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Serum Netrin-1 Level in Type 2 Diabetic Nephropathy

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Abstract

Background Diabetes-related nephropathy DN is a form of renal impairment that affects 40 % of DM patients. It is a permanent state that leads to inflammation and affects a large number of diabetes patients worldwide. Netrin-1 (NTN-1) is a protein that belongs to a group of proteins called neuronal guidance proteins and has a significant impact on the regulation of inflammation by impacting the migration of cells.

Objective To assess the relation of serum NTN-1 level with the early detection of nephropathy in type 2 diabetes mellitus (T2DM) patients.

Methods A total of 88 individuals were enrolled in this cross-sectional study, and subdivided into three groups: normoalbuminuria, microalbuminuria, and macroalbuminuria. Serum NTN-1 was measured by using an enzyme-linked immunosorbent test (ELISA).

Results The DN patients had significantly different serum levels of NTN-1; the macroalbuminuria group had the greatest value (1127.73±407.93 pg/ml). The prediction of DN was determined by ROC curve analysis to have 88% sensitivity and 63% specificity for serum netrin-1 at a cutoff point of 686.5 pg/ml with an AUC of 0.642 (P = 0.08).

Conclusion There is a progressive increase in serum NTN-1 levels with the severity of albuminuria in T2DM patients.

Keywords Netrin-1; Biomarkers, diabetic nephropathy, type 2 diabetes mellitus, albuminuria

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List of abbreviations: DN = Diabetic nephropathy, GFR = Glomerular filtration rate, NTN-1 = Netrin-1, T2DM = Type 2 diabetes mellitus

Introduction

Diabetes-related nephropathy (DN) is a form of renal impairment that affects 40% of diabetic patients. DN stands third among the biggest contributors to mortality among individuals diagnosed with type 2 diabetes mellitus (T2DM), after oncological disorders and cardiovascular conditions [1,2]. DN has a natural history characterized by stages: macroalbuminuria, microalbuminuria. Together with blood pressure and glucose, the glomerular filtration rate (GFR), which is related to the amount of albumin excretion, gradually decreases during the beginning of albuminuria [3].

Other variables that could lower GFR include obesity, hypertriglyceridemia, and female gender; these could help to explain why the typical histological lesions of DN are not directly linked to the heterogeneity of the DN phenotype. Some diabetic individuals have no renal issues, even with poor control of their diabetes. It suggests that other elements might

be involved in the beginning and progression of the illness [4,5].

Netrins are a class of proteins that can be recognized in both animals and humans, despite their differences [6]. There are six proteins that are excreted that make up the protein family. An N-terminal domain, followed by three epidermal growth factor (EGF)-like repeats (LE), and a shorter, less conserved, positively charged domain at the C-terminal end of the molecule are the elements that constitute the structure of netrins [7]. Netrin-1 (NTN-1) is a protein that is similar to laminin and is released into the surrounding environment. It is referred to as a neuronal guidance protein and has a significant impact on the movement of cells, particularly in the context of inflammatory regulation [8]. NTN-1 has contrasting effects with respect to its diverse roles, which are contingent upon its relative expression, concentration, receptor subtypes, cellular origins and tissues, inflammatory conditions, and diseases. NTN-1 and its receptor deleted in colorectal cancer (DCC), are essential for various cellular functions, including cell adhesion, cell death, cell division, cell survival, tissue arrangement, and cancer development [9,10]. A previous laboratory study found that UNC5B (Uncoordinated-5 homolog family, receptor of netrin-1) deletion in the proximal tubular epithelium increased epithelial cell death and inflammation, worsening acute kidney damage. UNC5B signaling is important, despite chronic renal failure. NTN-1 minimizes albuminuria and inflammation by interfering with COX-2 production and NFκB activation. NTN-1 also helps epithelial cells absorb albumin. Thus, NTN-1 may decrease nephropathy via UNC5B by improving lumen albumin clearance and decreasing tubular epithelial damage. This reduces inflammation and epithelial stress, influencing podocyte function [11,12]. There are a few likely explanations why DN is improving at increasing NTN-1; first, through the extracellular signal-regulated kinase (ERK) and protein kinase B pathways, high levels of albumin stimulate the proximal tubular epithelium's synthesis of NTN-1, therefore improving translation, second, the early stages of diabetes see the proximal tubules decreased functional capability being compensated for by the synthesis of NTN-1 [13]. A new discovery has found that the impaired reabsorption of albumin in the tubules is a significant factor in the development of DN. Furthermore, the release of inflammatory mediators by tubular epithelial cells can potentially hinder the functioning of other components of the kidney, third, NTN-1 might serve as a mechanism to counteract the upregulation of inflammatory cytokines [14].

This study aimed to assess the relation of serum NTN-1 level with the early detection of nephropathy in T2DM patients.

Methods

Study design

People with T2DM from the National Diabetes Centre for Research and Treatment (NDC)/Mustansiriyah University took part in a cross-sectional study that ran from October 2023 to April 2024. The study used 88 samples, with 33 being normoalbuminuria, 33 being microalbuminuria, and 22 being macroalbuminuria, depending on what was available. The samples were chosen based on the criteria for inclusion and exclusion, and all study participants gave written permission. People who had smoked, had cancer, had gestational diabetes, type 1 diabetes, heart disease, or any other problem in their history were not allowed to take part in the study. Based on the amount of albumin and creatinine in their urine, patients were categorized into microalbuminuria (albumin to creatinine ratio; ACR = 30–300 mg/g creatinine), and macroalbuminuria (ACR >300 mg/g creatinine).

Sample collection

Serum was separated using a 5 ml disposable syringe and discharged into plain tubes. The samples were collected from the antecubital

vein of all patients while they were fasting. The tubes were then left at room temperature (25 °C) for 15 minutes before being centrifuged at 2000–3000 rpm for 10 minutes. To prevent repeated refrigeration, they were melted and subsequently frozen. Aliquots of serum were used for the measurement of fasting blood sugar (FBS), glycated hemoglobin (HbA1c), insulin, lipid profile, urea, creatinine by using either enzymatic spectrophotometric methods or by enzyme linked immunosorbent assay (ELISA) using ELISA kits from USA which were assayed according to manufacturer instructions. Also, urine collected from each patient for albumin to creatinine ratio.

Statistical analysis

Data were analyzed using the statistical package for social sciences (SPSS) version 25. After assuring that the data was normally distributed, the data presentation was as simple measures, like mean, standard deviation of the mean and standard error. ANOVA method was used to test the presence of difference in means among groups. The P value of <0.05 was considered statistically significant. Sensitivity, specificity and measurement of the area under the curve (AUC) was analyzed for the NTN-1 results for all the study population, the results of (normoalbuminuria, microalbuminuria and macroalbuminuria) were presented to evaluate the use of NTN-1 as a differentiation marker between these three groups. The optimum cutoff was used for calculating the diagnostic sensitivity and the specificity.

Results

On comparison NTN-1 of DN groups (normoalbuminuria, microalbuminuria and macroalbuminuria), it was shown that NTN-1 had a highly significant differences between groups (P <0.001) as shown in table (1) and figure (1), that serum netrin-1 showed a highly significant increase in microalbuminuria group and macroalbuminuria group in comparison to the level in normoalbuminuria group.

In table (2), NTN-1 showed significant positive correlations with FBS (r = 0.398, P = 0.049) and with HbA1c (r = 0.421, P = 0.036), also high significant positive correlations with cholesterol (r = 0.532, P = 0.006) in normoalbuminuria group and a significant positive correlation with serum creatinine in macroalbuminuria group (r = 0.496, P = 0.022).

Table 1. Netrin-1 levels in normoalbuminuria, microalbuminuria and macroalbuminuria patients' groups

Variables	Type 2 Diabetes groups	Mean±SD	SE	Median	P value
Netrin-1 (pg/ml)	Normoalbuminuria	718.06±189.41	33.48	695	< 0.001*
	Microalbuminuria	812.97±331.52	57.71	736	
	Macroalbuminuria	1127.73±407.93	86.97	1104	

P value by ANOVA test

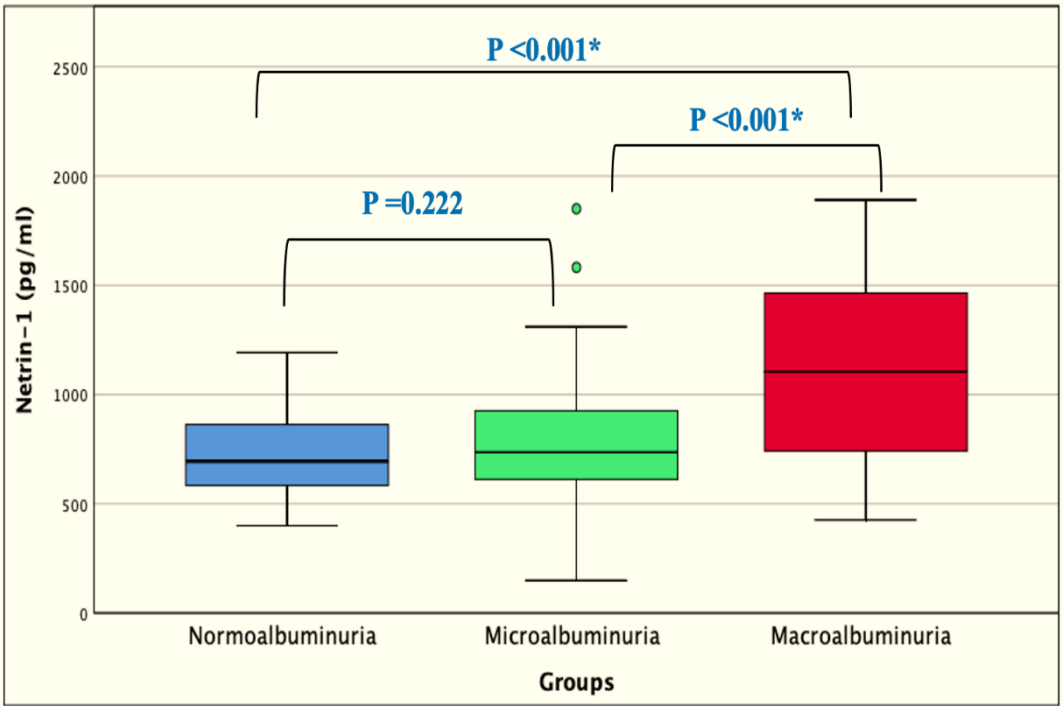

Figure 1. Median level of netrin-1 among subgroups of albuminuria

Table 2. Pearson correlation coefficient between Netrin-1 (pg/ml) and other variables in three different groups of type 2 diabetes mellitus

Variables	Type 2 Diabetes with Normoalbuminuria group		Type 2 Diabetes with Microalbuminuria group		Type 2 Diabetes with Macroalbuminuria group	
	r	*P*	*r*	*P*	*r*	*P*
Age (years)	-0.042	0.841	0.029	0.889	-0.383	0.086
Duration (years)	-0.126	0.548	-0.126	0.548	-0.067	0.774
FBS (mg/dl)	0.398*	0.049	-0.051	0.809	-0.096	0.678
HbA1c (%)	0.421*	0.036	-0.266	0.198	0.061	0.793
Insulin (U/ml)	-0.139	0.508	0.05	0.812	-0.024	0.919
IR (HOMA_IR)	0.22	0.291	-0.025	0.904	-0.103	0.656
Cholesterol (mg/dl)	0.532**	0.006	0.007	0.975	-0.176	0.445
Triglyceride (mg/dl)	-0.22	0.29	0.06	0.775	0.341	0.13
B. Urea (mg/dl)	0.219	0.292	-0.247	0.234	0.227	0.323
S. Creatinine (mg/dl)	-0.213	0.307	0.143	0.495	0.496*	0.022
GFR (ml/min/1.73 m^2)	0.131	0.532	-0.094	0.655	-0.339	0.133

FBS: Fasting blood sugar, HbA1c: Glycated hemoglobin, IR: Insulin resistance, B. urea: Blood urea, S. creatinine: Serum creatinine, GFR: Glomerular filtration rate

A ROC analysis to distinguish of NTN-1 in the three study groups, regulate the parameter depending on area under curve (AUC) that can be occupied and if this occupation is significant or not, the results of AUC were presented for the best discriminative cut-off values of NTN-1 (Table 3).

Table 3. ROC analysis criteria of netrin-1 versus GFR as differentiating between diabetic nephropathy groups

Combination	Parameter	Cutoff	Sensitivity	Specificity	AUC	Standard error	95% CI	P value
Normoalbuminuria vs Microalbuminuria	Netrin-1	686.5	0.88	0.63	0.642	0.078	0.488 to 0.795	0.086
	GFR	83.75	0.60	0.66	0.606	0.088	0.433 to 0.780	0.197
Microalbuminuria vs Macroalbuminuria	Netrin-1	948.5	0.72	0.56	0.597	0.084	0.432 to 0.763	0.261
	GFR	62.4	0.80	0.76	0.791	0.071	0.653 to 0.930	0.001*

Discussion

The results of the ANOVA test indicated there were highly significant differences in NTN-1 levels among the groups. The mean of NTN-1 for microalbuminuria and macroalbuminuria was significantly higher than for normoalbuminuria (P <0.001) and the mean of NTN-1 for macroalbuminuria was significantly higher than microalbuminuria (P <0.001). This is agreed by Ay et al., who reported that serum NTN-1 levels were raised in patients with diabetes whom mean HbA1c level was 8.1% compared to non-diabetic patients [15]. NTN-1 can be an early marker of tubular kidney damage [16,17]. During acute or chronic disease states, endogenous defense mechanisms that oppose excessive inflammation and cellular destruction have developed or are activated. An example of such a mechanism is the NTN-1 receptor. It has been established that this protective mechanism plays a vital role in numerous acute and chronic diseases [18]. A statistically significant positive connection goes with finding of Jung et al., who found Netrin-1 has the potential to serve as a novel biomarker for the early diagnosis of impaired fasting glucose [19,20]. A case-control study indicates NTN-1 is an inflammatory biomarker present at higher levels in individuals with obesity, indicating the presence of chronic inflammation and insulin resistance [6].

A significant positive correlation was observed between NTN-1 and S. creatinine in macroalbuminuria group patients; NTN-1 overexpression promoted tubular epithelial cell proliferation, indicating that NTN-1 may play a role in tubule regeneration and recovery following injury [21]. No other significant correlations were found.

However, ROC analysis showed that NTN-1 was not different from GFR in distinguishing DN in normoalbuminuria from microalbuminuria and inferior than GFR to distinguish between DN in microalbuminuria from macroalbuminuria in patients with T2DM.

In conclusion, there is a progressive increase in serum NTN-1 levels with the severity of albuminuria in T2DM patients, which suggests that NTN-1 may serve as a potential biomarker for the progression of DN, reflecting kidney

damage severity. However, NTN-1 is inferior than GFR in differentiating micro from macroabluminuria.

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Author contribution
All authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript

Conflict of interest
The authors declare there is no conflict of interest.

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References
1. Samsu N. Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. Biomed Res Int. 2021; 2021: 1497449. doi: 10.1155/2021/1497449.
2. Cai SS, Zheng TY, Wang KY, et al. Clinical study of different prediction models in predicting diabetic nephropathy in patients with type 2 diabetes mellitus. World J Diabetes. 2024; 15(1): 43-52. doi: 10.4239/wjd.v15.i1.43.
3. Patidar K, Deng JH, Mitchell CS, et al. Cross-domain text mining of pathophysiological processes associated with diabetic kidney disease. Int J Mol Sci. 2024; 25(8): 4503. doi: 10.3390/ijms25084503.
4. Rico Fontalvo JE. [Clinical practice guidelines for diabetic kidney disease]. Rev. Colomb. Nefrol. 2021; 8(2): e561.
5. Giglio RV, Patti AM, Rizvi AA, et al. Advances in the pharmacological management of diabetic nephropathy: A 2022 international update. Biomedicines. 2023; 11(2): 291. doi: 10.3390/biomedicines11020291.
6. Mentxaka A, Gómez-Ambrosi J, Ramírez B, et al. Netrin-1 promotes visceral adipose tissue inflammation in obesity and is associated with insulin resistance. Nutrients. 2022; 14(20): 4372. doi: 10.3390/nu14204372.
7. Ziegon L, Schlegel M. Netrin-1: A modulator of macrophage driven acute and chronic inflammation. Int J Mol Sci. 2021; 23(1): 275. doi: 10.3390/ijms23010275.
8. Xia X, Hu Z, Wang S, Yin K. Netrin-1: An emerging player in inflammatory diseases. Cytokine Growth Factor Rev. 2022; 64: 46-56. doi: 10.1016/j.cytogfr.2022.01.003.
9. Ke S, Guo J, Wang Q, et al. Netrin family genes as prognostic markers and therapeutic targets for clear cell renal cell carcinoma: Netrin-4 acts through the Wnt/β-Catenin signaling pathway. Cancers (Basel). 2023; 15(10): 2816. doi: 10.3390/cancers15102816.
10. Liu L, Liu KJ, Cao JB, et al. A novel Netrin-1-derived peptide enhances protection against neuronal death and mitigates of intracerebral hemorrhage in mice. Int J Mol Sci. 2021; 22(9): 4829. doi: 10.3390/ijms22094829.
11. Ranganathan P, Jayakumar C, Navankasattusas S, et al. UNC5B receptor deletion exacerbates tissue injury in response to AKI. J Am Soc Nephrol. 2014; 25(2): 239-49. doi: 10.1681/ASN.2013040418.
12. Xiao PY, Chen JY, Zeng Q, et al. UNC5B overexpression alleviates peripheral neuropathic pain by stimulating netrin-1-dependent autophagic flux in Schwann Cells. Mol Neurobiol. 2022; 59(8): 5041-55. doi: 10.1007/s12035-022-02861-z.
13. Liu C, Li Q, Feng X, et al. Elevated levels of netrin-1 in the serum of patients with diabetic nephropathy: relationship with renal function and inflammation. Eur J Inflam. 2018. http://dx.doi.org/10.1177/2058739218809288.
14. Miranda-Díaz AG, Pazarín-Villaseñor L, Yanowsky-Escatell FG, et al. Oxidative stress in diabetic nephropathy with early chronic kidney disease. J Diabetes Res. 2016; 2016: 7047238. doi: 10.1155/2016/7047238.
15. Ay E, Marakoğlu K, Kizmaz M, et al. Evaluation of netrin-1 levels and albuminuria in patients with diabetes. J Clin Lab Anal. 2016; 30(6): 972-7. doi: 10.1002/jcla.21965.
16. Övünç Hacıhamdioğlu D, Hacıhamdioğlu B, Altun D, et al. Urinary netrin-1: A new biomarker for the early diagnosis of renal damage in obese children. J Clin Res Pediatr Endocrinol. 2016; 8(3): 282-7. doi: 10.4274/jcrpe.2828.
17. Kamianowska M, Szczepański M, Chomontowska N, et al. Is urinary netrin-1 a good marker of tubular damage in preterm newborns? J Clin Med. 2021; 10(4): 847. doi: 10.3390/jcm10040847.
18. Aherne CM, Collins CB, Masterson JC, et al. Neuronal guidance molecule netrin-1 attenuates inflammatory cell trafficking during acute experimental colitis. Gut. 2012; 61(5): 695-705. doi: 10.1136/gutjnl-2011-300012.
19. Jung HI, Bae J, Han E, et al. Circulating netrin-1 as a novel biomarker for impaired fasting glucose and newly diagnosed type 2 diabetes mellitus. Diabetes. 2018; 67 (Supplement_1): 1535-P. doi: https://doi.org/10.2337/db18-1535-P.
20. Al-Shakour AA, Khalid HA, Naser NA, et al. Serum netrin-1 level and insulin resistance in type 2 diabetes mellitus. Anaesth Pain Int Care. 2024; 28(2):

341-6. doi: https://doi.org/10.35975/apic.v28i2.2422.

21. Ramesh G, Krawczeski CD, Woo JG, e al. Urinary netrin-1 is an early predictive biomarker of acute kidney injury after cardiac surgery. Clin J Am Soc Nephrol. 2010; 5(3): 395-401. doi: 10.2215/CJN.05140709.

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