

# Study the Possible Levels of Inflammatory Marker TNF- $\alpha$ and Microalbuminuria in Type 2 Diabetic Nephropathy Patients of Babylon Province/Iraq

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## Abstract

**Background:** Diabetic nephropathy is referred to as the kidney damage that occurs in people with diabetes developing it over many years characterized by gradually increasing urinary albumin excretion ( $>300\mu\text{g}/\text{mg}$ ) from microalbuminuria to proteinuria, blood pressure, and declining in glomerular filtration rate (GFR) (thickening in glomerulus) which represents the late event.

**Aim:** Study the relationship between the levels of urinary TNF- $\alpha$  and the progression of diabetic nephropathy in type2 diabetic patients.

**Objective:** Fasting blood and urine samples were collected from seventy type2 diabetic patients with enrollment the age, duration, blood pressure, and Body Mass Index (BMI) of each patient. the levels of urine TNF- $\alpha$  were established by ELISA.

**Results:** forty-patients have normal albuminuria (Albumin/Creatinine Ratio  $<30\mu\text{g}/\text{mg}$ ) and only thirty-patients have microalbuminuria (Albumin/Creatinine Ratio  $>300\mu\text{g}/\text{mg}$ ) with increased urinary TNF- $\alpha$  levels.

**Conclusions:** A significant positive relationship between urinary TNF- $\alpha$  and sever microalbuminuria.

**Keywords:** Diabetic nephropathy, Microalbuminuria, TNF- $\alpha$ , Hypertension.

## Introduction

Diabetic nephropathy is the chronic kidney disease (CKD) that occurs as a result of diabetes characterized by increased urinary albumin excretion (albuminuria) and decreased glomerular filtration rate (GFR)<sup>(1)</sup>. Microalbuminuria  $>300\text{mg}/24$  hour associated with hypertension is the first marker of this disease occurring in 20-40% of patients with diabetes leading to end stage renal disease (ESRD) or renal failure with glomerular filtration rate  $\text{GFR}<10\text{mg}/\text{mintypically}$  in patients with type 2 diabetes after a diabetes duration of about 10 years<sup>(2)</sup>, with some risk factors like genetic factors, blood pressure, dyslipidemia, metabolic syndrome, inflammation, growth factors, smoking, and others diabetes complications, the presence of these risk factors lead to dramatically increase of cardiovascular

disease and eventually progresses of renal failure<sup>(3)</sup>. Microalbuminuria is considered to be as a marker of both kidney disease and endothelial dysfunction (decreased glomerular filtration) because of diabetes or hypertension or both, is strongly associated with an increased risk for cardiovascular events<sup>(4)</sup>, increasing microalbuminuria in urine led to the formation of proteinuria which increase overtime to macroalbuminuria ( $300>\mu\text{g}/\text{mg}$ ) are linked with kidney disease and increased risk for progression to end stage renal disease. Impaired glycemic control is related with increasing in urinary microalbumin levels very low levels or absence of microalbuminuria is associated with low CVD, which also increases markedly with increasing albumin amount in urine<sup>(5)</sup>.

Tumore Necrosis Factore Alfa (TNF- $\alpha$ ) is synthesized and produced by stimulated kidney cells

and especially by glomerular, tubular, and active monocytes, causing the elevation of the levels of other inflammatory factors such as cytokines, chemokines, growth factors, and acute phase protein<sup>(6)</sup>. TNF- $\alpha$  plays an important role in the development of diabetic nephropathy by decreasing glomerular blood flow and a consequence reducing filtration rate, vasoconstriction, and damage of the glomerular filtration barrier leading to macroalbuminuria, in addition the elevated TNF- $\alpha$  levels leads to the elevation of free radicals increasing the apoptosis of glomerular cells<sup>(7)</sup>. In general, hypertension present in type2 diabetic patients before the development of kidney disease because of the risk factors such as glucose intolerance and obesity. Hypertension causes the progression of kidney disease and contributes to the increased cardiovascular disease in diabetic nephropathy patients because of the destruction of renal function. The distribution of hypertension in diabetic nephropathy were elevated at each step of chronic kidney disease, reaching to about 90% for end stage renal disease patients<sup>(8,9)</sup>.

### Subjects and Method

Seventy patients with type2 diabetes The study was conducted between March 2019-June 2019 in Marjan teaching hospital in AL-Hilla City/Babylon province-Iraq. Samples under study were divided according to the duration of disease and age, this study design focus on study some physiological and biochemical parameters.

Blood and urine samples were collected from type2 diabetic patients in the morning between 08:00 and 10:00 O'clock to minimize the effect of diurnal variation<sup>(10)</sup>, after a period of fasting 8-10 hours by vein puncture using 5 ml disposable syringes and it was divided into two parts: one part about 2 ml was put in EDTA containing tube; the blood was mixed gently for 3 minutes and then used for hematological tests and especially for HbA<sub>1c</sub> assay. The second part included 3 ml of blood was put in the centrifuge tube (glass tube)

and allowed to clot for 15 min then it was centrifuged for serum separation at approximately 3000 rpm for 15 minutes<sup>(11)</sup>, the separated sera were used for biochemical tests such as determination of fasting blood glucose.

First emorningeurinel samples werer collected aundersterilelconditionse, 10 ml of urinee were ecentrifugede at 1500 rpmr for 10' minute' and the supernatant' was stored' at -80°C' until' TNF- $\alpha$ ' determination by ELISA assay. This samples also used for measurement of Albumin/Creatinine Ratio ACR ( $\mu\text{g}/\text{mg}$ ), were ACR <30  $\mu\text{g}/\text{mg}$  were known as normal albuminuria, 30-300  $\mu\text{g}/\text{mg}$  as microalbuminuria, and > 300  $\mu\text{g}/\text{mg}$  as proteinuria. Body mass index (BMI) were calculated by dividing the weight in kilograms (kg) by the square of the height ( $\text{m}^2$ ). Blood pressure were determined by using mercury manometer, the blood pressure with systolic blood pressure SBP  $\geq$  140 mmHg and diastolic blood pressure DBP  $\geq$  90 mmHg were referred to as hypertension.

**Statistical Analysis:** The statistical analysis was performed by using the statistical package for social sciences (SPSS version 23.0) and found all of arithmetic mean and standard deviation ( $M \pm \text{S.D.}$ ) by using the T-test to know the signification between normal albumin and microalbumin groups at p value ( $p < 0.05$ ). Brivaraitte correlations were performed by using the Pearson correlation coefficient at p value ( $p < 0.05$ ) considered to be statistically significant.

### Results

The results of this study showed that the mean age and duration were 48.70 and 11.20 years, respectively. While, the mean of BMI was 27.70  $\text{kg}/\text{m}^2$ , with 133.90 and 81.53 mmHg for systolic and diastolic blood pressure. Laboratory results of Fasting blood glucose, HbA<sub>1c</sub>, ACR, and urinary TNF- $\alpha$  were shown in table (1).

**Table (1): Physiological-biochemical parameters of type 2 diabetic patients.**

Parameters	Mean $\pm$ Standard Deviation
Age (Years)	48.70 $\pm$ 9.84
Duration (Years)	11.20 $\pm$ 5.30
Fasting Blood Glucose FBG (mmol/ml)	13.15 $\pm$ 4.25
HbA <sub>1c</sub> %	8.56 $\pm$ 1.83
BMI (Kg/m <sup>2</sup> )	27.90 $\pm$ 2.02

Parameters	Mean±Standard Deviation
Albumin/Creatinine Ratio (ACR) (µg/mg)	40.15 ± 14.94
Urinary TNF-α (pg/mg)	6.88 ± 4.07
Systolic Blood Pressure (mmHg)	133.90 ± 5.67
Diastolic Blood Pressure (mmHg)	81.53 ±1.69

Forty type 2 diabetic patients with normal albuminuria, and only thirty patients with microalbuminuria. The mean values of age, duration, systolic and diastolic blood pressure were showed significant elevation ( $P \leq 0.01$  or  $P \leq 0.05$ ) in microalbumin group compared with normal

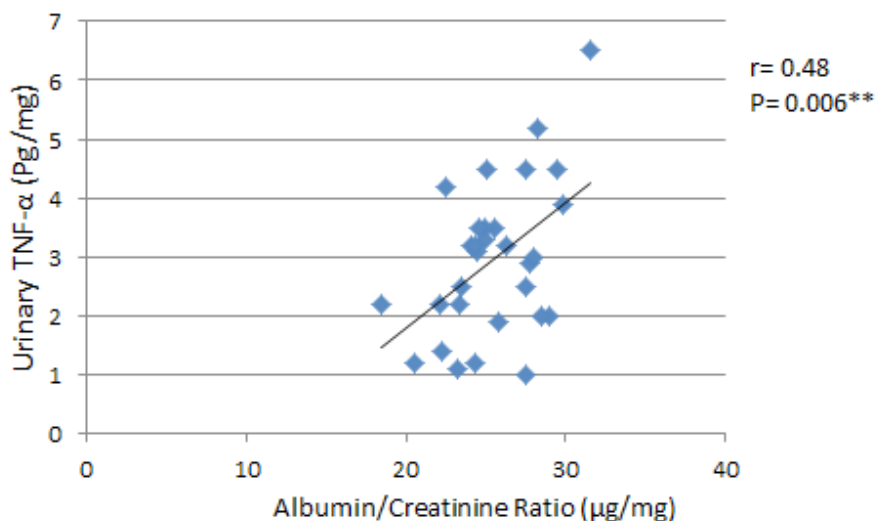
albumin group, also the mean levels of FBG, ACR, and urinary TNF-α were significantly higher ( $P \leq 0.01$  or  $P \leq 0.05$ ) in in microalbumin group compared with normal albumin group, as shown in table (2).

**Table (2): Some physiological and biochemical parameters in type 2 diabetic patients with normal albuminuria and microalbuminuria.**

Parameters	Normal albuminuria N = 40 (M±SD)	Microalbuminuria N = 30 (M±SD)	P Value
Age (Years)	40 ± 3.74	57.40 ± 4.95	0.0001**
Duration (Years)	6.40 ± 1.99	16.00 ± 2.23	0.0001**
Fasting Blood Glucose FBG (mmol/ml)	9.70 ± 2.32	16.60 ± 2.55	0.0001**
HbA <sub>1c</sub> %	8.08 ± 1.76	9.04 ± 1.83	0.15
BMI (Kg/m <sup>2</sup> )	28.40 ± 1.99	27.41 ± 1.89	0.18
Albumin/Creatinine Ratio (ACR) (µg/mg)	25.92 ± 3.56	54.38 ± 3.97	0.001**
Urinary TNF-α (pg/mg)	3.32 ± 1.49	10.44 ± 2.24	0.002**
Systolic Blood Pressure (mmHg)	130.60 ± 4.95	137.20 ± 4.34	0.001**
Diastolic Blood Pressure (mmHg)	80.80 ± 1.74	82.13 ± 1.55	0.03*

M±SD: Mean ± Standard Deviation, \*\*Signification at  $P \leq 0.01$ , \*Signification at  $P \leq 0.05$ .

On the other hand, the correlation study showed significant positive correlation between ACR and urinary TNF-α with ( $r = 0.48$ ,  $P = 0.006$ ), as shown in figure (1).



**Figure (1): Correlation between urinary TNF-α and Albumin/creatinine ratio in type2 diabetic patients with microalbuminuria**

## Discussion

Diabetic nephropathy is a chronic disease in which the renal structure and function probably distorted, many pathways that causes the pathological alterations in the kidney of diabetic patients have been suggested and are effectively being inspected. Where, it has been found that hyperglycemia generally lead to elevation the levels of oxidative stress and the stimulation a lot of inflammatory and apoptotic mechanisms in the renal, resulting in abnormal renal cells with excessive predisposition of extracellular matrix in the glomerulus' and tubulointerstitium<sup>(7,8)</sup>. Also it has been determined that high' glomerular' blood' flow is probably to result in glomerular capillary 'distention, additionally dysfunction of glomerular and mesangial cell. Moreover, it has been indicated that the loss of regulation of signaling' pathways comprising a number of inflammatory cytokines and growth factors, like protein kinase C (PKC) and transforming growth factor- $\beta$  (TGF- $\beta$ ), is also destructive for glomerular hyperperfusion and hyperfiltration<sup>(12)</sup>.

Glomerular injury is represents the early sign of diabetic nephropathy and microalbuminuria, which seem to be a strong predictor of renal damage progression, However, it has been proposed that the increased number of patients with normal albuminuria are probably to promote diabetic nephropathy, whereas not all patients with macroalbuminuria are perhaps to have advanced renal damage<sup>(13)</sup>. This refers that multiple pathways may participate in the pathogenesis of this disorder. Many of structural and pathological changes' may appear at the same time and proceed at different rates in the diabetic nephropathy causing high heterogeneity' of the disease. So that, biomarkers with elevated specificity to different abnormalities are needed to determine the onset and progression of nephropathy in different groups of diabetic patients. Lately, a number of possible biomarkers have been reported, comprising markers of glomerular 'injury, 'tubular injury, oxidative stress and inflammation<sup>(14)</sup>.

The results of this showed that there were a significant differences between microalbuminuria (nephropathy) and normal albuminuria (without nephropathy) groups in relation to age, duration of diabetes, FBG, ACR, urinary TNF- $\alpha$ , systolic and diastolic blood pressure were showed significant elevation in microalbuminuria than normal albuminuria, this study was in agreement with the study of<sup>(15)</sup>. TNF- $\alpha$  of human is a protein composed

of 157 amino acid and is differ from mouse TNF- $\alpha$  in that it is not glycosylated and act through two types of receptors TNF-R1 and TNF-R2, TNF- $\alpha$  mainly produced by inflammatory cells such as activated monocytes and by specific renal cells<sup>(16)</sup>. TNF- $\alpha$  play an important role in nephropathy pathogenesis and progression which stimulate the expression of adhesion molecules on the leukocytes and endothelial cells, increase the production of endothelin-1 leading to narrowing of blood vessels and reduction of blood flow and consequence decreasing glomerular filtration rate (GFR), TNF- $\alpha$  has an ability to stimulate glomerular cell apoptosis by damaging junctions between cells and increasing permeability resulting casing high levels of macroalbuminurea, urinary' TNF- $\alpha$  function as a biomarker of kidney inflammation<sup>(17,18)</sup>.

In this study, urinary TNF- $\alpha$  levels were significantly correlated with microalbuminurea where both levels higher in microalbuminurea than normal albuminuria group making urinary TNF- $\alpha$  as an independent indicator for the degree of microalbuminurea, some studies were showed that TNF-R2 inflammatory pathway is already participated in the progression of macroalbuminurea through the onset of diabetic nephropathy<sup>(19,20)</sup>.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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