

# Human Tumor Necrosis Factor- $\alpha$ as a Diagnostic marker for chronic Diabetic Nephropathy

## ABSTRACT

**Background:** diabetic nephropathy (DN) is a chronic main microvascular consequence of untreated hyperglycemia, affects a significant portion of the population. It's thought to be the main factor causing end-stage kidney disease. TNF- $\alpha$  is involved in the development and course of disease in DN.

**Aim:** This study aimed to evaluate plasma TNF- $\alpha$  as a new diagnostic marker for early DN.

**Patients and methods:** This study included 125 Egyptian subjects attending the out Patients Clinic of the Department of Internal Medicine, 10<sup>Th</sup> of Ramadan city Health Insurance Hospital and divided as follow :- Control group , Study groups (patient with diabetic mellitus (DM), DN, DN and other complications).

Control & study group were subjected to measurement of glycosylated hemoglobin (HbA1C), , creatinine, total cholesterol, triglycerides, F.B.G, GFR , HDL, LDL, ALT, AST, total protein, albumin, globulin, A/G ratio, HDL, LDL, ACR, TNF- $\alpha$ .

**Results:** results showed that plasma TNF- $\alpha$  was positive and significantly correlated with BMI, duration of DM, F.B.G, HbA1C, CR, GFR, CHOL, HDL, LDL, AST, A/G ratio, Meanwhile, plasma TNF- $\alpha$  was negatively correlated with ACR, TG, ALT, ALB, TP and GLB.

At cut-off level  $\geq 83.5$ , TNF- $\alpha$  had 96.7% sensitivity and 79.7% specificity for diagnosing diabetic nephropathy.

**Conclusion:** The study found that patients in study group had considerably higher plasma levels of TNF- $\alpha$ . This suggests that TNF- $\alpha$  may be used as a marker for predicting chronic DN in patients with type 2 diabetes.

**Key words:** TNF - $\alpha$ , DN.

## 1. Introduction

Diabetes mellitus type 2 (T2DM) is a chronic illness that is quite common. All microvascular and macrovascular problems in individuals with T2DM, including DN, which may manifest later in the disease, are primarily caused by prolonged hyperglycemia [1].

DM patients with DN, the most prevalent chronic microvascular consequence, have significantly reduced quality of life. The development of DN is aided by dedifferentiation, cell hypertrophy, and inflammation. Numerous variables, such as oxidative stress, elevated glucose, altered hemodynamic, and inflammatory processes, are linked to the development of diabetic ketoacidosis (DKA). While data shows increased macrophage infiltration and overproduction of leukocyte adhesion

molecules in kidneys, DN has always been regarded as a non-immune illness [2].

TNF- $\alpha$  is a cytokine that initiates the acute-phase response and is a cell signaling molecule associated with systemic inflammation. TNF- $\alpha$  is primarily involved in immune cell modulation [3]. Monocytes and macrophages are the main source of TNF- $\alpha$  synthesis, while intrinsic resident renal cells can also produce this cytokine. Some cell surface receptors mediate the effects of TNF- $\alpha$ . A variety of transcription factors, cytokines, growth factors, receptors, cell adhesion molecules, mediators of inflammatory processes, and acute-phase proteins are expressed when TNF- $\alpha$  binds to its receptors. It may also mediate necrotic and apoptotic cell death [4]. As a result, TNF- $\alpha$  speeds up the production and release of inflammatory cytokines and may contribute to the development of DN. Therefore, the purpose of this work is to investigate the function of TNF- $\alpha$  plasma level in DN.

### 1.1 Aim of the work

The present study aimed to evaluate plasma TNF- $\alpha$  as a diagnostic marker for DN.

## 2. Patients and Methods

### 2.1 Study Design

Cross sectional study aimed to evaluate plasma TNF- $\alpha$  as a diagnostic marker for DN.

### Study Setting

The study was conducted at the outpatient clinic of the Dept. of internal medicine, 10th of Ramadan City Health Insurance Hospital.

This study aid the clinicians in improving patient outcomes of DM by testing TNF- $\alpha$  as an early biomarker for DN particularly in Egypt where the prevalence of DM high.

### 2.2 Target Population

Clinically diagnosed Diabetic & chronic DN patients attending the outpatient clinic of the Dept. of internal medicine, 10th of Ramadan City Health Insurance Hospital. The study including 125 persons they were divided in to:-

- A. **Control group** :- 20 healthy subjects were taken as control group.
- B. **Study group** :- including 105 patients divided in to three group .
  - **Group I** :-20 with DM
  - **Group II**: - 65 with DN.
  - **Group III**:-20 with DN and other complications.

### 2.3 Inclusion criteria

- Age ranged between 30 -46 years old.
- Both genders.

### 2.4 Exclusion criteria

- Patients with T1DM,
- Pregnancy,
- Patients with congestive heart failure,
- Patients with systemic lupus erythematosus,

- Patients with polycystic kidney disease.

## 2.5 Methods

- The study and control group were subjected to collection of demographic data as required in the attached sheet including age, occupation, anthropometric measurements of height, weight, waist circumference, and history of disease.
- Collection of early morning urine samples in vacutainer cups and About **5 ml** of venous blood sample from overnight fasted subjects were collected on plain tubes and **5ml** blood were collected on **EDTA** tubes by vacutainer system under complete aseptic conditions and HbA1C first done and samples centrifuged for 10 min at 2.500g within 30min ,separated serum and plasma were stored at -20 degree to be used in the following testes:-Serum creatinine, serum total cholesterol, HDL, LDL and triglycerides, F.B.G,ALT,AST.albumin,total protein,gloubulin.
- Plasma concentration of TNF- $\alpha$  was examined by using an enzyme-linked immunosorbent assay (ELISA Kit).
- Spot urine sample from each patient for: Urinary albumin / creatinine ratio was measured.

The collected data was revised, coded, tabulated and introduced to a PC using

Statistical package for Social Science (**SPSS 26**). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. The following tests were used:

## 2.6 Biochemical

**Parameters:** **Glucose** was determined according to the method of Trinder (1969), **HbA1C** level was determined according to the method of Klenk (1991), **Creatinine** was determined according to the method of Larsen (1972), **cholesterol** were determined method of Roeschalu et al. (1974), The **triglycerides** level was estimated using method of Fossati et al. (1982), **high density lipoprotein** was estimated using method of Gordon (1977), **low density lipoprotein** was calculated according to Freidwald et al. (1972) **AST&ALT** activity was measured using the kinetically method of IFCC (1978) according to Bergmeyer & Horden (1980), **Total protein** was measured using the colorimetric method of Weichselbaum (1946), **albumin** level was measured using the colorimetric method of Doumas et al. (1971), **globulin** was calculated by subtracting the albumin value from the corresponding total protein value and albumin/globulin ratio (A/G) was

calculated according to Kingsley (1939),

**2.7 Determination of plasma TNF- $\alpha$  level:** plasma TNF- $\alpha$  level (ng/L) was measured using ELISA kit according to manufacturer instructions. ELISA kit for human TNF- $\alpha$  was used in this assay and procedure from (BT-LAB) Co., Ltd., china. Cat.NoE0082Hu

**2.8 Descriptive statistics:**

- Mean Standard deviation ( $\pm$  SD) and range for parametric numerical data.
- Median and Interquartile range (IQR) for non-parametric numerical data.
- Frequency and percentage of non-numerical data.

**2.9 Statistical analysis:**

1. **ANOVA test** of significance was used when comparing between means of more than two groups.
2. **Post-hoc test after ANOVA** for significance between each two groups.
3. **Chi-Square test** was used to examine the relationship between two qualitative variables.
4. **Correlation analysis (using Pearson's method)** to assess the strength of correlation between two quantitative variables.
5. **ROC curve** for prediction of independent value effect on the outcome.
6. **P-value: level of significance**

-P>0.05: Non significant (NS).

-P< 0.05: Significant (S).

### 3. Results

Table -1 showed that The mean age was 36.2 for group A, 41.7 for group B, 47.5 for group C, 46.9 for group D, there were 71 men and 54 women. BMI, Duration of D.M by year, F.B.G, HBAIC, ACR, Cholesterol, Triglycerides, LDL, AST and ALT, TNF- $\alpha$  were significantly highest While, GFR and HDL lower in study groups on contrary to control group.

Other parameters did not differ significantly between study & control groups.

Table 2 shows that Age, BMI, Duration of D.M, F.B.G, C.P.A, HBAIC, creatinine, Na, ACR, GFR, Cholesterol, Triglycerides, HDL, LDL, AST, ALT, ALB, T.P, AG ratio and TNF- $\alpha$  were significantly different between four groups.

Table 3 shows that plasma TNF- $\alpha$  was positive and significantly correlated with BMI, duration of DM, F.B.G, HbA1C, CR, GFR, CHOL, HDL, LDL, AST, A/G ratio, Meanwhile, plasma TNF- $\alpha$  was negatively correlated with ACR, TG, ALT, ALB, TP and GLB.

Table 4 and Fig. 1 show that At cut-off level  $\geq 83.5$ , TNF- $\alpha$  had 96.7% sensitivity and 79.7% specificity for diagnosing diabetic nephropathy .

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**Table 1: Baseline characteristics among control&studygroups.**

Comparison between groups						
	Group A	Group B	group C	GroupD	F-test	P value
Age	36.2±4.53	41.7±5.2	47.53±2.7	46.95±2.8	61.12	<0.001 ** <sup>a</sup>
Gender						
Male	12 (60%)	12 (60%)	34 (52.3%)	12 (60%)	0.749	0.86 ns
Female	8(40%)	8(40%)	31(47.7%)	8(40%)		
<b>BMI</b>	27.3±2.2	28.7±2.6	29.6±2.9	30.1±3.2	3.00	0.008 **
duration(year)	-	4.95±2.4	8.81±2.87	9.15±2.68	71.41	<0.001 ** <sup>a</sup>
<b>F.B.G</b>	88.5±9.0	167.6±31.8	151.7±170	178.5±29.0	83.92	<0.001 ** <sup>a</sup>
<b>HBAIC</b>	4.9±0.3	9.6±2.0	9.1±1.0	9.2±0.7	37.90	<0.001 ** <sup>a</sup>
<b>CR</b>	0.8±0.1	1.0±0.1	4.0±2.4	6.2±2.0	27.39	<0.001 ** <sup>a</sup>
<b>ACR</b>	10.9±1.9	21.6±3.4	324.1±28.3	3071.9±24.1	508.15	<0.001 ** <sup>a</sup>
<b>GFR</b>	129.0±27.0	110.5±8.8	20.9±10.3	10.6±4.5	57.87	<0.001 ** <sup>a</sup>
<b>CHOL</b>	169.2±13.4	170.9±12.1	181.0±20.5	238.8±27.2	35.94	<0.001 ** <sup>a</sup>
<b>TG</b>	127.0±8.9	156.2±15.9	146.6±16.1	191.3±38.5	23.18	<0.001 ** <sup>a</sup>
<b>HDL</b>	47.8±2.6	40.8±3.3	44.8±5.9	36.4±3.5	75.96	<0.001 ** <sup>a</sup>
<b>LDL</b>	96.9±13.1	99.2±11.8	107.6±19.8	170.3±22.5	86.57	<0.001 ** <sup>a</sup>
<b>AST</b>	26.9±3.7	37.2±7.7	37.2±6.8	64.7±13.1	123.55	<0.001 ** <sup>a</sup>
<b>ALT</b>	26.9±2.6	36.9±6.9	36.7±5.9	70.5±14.9	27.61	<0.001 ** <sup>a</sup>
<b>ALB</b>	4.0±0.2	4.0±0.2	3.8±0.2	3.5±0.2	40.70	<0.001 ** <sup>a</sup>
<b>T.P</b>	7.0±0.2	7.0±0.2	6.8±0.3	6.2±0.3	11.37	<0.001 ** <sup>a</sup>
<b>GLB</b>	3.0±0.2	3.0±0.2	3.0±0.2	2.7±0.3	49.64	<0.001 ** <sup>a</sup>
<b>A/G ratio</b>	1.8±0.2	1.4±0.1	1.3±0.1	1.3±0.2	71.14	<0.001 ** <sup>a</sup>
<b>HumanTNF-<math>\alpha</math></b>	94.5±35.4	281.0±76.6	524.1±56.4	713.7±18.5	99.30	<0.001 ** <sup>a</sup>

\*\*; means significant differences between groups at p <0.05, ns; no significant  
a; ANOVA test at P<0.05  
b; Chi square test at P<0.05

**Table 2: The comparison between the studied groups with control groups for different parameters.**

	group A	group B	Group C&D	F-test	P value
Years	36.2±4.5	41.7±5.2	47.4±2.7	91.88	<0.001** <sup>a</sup>
<b>Gender</b>					
Male	12 (60%)	12 (60%)	47(55.3)	2.46	0.88 <sup>b ns</sup>
Female	8(40%)	8(40%)	38(44.7)		
<b>BMI</b>	27.3±2.2	28.7±2.6	29.7±3.0	5.9	0.009 ** <sup>a</sup>
<b>Duration(year)</b>	-	4.95±2.4	8.89±2.81		<0.001 ** <sup>a</sup>
<b>F.B.G</b>	88.5±9.0	167.6±31.8	158.0±23.3	80.0	<0.001 ** <sup>a</sup>
<b>HBAIC</b>	4.9±0.3	9.6±2.0	9.1±0.9	126.7	<0.001 ** <sup>a</sup>
<b>CR</b>	0.8±0.1	1.0±0.1	4.5±2.5	40.5	<0.001 ** <sup>a</sup>
<b>ACR</b>	10.9±1.9	21.6±3.4	970.7±57.9	4.7	<0.001 ** <sup>a</sup>
<b>GFR</b>	129.0±27.0	110.5±8.8	18.5±10.2	713.3	<0.001 ** <sup>a</sup>
<b>CHOL</b>	169.2±13.4	170.9±12.1	194.6±33.0	10.3	<0.001 ** <sup>a</sup>
<b>TG</b>	127.0±8.9	156.2±15.9	157.1±29.9	11.2	<0.001 ** <sup>a</sup>
<b>HDL</b>	47.8±2.6	40.8±3.3	42.8±6.5	8.6	<0.001 ** <sup>a</sup>
<b>LDL</b>	96.9±13.1	99.2±11.8	122.3±33.6	9.8	<0.001 ** <sup>a</sup>
<b>AST</b>	26.9±3.7	37.2±7.7	43.6±14.6	15.1	<0.001 ** <sup>a</sup>
<b>ALT</b>	26.9±2.6	36.9±6.9	44.6±16.9	13.3	<0.001 ** <sup>a</sup>
<b>ALB</b>	4.0±0.2	4.0±0.2	3.7±0.3	20.7	<0.001 ** <sup>a</sup>
<b>T.P</b>	7.0±0.2	7.0±0.2	6.7±0.4	15.6	<0.001 ** <sup>a</sup>
<b>GLB</b>	3.0±0.2	3.0±0.2	2.9±0.3	1.0	<0.001 ** <sup>a</sup>
<b>A/G ratio</b>	1.8±0.2	1.4±0.1	1.3±0.2	74.1	<0.001 ** <sup>a</sup>
<b>Human TNF-α</b>	94.5±35.4	281.0±76.6	568.7±68.4	107.2	<0.001 ** <sup>a</sup>
<b>**;</b> means significant differences between groups at p <0.05 <b>a;</b> ANOVA test at P<0.05 <b>b;</b> Chi square test at P<0.05					

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Table 3: Correlations between the TNF- $\alpha$  and other parameters in patients with diabetes.

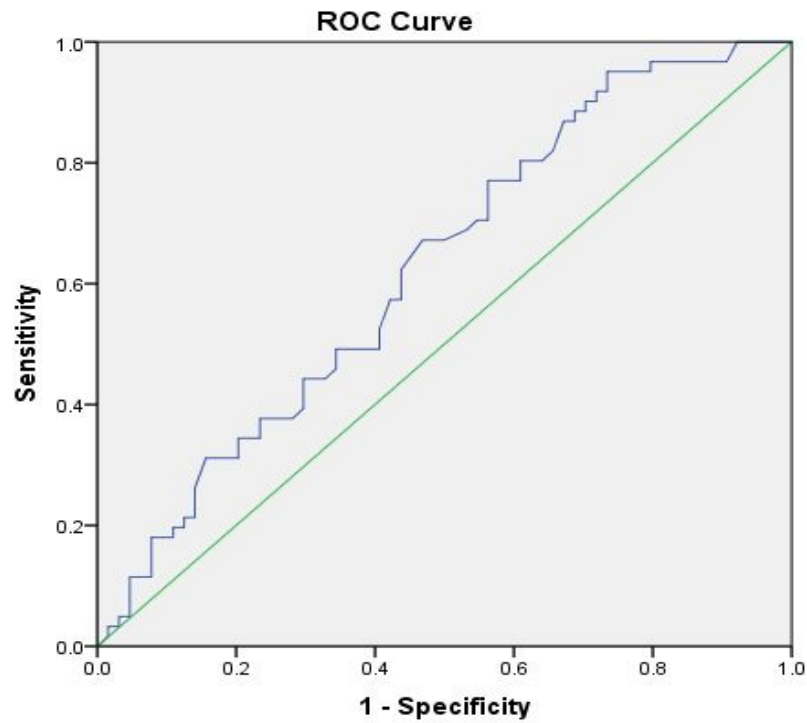
UNDER PEER REVIEW

<b>Human TNF-<math>\alpha</math></b>		
<b>Parameters</b>	<b>R</b>	<b>P value</b>
BMI	0.205	0.035*
duration	0.552	<0.001**
F.B.G	0.515	<0.001**
HBAIC	0.527	<0.001**
CR	0.427	<0.001**
ACR	-0.744	<0.001**
GFR	0.504	<0.001**
CHOL	0.425	<0.001**
TG	-0.284	<0.001**
HDL	0.504	<0.001**
LDL	0.519	<0.001**
AST	0.536	<0.001**
ALT	-0.470	<0.001**
ALB	-0.446	<0.001**
T.P	-0.224	0.012*
GLB	-0.498	<0.001**
A/G ratio	0.662	<0.001**

**Table 4: Validity of the TNF- $\alpha$ for DN.**

	AUC	Sensitivity	Specificity	Cut-off value
TNF- $\alpha$	0.626	96.7%	79.7%	83.5

**Figure 1: ROC curve of TNF- $\alpha$  for DN**



Diagonal segments are produced by ties.

UNDER

## 1. Discussion

One frequent diabetic microvascular consequence that might progress to end-stage renal disease is DN. Proinflammatory cytokine TNF- $\alpha$  is involved in the development and course of disease in

DN[5]. With no discernible difference between DM and DM-CKD, the

BMIs of both patient groups were significantly higher than those of the control group. These findings corroborated those of Doghish et al.[6], who found no difference in BMI between DM-CKD and non-DM patients. But according to Gupta et al.[7], there was no discernible change in BMI between the DM and DM-CKD groups when compared to the control group. According to Maric-Bilkan[8], interactions between a number of variables stimulate intracellular signalling, which in turn causes the generation of cytokines and growth factors and ultimately results in renal illness. These beginning processes are similar for renal disorders associated with diabetes and obesity.

It was discovered that DM-CKD had a longer history of diabetes than DM. This was in line with the findings of Mahfouz et al. [9], who discovered a substantial difference in the length of diabetes between DM and DM-CKD. On the other hand, Ochodnický et al.[10] and Motawi et al. (11)found no discernible variation in the length of diabetes between DM and DM-CKD.

Gallagher and Suckling[12]provided an explanation of the relationship between DN and the length of diabetes by stating that chronic exposure to hyperglycemia damages kidney structures either directly or indirectly through hemodynamic alterations. According to Anders et al.[13], hyperglycemia reduces the amount of sodium that is exposed at the macula densa. This, in turn, causes glomerular

hyperfiltration, dilates the afferent arteriole, suppresses tubuloglomerular feedback, and causes podocyte barotrauma, which ultimately leads to podocyte and nephron loss.

In a recent study, DM-CKD and DM had significantly greater FBG, 2 h PP, and HbA1c values than controls[14]. While Alnaggar et al. (15)and Gupta et al. [7]discovered that FBS and 2 h PP were considerably greater in T2DM with microalbuminuria compared with normoalbuminuria group, the obtained results were in agreement with Motawi et al. [11]. According to Saulnier-Blache et al.[16], there was no difference in HbA1c between DM and DM-CKD. Because hyperglycemia dysregulates multiple metabolic pathways, it has been proposed that hyperglycemia is the primary initiator of kidney damage linked to DN. According to Bedard and Krause [17], hyperglycemia exacerbates the formation of reactive oxygen species in the mitochondria, which damages DNA and promotes apoptosis. This, in turn, increases oxidative stress. Furthermore, as an inflammatory cytokine, TNF- $\alpha$ triggers the processes that lead to both cell death and survival. When TNF- $\alpha$ binds to TNF- $\alpha$ receptor-1, death domain protein is drawn to the site, activating more protein mediators and sending a signal from the active receptor to the signalling caspase cascade, which leads to apoptosis[11].

While there was no significant difference in BUN and creatinine between DM and controls, there was a difference in BUN, creatinine, and ACR between DM-

CKD and both DM and controls. These outcomes agreed with those of Dabhi and Mistry (18) and Doghish et al. [6]. In terms of lipid profile, there was no significant difference in total cholesterol and TG between DM and controls, while DM-CKD had significantly higher levels of these three markers than DM and controls. LDL-C and TG were also significantly higher. But compared to DM and controls, HDL-C was lower in DM-CKD; additionally, DM had lower HDL-C than controls. The outcomes obtained match the findings of Mahfouz et al. [9]. Furthermore, Motawi et al. (11) found that whereas total cholesterol, LDL-C, and HDL-C did not significantly differ between patient groups, TG was higher in DM-CKD than in DM and controls. In the meanwhile, Alnaggar et al. (15) found that the lipid profiles of DM and DM-CKD did not differ significantly. Under diabetic conditions, dyslipidemia increases the expression of extracellular matrix and activates macrophages in the glomeruli, which results in DN. Dyslipidemia is observed in diabetic patients with early stage renal damage, according to Doghish et al. [6]. It results in a rise in TG and a fall in HDL-C due to the compromised function of lipoprotein lipase, which is found in endothelial cells.

In this study, at cut-off level  $\geq 83.5$ , TNF- $\alpha$  had 96.7% sensitivity and 79.7% specificity for diagnosing DN

TNF- $\alpha$  concentration were considerably greater in both patient groups than in the control group, and DM-CKD was higher than DM. In the DM-CKD group, TNF- $\alpha$  level had a strong positive connection with FBG, creatinine, total cholesterol, LDL-C, HbA1c, and ACR.

These findings corroborated those of Chen et al. [19], who discovered that TNF- $\alpha$  was elevated in both DM and DM-CKD, but was higher in the former. This suggests that DN has an enhanced inflammatory load. According to Chen et al. [19], TNF- $\alpha$  is a pleiotropic cytokine that is essential for mediating inflammatory processes that are linked to glomerular and tubulointerstitial damage.

## 2. Conclusion and Recommendations

The study found that patients with DM and DM-CKD had considerably higher serum levels of TNF- $\alpha$ . This suggests that TNF- $\alpha$  may have a function in mediating changes in DN and may contribute to the progression of DM to DN. However, more investigation and clinical validation are required to confirm the link between TNF- $\alpha$  and the pathogenesis because of the intricacy of DN processes.

Disclaimer (Artificial intelligence)

we hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript. Yes

## 3. References

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