

Study of Serum Netrin-1 level in Prediabetics and Type 2 Diabetic Egyptian Patients

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Received for publication May 15, 2021; Accepted June 25, 2021; Published online June 25, 2021.

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doi: 10.21608/aimj.2021.74984.1468

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ABSTRACT

Background: Netrin-1 is a laminin-related protein that has anti-inflammatory, tissue-regenerating, and immune-modulating abilities. While inflammation is important in the pathogenesis of insulin resistance and type 2 diabetes, the possible association between netrin-1 and type 2 diabetes remains poorly understood.

Aim of the work: to evaluate the relationship between netrin-1 and prediabetes or newly diagnosed type 2 diabetes, and the role of netrin-1 as a novel biomarker in the pathogenesis of early-stage type 2 diabetes.

Subjects and methods: This cross-sectional study was conducted at Al-Hussein University Hospital of Al-Azhar University. Ninety Egyptian participants were enrolled and equally divided into three groups (prediabetes, recently diagnosed type 2 diabetes, and healthy controls). All participants aged 30-65 years. Netrin-1 levels were measured using an available commercially human enzyme-linked immunosorbent assay kit.

Results: The type 2 diabetes and prediabetes groups had significantly higher serum netrin-1 levels than the healthy control group (488.6, 408.4, and 228.6 pg/ml, respectively; $p < 0.001$). Additionally, netrin-1 demonstrated a highly statistically significant positive association with body mass index, fasting blood glucose, 2-hour postprandial blood glucose, hemoglobinA1c, and Homeostatic Model Assessment of Insulin Resistance in all studied groups ($p < 0.001$).

Conclusion: Serum netrin-1 levels were higher in people with prediabetes and recently diagnosed type 2 diabetes and were linked to higher body mass index, insulin resistance, and glucose homeostasis. Future research exploring the exact mechanism may provide new insights into diabetes prevention and treatment.

Keywords: Netrin-1; Insulin resistance; Inflammation; prediabetes; Type 2 diabetes mellitus.

Disclosure: The authors have no financial interest to declare in relation to the content of this article. The Article Processing Charge was paid for by the authors.

Authorship: All authors have a substantial contribution to the article.

INTRODUCTION

Diabetes affects 463.0 million adults aged 20–79 years globally, and it is predicted to affect over 570 million by 2030.¹

Egypt is the top 9 leading country with the highest incidence of diabetes worldwide. In 2019, the prevalence of diabetes was roughly 8.9 million, which accounts for 17.2% of adults aged 20–79 years, and the annual death was 76,262, which represents 41.6% of deaths at 60 years of age.¹

Generally, type 2 diabetes mellitus (T2DM) remains undiagnosed for many years because hyperglycemia develops gradually and at the early-stage, it is often not elevated enough to develop classic diabetes symptoms. Therefore, even individuals with undiagnosed diabetes will be at a higher risk of developing diabetic (macrovascular and microvascular) complications. Hence, the risk factors for prediabetes and T2DM must be early detected to direct healthcare providers in performing a diabetes diagnostic test.²

The immunologic and inflammatory aspects of T2DM pathogenesis have recently started to be explored. Previously, T2DM was considered to be purely a metabolic disease caused by numerous factors, such as genetics, environment, and lifestyle. However, researchers have now discovered that autoimmune and inflammatory aspects are involved in the pathogenesis of obesity-induced insulin resistance and T2DM.³

Along with semaphorins and ephrins, netrin-1 is involved in axon guidance. It controls axonal growth in the nervous system's development process and also regulates immune and inflammatory responses in the nervous system.⁴

Netrin-1 is also affected at varying degrees in numerous cancers, renal pathologies, and hepatic injuries.^{5,6}

Moreover, netrin-1 plays an inflammatory role in T2DM pathogenesis. According to Natura et al.,

netrin-1 participates in an inflammatory process that might negatively regulate insulin secretion and participate in β -cell dysfunction.⁷

This notion, coupled with netrin-1's inflammatory and immunomodulatory properties, prompted us to investigate whether serum netrin-1 levels are linked to prediabetes or T2DM development.

SUBJECTS AND METHODS

The current study conformed to the principles of the Declaration of Helsinki and was approved by Ethics Committee Unite, Faculty of Medicine, Al-Azhar University in Cairo, Egypt. The clinical pathway, steps, and potential side effects were clearly explained to all participants. Written informed consent was obtained from all participants or their legal representatives. The study was performed between May 2019 and April 2021 at Al-Hussien University Hospital.

This cross-sectional study included 90 participants, which were equally divided into three groups as follows: 30 in the prediabetes group (group A), 30 in the group with recently diagnosed T2DM (group B), and 30 in the healthy control group (group C). Diagnosis of prediabetes and T2DM was based on the American Diabetes Association 2019 diagnostic criteria. Patient age ranged between 30 and 65 years.

Meanwhile, participants with other diseases such as clinically significant kidney or liver disease, cancer, diabetes, clinically significant inflammatory disorder, or significant infection, and those taking diabetes drugs were all excluded.

The following data were obtained from all participants: thorough medical history and physical examination results, anthropometric measurements (height, weight, and body mass index [BMI]), and biochemical assays (fasting blood glucose [FBG], 2-hour postprandial blood sugar test [2-hour PP], and hemoglobin A1c [HbA1c]), insulin levels, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) ratio, alanine aminotransferase, aspartate aminotransferase, serum albumin, serum creatinine, erythrocyte sedimentation rate, and C-reactive protein. In addition, serum netrin-1 levels were measured using human enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Catalog No: E-EL-H2328, USA) according to the manufacturer's protocol.

Statistical analysis:

Data were analyzed using the Statistical Program for Social Science version 24. The quantitative results are expressed as mean and standard deviation (SD), whereas the qualitative results are expressed as frequency and percentage.

RESULTS

As mentioned, our study had 90 participants divided into three groups (30 per group). The mean age was 48.2 (36–58) years in group A (prediabetes), 44.7 (35–57) years in group B (recently diagnosed T2DM), and 44.3 (31–55) years in group C (healthy controls), with BMI values of 29.8, 33.9, 26.9, and kg/m², respectively (Table 1).

		Groups			Stat. test	p-value
		Group A (n = 30)	Group B (n = 30)	Group C (n = 30)		
Sex	Male	17 56.7%	16 53.3%	17 56.7%	$\chi^2 = 0.09$	0.956 NS
	Female	13 43.3%	14 46.7%	13 43.3%		
Age(years)	Mean	48.2	44.7	44.3	F = 3.18	0.046 S
	\pm SD	6.5	6.2	7.3		
BMI(kg/m ²)	Mean	29.8	33.9	26.9	F = 23.9	<0.001 HS
	\pm SD	3.0	4.7	3.9		

Table 1: Comparison between the study groups according to the demographic data.

Serum netrin-1 showed a highly statistically significant difference between the study groups ($p < 0.001$). The mean netrin-1 levels were 408.4, 488.6, and 228.6 pg/ml in groups A, B, and C, respectively (Table 2).

The serum netrin-1 levels demonstrated a statistically significant difference between groups A and B ($p = 0.004$) but revealed a highly statistically significant difference between group A or B and group C ($p < 0.001$) (Table 2).

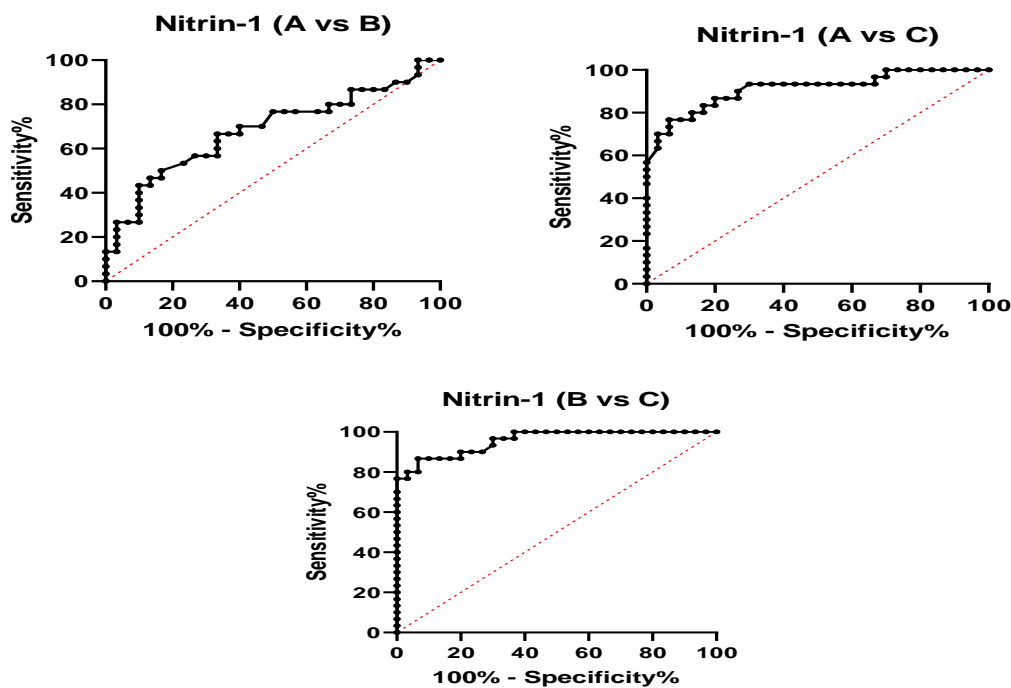
		Groups			F	P-value
		Group A (n = 30)	Group B (n = 30)	Group C (n = 30)		
Netrin-1	Mean	408.4	488.6	228.6	47.2	<0.001HS
	±SD	99.2	131.1	82.4		

Table 2: comparisons between studied groups as regard serum Netrin-1.

Serum netrin-1 levels at a cutoff value of >337.5pg/ml can be used to discriminate between groups A and C, with 76.7% sensitivity, 86.7% specificity, 85.2% positive predictive value (PPV), and 78.2% negative predictive value (NPV) (area under the curve [AUC] = 0.91; p < 0.001). Furthermore, serum netrin-1 levels at a cutoff value of >368pg/ml can be used to discriminate between groups B and C, with 80% sensitivity, 93.3% specificity, 92.3% PPV, and 82.4% NPV (AUC = 0.96; p < 0.001) (Table 3).

Netrin-1	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
A vs B	>451	0.68	63.3 %	66.7 %	65.5 %	64.5 %	0.015
A vs C	>337.5	0.91	76.7 %	86.7 %	85.2 %	78.2 %	< 0.001
B vs C	>368	0.96	80 %	93.3 %	92.3 %	82.4 %	< 0.001

Table 3: Diagnostic performance of netrin-1 in the discrimination of the study groups.



(Roc Curve) showing the diagnostic performance of Netrin-1 in the discrimination of the study groups.

Moreover, netrin-1 was positively related to FBG, 2-hour PP, HbA1c, BMI, and HOMA-IR in all groups, with a

Variables	Group A		Group B		Group C	
	r	p-value	r	p-value	r	p-value
Netrin-1 vs age	0.03	0.873	-0.162	0.393	0.19	0.314
Netrin-1 vs BMI	0.661	< 0.001	0.691	< 0.001	0.56	0.001
Netrin-1 vs FBS	0.53	0.003	0.787	< 0.001	0.77	< 0.001
Netrin-1 vs PPBS	0.693	< 0.001	0.831	< 0.001	0.77	< 0.001
Netrin-1 vs A1C	0.718	< 0.001	0.84	< 0.001	0.78	< 0.001
Netrin-1 vs F. Insulin	0.615	< 0.001	0.788	< 0.001	0.79	< 0.001
Netrin-1 vs HOMA-IR	0.649	< 0.001	0.839	< 0.001	0.83	< 0.001

highly statistically significant correlation ($p < 0.001$) (Table 4).

Table 4: Correlation between serum netrin-1 and other studied data in all groups.

Given that netrin-1 demonstrated a positive correlation with BMI, we also compared the netrin-1 levels between patients without obesity in group B and those in groups C and A to rule out the obesity factor. The netrin-1 levels showed a statistically significant increase in patients without obesity with diabetes compared with patients without both obesity and diabetes (Table 5).

		Groups		T	P-value
		Without obesity and diabetes (n = 38)	Without obesity, with diabetic (n = 8)		
Netrin-1	Mean	268.2	352.1	2.07	0.044 S
	±SD	106.7	89.1		

Table 5: Relationship between patients without both obesity and diabetes in groups A and C, and patients without obesity in group B (with diabetes) in terms of the netrin-1 levels.

DISCUSSION

The global incidence of diabetes and associated complications and the adverse influence of diabetes complications on the quality of life of the patients and their caregivers are alarming. Thus, a new approach for the prompt diagnosis and innovation of an effective and safe treatment option is needed. Thus, this study discussed the role of netrin-1 as a novel biomarker of early-stage diabetes for the early detection of diabetic complications and for the timely planning of a possible therapeutic goal using safe and effective anti diabetic agents.

Therefore, we investigated the plasma levels of netrin-1 and its associations with other clinical and laboratory parameters in three population groups: individuals with prediabetes, patients with newly diagnosed T2DM, and healthy controls.

According to our study results, the serum netrin-1 levels showed a statistically significant difference between groups A (prediabetes) and B (recently diagnosed T2DM) ($p = 0.004$) but revealed a highly statistically significant difference between group A or B and group C (healthy controls) ($p < 0.001$).

In addition, netrin-1 showed a highly statistically significant positive association with FBG, 2-hour PP, HbA1c, and HOMA-IR in all groups ($p < 0.001$).

Our results are similar to those in the study of Yim et al. They enrolled 218 subjects, with 41 healthy controls, 85 patients with impaired fasting glucose, and 92 patients with newly diagnosed T2DM without anti diabetic medications yet. They concluded that plasma netrin-1 levels were significantly higher in patients with T2DM or impaired fasting glucose than in the healthy controls (441.0, 436.6, and 275.9 pg/ml, respectively; $p < 0.001$). Plasma netrin-1 levels were also found to be significantly related to FBG, HbA1c, and HOMA-IR.⁸

Ay et al. conducted a study in the same field and found that patients with T2DM with a mean HbA1c of 8.1% had higher serum netrin-1 levels than those without T2DM. Serum netrin-1 levels also demonstrated a strong positive association with HbA1c. However, their sample size was relatively small and a third intermediate group between T2DM and normal controls (e.g., prediabetes) was not included in their study population.⁹

In other recent research, urinary netrin-1 level was increased at the earlier stages of diabetes in rats and humans.^{10, 11}

However, our findings were opposite to those of Liu et al., who performed a clinical trial on 56 subjects (30 patients were newly diagnosed with T2DM and the rest were the controls) to assess the level of plasma netrin-1 in patients with T2DM. They discovered that the patients with T2DM had significantly lower plasma netrin-1 levels than the healthy controls. Furthermore, the plasma netrin-1 levels were inversely linked to HOMA-IR, plasma glucose levels (FBG and 2-hour PP), and HbA1c.¹²

Furthermore, Nedeva et al. conducted a clinical study involving 163 subjects, who were divided into four groups: obesity without dysglycemia, prediabetes, diabetes, and healthy controls. Results showed that serum netrin-1 levels were substantially lower in individuals with obesity alone, as well as in those with prediabetes and T2DM than in the healthy controls.¹³

These results might be related to the varied characteristics of the study populations. During T2DM diagnosis, we measured the levels of plasma netrin-1 as well as other laboratory parameters. The mean values of HbA1c and HOMA-IR were 8.7% and 6.9%, respectively. However, Liu et al. included patients who were diagnosed with T2DM during the previous 6 months and had HbA1c and HOMA-IR mean values of 8.5% and 1.13%, respectively. Therefore, our study population showed slight hyperglycemia and higher insulin resistance.

Different characteristics and conditions of netrin-1 sampling, the use of different ELISA kits, and the serum measurement techniques for netrin-1 can all influence in the differences between findings; thus, such factors should be considered during data analysis.

As the status of diabetes and insulin resistance advances, no long-term analysis of shifts in netrin-1 levels have been performed, and the precise function of netrin-1 in the T2DM pathophysiology remains unknown.

Therefore, the potential advantageous compensatory response of netrin-1 to the shifts occurring in the initial stages of diabetes should be studied on a larger scale.

Ezzat M. et al. evaluated urinary netrin-1 excretion as an early marker for diabetic nephropathy and found that urinary netrin-1 levels were considerably higher in the DM group (1418 ± 3733.6 pg/mg creatinine) than in the control group (477.4 ± 283.6 pg/mg creatinine), with the highest value in the macroalbuminuria group (1919.4 ± 573.4 pg/mg creatinine) and the lowest value in the normoalbuminuria group (833.7 ± 595.3 pg/mg creatinine).¹⁴

From their experiments in both humans and animals, Cao et al., showed the important roles of classical neuronal guidance molecules such as netrin-1 and netrin-4 in detecting and correcting the pathology of diabetic retinopathy. The levels of both netrin-1 and netrin-4 decreased, while the vascular endothelial growth factor levels increased in patients with diabetic retinopathy and animal models.¹⁵

Thus, to study accurately the netrin-1 level in patients with diabetes, we recommend that future studies should consider the presence or absence of diabetic complications, such as diabetic retinopathy and nephropathy.

Our study results also clearly showed a highly statistically significant difference ($p < 0.001$) in the netrin-1 level between patients with both obesity and diabetes and patients with diabetes but without obesity, and a positive correlation between BMI and the netrin-1 levels in all study groups (mean BMI: 29.8, 33.9, and 26.9 kg/m² in prediabetes, recently diagnosed T2DM, and healthy controls, respectively).

Our finding is not in line with any previous studies describing the relationship between netrin-1 level and BMI.

Ramkhelawon B. et al., assessed the expression of netrin-1 and its receptor (uncoordinated-5 homologue family member [UNC5H2]) in human adipose tissue from the obesity and lean groups. The mRNA levels of netrin-1 and UNC5H2 (not the deleted in colorectal cancer) [DCC] receptors in the adipose tissue were higher in the obesity group than in the lean group, but netrin-1 plasma concentrations were less in the obesity group, indicating that netrin-1 is selectively elevated in the adipose tissue macrophages of individuals with obesity.¹⁶

In the study by Yim et al., BMI (mean BMI: 25.2 kg/m²) had no significant relationship with serum netrin-1 levels.⁸

Meanwhile, our study has limitations, such as the small sample size, limited study period (which prevented us from studying the netrin-1 levels in all diabetes stages), and lack of proper identification of diabetes complications among the participants. Hence, future research should consider these limitations.

CONCLUSION

Elevated serum netrin-1 levels significantly correlated with prediabetes and recently diagnosed T2DM. Netrin-1 also demonstrated a meaningful positive relationship with obesity. To fully understand the function of netrin-1 in T2DM pathogenesis and to explore its diagnostic and therapeutic potential, we need to conduct more prospective studies.

REFERENCES

- 1- International diabetes federation: IDF diabetes atlas 2019 - 9th edition.
- 2- American Diabetes Association: 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes. *Diabetes Care*. 2020, 43:S14–31
- 3- De Candia P, Prattichizzo F, Garavelli S, et al.: Type 2 diabetes; how much of an autoimmune disease? *Front Endocrinol (Lausanne)*. 2019;10:451
- 4- Lee WS, Lee W-H, Bae YC and Suk K.: Axon guidance molecules guiding neuroinflammation. *Exp. Neurobiol*. 2019; 28(3):311.
- 5- Kefeli U, Ucuncu Kefeli A, Cabuk D, et al.: Netrin-1 in cancer: Potential biomarker and therapeutic target. *Tumor Biology*. April 2017.
- 6- Ebrahim M. Yimer, Kaleab Alemayehu Zewdie, Hailemichael Zeru Hishe *J: Diabetes Res*. 2018; 8250521. Published online 2018 Sep 18.
- 7- Natura G, Bär K, Eitner A et al.: Neuronal prostaglandin E2 receptor subtype EP3 mediates antinociception during inflammation. *Proc. Natl. Acad. Sci USA.*, 2013;110(33):13648–53.
- 8- Yim J, Kim G, Lee B-W, Kang ES, Cha B-S, Kim J-H, Cho JW, Lee S-G and Lee Y-h.: Relationship Between Circulating Netrin Concentration, Impaired Fasting Glucose, and Newly Diagnosed Type 2 Diabetes. *Front. Endocrinol*. 2018; 9:691.
- 9- Ay E, Marakoglu K, Kizmaz M, and Unlu A.: Evaluation of Netrin-1 Levels and Albuminuria in Patients With Diabetes. *J Clin Lab Anal*. 2016; 30:972–7.
- 10- 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.
- 11- White JJ, Mohamed R, Jayakumar C, and Ramesh G.: Tubular injury marker netrin-1 is elevated early in experimental diabetes. *J Nephrol*. 2013; 26:1055–64.
- 12- C Liu, KeX, Wang Y, Feng X, Li Q, Zhang Y, et al.: The level of netrin-1 is decreased in newly diagnosed type 2 diabetes mellitus patients. *BMC Endocr Disord*. 2016; 16: 33
- 13- Iveta Nedeva, Antoaneta Gateva, Yavor Assyov, Vera Karamfilova, Tsvetelina Velikova and Zdravko Kamenov: Relationship between circulating netrin-1 levels, obesity, prediabetes and newly diagnosed type 2 diabetes. *Archives of Physiology and Biochemistry*, 2020. DOI: 10.1080/13813455.2020.1780453
- 14- Ezzat M. Mohammed, Fawzy A. El-Messallamy, Ahmed M. Mazroue, Islam A. Elsayed, and Amal A. Zidan: "Urinary Netrin-1 as an Early Marker for Diabetic Nephropathy". *The Egyptian Journal of Hospital Medicine*. October 2020; 81 (5), 1924-30
- 15- Bowen Cao, Xuxia Meng, Yudong Fu, Penghui Liu, Yu Lun, and Yangang Wang: "Neuron-derived netrin-1 and netrin-4 proteins are additional effective targets in diabetic retinopathy beyond VEGF" *Int J Clin. Exp. Pathol* 2017; 10(8):8174-86.
- 16- Ramkhelawon B, Hennessy EJ, Menager M, Ray TD, Sheedy FJ, Hutchison S, et al.: Netrin-1 promotes adipose tissue macrophage retention and insulin resistance in obesity. *Nat Med*. 2014; 20(4):377–84.