Original Research Article

The Role of Netrin-1 and Interleukin-6 in Diabetic Nephropathy in Patients with Type 2 Diabetes Mellitus

Abstract

Background: Netrin-1 is a laminin like protein highly induced after acute and chronic kidney injury, represent tubular damage and excreted in urine of both animals and humans. Netrin-1 is a potential biomarker predicting the development of diabetic kidney disease. Interleukin-6 (IL-6) is an inflammatory cytokine that has a role in the transformation from acute to chronic inflammation. The aim of this work was to evaluate the role of Netrin-1 and IL-6 in the development of diabetic nephropathy (DN) in patients with type 2 diabetes mellitus (T2DM).

Methods: Our study included 75 patients with T2DM and 25 healthy control group. The study duration started from October 2019 to January 2021. Participants were subdivided into four equal groups: group I: 25 healthy subjects as control group, group II: 25 diabetic patients with albumin/creatinine ratio < 30mg/g, group III: 25 diabetic patients with albumin /creatinine ratio 30-300mg/g, group IV: 25 diabetic patients with albumin/creatinine ratio > 300mg/g. All subjects underwent complete clinical examination, laboratory investigations and measurement of serum Netrin-1 and IL-6 by ELISA.

Results: Nertin-1 was significantly higher in group III and group IV than group II and control group ($P<0.001^{*}$). Netrin-1 was positively correlated with IL-6, fasting BG, 2HPP, HbA1C, B.urea, S.creatinine and urinary ACR, but it was negatively correlated with eGFR, Hb and S. albumin. IL-6 was significantly higher in group IV than group III, group II and control group (p<0.001*). There was a positive correlation between IL-6 and 2HPP, HbA1C, B.urea, S.creatinine and urinary ACR, but there was a negative correlation between IL-6 and

eGFR, Hb and S. albumin. Netrin-1 was more sensitive 96.5% and more specific 99.8% than IL-6 sensitivity 83.3% and specificity 85.0%.

Conclusions: Netrin-1 and IL-6 were significantly higher in diabetic nephropathy patients with macroalbuminuria than other groups. Netrin-1 was more sensitive and specific than IL-6 in predicting DN and its progression.

Keywords: Netrin-1, Interleukin-6, Diabetic Nephropathy, Diabetes Mellitus

Introduction:

Diabetes mellitus (DM) is a chronic metabolic syndrome of persistant hyperglycemia which may be due to absolute or relative insulin deficiency, resistance to peripheral actions of insulin or both[1].

It classified to type 1 DM, type 2 DM, gestational diabetes and other specific types. T2DM is the commonest form, that occur either due to impaired insulin secretion, insulin resistance or both. Strict glycemic control in patients with diabetes decreases the incidence of diabetic complications [2].

Diabetic patients are highly susceptible to various complications, short-term and long-term complications. Short term complications as diabetic ketoacidosis, hyperosmolar hyperglycemic state and hypoglycemia. Long term micro-angiopathic complications as (nephropathy, retinopathy and neuropathy) and macro-angiopathic complications as cardiovascular diseases(coronary heart disease, stroke, peripheral artery disease and heart failure) [3, 4].

Diabetic nephropathy (DN) is a common complication that is considered a major cause of end stage renal disease (ESRD) worldwide. Early diagnosis and treatment of DM may prevent progression to ESRD. Glomerular and tubular damage can occur even before microalbuminuria [5].

Netrin-1 is a laminin like protein that highly induced after acute and chronic kidney injury, represent tubular damage and excreted in urine of both animals and humans [6].

Netrin-1 is a potential biomarker predicting the development and progression of microvascular complications of DM [7, 8].

IL-6 is an inflammatory cytokine that has a role in the transformation from acute to chronic inflammation. Some studies show that IL-6 accelerates chronic inflammation and development of micro-angiopathic complications of DM[9].The aim of this work was to assess

the role of Netrin-1 and IL-6 in the development of diabetic nephropathy in patients with type 2 diabetes mellitus.

Patients and Methods:

This cross-sectional study was conducted on 75 patients with T2DM with and without nephropathy and 25 healthy subjects as a control group. The patients were recruited from the outpatient clinic and ward of Endocrinology unit of the Internal Medicine Department, Tanta University Hospital.

The study was done after being approved from the institutional Ethical Committee, Tanta University Hospital. An informed written consent was obtained from all included subjects.

Exclusion criteria: patients with severe uncontrolled hypertension, presence of acute infection, chronic inflammatory diseases, pregnancy and lactation, presence of malignancy, patients with chronic liver diseases, patients of type 1 DM, history of kidney diseases before onset of DM, estimated glomerular filtration rate <15 ml/min/1.73m^2

Participants were subdivided into four equal groups: Group I: 25 healthy subjects as control group, group II: 25 diabetic patients with albumin/creatinine ratio < 30mg/g, group III: 25 diabetic patients with albumin/creatinine ratio 30-300mg/g, group IV: 25 diabetic patients with albumin/creatinine ratio > 300mg/g.

All patients were subjected to: Full history taking, complete clinical examination, routine laboratory investigations, Pelvi-abdominal U/S and ECG, measurement of serum Netrin-1 and Interleukin-6 by ELISA.

Sample collection & storage:

Seven and half milliliters (7.5mL) of freshly blood were withdrawn from each subject under complete aseptic precaution; placed into sterile two K3 EDTA vacutainers tube and one clot activator tube. One EDTA vacutainers used for CBC measurement and hemoglobin A1C estimation and the other one was stored at -20 ℃ for Netrin-1 and IL-6 measurements by

ELISA. Serum was then separated by centrifugation at 1000 x g for 15 minutes for immediate assay of routine lab investigations. Frozen samples were allowed to thaw and brought to room temperature only before analysis. Hemolysed samples were discarded, repeated freezing and thawing was avoided.

Human Netrin-1 by ELISA:

The test used a double-antibody sandwich enzyme-linked immunosorbant assay (ELISA) to assay the level of human Netrin-1in samples. Ntn1 was added to monoclonal antibody enzyme well which was precoated with human Ntn1 monoclonal antibody, incubation, then added Ntn1 antibodies labeled with biotin, and combined with streptavidin-HRP to form immune complex; then carried out incubation and washing again to remove the uncombined enzyme. Then added Chromagen solution A, B, the color of the liquid was changed into the blue, and at the effect of acid, the color finally became yellow. The chroma of color and the concentration of the human substrate Ntn1 of sample were positively correlated.

Human IL-6 by ELISA:

The test used a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of human Interleukin-6 (IL-6) in samples. IL-6 was added to monoclonal antibody enzyme well which was precoated with human IL-6 monoclonal antibody, incubation, then added IL-6 antibodies labeled with biotin, and combined with streptavidin-HRP to form immune complex; then carried out incubation and washing again to remove the uncombined enzyme. Then added Chromagen solution A, B, the color of the liquid was changed into the blue, and at the effect of acid, the color finally became yellow. The chroma of color and the concentration of the human substrate IL-6 of sample were positively correlated.

Statistical analysis was done by SPSS v 20 (IBM Inc., Chicago, IL, USA). Quantitative variables were presented as mean and standard deviation (SD) and compared between the two

groups utilizing ANOVA (F) test. Qualitative variables were presented as frequency and percentage (%) and were analysed utilizing the Chi-square test. Correlations among variables were assessed using the correlation coefficient of pearson. The ROC curve were performed to test sensitivity and specificity. P value < 0.05 was considered statistically significant.

Results:

Regarding to demographic data (age, sex and BMI) there was no significant statistical difference between the studied groups (Table 1).

SBP and DBP were significantly higher in group IV with macroalbuminuria than group III, II and control group ($p < 0.001$). Hb and GFR in Group IV was statistically significantly lower than in group III, II, and control group ($p < 0.001$). FBG, 2HPP, HbA1C, urea, creatinine levels and Urinary ACR in Group IV were significantly higher than in group III, II, and control group ($p<0.001$). Serum albumin was significantly lower in diabetic group with advanced nephropathy than control group ($p<0.001$)(Table 2).

Nertin-1 was significantly higher in group III and IV than group II and control group ($P<0.001$*). IL-6 was statistically significantly higher in group IV (macroalbuminuria) than group III, II, and control group ($p<0.001$*)(Table 3).

There was a highly significant positive correlation between Netrin-1 and the following parameters: IL-6, fasting BG, 2HPP, HbA1C, B. urea, S.creatinine and urinary ACR. There was a highly significant negative correlation between Netrin-1 and eGFR, Hb and S.albumin. There was a positive correlation between IL-6 and the following parameters; (highly significant with HbA1C, B.urea, S.creatinine and urinary ACR) and (significant with 2HPP). There was a highly significant negative correlation between IL-6 and eGFR & Hb, and a significant negative correlation with S.albumin(Table 4).

ROC curve analysis of Netrin-1 in macroalbuminuria group with area under the curve was 0.931 at cutoff >1030.5 with sensitivity 96.5% and specificity 99.8%. ROC curve analysis of

IL-6 in macroalbuminuria group with area under the curve was 0.904 at cutoff >90.9 with sensitivity 83.3% and specificity 85.0%. (Figure 1)

Table 1: Demographic data of studied groups

		Group I (n=25)	Group II (n=25)	Group III (n=25)	Group IV (n=25)	P-value
Sex	**Female**	14 (56.0%)	12 (48.0%)	12 (48.0%)	12 (48.0%)	--
	Male	11 (44.0%)	13 (52.0%)	13 (52.0%)	13 (52.0%)	0.92
Age (years)		48.32 ± 8.21	48.16 ± 8.97	48.92 ± 8.25	52.80 ± 7.38	0.157
BMI (kg/m^2)		25.6 ± 6.23	27.04 ± 5.35	26.16 ± 6.18	25.30 ± 6.42	0.758

Data are presented as mean ± SD or frequency (%). BMI: Body mass index

Table 2: Comparison between studied groups according to SBP, DBP, CBC, FBG, 2HPP, HbA1C, ALT, AST, S. albumin, lipid profile and kidney function tests

	Group I	Group II	Group III	Group IV	P
SBP (mmHg)	122.4 ± 9.69	125.2 ± 10.04	130.8 ± 12.22	150 ± 12.90	0.001*
DBP (mmHg)	76.8 ± 6.27	79.20 ± 7.59	81.2 ± 6.65	88.40 ± 6.87	0.001*
Hb(gm/dl)	11.59± 1.0	10.44 ± 0.71	9.70 ± 0.68	9.10 ± 0.90	0.001*
WBCs (10^3)	6.87 ± 2.10	8.12 ± 2.35	7.10 ± 2.23	7.50 ± 2.25	0.303
Platelets (10^3)	284.6 ± 74.3	284.28 ± 55.27	235.88 ± 56.59	266.12± 81.3	0.161
FBG (mg/dl)	94.8 ± 14.11	128.56 ±41.41	131.0 ± 26.38	142.84 ± 33.44	0.001*
2HPP (mg/dl)	149.76 45.63	213.84 ±68.79	248.72 ± 47.9	265.52 ± 61.28	0.001*
HbA1C (%)	4.63 ± 0.52	6.42 ± 0.31	7.55 ± 0.62	8.27 ± 0.68	0.001*
ALT(U/L)	25.48 ± 3.34	21.16 ± 6.99	24.28 ± 6.22	23.36 ± 6.35	0.073
AST(U/L)	18.92 ± 3.56	19.96 ± 6.11	20.76 ± 5.98	20.88 ± 6.79	0.624
Albumin(g/dl)	4.26 ± 0.39	3.26 ± 0.47	3.25 ± 0.42	3.04 ± 0.38	0.001*

Total cholesterol (mg/dl)	183.28 ±27.6	188.12 ± 30.07	186.84 ± 26.67	192.44 ± 26.7	0.808
LDL (mg/dl)	118.48 ±16.8	123.28 ± 21.5	121.48 ± 23.2	128.0 ± 18.52	0.532
HDL (mg/dl)	50.32±8.65	51.52 ± 7.74	50.92 ±8.24	50.24 ± 8.47	0.942
TGs (mg/dl)	142.96 ±23.2	144.36 ± 20.0	145.24 ± 22.3	149.84 ± 23.0	0.650
Urea(mg/dl)	22.30 ± 7.75	27.94 ± 5.52	91.36 ± 30.52	116.04 ± 38.9	0.001*
Creatinine(mg/dl)	0.73 ± 0.15	0.80 ± 0.19	2.10 ± 0.48	3.14 ± 1.08	0.001*
e GFR (ml/min/1.73m2)	84.90 ± 17.1	82.0 ± 15.23	70.02 ± 11.88	29.82 ± 7.67	0.001*
Urinary ACR (mg/g)	12.0 ± 1.58	23.40 ± 5.2	120.44 ± 38.6	440.36 ± 111.4	0.001*

Data are presented as mean ± SD, * significant as P value < 0.05. SBP: Systolic blood pressure, DBP: Diastolic blood pressure, Hb: Haemoglobin, WBSs: White blood cells, FBG: Fasting blood glucose, 2HPP: Two- hour postprandial plasma glucose, HbA1C: Hemoglobin A1c, ALT: Alanine transaminase, AST: Aspartate aminotransferase, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TGs: Triglycerides, e GFR: Estimated glomerular filtration rate, ACR: Albumin to creatinine ratio.

Table 3: Comparison between studied groups according to Nertin-1 and Il-6

	Group I	**Group II**	**Group III**	**Group IV**	**P**
Netrin_1	600.12 ± 131.6	770.51 ± 36.7	979.85 ± 41.34	1604.83 ± 516.6	0.001*
P_0		0.084	0.001*	0.001*	
$P_1<0.046^*$, $p_2<0.001^*$, $p_3<0.001^*$					
IL-6 (pg/ml)	32.24 ± 21.6	33.43 ± 12.63	36.22 ± 27.63	215.48 ± 139	0.001*
P_0		0.813	0.328	0.001*	
$P_1<0.225$, $p_2<0.001^*$, $p_3<0.001^*$					

Data are presented as mean ± SD, * significant as P value < 0.05. IL-6: Interleukin-6.

Table 4: Correlation between Netrin-1 and IL-6 with different parameters in total patient's groups

	Netrin-1		IL-6	
	R	**P**	**R**	**P**
IL-6	0.563**	<0.001**	1	
BMI	0.096	0.334	0.049	0.629
Hb	-0.381**	<0.001**	-0.359**	<0.001**
WBCS	0.061	0.544	0.147	0.145
Platelets	-0.068	0.502	-0.007	0.948
Fasting BG	0.356**	<0.001**	0.121	0.230
2H postprandial	0.456**	<0.001**	0.243*	0.015*
HbA1C	0.563**	<0.001**	0.400**	<0.001**
S. Albumin	-0.398**	<0.001**	-0.209*	0.037*
B. Urea	0.417**	<0.001**	0.457**	<0.001**
S. Creatinine	0.538**	<0.001**	0.570**	<0.001**
eGFR	-0.531**	<0.001**	-0.462**	<0.001**
Urinary ACR	0.658**	<0.001**	0.682**	<0.001**
Total cholesterol	0.151	0.133	0.127	0.207
LDL	0.121	0.229	0.089	0.381
HDL	-0.113	0.261	-0.142	0.158
TG	0.174	0.084	0.127	0.209

*Significant correlation, ** Highly significant correlation, *P statistically significant < 0.05, ** P highly significant < 0.001.

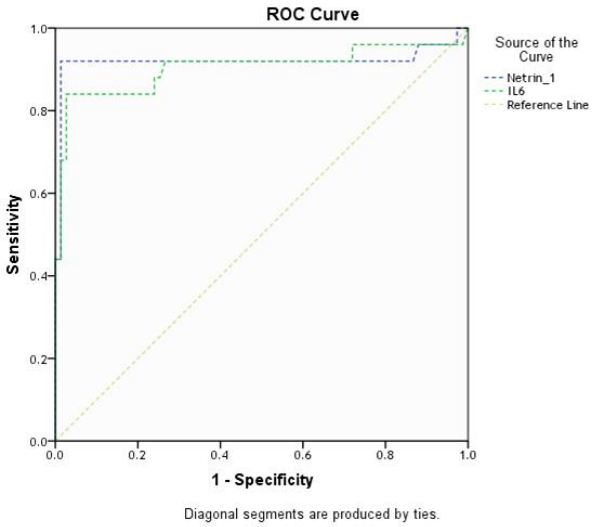

Figure 1: ROC Curve for detection of Netrin-1 and IL-6 cutoff regard macroalbuminuria group (IV)

Discussion

Diabetic nephropathy is one of the most common microvascular complications of DM that leads to ESRD. Early diagnosis and management of T2DM is of critical importance to delay its complications. Thus, reliable biomarkers are needed for early diagnosis of these complications [10].

Chronic inflammatory process participates in the development of microvascular complications of diabetes. IL-6 is an inflammatory cytokine that has a role in the transformation from acute to chronic inflammation [11]. Netrin-1 is highly induced after acute and/or chronic kidney injury and excreted in urine in both human and animal experiments[12,13].

The current study showed that, SBP and DBP were significantly higher in group IV (with macroalbuminuria) than group III, II and control group ($p<0.001$).

This coincided with, Liu et al.[12] study which found that with increasing albuminuria, T2DM patients had higher SBP than control group.

Regarding to FBG, 2HPP and HbA1C in the current study, group IV (with macroalbuminuria) was significantly higher than group III, II, and control group ($p<0.001$).

In agreement with our results, Mohammed et al.[14] documented that there was a statistically significant increase in FBG & HbA1C in diabetic group with macroalbuminuria when compared to other groups ($p<0.001$).

In our study, there was no statistical significant difference between studied groups regarding ALT and AST (p: 0.073, 0.624) respectively, but S.albumin was significantly lower in diabetic groups with advanced nephropathy than control group ($p<0.001$) and this agreed with Liu et al.[12] who found that T2DM patients with macroalbuminuria had lower levels of S. albumin in comparison to control group.

In the current study, Total cholesterol, LDL, HDL and TGs showed no statistically significant difference between studied groups (p: 0.808, 0.532, 0.942 and 0.650) respectively.

The present study showed that, group IV (macroalbuminuria) was significantly higher regarding B. urea, S. creatinine and urinary ACR than group III, II and control group ($p<0.001$), but as regard eGFR, group IV was significantly lower than group III, II, and control group ($p<0.001$).

This coincided with Liu et al.[12]& Mohammed et al.[14] that found T2DM patients with macroalbuminuria had lower levels of eGFR than other groups.

In accordance with our study Lin et al.[15] reported that there was a significant increase in urinary ACR in diabetic microalbuminuric group than normoalbuminuric and control group this elevation was due to thickening of the glomerular basement membrane[15] .

Regarding to serum Netrin-1, our study showed that mean serum Netrin-1 in control group was 600.12±131.6 pg/ml, group II was 770.51±36.7 pg/ml, group III was 979.85±41.34 pg/ml and group IV was 1604.83±516.6 pg/ml so, Nertin-1 was significantly higher in group III and IV than group II and control group ($P<0.001$).

Our results coincided with, Liu et al.[12] who found that serum Netrin-1 was significantly higher in diabetic patients with macroalbuminuria than diabetic patients with normoalbuminuria (P<0.001).

On the other hand, Nedeva et al.[16] study involved 163 subjects, who were divided into four groups: obesity without dysglycemia, prediabetes, diabetes, and healthy control, showed that serum Netrin-1 levels were lower in individuals with obesity alone, as well as in those with prediabetes and T2DM than in the healthy control.

In the present study, Mean IL-6 in group II was 33.43±12.63 pg/ml, group III was 36.22±27.63 pg/ml and group IV was 215.48±139 pg/ml, while in control group it was 32.24±21.6 pg/ml. So, group IV (macroalbuminuria) was significantly higher than group III, II, and control group (p<0.001). Furthermore, there was a positive correlation between IL-6 and the following parameters; (highly significant with HbA1C, B.urea, S.creatinine and urinary ACR) and (significant with 2HPP), but there was a highly significant negative correlation between IL-6 and eGFR & Hb, and a significant negative correlation with S. albumin.

This coincided with Liu et al.[12] study in which patients with macroalbuminuria had higher IL-6 than other groups (p<0.05).

DKD patients showed an elevated serum level of inflammatory cytokines, including IL-6, which positively correlated with the extent of proteinuria [11].

In our study, there was a highly significant positive correlation between Netrin-1 and IL-6, FBG, 2HPP, HbA1C, B.urea, S.creatinine and urinary ACR, but there was a highly significant negative correlation between Netrin-1 and eGFR, Hb and S.albumin.

This was in agreement with Shalaby et al. [18] study which found Netrin-1 had a highly significant positive correlation with FBG, 2 HPP, HbA1C and BMI (p< 0.001).

Furthermore, in our study, Netrin-1 level in macroalbuminuria group had optimal cutoff value >1030.5 with area under the ROC curve at 0.931. Netrin-1 had sensitivity 96.5% and specificity 99.8% and statistically high significant difference between the studied groups ($p<0.001$). While IL-6 had optimal cutoff value >90.9 with area under the ROC curve at 0.904. IL-6 had sensitivity 83.3% and specificity 85.0% and statistically high significant difference between the studied groups ($p<0.001$).

So, Netrin-1 was more sensitive and specific in predicting DKD than IL-6, which support considering Netrin-1 as a sensitive and early indicator of inflammation and progression of disease process.

Conclusions:

Netrin-1 was significantly higher in diabetic patients with macroalbuminuria and microalbuminuria than normoalbuminuric and control groups. IL-6 was significantly higher in diabetic patients with macroalbuminuria than other groups. Netrin-1 was more sensitive and specific in predicting DKD than IL-6. Netrin-1 can be used as a sensitive and early indicator of inflammation and progression of disease process.

Consent and Ethical approval: As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors. The Ethical Committee of the Faculty of Medicine of Tanta University approval this trial.

References:

1. Goyal R, Jialal I. Diabetes Mellitus Type 2. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC.; 2022. p. 1-9.

2. Patil PD, Mahajan UB, Patil KR, Chaudhari S, Patil CR, Agrawal YO, et al. Past and current perspective on new therapeutic targets for Type-II diabetes. Drug Des Devel Ther. 2017;11:1567-83.

3. Thompson AM, Linnebur SA, Vande Griend JP, Saseen JJ. Glycemic targets and medication limitations for type 2 diabetes mellitus in the older adult. Consult Pharm. 2014;29:110-23.

4. Toque HA, Fernandez-Flores A, Mohamed R, Caldwell RB, Ramesh G, Caldwell RW. Netrin-1 is a novel regulator of vascular endothelial function in diabetes. PLoS One. 2017;12:18-34.

5. Amsellem S, Gburek J, Hamard G, Nielsen R, Willnow TE, Devuyst O, et al. Cubilin is essential for albumin reabsorption in the renal proximal tubule. J Am Soc Nephrol. 2010;21:1859-67.

6. Brian Reeves W, Kwon O, Ramesh G. Netrin-1 and kidney injury. II. Netrin-1 is an early biomarker of acute kidney injury. American Journal of Physiology-Renal Physiology. 2008;294:F731-F8.

7. Fadel MM, Abdel Ghaffar FR, Zwain SK, Ibrahim HM, Badr EA. Serum netrin and VCAM-1 as biomarker for Egyptian patients with type II diabetes mellitus. Biochem Biophys Rep. 2021;27:10-45.

8. Jayakumar C, Nauta FL, Bakker SJ, Bilo H, Gansevoort RT, Johnson MH, et al. Netrin-1, a urinary proximal tubular injury marker, is elevated early in the time course of human diabetes. J Nephrol. 2014;27:151-7.

9. Jialal I, Kaur H. The Role of Toll-Like Receptors in Diabetes-Induced Inflammation: Implications for Vascular Complications. Curr Diab Rep. 2012:11-9.

10. Yim J, Kim G, Lee BW, Kang ES, Cha BS, Kim JH, et al. Relationship Between Circulating Netrin-1 Concentration, Impaired Fasting Glucose, and Newly Diagnosed Type 2 Diabetes. Front Endocrinol (Lausanne). 2018;9:6-9.

11. Navarro JF, Milena FJ, Mora C, León C, García J. Renal pro-inflammatory cytokine gene expression in diabetic nephropathy: effect of angiotensin-converting enzyme inhibition and pentoxifylline administration. Am J Nephrol. 2006;26:562-70.

12. Liu C, Li Q, Feng X, Li Q. Elevated levels of netrin-1 in the serum of patients with diabetic nephropathy: relationship with renal function and inflammation. Eur J Inflamm. 2018;16:20-8.

13. Ay E, Marakoğlu K, Kizmaz M, Ünlü A. Evaluation of Netrin-1 Levels and Albuminuria in Patients With Diabetes. J Clin Lab Anal. 2016;30:972-7.

14. Mohammed EM, El-Messallamy FA, Mazroue AM, Elsayed IA, Zidan AA. Urinary Netrin-1 as an Early Marker for Diabetic Nephropathy. Egypt J Hosp Med. 2020;81:1924-30.

15. Lin YC, Chang YH, Yang SY, Wu KD, Chu TS. Update of pathophysiology and management of diabetic kidney disease. J Formos Med Assoc. 2018;117:662-75.

16. Nedeva I, Gateva A, Assyov Y, Karamfilova V, Velikova T, Kamenov Z. Relationship between circulating netrin-1 levels, obesity, prediabetes and newly diagnosed type 2 diabetes. Arch Physiol Biochem. 2020:1-6.

17. Rehman K, Akash MSH, Liaqat A, Kamal S, Qadir MI, Rasul A. Role of Interleukin-6 in Development of Insulin Resistance and Type 2 Diabetes Mellitus. Crit Rev Eukaryot Gene Expr. 2017;27:229-36.

18. Shalaby A, El Mancy IM. Study of serum Netrin-1 level in prediabetics and type 2 diabetic Egyptian patients. AIMJ. 2021;2:17-22.