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Association of Serum Netrin-1 Level with Metabolic Syndrome Components in Gorgan

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Abstract: **Introduction:** Metabolic syndrome (MetS) is a complex and multi-factorial condition that affects millions of people worldwide. Netrin-1 may have an important function in metabolic processes. **Aim:** There are controversial studies about the role of netrin-1 in MetS. Therefore, the aim of this study was to assess the serum level of netrin-1 and its association with MetS components in our study subjects in this area. **Materials and Methods:** The National Cholesterol Education Program, Adult Treatment Panel III (NCEP, ATP III) criteria were used to include individuals with MetS. **Results:** Waist circumference, Systolic blood pressure, diastolic blood pressure, fasting blood glucose(FBG), Triglyceride (TG), High density lipoprotein cholesterol (HDL-C) and netrin-1 were significantly higher in subjects with MetS than those without MetS (Except HDL-C). FBG and TG were significantly higher in type 2 diabetic patients with MetS than those without MetS (Except HDL-C). Netrin-1 showed a significant positive correlation with FBG in type 2 diabetic patients without MetS. **Conclusion:** This study may be provided that a future research should focus on investigating the potential therapeutic role of netrin-1 in the management and prevention of T2DM, and its potential impact on the diagnosis and management of this condition should not be overlooked.

Key Words: Netrin-1, Metabolic Syndrome, Components, Gorgan

I. INTRODUCTION

Metabolic syndrome (MetS) is a complex and multi-factorial condition that affects millions of people worldwide. It is characterized by a cluster of metabolic abnormalities, including insulin resistance, obesity, dyslipidemia, and hypertension [1]. Many researchers believe that MetS is a precursor to cardiovascular diseases and type 2 diabetes. People with syndrome MetS have double and five times the risk of developing cardiovascular disease and type 2 diabetes mellitus (T2DM) when compared to healthy people [2], [3], and due to its high prevalence, it has attracted the attention of many researchers. The prevalence of MetS varies from 7 to 58 percent depending on age, gender, race/ethnicity, and diagnosis criteria [4]–[6]. This difference may be explained by race and multiple definitions of this syndrome [7], [8]. Until now, the main cause of MetS has not been fully determined, but it is believed that hyperinsulinemia and insulin resistance may play a major role in its mechanism. Although insulin resistance plays an important role in MetS, not all people who have insulin resistance have MetS, and this indicates that other factors are also involved in MetS, which include obesity, especially abdominal obesity is involved in the metabolic pathophysiological framework [9], [10].

Netrin-1 plays a critical role in the development of the nervous system. However, recent research has demonstrated that netrin-1 also has important functions outside the nervous system, including its involvement in metabolic processes [11]. Numerous research studies have investigated the role of Netrin-1 in MetS [12]. The netrin family is extracellular proteins related laminin. This protein includes netrin-1, -3, and -4 and double membrane peptides attached to glycosyl phosphatidylinositol. Netrin-1 is the first known protein of this family [13], [14]. Netrin-1 was the first Netrin member to be identified and prominently presented. This peptide is made up of almost six hundred amino terminal residues, followed by three laminin type epidermal growth factor replications and a carboxy-terminal domain [15], [16].There is a number of studies showed the role of netrin in various pathologies including rapid detection of diabetes-related cardiovascular diseases [17]. Some study indicated that netrin-1 may be used as a new biomarker for early detection of T2DM. Increased serum netrin-1 level was considered in type 2 diabetic patients compared to the control group, and also serum netrin-1 levels had a significant positive correlation with fasting blood glucose while a statistically negative correlation was found between netrin-1 and high density lipoprotein cholesterol

(HDL-C). Serum netrin-1 levels may be independently linked with the presence of T2DM [18]. Liu et al suggested that the serum netrin level in type 2 diabetic patients was significantly lower compared with controls. In addition, netrin-1 was found to be negatively correlated with plasma glucose and triglyceride (TG). Netrin-1 may affect the metabolism of cholesterol and triglycerides, further contributing to dyslipidemia in individuals with MetS [19]. Several studies have suggested that netrin-1 may play a role in the development of obesity and its related conditions [20], [21].

Some findings indicate that netrin-1 may play a role in regulating blood pressure. A study conducted on mice found that the loss of netrin-1 leads to high blood pressure [22]. It suggests that netrin-1 may play a role in lipid metabolism and could potentially be involved in the development of abnormal lipid levels [23]. Overall, these findings suggest that netrin-1 may be involved in the regulation of MetS components and may play a role in the development of some diseases associated with MetS. According to contradictory findings in netrin-1 level and its relation to some metabolite alteration, we supposed that netrin-1 may be different in subjects with and without MetS in this area. Therefore, the aim of this study was to assess the serum level of netrin-1 and its association with MetS components in our study subjects.

II. MATERIALS AND METHODS

The study was done at the Metabolic Disorder Research Center of Gorgan, Golestan province (Southeast of Caspian Sea). The ethical committee of Golestan University for medical sciences approved the study (IR.GOUMS.REC.1402.037). A written consent was obtained from all subjects after being informed about the study aims and procedures. Ninety subjects with MetS were compared with ninety age-matched who did not have MetS. A five ml of blood was provided from each subject at Gorgan non-governmental laboratory and serum collected from all subjects. An ELISA (Enzyme-linked Immunosorbent Assay) kit was used to measure the levels of Netrin-1 (Catalog No: YLA1764HU, LOT: YL8238761648). The MetS criteria such as fasting blood glucose (FBG), high density lipoprotein-cholesterol (HDL-C) and triglycerides (TG) (all measured using commercial kits). A digital blood pressure monitor was used to measure systolic and diastolic blood pressures (Omron 70JCP; Omron Maussaka, Mie-Ken, Japan). A tape in centimeters was used to measure waist circumference (WC). Waist circumference (WC) was determined midway between the iliac crest and the lower rib. Body mass index (BMI) (units of kg/m2) was determined using the formula: Weight (in kilograms, kg) / body height (in meters, m) exponent of 2. In order to identify those subjects with and without MetS, we applied NCEP's ATP III method to identify the MetS status of participants [24]. The National Cholesterol Education Program, Adult Treatment Panel III (NCEP, ATP III) criteria were used to include individuals with MetS. Metabolic syndrome was determined if the subjects had any three or more of the following criteria:

1) WC: >102 cm (male), >88 cm (female)
2) TG levels: >150 mg / dl
3) HDL-cholesterol levels: <40 mg / dl (male), <50 mg / dl (female)
4) Blood pressure: >130/85 mmHg
5) Fasting blood glucose levels: >110 mg / dl

A. STATISTICAL ANALYSIS

The data of this study was analyzed using the software SPSS 22.0. An unpaired sample t-test was used to compare the variables between the two groups. Pearson's test was used to determine the correlation between the groups with normal distribution. The Spearman test was also employed as a method to determine the correlation between groups that did not have normal distributions. Normality check was assayed by Kolmogrov-Smirnov test. Results were expressed as mean $\pm$ standard deviation. P-values < 0.05 were considered statistically significant.

III. RESULT

Table 1 shows demographic and biochemical characteristics of subjects with and without MetS. The study included ninety subjects with MetS (47.16 $\pm$13.67 years) and age matched ninety subjects without MetS (48.91 $\pm$8.05 years). WC, FBG, SBP, DBP, FBG,TG and netrin-1 were significantly higher in subjects with MetS than those without MetS (Except HDL-C).

Table 2 shows demographic and biochemical characteristics of type 2 diabetic patients with and without MetS (48.52$\pm$5.50 years and 48.91 $\pm$8.05 years, respectively). FBG and TG were significantly higher in type 2 diabetic patients with MetS than those without MetS (Except HDL-C). Netrin-1 showed no significant differences in both groups.

Table 3 shows the correlation of netrin-1 level with MteS components in subjects with and without MetS. Netrin-1 level did not show any significant correlation with MteS components in both subjects.

P-value < 0.05 was significant. T2DM: Type diabetes mellitus, MetS: Metabolic syndrome, BMI: body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol.

The correlation between netrin-1 level and MteS components in type 2 diabetic patients with and without MetS is shown in Table 4. There were no correlations between netrin-1 and MteS components in both groups. However, netrin-1 showed a significant positive correlation with FBG in type 2 diabetic patients without MetS.

P-value < 0.05 was significant. T2DM: Type diabetes mellitus, MetS: Metabolic syndrome, BMI: body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol.

Parameters	Subjects with MetS (n= 90)	Subjects without MetS (n=90)	P-value
Age (Year)	47.16 ±13.67	48.91 ±8.05	0.295
BMI (Kg/m2)	26.43± 3.92	26.53 ± 4.20	0.869
WC (cm)	106.20 ± 12.85	99.20 ± 11.15	<0.001
SBP (mmHg)	131.20 ± 10.71	116.10 ± 12.44	<0.001
DBP(mmHg)	88.30 ± 15.18	76.87± 13.85	<0.001
FBG (mg/dl)	136.14 ± 14.63	95.81 ± 13.42	<0.001
TG (mg/dl)	164.25 ± 37.92	109.28 ± 24.04	<0.001
HDL-C (mg/dl)	45.22 ±7.90	50.29 ±7.85	0.01
Netrin-1 (pg/ml)	420.9 ± 9.42 and	345.4 ± 10.41	<0.001

TABLE 1: Demographic and biochemical characteristics of subjects with and without MetS [P-value < 0.05 was significant. MetS: Metabolic syndrome, BMI: body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol.]

Parameters	T2DM patients with MetS (n= 36)	T2DM pateints without MetS (n=54)	P-value
Age (Year)	48.52±5.50	46.94 ±9.22	0.387
BMI (Kg/m2)	25.68± 4.11	24.98± 2.04	0.267
WC (cm)	107.69 ± 6.91	101.94 ± 8.68	0.127
SBP (mmHg)	135.97 ± 17.02	124.70 ± 14.19	0.219
DBP(mmHg)	88.33 ± 12.28	82.92± 10.44	0.662
FBG (mg/dl)	152.61± 38.29	117.17±31.66	<0.001
TG (mg/dl)	187.94 ±75.04	152.91±62.67	0.009
HDL-C (mg/dl)	38.27 ±7.66	49.55 ±8.21	0.032
Netrin-1 (pg/ml)	428.78 ± 17.84	402.24± 11.84	0.085

TABLE 2: Demographic and biochemical characteristics of type 2 diabetic subjects with and without MetS [P-value < 0.05 was significant. T2DM: Type diabetes mellitus, MetS: Metabolic syndrome, BMI: body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol.]

Parameters	Subjects with MteS		Subjects without MteS	
	r	P-value	R	P-value
Age (years)	0.062	0.606	0.044	0.705
BMI (Kg/m^2)	0.125	0.303	0.172	0.138
WC (cm)	0.072	0.514	0.069	0.563
SBP (mmHg)	0.052	0.674	0.158	0.179
DBP(mmHg)	0.116	0.336	0.025	0.832
FBG (mg/dl)	0.001	0.993	0.183	0.113
TG (mg/dl)	-0.098	0.427	- 0.309	0.09
HDL-C (mg/dl)	-0.146	0.236	0.146	0.229

TABLE 3: Correlation of netrin-1 level with MteS components in subjects with and without MetS

Parameters	T2DM patients with MetS		T2DM patients without MetS	
	r	P-value	R	P-value
Age (years)	0.058	0.588	0.007	0.970
BMI (Kg/m^2)	-0.084	0.443	0.057	0.752
WC (cm)	0.048	0.655	-0.102	0.598
SBP (mmHg)	0.121	0.254	-0.900	0.612
DBP(mmHg)	0.183	0.084	0.026	0.884
FBG (mg/dl)	-0.011	0.921	0.350	0.042
TG (mg/dl)	-0.122	0.251	-0.107	0.565
HDL-C (mg/dl)	-0.052	0.623	-0.104	0.563

TABLE 4: Correlation of netrin-1 level with MteS components in type 2 diabetic patients with and without MetS

IV. DISCUSSION

Several clinical studies have investigated the association between serum netrin-1 levels and MetS. These studies have provided valuable insights into the potential use of netrin-1 as a biomarker for the condition. Present study showed that netrin-1 was significantly higher in subjects with MetS than those without MetS (Table 1). Netrin-1 showed no significant differences in type 2 diabetic patients in both mentioned groups (Table 2). Netrin-1 level did not show any significant correlation with MetS components in subjects with and without MetS (Table 3).

However, our present study showed that there is a significant positive correlation between netrin-1 and FBG in type 2 diabetic patients without MetS (Table 4). The findings are not in agreement with the results of Liu et al, which reported that netrin-1 level was significantly low in T2DM patients compared to controls 19. A study indicated the relationship between netrin-1 levels in type 2 diabetes and its role as a new biomarker in pathogenesis of some disease in Egyptian Patients. The results of their study showed that the level of netrin-1 in the type 2 diabetic patients was significantly increased compared to the control group and netrin-1 showed a strong positive correlation with FBG in type 2 diabetic patients [25], which this correlation was in accordance with our finding. Another study revealed that the netrin-1 level was significantly lower in the diabetic subjects compared to control group. There were also significantly higher level of TG and lower HDL-C levels in diabetic individuals [26].

Another finding was shown the relationship between prognostic parameters of type 2 diabetes and serum intrin-1 levels. Their study showed that serum netrin-1 levels were significantly lower in control subjects compared to T2DM. Netrin-1 serum level had a significant positive correlation with FBG. Their results also showed that the serum netrin-1 elevation is significantly connected to the occurrence of T2DM [12], which were in accordance with our results [12], [26], while other study was indicated that there was no significant difference in netrin-1 levels among controls and diabetic patients [27], which was in accordance with our finding. Study of Liu C et al. on patients with T2DM evaluated the netrin-1 level and the netrin-1 role as an important factor in the T2DM development. The results showed that the level of netrin-1 in healthy subjects was significantly higher than that of T2DM patients. The level of netrin-1 was associated with FBG. Plasma levels of netrin-1 have decreased in T2DM patients and there was a negative correlation between netrin-1 levels and glucose homeostasis [19], which was not in accordance with our finding. Wang et al. reported that serum netrin-1 levels were elevated in subjects with MetS and were positively associated with waist circumference, fasting blood glucose, and lipid parameters. The study suggested that netrin-1 may be involved in the development of MetS through its effects on glucose and lipid metabolism [28], which was in agreement with our results except for FBG correlation. We found that the serum level of netrin-1 may be a sensitive indicator for type 2 diabetes. In addition, serum netrin-1 levels were significantly positively correlated with FBG. The mean levels of netrin-1 were 428.78$\pm$17.84 pg/ml and 402.24$\pm$11.84 pg/ml in type 2 diabetic patients with and without MetS, respectively. According to Natura et al study, netrin-1 is capable of influencing insulin secretion and contributing to β -cell dysfunction [29]. Some other studies have been indicated that netrin-1 may play an important function in pancreatic development [30]. Netrin may inhibit pancreatic epithelial cell adhesion and migration [31]. De Breuck et al. revealed that netrin-1 is secreted in the pancreas and plays important role in pancreatic morphogenesis in the regenerating pancreas. The reduction of apoptotic β-cells and netrin-1 secretion in the injured is significantly decreased in patients with newly diagnosed T2DM by encouraging β -cells. Their study also demonstrated that netrin-1 enhances pancreatic islet cell mass and density in T2DM. They found also that netrin-1 possibly has a protective role in β-cell to delay the progression of this disease [32]. Some other studies revealed that netrin is expressed in vascular epithelium [33]. The hyperglycemic condition may decrease vascular netrin level in diabetes which resulted in a reduced endothelial function where as the highest expression of vascular netrin in hyperglycemic animal prevented diabetes-induced endothelial impairment [34].Therefore, we believe that the unchanged netrin-1 level in type 2 diabetic patients probably was a reflection of the diabetes and not MetS. In addition, there were positive correlations only between serum netrin-1 and FBG in our study. The data suggests that diabetes appears to be an effective factor for netrin-1 excretion. Thus, the findings of this study confirmed our hypothesis and showed that netrin-1 levels are increased in subjects with MetS when compared those without MetS, while it is not confirmed for type2 diabetic patients. However, more studies are required to investigate the relationship between netrin-1 and type 2 diabetes. However, there is little study of changes in netrin-1 levels during the development of diabetes and MetS, and the role of netrin-1 in the patho-physiology of MetS and type 2 diabetes are not exactly clear. There are several distinguishing aspects of this study. First, we identified that serum netrin-1 levels were not as a possible predictor of MetS. When we compared netrin-1 levels in type 2 diabetic patients with and without MetS, we found that the levels of netrin-1 were not changed in patients with type 2 diabetes with MetS compared with those without MetS. Moreover, investigation of the mechanism of action between netrin-1 level and pathogenesis of FBG in type 2 diabetic patients is needed to further our understanding.

V. CONCLUSION

We showed that increased serum netrin-1 levels are significantly associated with FBG in type 2 diabetic patients. This study provided valuable insights into the underlying the development of T2DM and identified netrin-1 as a possible potential therapeutic target for diabetic people. However, this study may be provided that a future research should focus on investigating the potential therapeutic role of netrin-1 in

the management and prevention of T2DM, and its potential impact on the diagnosis and management of this condition should not be overlooked.

DATA AVAILABILITY STATEMENT

All of the data is included in the article/Supplementary Material.

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COMPETING INTERESTS

Authors state no conflict of interest.

INFORMED CONSENT

Informed consent was obtained from all individuals included in this study.

ETHICAL APPROVAL

The Golestan University of Medical Sciences Ethics Committee (Ethic number: (IR.GOUMS.REC.1402.037) was approved this study, according to the Declaration of Helsinki and Good Clinical Practice guidelines.

AUTHOR CONTRIBUTIONS

All authors have accepted responsibility and approved its submission. A.M. Conceived and designed the experiments. A.M. Analyzed and interpreted the data and wrote the article. H.A. S.A.K. and M.D. Performed the experiments. M.T. Analyzed and interpreted the data.

DECLARATION OF STATEMENT

There is nothing to disclose to add a statement.

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