HumanTumorNecrosisFactor-αasa DiagnosticMarkerforChronicDiabetic Nephropathy

ABSTRACT

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

Aim: This study aimed to evaluate plasma TNF-α as a new diagnostic marker for early DN. **Patients and Methods:**Thisstudyincluded125EgyptiansubjectsattendingtheoutPatientsClinic oftheDepartmentofInternalMedicine,10ThofRamadancityHealthInsuranceHospitaland divided as follow:- Control group , Study groups(patient with diabetic mellitus(DM),DN, DN and other complications).
Control& study group were subjected to measurement ofglycosylated hemoglobin (HbA1C), creatinine,totalcholesterol, triglycerides,F.B.G,GFR,HDL, LDL, ALT, AST,totalprotein, albumin, globulin, A/G ratio, HDL, LDL, ACR,TNF-α.
Results: results showed that plasma TNF-α was positive and significantly correlated with BMI, duration of DM, F.B.G, HbAIC, CR, GFR, CHOL, HDL, LDL, AST, A/G ratio, Meanwhile, plasma TNF-α was negatively correlated with ACR, TG, ALT, ALB, TP and GLB.
At cut-off level ≥83.5, TNF-α had 96.7% sensitivity and 79.7% specificity for diagnosing diabetic nephropathy**.**
Conclusion: Thestudyfoundthatpatientsinstudygrouphadconsiderably higherplasma levelsof TNF-α.This suggests that TNF-αMay be used as a markers for predicting chronic DN in patients with type 2 diabetes.

Keywords:Chronicdiabeticnephropathy;diagnosticmarker;TNF-α,DN;diabeticcomplications.

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 diabeticketoacidosis (DKA). While data shows increased macrophage infiltration and overproduction of leukocyte adhesion molecules in kidneys, DNhas always been regarded as a nonimmune illness [2].

TNF-αisacytokinethatinitiatestheacute-phase response and is a cell signaling molecule associatedwithsystemicinflammation.TNF-α is primarily involved in immune cell modulation [3]. Monocytes and macrophages are the main source of TNF-αsynthesis, while intrinsic resident renal cells can also produce this cytokine. Some cell surface receptors mediate the effects of TNF-α.A variety of transcription factors, cytokines, growth factors, receptors, cell adhesion molecules, mediators of inflammatory processes, and acute-phase proteins are expressed when TNF-α binds to its receptors. It may also mediate necrotic and apoptotic cell death[4].Asaresult,TNF-αspeedsupthe productionandreleaseofinflammatorycytokines and may contribute to the development of DN. Therefore, the purpose of this work is to investigatethefunctionofTNF-α plasmalevelin DN.

AimoftheWork

The present study aimed to evaluates plasma TNF-α as a diagnostic marker for DN

2. PATIENTSANDMETHODS

StudyDesign

Crosssectionalstudy aimedtoevaluates plasma TNF-α as a diagnostic marker for DN**.**

StudySetting

Thestudywasconductedattheoutpatient clinic of the Dept. of internal medicine, 10th of Ramadan City Health Insurance Hospital.

This study aid theclinicians in improving patient outcomes of DM by testing TNF-αas an early biomarkerforDNparticularlyinEgyptwherethe prevalence of DM high.

TargetPopulation

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. Controlgroup:-20healthysubjects were taken as control group.

B. Study group:- including 105 patientsdivided in to three group .

- **GroupI:-**20withDM
- **GroupII**:-65with DN.
- **Group III:-**20 with DN and other complications.

InclusionCriteria

- Agerangedbetween30-46yearsold.
- Bothgenders.

ExclusionCriteria

- PatientswithT1DM,
- Pregnancy,
- Patientswithcongestivehardfailure,
- Patientswithsystemiclupuserythematous,
- Patientswithpolycystickidneydisease.

Methods

- The study and control group were subjected to collection of demographicdata as required in the attached sheet including age, occupation, anthropometric measurements of height, weight, waistcircumference,andhistoryofdisease.
- Collection of early morningurine samples in vacutainer cupsand About 5 ml of venous blood samplefrom overnightfasted subjects were collected on plain tubesand 5mlblood were collectedon EDTA tubesby vacutainer system under complete aseptic conditionsand 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-αwas examined be using an enzyme-linked immunosorbent assay (ELISA Kit).
- Spot urine sample from each patient for: Urinary albumin / creatinine ratio was measured.

Thecollecteddatawasrevised,coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 26). Data was presentedandsuitableanalysiswasdone according to the type of data obtained for each parameter. The following tests were used:

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 usingmethod of Fossati et al. (1982), high density lipoprotein was estimated using method of Gordon (1977),low density lipoproteinwascalculatedaccordingtoFreidwald et al. (1972) , AST&ALTactivity 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),

Determination of plasma TNF-α level: plasma TNF-αlevel (ng/L) was measured using ELISA kit according to manufacturer instructions. ELISA kit for human TNF-α was used in this assay and procedure from (BT-LAB) Co., Ltd., china.Cat.No E0082Hu

Descriptivestatistics:

- MeanStandarddeviation(±SD)andrange for parametric numerical data.
- MedianandInterquartilerange(IQR)for non-parameteric numerical data.
- Frequency and percentage of non-numerical data.

StatisticalAnalysis

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. ***ROCcurve***forpredictionofindependent value effect on the outcome.
6. ***P-value:levelofsignificance***

-P>0.05:Nonsignificant(NS).
-P<0.05:Significant(S).

3. RESULTS

Table1showedthatThemean agewas36.2for groupA,41.7forgroupB,47.5forgroupC,46.9 for group D, there were 71 men and 54 women. BMI,DurationofD.Mbyyear,F.B.G,HBAIC, ACR, Cholesterol, Triglycerides, LDL, AST and ALT, TNF-αwere 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-α were significantly different between four groups.

Table1.Baselinecharacteristicsamongcontrol&studygroups.

	Comparisonbetweengroups					
	GroupA	**GroupB**	**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**[a]
Gender						
Male	12(60%)	12(60%)	34(52.3%)	12(60%)	0.749	0.86ns
Female	8(40%)	8(40%)	31(47.7%)	8(40%)		
BMI	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**[a]
F.B.G	88.5±9.0	167.6±31.8	151.7±170	178.5±29.0	83.92	<0.001**[a]
HBAIC	4.9±0.3	9.6±2.0	9.1±1.0	9.2±0.7	37.90	<0.001**[a]
CR	0.8±0.1	1.0±0.1	4.0±2.4	6.2±2.0	27.39	<0.001**[a]
ACR	10.9±1.9	21.6±3.4	324.1±28.3	3071.9±24.1	508.15	<0.001**[a]
GFR	129.0±27.0	110.5±8.8	20.9±10.3	10.6±4.5	57.87	<0.001**[a]
CHOL	169.2±13.4	170.9±12.1	181.0±20.5	238.8±27.2	35.94	<0.001**[a]
TG	127.0±8.9	156.2±15.9	146.6±16.1	191.3±38.5	23.18	<0.001**[a]
HDL	47.8±2.6	40.8±3.3	44.8±5.9	36.4±3.5	75.96	<0.001**[a]
LDL	96.9±13.1	99.2±11.8	107.6±19.8	170.3±22.5	86.57	<0.001**[a]
AST	26.9±3.7	37.2±7.7	37.2±6.8	64.7±13.1	123.55	<0.001**[a]
ALT	26.9±2.6	36.9±6.9	36.7±5.9	70.5±14.9	27.61	<0.001**[a]
ALB	4.0±0.2	4.0±0.2	3.8±0.2	3.5±0.2	40.70	<0.001**[a]
T.P	7.0±0.2	7.0±0.2	6.8±0.3	6.2±0.3	11.37	<0.001**[a]
GLB	3.0±0.2	3.0±0.2	3.0±0.2	2.7±0.3	49.64	<0.001**[a]
A/Gratio	1.8±0.2	1.4±0.1	1.3±0.1	1.3±0.2	71.14	<0.001**[a]
HumanTNF-α	94.5±35.4	281.0±76.6	524.1±56.4	713.7±18.5	99.30	<0.001**[a]

***;meanssignificantdifferencesbetweengroupsatp<0.05,ns;nosignificant a; ANOVA test at P<0.05*
b;ChisquaretestatP<0.05

Table2.Thecomparisonbetweenthestudiedgroupswithcontrol groupsfordifferent parameters.

	group A	**group B**	**GroupC&D**	**F-test**	**P value**
Years	36.2±4.5	41.7±5.2	47.4±2.7	91.88	<0.001**[a]
Gender					
Male	12(60%)	12(60%)	47(55.3)	2.46	0.88[b ns]
Female	8(40%)	8(40%)	38(44.7)		
BMI	27.3±2.2	28.7±2.6	29.7±3.0	5.9	0.009 **[a]
Duration(year)	-	4.95±2.4	8.89±2.81		<0.001**[a]
F.B.G	88.5±9.0	167.6±31.8	158.0±23.3	80.0	<0.001**[a]
HBAIC	4.9±0.3	9.6±2.0	9.1±0.9	126.7	<0.001**[a]
CR	0.8±0.1	1.0±0.1	4.5±2.5	40.5	<0.001**[a]
ACR	10.9±1.9	21.6±3.4	970.7±57.9	4.7	<0.001**[a]
GFR	129.0±27.0	110.5±8.8	18.5±10.2	713.3	<0.001**[a]
CHOL	169.2±13.4	170.9±12.1	194.6±33.0	10.3	<0.001**[a]
TG	127.0±8.9	156.2±15.9	157.1±29.9	11.2	<0.001**[a]

	group A	group B	GroupC&D	F-test	P value
HDL	47.8±2.6	40.8±3.3	42.8±6.5	8.6	<0.001**a
LDL	96.9±13.1	99.2±11.8	122.3±33.6	9.8	<0.001**a
AST	26.9±3.7	37.2±7.7	43.6±14.6	15.1	<0.001**a
ALT	26.9±2.6	36.9±6.9	44.6±16.9	13.3	<0.001**a
ALB	4.0±0.2	4.0±0.2	3.7±0.3	20.7	<0.001**a
T.P	7.0±0.2	7.0±0.2	6.7±0.4	15.6	<0.001**a
GLB	3.0±0.2	3.0±0.2	2.9±0.3	1.0	<0.001**a
A/Gratio	1.8±0.2	1.4±0.1	1.3±0.2	74.1	<0.001**a
HumanTNF-α	94.5±35.4	281.0±76.6	568.7±68.4	107.2	<0.001**a

***;meanssignificantdifferencesbetweengroupsatp<0.05 a; ANOVA test at P<0.05 b;ChisquaretestatP<0.05*

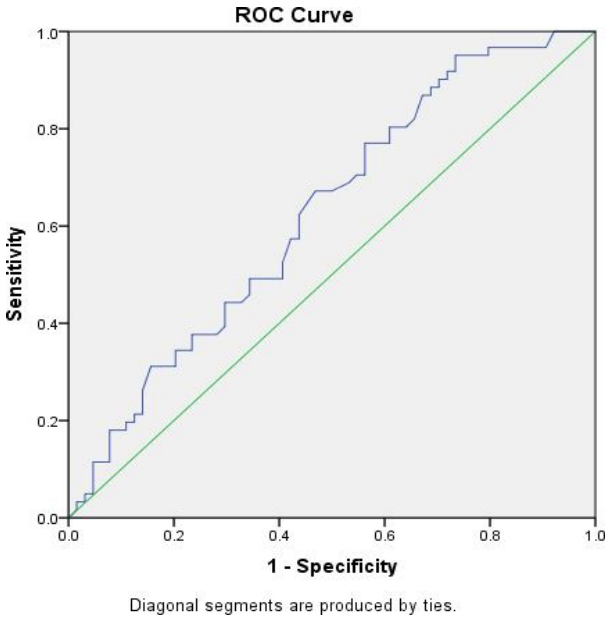

Fig.1.ROCcurveofTNF-αfor DN

Table3.CorrelationsbetweentheTNF-αandotherparametersinpatientswithdiabetes.

	HumanTNF-α	
Parameters	**R**	**Pvalue**
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/Gratio	0.662	<0.001**

Table4.ValidityoftheTNF-αfor DN

	AUC	Sensitivity	Specificity	Cut-offvalue
TNF-α	0.626	96.7%	79.7%	83.5

Table 3 shows that plasma plasma TNF-αwas positive and significantly correlated with BMI, durationofDM,F.B.G,HbAIC,CR,GFR,CHOL, HDL, LDL, AST, A/G ratio,Meanwhile, plasma TNF-α was negatively correlated with ACR, TG, ALT, ALB, TP and GLB.

Table4andFig.1showthatAtcut-offlevel ≥83.5, TNF-αhad 96.7% sensitivity and 79.7% specificity for diagnosing diabetic nephropathy.

4. DISCUSSION

One frequent diabetic microvascular consequence that might progress to end-stage renal disease is DN.Proinflammatory cytokine TNF-αisinvolvedinthedevelopmentandcourse of disease in DN [5]. With no discernible difference between DM and DM-CKD, theBMIs 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-CKDand 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 similarfor renal disorders associated with diabetes and obesity.

It was discovered that DM-CKD had a longer history of diabetes than DM. This was inlinewith thefindings of Mahfouzet al.[9],whodiscovered a substantial difference in the length of diabetes between DM and DM-CKD. On the other hand, Ochodnicky 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 hemodynamicalterations.AccordingtoAnderset al. [13], hyperglycemia reduces the amount of sodiumthatisexposedatthemaculadensa. This, in turn, causes glomerular hyperfiltration, dilates the afferent arteriole, suppresses tubuloglomerularfeedback,andcausespodocyte 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 valuesthancontrols[14].WhileAlnaggaretal. [15] andGuptaetal. [7] discoveredthat FBSand 2 h PP were considerably greater in T2DM with microalbuminuria compared withnormoalbuminuria 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- αtriggers the processes that lead to both cell deathandsurvival.WhenTNF-αbindstoTNF-α 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] andDoghishet al.[6].Intermsof 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 significantlydifferbetweenpatientgroups,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 notdiffer significantly. Under diabetic conditions, dyslipidemia increases the expression of extracellular matrix and activates macrophagesin the glomeruli, which results in DN. Dyslipidemiais observedindiabetic patientswith 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 ≥83.5, TNF-αhad 96.7% sensitivity and 79.7% specificity for diagnosing DN TNF-αconcentration were considerably greater in both patient groups than in the control group, and DM-CKD was higher thanDM.IntheDM-CKDgroup,TNF-αlevelhad 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-αwas 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-α is a pleiotropic cytokine that is essential for mediating inflammatory processes that are linked to glomerular and tubulointerstitial damage.

5. CONCLUSIONANDRECOMMENDA-TIONS

The study found that patients with DM and DM-CKD had considerably higher serum levels of TNF-α.This suggests that TNF-αmay 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-αand the pathogenesis because to the intricacy of DN processes.

DISCLAIMER(ARTIFICIALINTELLIGENCE)

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.

COMPETINGINTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Martinez LC, Sherling D, Holley A. The screening and prevention of diabetes mellitus. Prim Care Clin Off Pract. 2019; 46(1):41–52.
   Available:http://dx.doi.org/10.1016/j.pop.2018.10.006
2. Kitagawa N, Kitagawa N, Ushigome E, Ushigome H, Yokota I, Hamaguchi M, etal. Impact of isolated high home systolic blood pressure on diabetic nephropathy in patients with type 2 diabetes mellitus: A 5-year prospective cohort study. Research Square Platform LLC; 2020. Available: http://dx.doi.org/10.21203/rs.3.rs-66901/v1
3. Holbrook J, Lara-Reyna S, Jarosz-Griffiths H,McDermottM.Tumournecrosisfactor signalling in health and disease. F1000Research[Internet].2019;8:F1000 Faculty Rev-111.
   Available:https://pubmed.ncbi.nlm.nih.gov/30755793
4. White JJ, Mohamed R, Jayakumar C, Ramesh G. Tubular injury marker netrin-1 is elevated early in experimental diabetes. J Nephrol. 2013/08/07. 2013;26(6):1055–64.
   Available:https://pubmed.ncbi.nlm.nih.gov/24052471
5. Kushwaha K, Sharma S, Gupta J. Metabolic memory and diabetic nephropathy: Beneficial effects of natural epigenetic modifiers. Biochimie. 2020;170: 140–51.
   Available:http://dx.doi.org/10.1016/j.biochi.2020.01.007
6. Doghish AS, Bassyouni AA, Mahfouz MH, Abd El-Aziz HG, Zakaria RY. Plasma endoglin in Type2 diabetic patients with nephropathy. Diabetes & MetabSyndr Clin Res & Rev [Internet]. 2019;13(1):764–8.
   Available:http://dx.doi.org/10.1016/j.dsx.2018.11.058
7. GuptaS,MehndirattaM,KalraS,Kalra OP, Shukla R, Gambhir JK. Association of TumorNecrosisFactor(TNF)promoter polymorphismswithplasmaTNF-αlevels andsusceptibilitytodiabeticnephropathy inNorthIndianpopulation.JDiabetes Complications. 2015;29(3):338–42.
   Available:http://dx.doi.org/10.1016/j.jdiacomp.2015.01.002
8. Maric-Bilkan C. Obesity and diabetickidney disease. Med Clin North Am [. 2012/11/27. 2013;97(1):59–74.

Available:https://pubmed.ncbi.nlm.nih.gov/23290730

9. MahfouzMH,AssiriAM,MukhtarMH. Assessment of Neutrophil Gelatinase-AssociatedLipocalin(NGAL)andRetinol-BindingProtein4(RBP4)inType2Diabetic Patients with Nephropathy. BiomarkInsights[Internet].2016;11:31–40. Available:https://pubmed.ncbi.nlm.nih.gov/26917947
10. Ochodnicky P, Lattenist L, Ahdi M, Kers J, Uil M, Claessen N, et al. Increased Circulating and Urinary Levels of Soluble TAM Receptors in Diabetic Nephropathy. Am J Pathol [Internet]. 2017;187(9):1971–83. Available:http://dx.doi.org/10.1016/j.ajpath.2017.05.004
11. MotawiTK,ShehataNI,ElNokeetyMM,El-Emady YF. Potential serum biomarkers for earlydetectionofdiabeticnephropathy. Diabetes Res Clin Pract. 2018;136:150–8. Available:http://dx.doi.org/10.1016/j.diabres.2017.12.007
12. Gallagher H, Suckling RJ. Diabetic nephropathy: where are we on the journey from pathophysiology to treatment? Diabetes, Obes Metab. 2016;18(7):641–7. Available:http://dx.doi.org/10.1111/dom.12630
13. Anders H-J, Davis JM, Thurau K. Nephron Protection in Diabetic Kidney Disease. N Engl J Med [Internet]. 2016;375(21):2096–8. Available:http://dx.doi.org/10.1056/nejmcibr1608564
14. El-Edel RH, Fathy WM, Abou-Elela DH, Emara MM, El-Khair NTA. Role of tumor necrosisfactoralphaintype2diabetic nephropathy. Menoufia Med J. 2020;33(3):920.
15. Alnaggar ARLR, Sayed M, El-deena KE, Gomaa M, Hamed Y. Evaluation of serum adiponectin levels in diabetic nephropathy. Diabetes & Metab Syndr Clin Res & Rev. 2019;13(1):128–31. Available:http://dx.doi.org/10.1016/j.dsx.2018.04.035
16. Saulnier-Blache J-S, Feigerlova E, Halimi JM, Gourdy P, Roussel R, Guerci B, et al. Urinary lysophopholipids are increased in diabetic patients with nephropathy. J Diabetes Complications [Internet]. 2017;31(7):1103–8. Available:http://dx.doi.org/10.1016/j.jdiacomp.2017.04.024
17. Bedard K, Krause K-H.TheNOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. Physiol Rev. 2007;87(1):245–313. Available:http://dx.doi.org/10.1152/physrev.00044.2005
18. Dabhi B, Mistry KN. Oxidative stress anditsassociationwithTNF-α-308G/CandIL-1α-889 C/T gene polymorphisms in patientswithdiabetesanddiabetic nephropathy. Gene. 2015;562(2): 197–202. Available:http://dx.doi.org/10.1016/j.gene.2015.02.069
19. ChenY,QiaoY,XuY,LingW,PanY, Huang Y, et al. Serum TNF-α concentrationsintype2diabetesmellitus patientsanddiabeticnephropathypatients: Asystematicreviewandmeta-analysis. Immunol Lett. 2017;186:52–8. Available:http://dx.doi.org/10.1016/j.imlet.2017.04.003