberrant levels of this cytokine correlate with increased expression of tubular cell injury markers such as NADPH oxidase and Reactive oxygen species (ROS) (4).

These actions are mediated via binding to TNF-α receptors (5). Clinical studies have reported higher serum and urinary levels of TNF-α in diabetic patients with renal dysfunction, which further increase with progression of the disease (6) In addition, circulating levels of TNF-α receptors 1 and 2 are elevated in diabetic patients independent of albuminuria status, suggesting that assessing TNF-α or their receptor levels in the serum and urine could predict the progression of DN to ESRD in the presence or absence of proteinuria (7).

Our study aimed to evaluate serum TNF α as an early marker of DN in children and adolescents with T1DM and to assess the relation between the level of serum TNF α and the age, age of onset, duration of diabetes and glycemic control.

PATIENTS AND METHODS

This cross-sectional study was carried out at the Diabetic Endocrine and Metabolic Pediatric Unit (DEMPU), Pediatric Hospital, Cairo University, Egypt, during the period from May 2016 to May 2017. It included 50 children and adolescents with (T1DM) for more than 5 years' duration, together with 30 age and sex-matched healthy children and adolescents as a control group.

The patients were further subdivided into two groups according to their (ACR):

Group I: T1DM patients with norm albuminuria (ACR < 30 mg/g creatinine): It included 25 patients their ages ranged between 8 and 18 years. They were 9 (36%) females and 16 (64%) males.

Group II: T1DM patients with albuminuria (ACR 30-300 mg/g creatinine): It included 25 patients; their ages ranged between 7 and 18 years. They were 18 (72%) females and 7 (28%) males.

Controls (Group III): It included 30 age and sex-matched healthy children and adolescents without clinical manifestations or family history suggestive of diabetes or renal diseases. Their ages ranged between 8 and 18 years. Albuminuria was confirmed by two ACR > 30 mg/g creatinine (on 2 separate occasions) (8). The study protocol was accepted by the Local Ethical Committee, Pediatric Hospital, Cairo University. An informed consent was obtained from the parents or care-givers prior to enrollment.

Study population

Patients: They were diagnosed as T1DM according to the clinical practice consensus guidelines of International Society for Pediatric and Adolescent Diabetes (ISPAD) (9).

The patients were included according to the following criteria:

Inclusion criteria: Age at enrollment younger than 18 years and Patients with T1DM for more than 5 years' duration.

Exclusion criteria: Patients with cardiac or hepatic diseases and Patients with renal diseases other than DN.

Study methodology

All patients were subjected to the following:

Measurement of serum TNFα levels:

Serum TNFα concentration was measured using a commercially available human ELIZA kit (10).

TNFα Detection by ELIZA:

Test principle:

The ELISA is based on the competitive binding enzyme immunoassay technique. 2 ml of venous blood were collected from all patients and control. Serum was separated after clotting and stored at -20 c tell the time of assay. Wuhan Elaab Science source ELISA kits for TNFα is so called sandwich-assay using two specific and highly affine antibodies. The TNFα in the samples binds to the first biotin conjugated antibody coated on the microliter plate. Avidin conjugated to horseradish peroxidase is added to each microplate well and incubated. Then TMB substrate solution is added to each well. only those wells that contain target antigen will exhibit a change in color (Wuhan Elaab Science Co., Ltd, Wuhan, China,430074).

In Urine:

Morning mid-stream urine samples were obtained from studied patients and controls in plastic sterile containers after instructing them to clean the genital area with soap and tap water, and subdivided as follows: Ten mL of urine used for urine analysis and ACR determination.

The following investigations were done: Complete urine analysis: a urine specimen was submitted for chemical analysis using dipsticks and microscopic examination with special emphasis on the presence or absence of albuminuria, hematuria, pyuria and urinary casts. Urine culture and sensitivity was done in cases with pyuria to exclude urinary tract infection. Assessment of albuminuria was done in absence of confounders namely urinary tract infections, exercise and menstrual bleeding. ACR was measured by immune nephelometric method on Prospect Siemens, Siemens Healthcare Diagnostic Inc. Newark, DE 19714 U.S.A (11). Albuminuria was confirmed by two ACR > 30 mg/g creatinine (on 2 separate occasions).

RESULTS

Table (1): Comparison between the two patient groups and controls as regard age and sex

Variables		Cases (n=50)				Control(n=30)		P*
		Group I (n=25)		Group II (n=25)		Group III (n=30)		
		Mean ±SD	IQR	Mean ±SD	IQR	Mean ±SD	IQR	
Age (years)		13.62 ± 1.89	10 - 16	13.60 ± 1.83	10- 16	12.42 ± 1.58	10- 15	0.064
		Count	%	Count	%	Count	%	
Sex	Male	16	64.0 %	7	28.0 %	16	53.3 %	0.032 **
	Female	9	36.0 %	18	72.0 %	14	46.7 %	

Kruskal-Wallis test, IQR= Interquartile Range. Chi square test, *Significant

There was no statistically significant difference in age, however, the female sex was significantly higher in the microalbuminuric group (Table 1).

Table (2): Comparison between the two patient groups as regard the duration of diabetes, the age at its onset and insulin dose

	Group I n=25	Group II n=25	P*
	Mean± SD	Mean± SD	
Age of onset (yrs.)	6.26 ± 2.38	5.12± 2.68	0.082
Duration (yrs.)	6.58 ± 2.13	7.84± 2.61	0.058
Insulin (IU/kg/day)	2.68 ± 6.96	1.18± 0.42	0.249

*=Student t test, yrs. =years, IU= international unit.

There was no significant difference between the two patient groups regarding the duration of diabetes, age of onset and insulin dose (Table 2).

Table (3): Comparison of serum TNFα level between the diabetic and albuminuric patients

	Group 1 n=25	Group 2 n=25	P*
	Mean ±SD	Mean ±SD	0.401
TNF & (ug/ml)	5.64 ± 2.47	5.68±0.97	

TNFα level was not statistically different between diabetic and albuminuric patients (Table 3).

Table (4): Correlation between serum TNFα level and the patient age, age at onset, duration of diabetes and the insulin dose among the two patient groups.

TNFα	Group I		Group II	
	r	P	r	P
Age (yrs.)	0. 188	0. 368	0. 377	0. 063
Age of onset (yrs.)	0.112	0. 593	-0.017	0. 936
Duration (yrs.)	-0.194	0. 352	0. 228	0. 274
insulin(IU/kg/d)	0.411	0. 041	0. 045	0. 831

yrs.= years, IU= international unit.

There was no correlation between serum TNF level and the patients' age, age at onset duration of diabetes and the insulin dose among the two patient groups except for insulin dose and age (Table 4).

Table (5): Correlation between serum TNF α level and clinical data among the two patient groups

TNFα	Group I		Group II	
	R	P	r	P
Height (cm)	-0. 309	0. 132	0. 399	**0. 048****
Height SDS	-0. 185	0. 376	-0. 027	0.899
Weight (kg)	-0.156	0. 456	0. 130	0. 537
Weight SDS	-0.032	0. 879	0. 179	0. 391
BMI (kg/m^2)	0.064	0. 760	0. 065	0. 759
BMI SDS	-0.025	0. 904	0. 174	0. 405
Systolic Bp	-0.068	0. 747	0. 295	0. 153
Diastolic Bp	0. 161	0. 441	0. 053	0. 803
waist circumference (cm)	0.114	0.588	0.178	0.395

SDS= standard deviation score, BMI=body mass index, Bp= blood pressure.

There was no significant correlation between serum TNF α level and clinical data including the anthropometric measures in terms of height SDS (standard deviation score), weight SDS and body mass index (BMI) SDS, and the BP among the two patient groups except for significant correlation between two groups in height p value 0.048 (Table 5).

Table (6): Correlation between serum TNF α level and laboratory data among the two patient groups

TNF α	Group I		Group II	
	R	P	r	P
Mean FPG (mg/dl)	.027	0.898	-.101-	0.632
Mean PP (ml)g/d	-0.314	0.127	-0.108	0.607
Creatinine (mg/dl)	0.146	0.485	-.003-	0.989
HbA1C (%)	-.029-	0.889	-.289-	0.162
HDL (mg/dl)	0.122	0.561	-.128-	0.543
LDL mg/dl)	-.019-	0.928	-.152-	0.469
Cholesterol (mg/dl)	-.308-	0.134	-.243-	0.241
TGs (mg/dl)	-.112-	0.595	-.062-	0.769
ACR (gm/dl)	0.394	0.051	-.286-	0.166

FPG= fasting plasma glucose, PP= postprandial plasma glucose, HbA1C= glycated hemoglobin A1C, TGs= Triglycerides, HDL= High density lipoproteins, LDL= Low density lipoproteins, ACR=albumin creatinine ratio.
There was no significant correlation between serum TNF α level and laboratory data among the two patient groups (Table 6).

Table (7): Comparison of serum TNFα level between the patients and controls.

	Patients n=50	controls n=30	P*
	Mean ±SD	Mean ±SD	**0.036***
TNF & (ug/ml)	5.66 ±1.86	5.16±0.91	

*=Kruskal Wallis test.

TNFα level was a statistically different between patients and controls (Table 7).

ROC CURVE

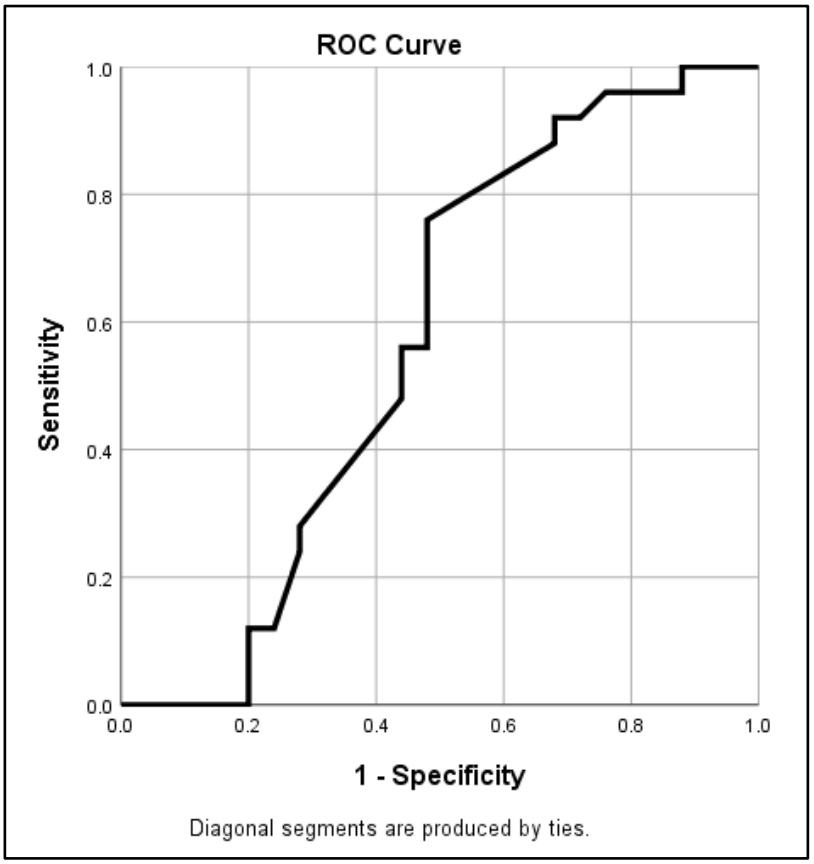

Figure (1): Represents the receiver operating characteristic curve (ROC) of TNFα at a p value of (0,033**) to detect the cutoff point (5.1) at which the patient will be at risk to microalbuminuria with a sensitivity of (76%) and a specificity of (63,6%) in which the area under the curve (AUC) (95% confidence interval) was 0.649. It means that the TNFα was strong differentiating marker between patients and controls.

DISCUSSION

There was no statistically significant difference in age, however, the female sex was significantly higher in the microalbuminuric group

This was in accordance with Youssef and Fawzy, (12) in their study on 25 cases with T1DM, the mean for age was 10.8 ± 2.2 years and for the duration of diabetes was 5 ± 1.1 years and 20 control healthy children, reported that there was no significant difference regarding age and sex between them.

There was no significant difference between the two patient groups regarding the duration of diabetes, age of onset and insulin dose

This was in accordance with Pestana et al., (13) in his study on 125 individuals with T1DM reported that; there was no significant difference between the groups' normoalbuminuria / micro and macroalbuminuria regarding to duration of diabetes & with Suh et al., (14) who reported that; the duration of diabetes did not differ between the normoalbuminuria and microalbuminuria groups.

TNFα level was not statistically different between diabetic and albuminuric patients and TNFα level was a statistically different between patients and controls

On the other hand, Navarro et al., (15) stated that serum TNF-α has been elevated in diabetic patients, and even in patients with only impaired glucose tolerance, compared with healthy individuals.

There was no correlation between serum TNF level and the patients' age, age at onset duration of diabetes and the insulin dose among the two patient groups except for insulin dose and age

On the opposite hand, Dogan et al., (16) conducted a study on population consisted of 27 children with T1DM and 25 healthy controls, and they found that there was a positive significant correlation between level of HbA1c and inflammatory markers.

Rashad et al., (17) reported that serum and urinary TNFα levels were significantly higher in patients with T2DM compared to control group. Moreover, when stratified diabetic nephropathy patients according to albumin excretion ratio, they found that patients with macroalbuminuria had higher levels of serum and urinary TNF α than normoalbuminuric and microalbuminuric patients.

There was no significant correlation between serum TNF α level and clinical data including the anthropometric measures in terms of height SDS (standard deviation score), weight SDS and body mass index (BMI) SDS, and the BP among the two patient groups except for significant correlation between two groups in height p value 0.048

Similar results detected by Suh et al., (14) who reported that the clinical characteristics including sex, age and BMI did not differ between the normoalbuminuria and microalbuminuria groups & Fares et al., (18) who reported that; there were no significant group differences in the age at onset of diabetes, duration of diabetes and BMI.

Also, Cherney et al., (19) recorded that no statistically significant differences were detected in gender, weight and BMI between microalbuminuric & normoalbuminuric groups.

There was no significant correlation between serum TNF α level and laboratory data among the two patient groups
According to ROC curve our results revealed that TNF& was good differentiating marker between patients and controls.
In agreement with our results also, Wong et al., (20) reported that the serum and urinary concentrations of TNF-α were elevated in DN as compared with non-diabetic individuals or with diabetic subjects without kidney disease and that these concentrations increase concomitantly with the progression of DN. These results indicate the role of elevated levels of this inflammatory cytokine with the development and progression of renal injury in DM.

CONCLUSION

Our results provide evidence serum TNFα was not significantly different between microalbuminuric and normoalbuminuric groups, also it is not sensitive or specific between two patient groups. TNFα was significantly different between patients and controls and a strong differentiating marker between patients and controls in correlation to microalbuminuria.

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