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Relationship between Circulating Netrin-1 Concentration and Type 2 Diabetes Mellitus

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Abstract

Diabetes mellitus is one of the most common non-communicable diseases of the current era. The burden of this disease is immense owing to transition in lifestyle and dietary habits, ageing of the population and urbanization in the setting of a genetically predisposed environment. Aim of the Work: The aim of current study was to study the plasma netrin 1 level in type II diabetes mellitus patients with and without proteinuria to elucidate the role of netrin-1 in the pathogenesis of type 2 diabetes and expand its potential for diagnosis. A case control study was conducted on (54) subjects including 26 male and 28 female they were classified into 2 groups as follows: Patient group: include 34 patients with type 2 diabetes mellitus based on Hb A1c 6.5 % according to ADA 2020 diagnostic criteria for diabetes. Control group: include 20 age and sex matched healthy control subjects. Patients were subdivided into 2 subgroups: Subgroup(A): includes 17 patients with type 2 diabetes mellitus without proteinuria. Subgroup(B): includes 17 patients with type 2 diabetes mellitus with proteinuria. This study showed that there was a high statistically significant increase in serum netrin 1 in patient group compared to control group. The study revealed no statistically significant correlation between serum netrin 1 and other studied parameters in patient group. Our study revealed that there was a high statistically significant increase in serum ALT, AST and albumin creatinine ratio in patient group than control group, also, there was a high statistically significant increase in lipid profile including serum cholesterol, triglycerides and LDL in patient group than control group & a high significant decrease in HDL in patient group than control group. The study showed that there was a high statistically significant increase in fasting blood sugar (FBS), 2 hours post prandial blood sugar (2hppbs), HbA1C, fasting insulin & HOMA IR in patient group than control group. This study showed that, there was a statistically significant increase in disease duration in subgroup B compared to subgroup A. There was a high statistically significant increase in serum netrin 1 in subgroup B than other studied groups. There was a non - significant increase in serum netrin 1 in subgroup B compared to subgroup A, a high significant increase in serum netrin 1 in subgroup A compared to control group and a high significant

increase in serum netrin 1 in subgroup B in comparison to control group. Conclusion: From the results of the current study, we concluded that plasma level of Netrin 1 is significantly higher in patients with type 2 diabetes mellitus than control group, there was a non-significant increase of serum netrin 1 in diabetic patients with proteinuria in comparison to type 2 DM patients without proteinuria. This could focus the light on the importance of Netrin 1 on the pathogenesis of nephropathy in diabetes.

Keywords: Circulating; Netrin-1; Type 2 Diabetes Mellitus.

1. Introduction

Diabetes mellitus is one of the most common non communicable diseases of current era [1]. The burden of this disease is immense owing to transition in lifestyle and dietary habits, ageing of the population and urbanization in the setting of a genetically predisposed environment [2].

Netrin-1 is a multifunctional diffusible protein that plays an active role in regulating angiogenesis, cell migration and inflammation [3]. After initial research that focused on its functions in axon patterning, Netrin-1 was later implicated in development of pancreas [4]. Many studies proposed a protective role of Netrin-1 against pancreatitis [5] and islet β-cell apoptosis [6]. A positive impact of Netrin-1 application was also implicated in diabetic nephropathy [7] diabetic retinopathy [8] and diabetes-associated myocardial infarction [9]. Diabetic nephropathy is a common and often devastating complication of type 2 diabetes and is associated with increased cardiovascular (CV) mortality and a reduction in quality of life [10]. Previous studies demonstrated that netrin-1 signaling dampens hypoxia-induced inflammation of different organs, including the kidneys, the intestine, and the lungs [11]. Also, they showed a functional role for netrin-1 in acute renal disease models. Netrin-1 is highly expressed in the kidneys and functions to attenuate ischemic AKI [12]. The aim of our work was to study the plasma netrin 1 level in type II diabetes mellitus patients with and without proteinuria to elucidate the role of netrin-1 in the pathogenesis of type 2 diabetes and expand its potential for diagnosis.

2. Patients and Methods

A case control study was conducted on (54) subjects including 26 male and 28 female they were classified into 2 groups as follows:

2.1 Patient group

This group included 34 patients with type 2 diabetes mellitus based on Hb A1c 6.5 % according to ADA 2020 diagnostic criteria for diabetes including 17 male and 17 female their age ranged from 37 to 78 years with Mean ± SD (53.24 ± 11.76) & their BMI ranged between (26.5-36) with Mean ± SD (31.03 ± 2.80).

2.2 Patients were subdivided into 2 subgroups

Subgroup(A): This subgroup includes 17 patients with type 2 diabetes mellitus without proteinuria they were 7 males and 10 females their age ranged between (37-71) years with Mean ± SD (48.24 ± 9.83) & their BMI ranged between 26.5-36 with Mean ± SD (31.22 ± 2.97), **subgroup(B) :**This subgroup includes 17 patients with type 2 diabetes mellitus with proteinuria they were 10 males and 7 females their age range between (42-78) years with Mean ± SD (58.24 ± 11.64) & their BMI ranged between (26.5-37.5) with Mean ± SD (30.84 ± 2.68), **control group :**They include 20 age and sex matched healthy

control subjects, 9 males and 11females. Their age ranged between (27-64) years with Mean ± SD (45.60 ± 10.60) & their BMI ranged between (19-27) with Mean ± SD (22.58 ± 2.21).

2.3 Patients selection

All patients are selected from the Internal Medicine and Endocrine departments Al-Zahraa University Hospital. All selected individuals were enrolled in the study after informed consent has been obtained during the period from September 2020 to May 2021. All procedures performed in the study were in accordance with the ethical standards of the Faculty of Medicine for girls, Al-Azhar University Research committee and the 1964 Helsinki Declaration and its later amendments or ethical standards.

2.4 Exclusion criteria:

Subjects with current renal or hepatic disease, malignancy, diabetes other than type 2 diabetes mellitus, any acute inflammation or infection and current significant cardiovascular disease

2.5 All patients were subjected to:

Full and detailed history taking, complete clinical examination, BMI (body mass index) was calculated using the equation: - weight (in kg) / height (in meter)^2, Laboratory investigations in the form of:
Complete blood count, blood urea, serum creatinine, serum sodium and potassium, albumin/creatinine ratio, serum ALT, AST, lipid profile (serum cholesterol, triglycerides, LDL and HDL), fasting and postprandial blood glucose level, glycosylated hemoglobin (HbA1c), fasting insulin level from which insulin resistance (HOMA_ IR) can be calculated HOMA-IR was calculated according to the formula: fasting insulin (micro U/L) x fasting glucose (nmol/L)/22.5 and serum netrin- 1 concentration.

2.6 Methods:

Determination of Netrin 1: Netrin1 is estimated in serum by enzyme linked immunosorbent Assay (ELISA) sandwich technique with (Human Netrin 1(Ntn1) ELISA Kit Catalog NO: SG 11227) with assay range of 8 pg/ml - 400 pg/ml.

2.7 Statistical Analysis:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative variables were presented as mean, standard deviations (SD), range, median and interquartile range (IQR) while qualitative variables were presented as number and percentages. The comparison between two independent groups with quantitative data and parametric distribution was done using an independent t-test. The comparison between two independent groups with quantitative data and non-parametric distribution was done using the Mann-Whitney test. The comparison between more than two independent groups with quantitative data and parametric distribution was done using One Way ANOVA Test. Spearman correlation coefficient test were used to assess the correlation between two quantitative variables in the same group.
The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:
P-value > 0.05: Non-significant (NS)
P-value < 0.05: Significant (S)
P-value < 0.01: Highly significant (HS)

3. Results

As shown in Table .1 there was a high statistically significant increase in serum netrin 1 in the patient group in comparison to the control group. As shown in Table .2 there was 1/ a high statistically significant increase in serum ALT, AST & albumin creatinine ratio in the patient group than the control group, 2/ a high statistically

significant decrease in hematocrit in the patient group than the control group, 3/ a significant increase in serum urea in the patient group compared to the control group, 4/ no statistically significant difference between 2 groups as regard WBCs, RBCs, HB, PLT, Creatinine, Na and K As shown in Table 3 there was a high significant increase in lipid profile including serum cholesterol, triglycerides & LDL cholesterol in patient group compared to control group & a high significant decrease in HDL cholesterol in patient group in comparison to control group. As shown in Table .4 there was a high statistically significant increase in FBS, 2hppbs, HbA1C, F insulin, HOMA IR in patient group than control group. As shown in Table .5 there was a high statistically significant increase in serum netrin 1 in subgroup B than in other studied groups. There was a non -significant increase in serum netrin 1 in subgroup B compared to subgroup A, a high significant increase in serum netrin 1 in subgroup A compared to the control group and a high significant increase in serum netrin 1 in subgroup B in comparison to control group. As shown in Table .6 there was a statistically significant increase in disease duration in subgroup B than subgroup A. As shown in Table .7 there was a non-significant positive correlation between serum netrin 1 and (age, hematocrit, MCV, MCH, PLT, serum creatinine, K, ALT, AST, cholesterol, LDL, HDL, fasting insulin, HOMA IR and c-peptide). There was a non-significant negative correlation between serum netrin 1 and (disease duration, WBCs, RBCs, HB, serum urea, Na, triglycerides, albumin creatinine ratio, FBS,2hppbs, HbA1C).

Table (1): Comparison between patient group and control group as regard serum netrin 1

V		Patients group	Control group	Test value	P-value	Sig.
		No. = 34	No. = 20			
Netrin 1(pg/ml)	Mean ± SD	172.78 ± 24.82	144.71 ± 15.18	4.571•	0.000	HS
	Range	141.35 – 251.35	124.05 – 181.29			

Table (2): Comparison between patient group and control group as regard CBC, serum urea, creatinine, Na, K, ALT, AST and albumin creatinine ratio

		Patients group	Control group	Test value	P-value	Sig.
		No. = 34	No. = 20			
WBC x1000/cmm	Mean ± SD	7.19 ± 2.05	6.64 ± 1.47	1.056	0.296	NS
	Range	3.5 – 13.5	4 – 9.4			
RBC (Mill/cmm)	Mean ± SD	4.52 ± 0.73	4.41 ± 0.62	0.616	0.541	NS
	Range	2.9 – 5.7	3.5 – 5.5			
HB(g/dl)	Mean ± SD	11.78 ± 2.09	12.25 ± 1.12	-0.914	0.365	NS
	Range	8.2 – 15.5	10.6 – 14.1			
HCT (%)	Mean ± SD	34.11 ± 5.40	38.90 ± 3.65	-3.518	0.001	HS
	Range	25 – 46	31 – 45			
	Range	17.5 – 36	24 – 35			
PLT x1000/cmm	Mean ± SD	244.53 ± 68.25	275.70 ± 75.95	-1.554	0.126	NS
	Range	125 – 438	167 – 421			
Urea (mg/dl)	Mean ± SD	34.91 ± 13.41	27.55 ± 7.83	2.236	0.030	S
	Range	17 – 80	15 – 44			
Creat (mg/dl)	Mean ± SD	0.81 ± 0.40	0.68 ± 0.25	1.330	0.189	NS
	Range	0.4 – 1.9	0.3 – 1.1			
Na (mEq/L)	Mean ± SD	138.21 ± 4.80	136.55 ± 4.43	1.259	0.214	NS
	Range	131 – 150	130 – 144			
K (mEq/L)	Mean ± SD	4.43 ± 0.57	4.49 ± 0.56	-0.346	0.731	NS
	Range	3.3 – 5.4	3.6 – 5.4			
ALT(U/L)	Mean ± SD	24.88 ± 7.03	18.95 ± 5.78	3.190	0.002	HS
	Range	14 – 44	7 – 28			
AST(U/L)	Mean ± SD	28.56 ± 9.72	19.65 ± 6.95	3.589	0.001	HS
	Range	16 – 59	8 – 34			
Alb/ Creat ratio	Mean ± SD	130.29 ± 169.10	19.05 ± 9.08	2.925	0.005	HS
	Range	5.5 – 734	5 – 28			

Table (3): Comparison between the patient group and control Group as regard lipid profile.

		Patients group	Control group	Test value	P-value	Sig.
		No. = 34	No. = 20			
Cholesterol (mg/dl)	Mean ± SD	229.32 ± 59.37	126.30 ± 27.37	7.296	0.000	HS
	Range	123 – 340	75 – 178			
TG (mg/dl)	Mean ± SD	177.50 ± 71.17	103.20 ± 27.81	4.458	0.000	HS
	Range	89 – 396	66 – 145			
LDL (mg/dl)	Mean ± SD	145.53 ± 40.77	101.60 ± 22.87	4.416	0.000	HS
	Range	59 – 249	63 – 149			
HDL (mg/dl)	Mean ± SD	46.50 ± 6.82	60.41 ± 14.16	4.111	0.000	HS
	Range	34 – 58	39 – 94			

Table (4): Comparison between patient group and control group as regard FBS, 2hppbs, F insulin, HbA1C and HOMA IR

		Patients group	Control group	Test value	P-value	Sig.
		No. = 34	No. = 20			
FBS (mg/dl)	Mean ± SD	199.03 ± 72.54	95.90 ± 8.77	6.307•	0.000	HS
	Range	101 – 450	78 – 111			
2hppbs (mg/dl)	Mean ± SD	248.79 ± 86.32	120.95 ± 9.90	6.572•	0.000	HS
	Range	144 – 513	107 – 138			
F Insulin (uIU/ml)	Median (IQR)	15.8 (9.06 - 24.5)	6.99 (6.44 - 8)	-3.904‡	0.000	HS
	Range	4.27 – 52.8	5.59 – 12			
HbA1C (%)	Mean ± SD	9.79 ± 2.08	5.17 ± 0.78	9.539•	0.000	HS
	Range	6.5 – 14.6	3.5 – 6.1			
HOMA IR	Median (IQR)	5.69 (4.52 - 10.33)	1.69 (1.48 - 1.95)	-5.315‡	0.000	HS
	Range	2.03 – 65.5	1.22 – 3			

Table (5): Comparison between studied groups as regard serum netrin 1

		Subgroup A	subgroup B	Control group	P1	P2	P3	Sig.
		No. = 17	No. = 17	No. = 20				
Netrin 1 (pg/ml)	Mean ± SD	165.93 ± 17.86	179.64 ± 29.18	144.71 ± 15.18	0.066	0.004	0.000	P1: NS P2: HS P3: HS
	Range	141.35 – 202.02	146.69 – 251.35	124.05 – 181.29				

P1: p value subgroup B Vs subgroup A P2 : p value subgroup A Vs control group P3 : p value subgroup B Vs control group

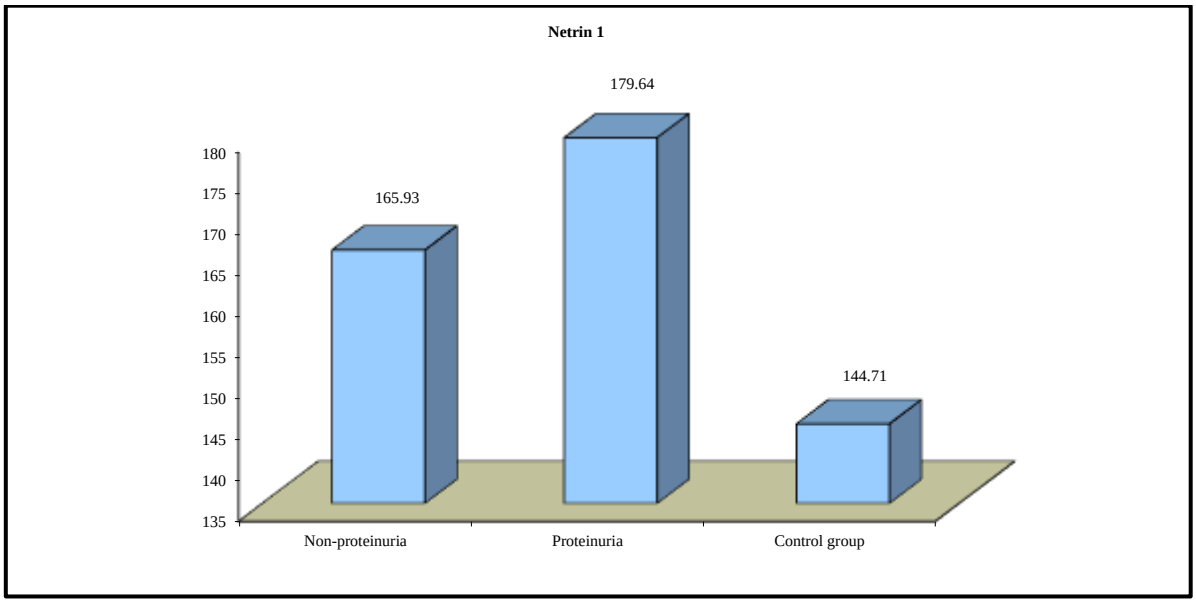

Figure (1): comparison between studied groups as regard serum netrin

Table (6): Comparison between subgroup A and subgroup B as regards disease duration

		Subgroup A	Subgroup B	Test value	P-value	Sig.
		No. = 17	No. = 17			
Disease duration (year)	Median (IQR)	4 (2 - 10)	12 (7 - 17)	-2.316‡	0.021	S
	Range	1 – 20	1 – 40			

Table (7): Correlation between serum Netrin1 and other studied parameters in the patient group

	Netrin 1	
	R	**P-value**
Age	-0.124	0.635
BMI	0.040	0.821
Disease duration (year)	0.306	0.232
WBC	0.226	0.384
RBC	0.385	0.127
HB	0.081	0.757
HCT	-0.190	0.465
MCV	-0.166	0.524
MCH	-0.223	0.390
PLT	-0.096	0.715
Urea	0.150	0.565
Creat	-0.085	0.747
Na	0.255	0.323
K	-0.445	0.074
ALT	-0.161	0.537
AST	-0.175	0.501
CHOL	-0.083	0.751
TG	0.115	0.660
LDL	-0.013	0.959
HDL	-0.102	0.697
Alb/ Create rat	0.424	0.090
FBS	0.219	0.397
2hppbs	0.115	0.660
F Insulin	-0.061	0.844
HbA1C	0.136	0.602
HOMA IR	-0.082	0.789
C-peptide	-0.949	0.051

4. Discussion

Type 2 diabetes is recognized as a serious public health concern with a considerable impact on human life and health expenditures. Rapid economic development and urbanization have led to a rising burden of diabetes in many parts of the world [13]. Diabetes has a multifaceted pathogenesis that occurs either due to impaired insulin secretion or due to development of insulin resistance at target tissues and/or wide-ranging destruction of pancreatic β-cells [14].

Concerns have been raised that more than one-third of diabetes-related deaths occur in people under the age of 60 [15].

Increased consumption of unhealthy diets and sedentary lifestyles, resulting in elevated Body Mass Index and fasting

plasma glucose, have been blamed for these trends [16].

Diabetes mellitus (DM) is the leading cause of chronic renal failure in developing countries and is increasing as a cause of morbidity and mortality worldwide [17]. Diabetic nephropathy affects approximately 25% of patients with T2D and represents the leading cause of end stage renal disease (ESRD) in high-income countries [18]. Moreover, patients with diabetic nephropathy have very high cardiovascular risk, which is comparable with the cardiovascular risk of patients with coronary heart disease [19].

Netrin-1, the axon-guidance molecule has recently become an investigational protein in modulating inflammation, apoptosis, and many other pathological alterations in renal tubular epithelial cells. For instance, Netrin-1 anti-inflammatory actions were mediated through diabetes induced COX-2 expression and PGE2 production. This suppressive effect of COX-2 was expedited through inhibition of NFκB activation. These inflammatory suppressant actions of Netrin-1 were proposed to modulate not only diabetic nephropathy but also the progression of various microvascular diabetic complications [20].

In addition, Netrin-1-mediated reduction in albuminuria occurs by enhancing the uptake of albumin by proximal tubular epithelial cells through the activation of phosphatidylinositol 3 kinase (PI3k) and extracellular regulated receptor kinase (ERK) pathways. In various animal and human studies, it has been reported that Netrin-1 was highly secreted later on both acute and chronic kidney diseases [21],[22]. Our study showed that serum netrin 1 level was significantly increased in all diabetic patients (mean 172.78 ± 24.82) compared to control group (mean 144.71 ± 15.18) (p value <0.01) But there was a highly significant increase of serum netrin 1 level in Subgroup B (mean 179.64 ± 29.18) than other studied groups.

Our result showed no statistically significant correlation between serum netrin 1 and other studied parameters in the patient group.

There was a non- significant positive correlation between serum netrin 1 and (disease duration, WBCs, RBCs, HB, serum urea, Na, triglycerides, albumin creatinine ratio, FBS,2hppbs, HbA1C)

There was a non-significant negative correlation between serum netrin 1 and (age, hematocrit, MCV, MCH, PLT, serum creatinine, K, ALT, AST, cholesterol, LDL, HDL, fasting insulin, HOMA IR and c-peptide)

[23] agreed with our result, they found that Serum netrin-1 levels in subjects with newly diagnosed type 2 diabetes or IFG were significantly higher compared to normal controls. In contrast to our result, they found that Serum netrin-1 levels had strongly positive correlations with age and male gender. HbA1c, fasting glucose, insulin, C-peptide, HOMA IR, AST, and ALT values were also significantly positively correlated with serum netrin-1 levels. Meanwhile, statistically inverse correlations were found between netrin-1 and HDL cholesterol.

Our result agreed with [24] who claimed that Netrin- 1 may be a new biomarker for early detection of impaired fasting glucose (IFG) or T2DM. they found a significant increment of serum Netrin-1 level in subjects with IFG or type 2 DM compared to the control group. they disagreed with our result as they reported that Serum netrin-1 levels had a significant positive correlation with fasting glucose, HbA1c, HOMA-IR, AST and ALT. Meanwhile, a statistically inverse correlation was found between netrin-1 and HDL cholesterol.

[25] conducted a study on 116 individuals, they were divided into two groups: 60 diabetic and 56 nondiabetic patients. Diabetic patients were divided as 30 normoalbumiunric diabetic patients, and 30 microalbumiunric diabetic patients, they reported that Plasma netrin-1level was higher when comparing diabetic group to non-diabetics. There was no statistical significance between the group without

diabetes and normoalbumiunric diabetic patients. There was also a positive statistically significant correlation between netrin-1 level and the duration of diabetes and HbA1c.

[26] was in agreement with our result, they demonstrate a protective role of netrin 1 during diabetic nephropathy. They induced diabetes in mice at the age of 8 weeks by streptozotocin (STZ) treatment. Sixteen weeks after STZ treatment, they examined the kidneys. Initial studies in wild-type mice demonstrated robust induction of renal, urinary, and plasma netrin-1 protein levels during diabetic nephropathy. Subsequent genetic studies in mice with partial netrin-1 deficiency (Ntrn1+/− mice) revealed a more severe degree of diabetic nephropathy, including more severe loss of kidney function (albuminuria, glomerular filtration rate, histology). They subsequently performed pharmacologic studies with recombinant netrin- 1 treatment given continuously via osmotic pump. Indeed, netrin-1 treatment was associated with attenuated albuminuria and improved histologic scores for diabetic nephropathy compared to controls.

[27] was in agreement with our result, they suggest that serum netrin-1 concentrations are increased with diabetic nephropathy progression, particularly in patients with macroalbuminuria, which are associated with renal insufficiency and compensatory responses after inflammation they investigate the level of serum netrin 1 in Type 2 diabetic patients at different degrees of albuminuria, The results showed that plasma netrin-1 level in patients with macroalbuminuria was significantly higher than that in those with microalbuminuria and normoalbuminuria, they have several possible explanations for this First, the high levels of albumin trigger production of netrin-1 from proximal tubular epithelial cells via extracellular signal– regulated kinase (ERK) and Akt kinase pathways to enhance translation [28].

Second, netrin-1 production is a compensatory mechanism for defective reduced capacity of the proximal tubules in early diabetes. More recently, impaired tubular reabsorption of albumin plays a major role in the development of DN [29].

Third, inflammation from tubular epithelial cells can deteriorate other areas of the kidney via inflammatory mediators [30]. In addition, netrin-1 is possible a negative feedback loop for increased inflammation

Our result was in contrast to study made by [31] they reported that Circulating serum Netrin-1 was significantly lower in patients only with obesity, as well as with those with prediabetes and diabetes in comparison to the control group.

Our result was in contrast to study made by [32], they reported that the netrin-1 level of T2DM patients was significantly lower than that of healthy controls they have 3 possible explanations for this finding. First netrin-1 has a role in inflammation, which is implicated in the pathogenic mechanism of T2DM . [33] showed that netrin-1 could regulate inflammation, which might negatively regulate insulin secretion and contribute to β-cell dysfunction

Second, netrin-1 is also associated with islet dysfunction in diabetes and negatively correlated with hyperglycemia. It is understandable that repeated and prolonged exposure to hyperglycemia leads to β-cell degradation, reduces glucose-stimulated insulin secretion and eventually causes β-cell apoptosis [34].

[35] had suggested that netrin-1 is expressed and secreted in the pancreas where it plays a major role in pancreatic morphogenesis in the regenerating pancreas. In patients with newly diagnosed T2DM, secretion of netrin-1 in the injured and apoptotic β cells is significantly reduced, in turn promoting β-cell function failure

Third, IR disrupts the circulation of netrin-1. [36] have suggested that netrin-1 is only selectively modestly upregulated in the visceral white adipose tissue, and that significant reductions in circulating levels

of netrin-1 occur in obese individuals compared with lean individuals

In our study, we found that there was a high significant increase in lipid profile including total Cholesterol, triglycerides & LDL-C in patient group compared to control group & a high significant decrease in HDL -C in patient group compared to control group (P value <0.01).

Our result was in agreement to study made by [37] they found that triglycerides level was significantly increased in subjects with type 2 diabetes compared to normal subjects also the levels of HDL cholesterol were markedly lower in individuals with type 2 diabetes, compared to normal subjects.

Our result was in contrast to study made by [32] they found no significant differences in triglyceride (TG), total cholesterol (TC), high-density lipid (HDL) cholesterol, and LDL cholesterol levels between the two groups.

Our result showed that there was a high statistically significant increase in fasting blood glucose level, 2 hours post prandial blood glucose level, HbA1C, fasting insulin and HOMA IR in patient group compared to control group (P value <0.01).

[38] was in agreement with our results, who found a high significant increase in fasting blood glucose level, 2 hours post prandial blood glucose level, HbA1C in type 2 diabetic patient group compared to control group.

[39] was in agreement with our results, who found that high HbA1C levels are associated with increased risk of diabetic microvascular complications.

5- CONCLUSION

From the results of the current study we concluded that plasma level of Netrin 1 is significantly higher in patients with type 2 diabetes mellitus than control group, there was a non-significant increase of serum netrin 1 in diabetic patients with proteinuria in comparison to type 2 DM patients without proteinuria. This could focus the light on the importance of Netrin 1 on the pathogenesis of nephropathy in diabetes.

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