The pro-inflammatory cytokine Tumor Necrosis Factor alpha (TNF-α) in Diabetic Nephropathy

Alia KH Mahmoud[a] *, Muhammed Ahmed Abd El Moety[a] ,Amal Khalifa Ahmed[b] , Samer A. El-Sawy [a,c]

[a]Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Sohag University, Sohag, Egypt.

[b]Department of Internal Medicine, Faculty of Medicine, Sohag University, Sohag, Egypt.

[c]Department of Restorative Dentistry and Basic Medical Sciences, Faculty of Dentistry, University of Petra, Amman 11196, Jordan.

Abstract

Background: Diabetic nephropathy has become the main cause of renal failure, and furthermore, is associated with a dramatic increase in cardiovascular risk**.** Regretfully, we still don't fully understand the mechanisms underlying the onset and progression of renal injury in diabetes. There is now proof that inflammation and activated innate immunity play important roles in the etiology of problems with diabetes. Furthermore, a variety of inflammatory chemicals, including pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α).

Objectives: is to detect the role of TNF-α as a pathogenic factor in diabetic nephropathy.

Patients and methods:: Between January 2022 and June 2022, 50 diabetic study participants sought medical care at the Sohag University Hospital's outpatient diabetes clinic. They were compared to 25 people who appeared to be in good condition. TNF-α level in the serum was measured in 25 individuals with diabetes, 25 individuals with diabetic nephropathy, and 25 healthy controls by enzyme-linked immun-osorbent assay (ELISA).

Results: As compared to healthy controls, the diabetes and DN groups had significantly higher serum levels of TNF-α. TheTNF-α level and the duration of diabetes have a significant (positive) association, ($P<0.05$), a strong (positive) correlation ($P<0.05$) between TNF-α and the albumin creatinine ratio. A moderately positive correlation was seen between TNF-α and HBA1C and demonstrating a significant inverse relationship ($P<0.05$) between TNF-α and eGFR.

Conclusion: Our results indicated that serum levels of TNFα were significantly higher in diabetics and diabetic nephropathy group compared to the control group.

Keywords; DM; DKD; TNF-α .

DOI: 10.21608/SVUIJM.2024.255031.1758

***Correspondence**: aliakhairy2@gmail.com

Received: 28 December,2023.

Revised: 11 January,2024.

Accepted: 25 January,2024.

Published: 27 September, 2024

Cite this article as: Alia KH Mahmoud, Muhammed Ahmed Abd El Moety ,Amal Khalifa Ahmed , Samer A. El-Sawy.(2024). The pro-inflammatory cytokine Tumor Necrosis Factor alpha (TNF-α) in Diabetic Nephropathy. SVU-International Journal of Medical Sciences. Vol.7, Issue 2, pp: 545-555.

Introduction

DM is typified by elevated plasma glucose levels and insufficient insulin synthesis or use **(Elsisi et al., 2022) .** A severe micro vascular consequence of diabetes, diabetic nephropathy (DN) is one of the main causes of end-stage renal failure **(Dagar et al., 2021).** Unfortunately, due to its intricate pathogenic processes, DN cannot be stopped from getting worse **(Tang et al., 2021).**

Tumor necrosis factor also called cachexin, cachectin, or tumor necrosis factor alpha, or TNF-α) is a cytokine and an adipokine. It belongs to the TNF superfamily, which is made up of several trans-membrane proteins with homologous TNF domains. TNF-α is a pleiotropic cytokine that has many effects and can have multiple mechanisms for contributing to the development of DN including vasoconstriction brought on by an increase in endothelin-1 production, a decrease in glomerular blood flow and glomerular filtration rate, and a breakdown of the glomerular filtration barrier, which is caused by interaction with intercellular junctions and results in proteinuria. Oxidative stress can also be caused by increased TNF-α production as mesangial cells' nicotinamide adenine dinucleotide phosphate (NADPH) is activated. Ultimately, it seems that TNF-α directly induces apoptosis and cytotoxicity in glomerular cells (**Shan et al.**, **2018**).

Patients and methods

A case control study has been conducted between January; 2022 and June; 2022.

Subjects: Seventy-five adult Egyptian volunteers were enrolled in this case control study (46 females and 29 males); fifty diabetic patients (both T1DM&T2DM) who sought medical advice at the DM Outpatient Clinic at Sohag University Hospital – Sohag-Egypt ,and twenty-five healthy volunteers were included in this case-control study. The study plan was approved by the research ethics committee of the Sohag faculty of medicine**.**

Participants in the study were divided into three groups:

Group I: includes twenty-five cases of DM 12 males and 13 females, with a mean age of 53.24±10 years.

Group II:There were twelve male and thirteen female DKD cases, with a mean age of 54.92±13 years.

Group III: Twenty-five fit, non-diabetic volunteers As the control group, twenty males and five females with a mean age of 31.24±13 years.

DM was diagnosed in accordance with the American Diabetes Association (ADA) guidelines (fasting blood glucose level > 126 mg/dl or HbA1C >6.5%) **(ADA, 2019)** Patients diagnosed with diabetic kidney disease must meet the enrollment requirements for DN diagnostic standards; These requirements include a high urine A/C ratio, persistent micro-albuminuria.

Exclusion criteria: Any other significant medical conditions that could affect glycemia or albuminuria, such as pregnancy, cancer, infections, or pharmaceutical therapy, additional potential nephropathy causes including: gout, systemic lupus, or cardiac problems. We exclude diabetes by measuring HbA_1C and fasting blood glucose in the participants in the control group**.**

Methodology

1- Biochemical parameters: It was instructed that study participants not eat or exercise when they arrived. All trial participants had midstream urine samples and peripheral blood drawn early in the morning. Every study participant supplied basic data, such as age, height, body weight, and the type and duration of their diabetes mellitus.

A medical assessment was performed to measure blood pressure and rule out other potential causes of nephropathy, such as acute infections or cardiovascular disease. Patients have abdominal sonography exams to evaluate the condition of their kidneys**.**

Biological investigation

Samples and assays: 1- three milliliters of venous blood was utilized for the colorimetric reaction (Jaffe reaction) to measure serum creatinine (Cr) and glycated haemoglobin (HbA_1c) in a laboratory setting. The sandwich immuno-detection method was employed in conjunction with fluorescence immunoassay technology to ascertain the percentage of HbA1c in the blood that had received EDTA treatment **(**Fine care, CAT NO.0350 from Texas).

Estimation of GFR: Modification of Diet in Renal Disease or MDRD has the following formula: (s creatinine/88.5) x 186 is the eGFR (ml/min/1.73m^2). - 0.203 x 0.742 (if female) - 1.154 x (Age).

2-Sample of fasting midstream urine was used for estimating Computing urine creatinine, and micro-albumin (UMA), After taking, the mixture was centrifuged for ten minutes at 3000 rpm to extract the supernatant. Next, to quantify micro-albuminuria, the One Step- MAU Quick Quantitative Test was utilized. (MAU) (Fine care) catalog No.W206 and urine Creatinine chemically measured.

Next, the ACR calculation was applied: ACR readings exceeding 3 mg/g were considered aberrant (high), whereas those below 3 mg/g were considered normal **.**

3. Measuring TNF-α serum level: We measured serum TNFα level by (ELISA) bio assay kit for TNF-α catalog no = 201-12-0083 (Sun red Biotechnology Company, Shanghai, China).The kit uses a double-antibody sandwich enzyme-linked immuno-sorbent assay (ELISA) to assay the level of Human (TNF-α) in samples. Add (TNF-α) to monoclonal antibody enzyme well which is pre-coated with Human (TNF-α) monoclonal antibody, incubation; then, add (TNF-α) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogenic Solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human Substance (TNF-α) of sample were positively correlated.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 23 was used. The Kolmogorov-Smirnov test was used to determine data normality. The chart creator was used to create the graphs. For parametric data, use the Student t test; for non-parametric data, Apply the Mann Whitney, The parameters' correlation was ascertained using the Spearman's rho test. P 0.05 indicated each analysis's statistical significance**.**

Results

Clinical and socio-demographic characteristics of the group under study: The three study groups differ in terms of age as shown in **(Table .1)**, additionally; there is a substantial variation in the length of (DM) between diabetics and patients with DN. The length of diabetes and the A/C ratio in the urine of the two research groups (diabetics and patients with DN) differ very highly significantly ($P<0.001$) as seen in **(Table.2)**

(Table.3) demonstrates that between controls and diabetic nephropathy, there was a significant

difference in HbA1C ,serum creatinine, eGFR and TNF-α (P<0.05), there was no statistically significant change in eGFR (P > 0.05) between patients with DN and those with diabetes alone, there was a significant median difference of serum TNF-α level between study groups, as the DN group and diabetics had considerably greater serum levels of TNF-α than the control group as showed by (**Fig.1**) .

Table.1. Variations in the research groups' age, gender as well as systolic and diastolic blood pressure

Parameters		Controls N=25	Diabetics N= 25	Diabetic nephropathy N= 25	p- value
Age by years	Mean ± SD	**31.24±13.206**	**53.24±10.91**	**54.92±13.276**	<0.0003
	RANGE	**(15-64)**	**(35- 77)**	**(27-83)**	
Gender No. (%)	Female	20(80%)	13 (52%)	13 (52%)	0.064
	Male	5 (20.4%)	12 (48%)	12 (48%)	
SBP[1](mmHg)	Mean ± SD	**117±14.8**	**119.8±18.6**	**126.8±19.7**	0.68
DBP[2](mmHg)	Mean ± SD	**76.8±11.4**	**76.8±11.4**	**80.2±9.06**	0.53

By one-way ANOVA, Independent T test, or CHI Square test as appropriate**.** SBP: systolic blood pressure. DBP: diastolic blood pressure

Table .2. Mann Whitney test of Duration of diabetes in years and A/c between the study groups

Parameters		Controls N=25	diabetics N=25	DN patients N=25	P value
Duration Of Diabetes in years	**mean ±SD median**	_____ _____	3.8±3.8 3 (1-5)	11.7±7.7 12 (4-17)	0.001
Urine A/C mg/gm.	**mean ±SD median**	0.16±0.18 0.15(0.1-0.2)	0.18±0.17 0.14 (0.12-0.2)	0.52±0.16 0.5 (0.4-0.6)	0.001

Mann Whitney between study groups * Significant (P < 0.05)

Table 3. Comparison of HBA1C, serum creatinine, eGFR and TNFα levels between the studied groups

Parameter		Control N=25	diabetics n=25	Diabetic nephropathy N=25	P value between control and diabetic	P value between control and diabetic nephropathy	P value between diabetic and diabetic-nephropathy
HbA1C %	**mean ±SD Range**	5.06 ±1.08 4.5 (4.5-6.1)	7.7 ±2.02 7.1 (6.8-8.2)	9.6 ±1.6 9.95 (8.5-10.9)	0.001	0.001	0.001

S. Creatinine (mg/dl)	**mean ±SD Range**	1.26±1.4 1 (0.9-1.0)	1.0±0.2 1.0 (0.9-1)	1.2±0.317 1.3 (1-1.4)	0.74	0.009	0.006
eGFR (ml/min/1.73m²)	**mean ± SD Range**	106.2 ±5.99 105 (100-112)	99.04 ±7.3 99.3 (96.2-100)	95.8 ±6.9 96.3 (89.0-100)	0.001	0.001	0.113
TNF-α (mg/dl)	**mean ±SD Range**	62.7 ±137.1 25 (19-41.9)	190 ±264.2 53 (36.3-209.5)	271.7 ±171.6 212 (161.0-362.5)	0.001	0.001	0.003

MannWhitney*P<0.05(significant)

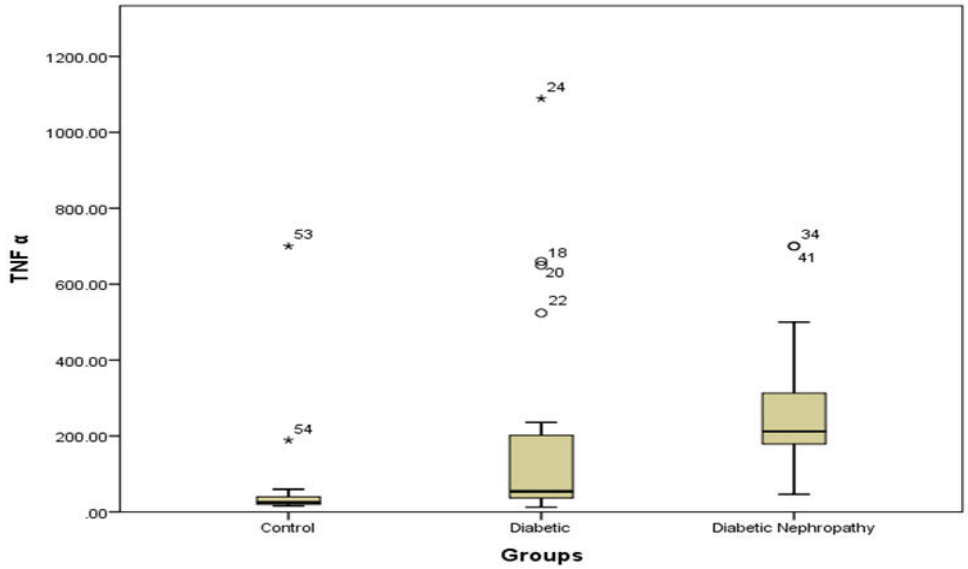

Fig.1. Box plot of TNF-α median variation between research groups

Also there was significant positive strong correlation between TNF-α and the length of the diabetes P =0.00, r= 0.643 as shown in (**Table. 4** , **Fig.2**, demonstrating a substantial positive association between urine A/C ratio and TNF-α as P =0.005 ,r= 0.387 as shown in **(Table. 4 , Fig.3)**, demonstrating a substantial positive association between TNF-α and HBA_1C as P =0.03,r=0.277 as shown in **(Table. 4 , Fig.4)** showed substantial positive mild association between TNF-α and S. creatinine as P p=0.04,r=0.239 as shown in (**Table. 4** , **Fig.5).**While there was substantial negative moderate association between TNF-α and eGFR, P =0.00, r=0.398 as showed in (**Table. 4** , **Fig.6).**

Table 4. Spearman correlation coefficient between TNF-α and Clinical, laboratory parameters, across all research participants

Correlation with TNF- α	R value	P value
Urine A/C ratio (mg/gm.)	0.387**	0.005
HBA_1C	0.277*	0.037
S.creatinine (mg/dl)	0.239*	0.040
Duration of diabetes in years	0.643**	0.000
eGFR (ml/min/1.73m^2)	0.398**	0.000

At the 0.01 level correlation is of significance.

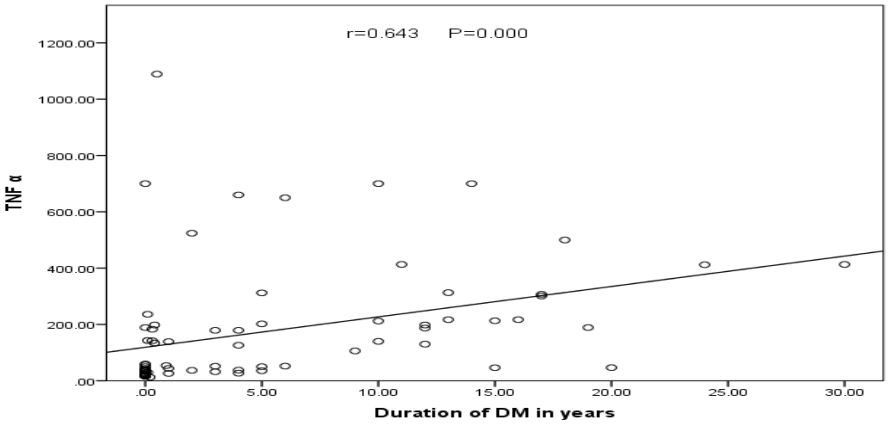

Fig.2. Correlation between TNF- α and duration of DM

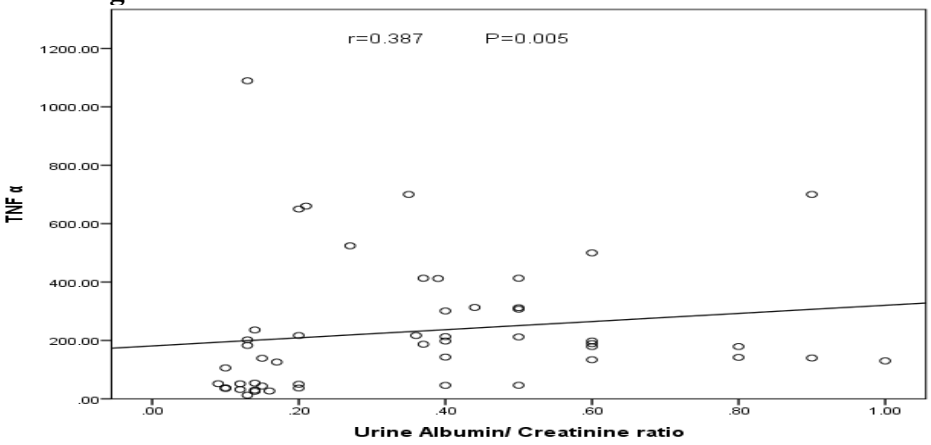

Fig.3. Correlation between TNF-α and urine A/C ratio

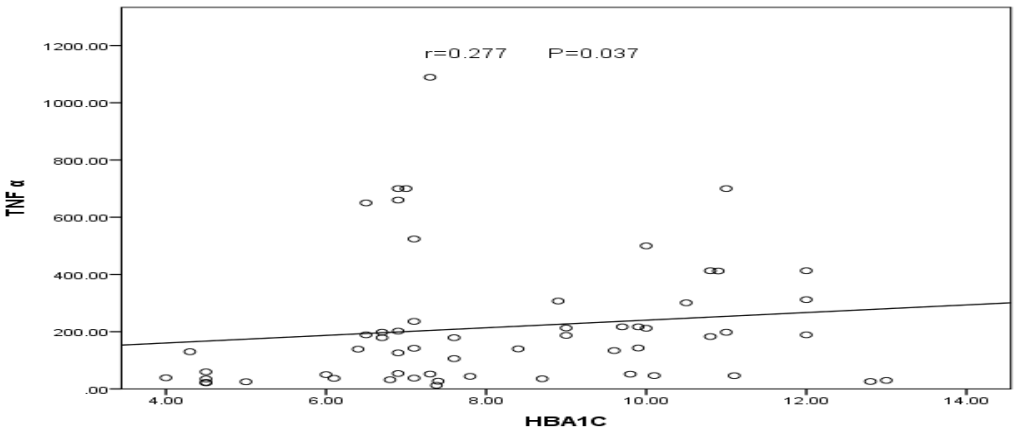

Fig.4. Correlation between TNFα and HbA_1C

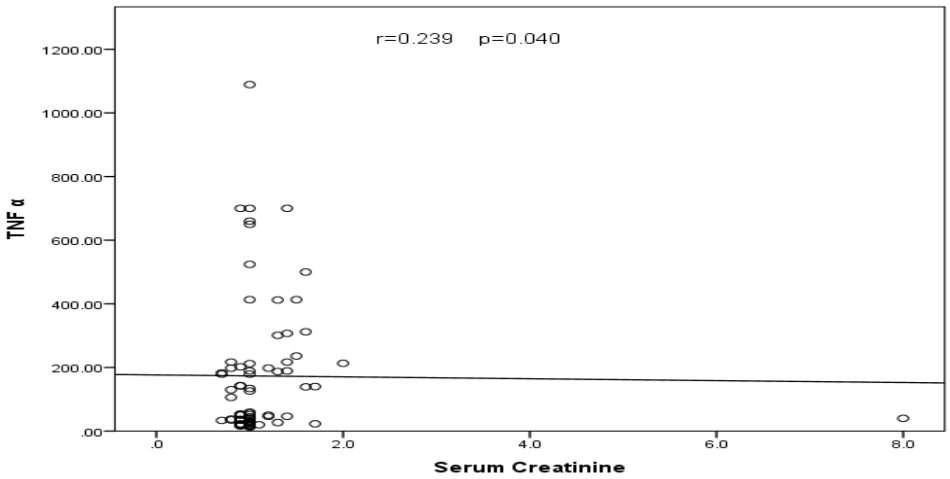

Fig.5.Correlation between TNFα and serum creatinine

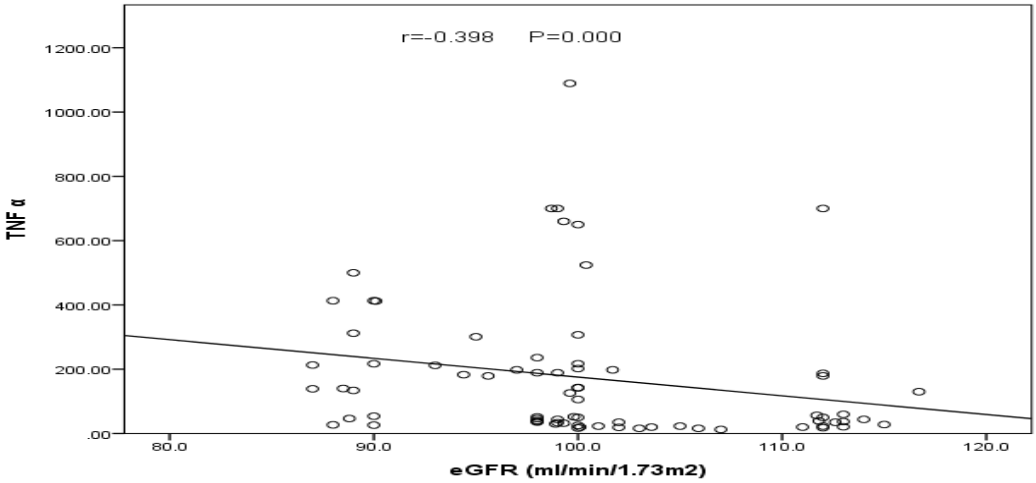

Fig.6. Correlation between TNF-α and eGFR .

Discussion

One of the main causes of end-stage renal disease worldwide is diabetic nephropathy, a significant micro vascular consequence of diabetes. **(Dagar et al., 2021).** DN cannot be stopped from progressing because of its complex pathogenic processes, which puts a tremendous financial burden on many families and society. **(Tang et al., 2021).** The pathophysiology of DN must thus be urgently investigated in order to develop appropriate treatments. Prior research has shown that the primary pathogenic mechanisms of DN are apoptosis and inflammation, which play critical roles in speeding DN development and worsening. **(Yang et al., 2022).** TNF-α is a pleiotropic cytokine that has many effects and can have multiple mechanisms for contributing to the development of DN including vasoconstriction brought on by an increase in endothelin-1 production, a decrease in glomerular blood flow and glomerular filtration rate, and a breakdown of the glomerular filtration barrier, which is caused by interaction with intercellular junctions and results in proteinuria. Oxidative stress can also be caused by increased TNF-α production as mesangial cells' nicotinamide adenine

dinucleotide phosphate (NADPH) is activated. Ultimately, it seems that TNF-α directly induces apoptosis and cytotoxicity in glomerular cells (**Shan et al.**, **2018**).

In this case-control study, 75 Egyptians—25 with diabetic nephropathy and 25 with diabetes—as well as 25 controls were involved , Every participant underwent a comprehensive medical history, clinical examination, and ELISA technique measurement of serum TNFα levels**.**

Blood pressure in the investigated groups was similar in the present investigation, In line with what we detected**;** Fawzy et al. (2020) reported that Diabetics and diabetics with nephropathy didn't differ in blood pressure in a statistically meaningful way **(Fawzy et al., 2020)**.

In the current study, individuals with diabetes and nephropathy had substantially higher urine A/C ratios and longer DM duration in years than individuals with diabetes alone. (P =0.001).Our results are in the same line as those with **Mariye Zemicheal et al. (2020**), who provided evidence of the factors influencing DN in Ethiopian diabetic patients. 168 cases and 672 controls, with corresponding mean ages of 45.18 years and 62.12 years, were included in the unmatched case-control study , DN was found to be determined by the length of time a person has had DM since being diagnosed **(Mariye Zemicheal et al., 2020)**.

In the present study, HbA1C wes significantly higher in diabetic with nephropathy patients compared to only diabetic patients and controls and was significantly higher in only diabetic patients compared to healthy volunteers (P =0.001).Present study results were in harmony with **Ashjari et al. (2022)** who discovered that, in comparison to type 2 diabetes mellitus, the HbA1C was considerably greater in DN **(Ashjari et al., 2022)**. Our findings are consistent with a cross-sectional comparative study conducted by Sahu et al. (2021) on 180 healthy controls (group I), Group II consisted of the 205 individuals with DM who did not have micro albuminuria, while group III consisted of those who did, These results proved that HbA1c in controls was considerably lower than those with diabetes and DN (p = 0.00) **(Sahu et al., 2021)**.

In our study serum levels of TNFα were notably higher in diabetics and DN patients compared to healthy volunteers, Szabo et al., (2020) examined how adiponectin and TNF-α) contribute to the development of type 1 diabetes. With 52 diabetes patients and 66 controls, the research was set up as **a** case-control observational study. Compared to the control group, the type 1 diabetes group has considerably higher levels of serum TNF-α and a greater percentage of patients who were considered high-risk for inflammation since they had higher TNF-α values **(Szabo et al., 2020).**Also In their research **;El-Edel et al.(2020),** discovered that the DM group's TNF-α values in serum were considerably greater than those of the healthy volunteers (P = 0.001), and that the level in the DN group was considerably greater than that of the healthy volunteers and diabetics (P = 0.001**)** (**El-Edel et al**.,**2020)** .

Additionally**, Awad et al.(2015)** reported that TNF-α, which is mainly produced by macrophages and monocytes and affects peripheral insulin resistance in addition to insulin secretion, plays a crucial role in the course of DN . This cytokine is cytotoxic to mesangial, epithelial, and glomerular cells, which may cause direct renal injury **(Awad et al., 2015**). Nevertheless, TNF-α in the groups they evaluated did not reach statistical significance, according to **Gupta et al.(2015)** The present investigation revealed a statistically significant positive association between TNF-α serum levels and (HBA1C, duration of diabetes)**. Szabo et al.'s** findings, which showed a substantial positive association between TNF-α and HBA1C and the length of diabetes **(Szabo et al., 2020).** In the current study, Additionally , there was a strong positive connection between serum creatinine and urine albumin creatinine ratio and TNF-α levels , These results were consistent with **El-Edel et al.(2020**) who reported that among the DM and DN group, there was a significant positive relationship between serum levels of TNF-α and fasting blood glucose, creatinine, total cholesterol, low-density lipoprotein-cholesterol, HbA1C, and the micro albumin/creatinine ratio were found to be correlated (r = 0.042, <0.001, <0.001, <0.001, 0.027, and 0.043, respectively) **(El-Edel et al., 2020)**

In the ongoing examination, serum creatinine wasn't statistically different between only diabetic patients and controls, but it was higher in patients with DN compared to healthy volunteers (P = 0.009) and diabetics (P = 0.006). Our results agree with those of Sahu and his colleagues (2021), who found that the non-diabetic control group possessed the least blood creatinine ($p < 0.01$) compared to the DM without micro-albuminuria and the micro- albuminuria group **(Sahu et al., 2021)**. Furthermore Fawzy and his collegues .,(2020) observed that the serum creatinine level in diabetic patients with nephropathy was substantially higher than that in diabetes patients alone ($p < 0.001$) **(Fawzy et al., 2020)**. Asmamaw and his colleagues.(2020) conducted a cross-sectional study to find out how important blood creatinine levels are for detecting renal disease in people with type 2 diabetes early on, which is in contradiction to our findings. Sixty-two T2DM patients and sixty healthy controls were included .They found that, in comparison to healthy controls .patients with type 2 DM exhibited significantly elevated serum creatinine **(Asmamaw et al., 2020)**. The observed discrepancy between our results and theirs could be explained by the fact that our patients had a mean duration of T2DM of only 3.8 ± 3.8 years, while their patients had a mean duration of 12.3 ± 8.6 years. Additionally, other researcher's studies have shown that the risk of nephropathy increases with the duration of diabetes. **(Tziomalos and Athyros.,2015)**.

eGFR did not significantly differ in the current study between patients with diabetes and nephropathy and those with diabetes alone, but it was significantly lower in patients with nephropathy and in diabetics compared to healthy volunteers (P =0.001),

Fawzy et al.(2020)'s research proved that ESRD in diabetic patients had an eGFR that was considerably lower than that of solely diabetic patients (5 (4.25–7.0) vs. 98.6 (91.1–127.3); p= <.001), which is in contrast to our findings. A different study methodology, a larger sample size, and they included ESRD patients receiving dialysis may be suitable explanations for this discrepancy **(Fawzy et al., 2020)**.

Study limitations were small sample size and the exclusion of patients on dialysis.

Conclusion

Our results indicated that serum levels of TNFα were considerably higher in diabetics and DN group compared to control group ,TNF- α showed significant positive correlation with s.creatinine, HBA1C, urine A/c ratio and duration of diabetes and a substantial inverse relationship with the eGFR , this findings suggesting involvement of TNF-α in the pathogenesis of DN. High serum TNF-α may be a positive marker as a predictor of the initiation of complications such; nephropathy in diabetics.

References

- **Ashjari D , Karamali N , Rajabinejad M , Hassani SS , Afshar LH , Afshari D et al . (2022)**. The axis of long non-coding RNA MALAT1/miR-1-3p/CXCR4 is dysregulated in patients with diabetic neuropathy. Heliyon, 8(3)**:** 917-8.
- **Asmamaw T , Genet S , Menon M , Tarekegn G , Chekol E , Geto Z , et al . (2020)**. Early Detection of Renal Impairment Among Patients with Type 2 Diabetes Mellitus Through Evaluation of Serum Cystatin C in Comparison with Serum Creatinine Levels: A Cross-Sectional Study. Diabetes Metab Syndr Obes, 13**:** 4727-35.
- **American Diabetes Association (2019)** Micro vascular complications and foot care: standards of medical care in diabetes. Diabetes Care, (Vol: 42, pp. S124-S138).
- **Awad H, You H, Gao T, Cooper T, Nedospasov S, Vacher J, et al.(2015).** Macrophage-derived tumor necrosis factor-alpha mediates diabetic renal injury. Kidney Int, 88:722–733**.**
- **Dagar N , Das P, Bisht P , Taraphdar AK , Velayutham R , Arumugam S (2021)**. Diabetic nephropathy: A twisted thread to unravel. Life Sci, 278**:** 119635.
- **El-Edel RH, Fathy WM, Abou-Elela DH, Emara MM, Abo El-Khair NT (2020).** Role of tumor necrosis factor alpha in type 2 diabetic nephropathy. Menoufia Med J 2020,33:920-925.
- **Elsisi G. H , Afify A , Abgad A , Zakaria I , Nasif N , Ibrahim H N et al., (2022).** A budget impact analysis of substituting sitagliptin with liraglutide in type 2 diabetes from a private health insurance perspective in Egypt. Cost Eff Resour Alloc; 20(1): 1-1.
- **Fawzy MS, Abu AlSel BT, Al Ageeli E, Al-Qahtani SA, Abdel-Daim MM and Toraih EA. (2020).**Long non-coding RNA MALAT1 and microRNA-499a expression profiles in diabetic ESRD patients undergoing dialysis: a preliminary cross-sectional analysis. Arch Physiol Biochem;126(2):172-82.
- **Fawzy M , Als B , Al Ageeli E , Al-Qahtani S , Abdel Daim M , Toraih E. (2018).** Long non-

coding RNA MALAT1 and microRNA-499a expression profiles in diabetic ESRD patients undergoing dialysis: apreliminary cross-sectional analysis.Archives of Physiology and Biochemistry, 126: 1-11 .

- **Mariye ZT , Bahrey TD , Tasew AH , Teklay WG , Gebremichael GB , Tesfay HN , et al. (2020).** Determinants of diabetic nephropathy among diabetic patients in general public hospitals of tigray, Ethiopia, 2018/19. Int J Endocrinol, 2020**:** 483-6.
- **Sahu S , Taywade M , Saharia GK , Ramadass B. (2021).** Uromodulin-Glycated Hemoglobin Ratio Plays The Trump In Diabetic Nephropathy Detection-A Case Control Study. Natl J Integr Res Med 12**,** 120-3
- **Shan B , Pan H, Najafov A , Yuan, J.(2018).** Necroptosis in development and diseases. Genes & development, 32(5-6): 327-340.
- **Szabo CE, Man OI, Istrate A, Kiss E, Catana A, Creț V. (2020**) Role of adiponectin and tumor necrosis factor-alpha in the pathogenesis and evolution of type 1 diabetes mellitus in children and adolescents. Diagnostics ;10(11):945-6.
- **Tang G , Li S , Zhang C , Chen H , Wang N , Feng Y. (2021).** Clinical efficacies, underlying mechanisms and molecular targets of Chinese medicines for diabetic nephropathy treatment and management. Acta Pharm Sin B, 11**:** 2749-2767.
- **Tziomalos K , Athyros V G . (2015).** Diabetic Nephropathy: New Risk Factors and Improvements in Diagnosis. Rev Diabet Stud, 12**:** 110-8.
- **Yang Z , Song D , Wang Y, Tang L. (2022) .** lncRNA MALAT1 promotes diabetic nephropathy progression via mir-15b-5p/tlr4 signaling axis. J Immunol Res, 2022**,** 201-5.