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ORIGINAL RESEARCH

Plasma Nesfatin-I: Potential Predictor and Diagnostic Biomarker for Cognitive Dysfunction in T2DM Patient

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Purpose: Nesfatin-1 plays a crucial role in glucose metabolism and cognitive function. This study aimed to investigate the correlation between plasma nesfatin-1 levels and clinical indicators and cognitive function in patients with type 2 diabetes mellitus (T2DM).

Methods: Demographic and medical history data, physical examination, and biochemical test results of 132 T2DM patients were collected. The plasma concentrations of nesfatin-1, C-reactive protein (CRP), interleukin-6 (IL-6), soluble triggering receptors expressed on myeloid cells 1 (sTREM1), and sTREM2 in T2DM patients were measured. Cognitive function was evaluated using the Behavior Rating Inventory of Executive Function-Adult (BRIEF-A). The patients were divided into two groups: a low-nesfatin-1 group ($n = 75$) and a high-nesfatin-1 group ($n = 57$) based on a plasma nesfatin-1 concentration less than or above the 50th percentile value of all the samples.

Results: The results showed that plasma HbA1c levels were positively correlated with CRP, IL-6, sTREM1, and sTREM2 levels in patients with T2DM ($P < 0.05$). Plasma nesfatin-1 concentrations were positively associated with diabetes-related biochemical indicators including glycated haemoglobin (HbA1c), insulin, and homeostatic model assessment of insulin resistance (HOMA-IR), and inflammation-related indicators including CRP, IL-6, sTREM1, and sTREM2 among patients with T2DM ($P < 0.05$). Moreover, T2DM patients with high nesfatin-1 levels showed higher HbA1c and fasting plasma glucose (FPG) levels ($P < 0.05$). Furthermore, T2DM patients with high nesfatin-1 levels also showed higher BRIEF-A scores ($P = 0.01$). Additionally, T2DM patients with high total scores of BRIEF-A (scores $>$ 50th percentile) could be identified with a sensitivity of 59.1% and a specificity of 72.7% by nesfatin-1.

Conclusion: These findings indicate that plasma nesfatin-1 might be involved in the T2DM-associated comorbidities and the development of cognitive dysfunction, and the mechanism underlying this involvement is related to the imbalance in the expression of CRP, IL-6, sTREM1, and sTREM2 levels.

Keywords: type 2 diabetes mellitus, cognitive dysfunction, nesfatin-1, inflammatory response, sTREM1/2

Introduction

Epidemiological data shows that the estimated global direct health expenditure on diabetes was USD 760 billion in 2019 and is projected to increase to USD 825 billion by 2030 and USD 845 billion by 2045.¹ Cognitive dysfunction is increasingly considered a significant comorbidity and complication of diabetes, affecting personal health and diabetes management.² It has been reported that the prevalence of cognitive dysfunction, including dementia and cognitive impairment,

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among people with diabetes was 13.1% for individuals aged 65 to 74 years and 24.2% for those aged 75 years and older.³ Moreover, recent guidelines strongly recommend screening for cognitive dysfunction in elderly patients with type 2 diabetes mellitus (T2DM).⁴ Several possible mechanisms contributing to cognitive decline, including vascular damage, negative consequences of hypo- and hyperglycemia, and various dysfunctions in insulin action, summarized as insulin resistance, have been proposed.⁵ However, the exact mechanism that links T2DM and cognitive dysfunction, as well as predictors of T2DM-associated cognitive dysfunction remain unclear.

Nesfatin-1 is an energy-regulating peptide that is widely expressed in the central and peripheral tissues. Apart from its crucial role in regulating glucose homeostasis,⁶ lipid metabolism,⁷ gastrointestinal,⁸ and cardiovascular function,⁹ nesfatin-1 is reported to be associated with neuropsychiatric diseases, including epilepsy,¹⁰ stress,¹¹ sleep disorders,¹² anxiety,¹³ and depression.¹⁴ In line with these findings, the results of our previous studies showed that increased nesfatin-1 concentration in stressed or high-fat diet-fed rats,^{15,16} and intraperitoneal injection of nesfatin-1 induces depression-like behavior together with the BDNF-associated alteration of synaptic plasticity.¹⁷ Thus, suggesting that nesfatin-1 may play a role in linking glucolipid dysfunction and neuropsychiatric injuries.

Recent studies have found aberrant expressions of nesfatin-1 in the blood of patients with diabetes-related diseases. Although it has been reported that the plasma nesfatin-1 level was significantly decreased in patients with T2DM compared with healthy controls,¹⁸ increasing evidence has indicated a higher level of blood nesfatin-1 in T2DM patients and a close relationship between nesfatin-1 concentration and clinical parameters known to be associated with insulin resistance.^{19–21} These findings suggest a potential role of nesfatin-1 in T2DM.

Inflammation is a common symptom or complication in both T2DM and neuropsychiatric diseases, including cognitive dysfunction.^{22,23} A number of studies have shown a relationship between inflammation markers and increased risks for dementia, mild cognitive impairment, and cognitive decline.^{24,25} It has been reported that serum IL-6 concentrations were significantly high in patients with mild cognitive impairment, indicating that inflammatory factors may be involved in the early stages of Alzheimer's disease (AD).²⁶ Recently, epidemiological studies have demonstrated that CRP and IL-6 are

major risk factors for mild cognitive impairment in patients with T2DM, suggesting an association between inflammatory markers and the risk of mild cognitive impairment in T2DM patients.^{27,28} In our previous study, rats injected with nesfatin-1 presented not only anxiety and depression-like behavior, but also increased plasma concentrations of CRP and IL-6, with a significant correlation.¹⁷ Consistently, results of clinical studies also demonstrated a positive correlation between plasma nesfatin-1 and IL-6 or CRP concentrations in patients with depression.²⁹ Therefore, it is assumed that the underlying mechanism of nesfatin-1 in the neuropsychiatric injury of T2DM might be involved in regulating the inflammatory response.

As members of the triggering receptors expressed on myeloid cells (TREM) family, TREM1 and TREM2 participate in the regulation of inflammation and immune response by modulating the secretion of inflammatory cytokines such as TNF- α , IL-1 β , and IL-6.³⁰ Recent studies have indicated that the missense mutation and expression changes of TREM1 and TREM2 are closely related to cognitive dysfunction in patients with AD.^{31,32} Clinical studies have shown that the level of TREM2 in the peripheral blood of patients with AD increased significantly.³³ Animal experiments have found that knock-down of TREM2 expression in the brains of SAMP8 mice, a rapidly aging animal model, can cause neuronal and synaptic losses and cognitive impairment, accompanied by increased secretion of inflammatory factors such as TNF- α and IL-6.³⁴ Our recent study has shown that abnormal expression of TREM1 and TREM2 in the hippocampus and prefrontal cortex may be involved in lipopolysaccharide (LPS)-induced depression-like behaviors and impaired learning and memory in rats.³⁵ Collectively, these findings suggest that TREM1 and TREM2 are involved in the regulation of cognitive impairment, which may be related to inflammation. Considering that nesfatin-1 exhibits anti-inflammatory properties,³⁶ the plasma concentrations of TREM1 and TREM2 and its correlation between plasma nesfatin-1 were investigated in patients with T2DM in the present study.

Materials and Methods

Study Subjects

A total of 132 patients diagnosed with T2DM were recruited consecutively from the Department of Endocrinology, the Fourth Affiliated Hospital of Anhui Medical University, from April 2017 to April 2018. All diabetic subjects met Guidelines for the Prevention and

Treatment of Type 2 Diabetes in China (2017 edition) based on the World Health Organization diagnostic criteria in 1999 with a fasting plasma glucose ≥ 7.0 mmol/l or a 2-h plasma glucose ≥ 11.1 mmol/l, determined by oral glucose tolerance cation barriers. The inclusion criteria were as follows: 1) patients diagnosed with T2DM based on the World Health Organization diagnostic criteria in 1999; 2) adult patients older than 18 years; 3) currently not receiving any medication (blood was collected when the patient was admitted to the hospital for treatment); and 4) no language communication barriers and could actively cooperate with the completion of the evaluation. The exclusion criteria were as follows: 1) suffering from acute diabetic complications, such as diabetic ketoacidosis and severe hypoglycemia coma; 2) suffering from acute cardiovascular and cerebrovascular events, epilepsy, head injury, moderate depression, or other psychiatric illnesses; 3) severe systemic disease (ie, thyroid disease, serious infection, and anemia); and 4) diagnosed with alcohol or other substance-dependent diseases. The study protocol was approved by the ethical committee of Anhui Medical University (No. 20170245). All subjects provided written informed consent in accordance with the Declaration of Helsinki.

Measurements

The height and weight of all subjects were measured, and body mass index (BMI) was calculated accordingly. To avoid the influence of daily energy intake on the plasma levels of nesfatin-1, all patients received the same food provided by the cafeteria of the Fourth Affiliated Hospital of Anhui Medical University. To avoid the influence of fasting time, blood samples were collected between 8:00 a.m. and 9:00 a.m. from the participants' veins after a fasting period of 10 to 11 h. The blood samples were centrifuged at 2500 rpm for 20 min at 4°C, and the plasma was collected and stored at -80°C until detection. The concentrations of nesfatin-1, CRP, IL-6, sTREM1, sTREM2, and insulin were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer's instructions (nesfatin-1, CRP, and IL-6: Huamei Bio, Wuhan, China; and sTREM1, sTREM2, and insulin: Meilian Bio, Shanghai, China). Plasma concentrations of total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein cholesterol (LDL), alanine aminotransferase (ALT), aspartate transaminase (AST), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), serum creatinine (SCR), blood urea nitrogen (BUN), fasting plasma

glucose (FPG), postprandial glucose (PPG); blood samples taken to measure PPG 2 h after breakfast, and glycated hemoglobin (HbA1c), were measured by the Department of Clinical Laboratory of the Fourth Affiliated Hospital of Anhui Medical University.

Self-Reported Executive Functions with Behavior Rating Inventory of Executive Function-Adult (BRIEF-A)

BRIEF-A was used to evaluate cognitive function in T2DM patients. BRIEF-A includes two composite scores, including sets of subscales. Specifically, the inhibit, shift, self-monitor, and emotional control subscales were used to calculate the behavioral regulation index (BRI). The initiate, plan/organize, working memory, organization of materials, and task monitor subscales were used to calculate the metacognition index.

Statistical Analysis

Data were entered into EpiData version 3.1, and analyzed using SPSS version 17.0 statistical software. A one-sample Kolmogorov-Smirnov test showed a normal distribution of continuous variables. The statistical results of the continuous variables are presented as means \pm standard deviation (SD) for normal distribution and medians (first quartile to third quartile) for abnormal distribution. For variables with abnormal distribution, non-parametric tests were applied (Mann-Whitney U-test), and parametric tests were used for normal distribution (Student's t-test). The chi-square test was used for dichotomous variables. Power calculations were performed using G* Power 3.1 software. Correlation analysis was performed using Pearson's test or partial correlational analysis. Receiver operating characteristic (ROC) curve analysis was used to determine the area under the curve (AUC) and cut-off values of nesfatin-1 in the identification of T2DM patients with high total scores of BRIEF-A (scores $>$ 50th percentile) from T2DM patients with low total scores of BRIEF-A (scores \leq 50th percentile). Statistical significance was set at $P < 0.05$.

Results

Comparison of Scales of BRIEF-A

Between the HbA1c $\leq 7\%$ Group and the HbA1c $> 7\%$ Group

According to the recommendation that a target HbA1c goal of $< 7\%$ for patients with diabetes should not be higher than 7% to avoid diabetes-related complications, the patients were

divided into two groups according to HbA1c < 7% (HbA1c ≤ 7%) or above (HbA1c > 7%). There were 32 patients in the HbA1c ≤ 7% group and 100 patients in the HbA1c > 7% group. Table 1 shows that the total scales and all the terms of BRIEF-A were significantly higher in the HbA1c > 7% group than in the HbA1c ≤ 7% group ($P < 0.05$).

Post hoc power analyses using G*power 3.1, demonstrated that sufficient power was available to distinguish the above significant differences (BRI: power = 0.98; inhibit: power = 0.97; shift: power = 0.69; emotional control: power = 0.85; self-monitor: power = 0.86; metacognition index: power = 0.96; initiate: power = 0.93; plan/organize: power = 0.95; organization of materials: power = 0.92; task monitor: power = 0.95; and total scores: power = 0.99).

As presented in Figures 1 and 2A–D, the results of correlation analysis showed that the plasma HbA1c concentration was positively correlated with CRP ($r = 0.237$, $P = 0.006$), IL-6 ($r = 0.216$, $P = 0.013$), sTREM1 ($r = 0.211$, $P = 0.015$), and sTREM2 ($r = 0.189$, $P = 0.030$) levels in patients with T2DM. Additionally, the plasma HbA1c concentration was positively correlated with the total scores of BRIEF-A ($r = 0.269$, $P = 0.002$), BRI ($r = 0.201$, $P = 0.021$), and metacognition index ($r = 0.265$, $P = 0.002$) (Figures 1 and 2E–G).

Relationship Between Plasma Nesfatin-I Levels and Biochemical Indicators, Inflammatory Cytokines, or BRIEF-A in Patients with T2DM

As shown in Figures 1 and 3, the results of the correlation analysis showed that plasma nesfatin-1

concentration was positively correlated with HbA1c ($r = 0.184$, $P = 0.035$), insulin ($r = 0.234$, $P = 0.007$), homeostatic model assessment of insulin resistance (HOMA-IR) ($r = 0.244$, $P = 0.005$), CRP ($r = 0.535$, $P < 0.001$), IL-6 ($r = 0.627$, $P < 0.001$), sTREM1 ($r = 0.352$, $P < 0.001$), and sTREM2 ($r = 0.316$, $P < 0.001$) in patients with T2DM.

As shown in Figure 1, no correlation was observed between nesfatin-1 levels and cognitive function ($P > 0.05$). Because age and nutrition are factors influencing cognitive function, a partial correlational analysis was used to explore the relationship between nesfatin-1 levels and cognitive function after controlling for age and BMI as covariates. The correlation between nesfatin-1 level and cognitive function was still not statistically significant ($P > 0.05$).

Comparison of Demographic Values and Biochemical Indicators of the Low-Nesfatin-I Group and the High-Nesfatin-I Group

The patients were divided into two groups: a low-nesfatin-1 group, and a high-nesfatin-1 group based on the plasma nesfatin-1 concentration less than or above the 50th percentile value of all the samples. There were 75 and 57 patients in the low-nesfatin-1 group and in the high-nesfatin-1 group, respectively. As shown in Table 2, there were no significant differences in age, BMI, waist-hip ratio, year of diagnosis of diabetes, sex, or smoking between the two groups. In terms of the biochemical indicators, there were no

Table 1 Comparison of Scales of BRIEF-A Between the HbA1c≤7% Group and the HbA1c>7% Group

BRIEF-A	HbA1c ≤ 7% Group	HbA1c > 7% Group	Statistics (t)	P-value	Power
Behavioral regulation index	194.38±21.26	217.65±32.22	-4.70	<0.001	0.98
Inhibit	46.81±5.73	53.38±10.11	-4.59	<0.001	0.97
Shift	51.97±8.98	56.95±10.29	-2.46	0.02	0.69
Emotional control	49.13±6.66	55.19±11.81	-3.64	<0.001	0.85
Self-monitor	46.47±7.64	52.13±10.06	-3.36	0.001	0.86
Metacognition index	257.00±40.79	293.90±54.95	-3.50	0.001	0.96
Initiate	51.38±11.14	60.84±14.81	-3.32	0.001	0.93
Working memory	61.69±12.80	67.25±13.99	-2.00	0.05	
Plan/organization	52.56±10.03	61.74±14.46	-4.01	<0.001	0.95
Organization of materials	42.75±6.29	48.29±9.12	-3.85	<0.001	0.92
Task monitor	48.63±7.70	55.78±11.16	-4.07	<0.001	0.95
Total scores	451.38±56.46	511.55±76.99	-4.08	<0.001	0.99

Note: Bold results are statistically significant.

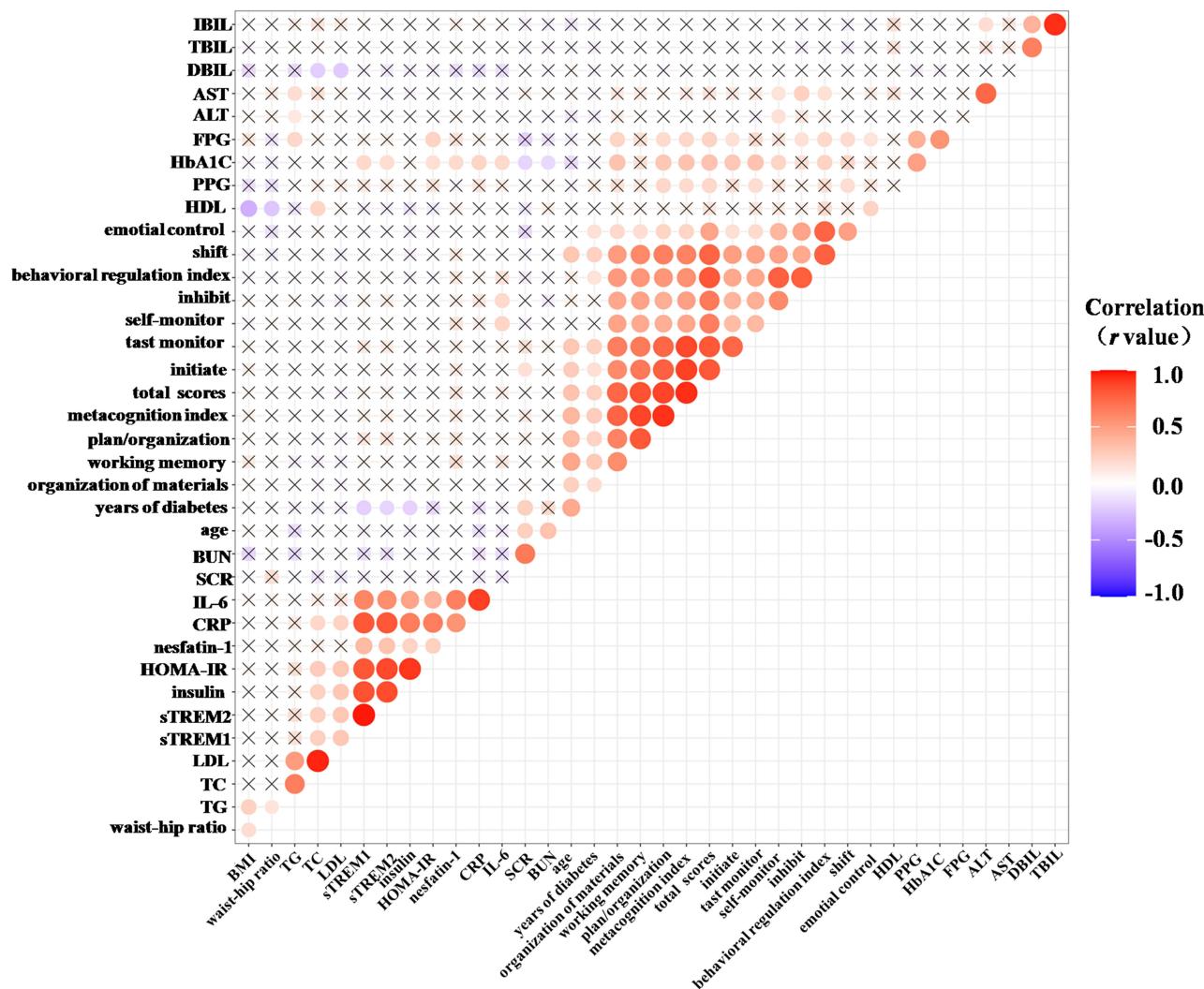


Figure 1 Correlation between plasma HbA1c or nesfatin-1 levels and biochemical indicators, inflammatory cytokines, or BRIEF-A in patients with T2DM.

Note: The size of the circle represents the size of the *P* value; the symbol X represents no statistical significance.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; BUN, blood urea nitrogen; CRP, C-reactive protein; DBIL, direct bilirubin; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; IBIL, indirect bilirubin; IL-6, interleukin-6; LDL, low-density lipoprotein cholesterol; PPG, postprandial glucose; SCR, serum creatinine; sTREM1, soluble triggering receptors expressed on myeloid cells 1; TBIL, total bilirubin; TC, total bilirubin; TG, triglyceride.

significant differences in TC, TG, HDL, LDL, ALT, AST, TBIL, DBIL, IBIL, PPG, SCR, BUN, insulin, or HOMA-IR between the two groups. The mean HbA1c ($t = -2.07$, $P = 0.04$) and FPG ($t = -2.43$, $P = 0.02$) levels were significantly higher in the high-nesfatin-1 group than in the low-nesfatin-1 group (Table 2). In terms of inflammation-related indicators, there were no significant differences in CRP, IL-6, sTREM1, or sTREM2 between the two groups ($P > 0.05$).

Receiver Operating Characteristic (ROC) Curve of Plasma Nesfatin-1 in Identification of T2DM Patients with High Total Scores of BRIEF-A (Scores $>$ 50th Percentile) from T2DM Patients with Low Total Scores of BRIEF-A (Scores \leq 50th Percentile)

Figure 4 shows the results of ROC curve analysis, indicating the potential diagnostic value of plasma nesfatin-1 in discrimi-

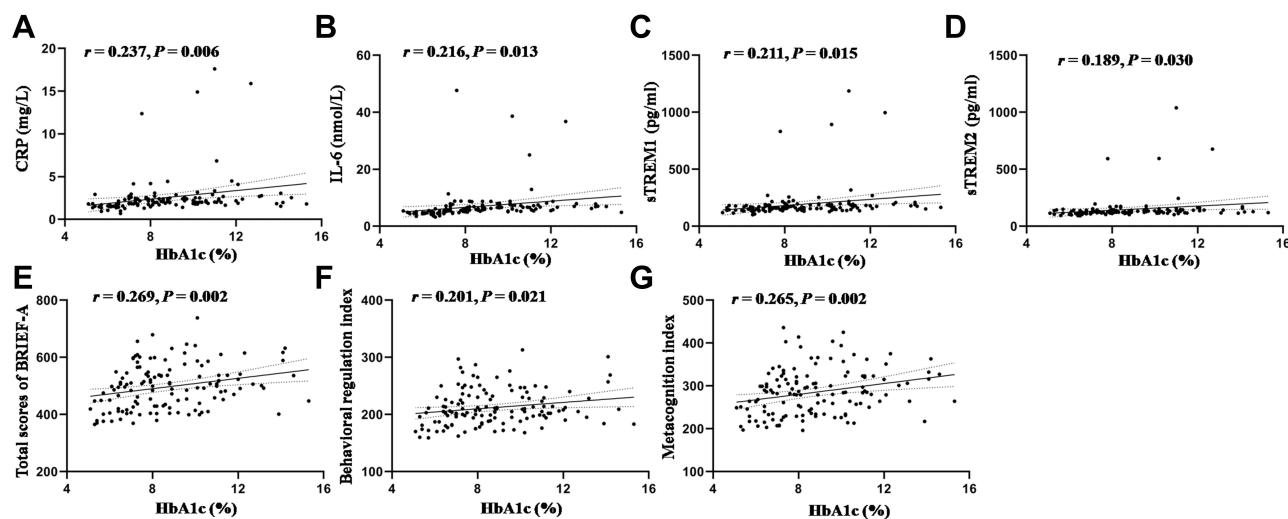


Figure 2 Positive relationship between plasma HbA1c and CRP, IL-6, sTREM1, sTREM2, or BRIEF-A in patients with T2DM.

Notes: (A) Correlation between plasma HbA1c and CRP levels; (B) Correlation between plasma HbA1c and IL-6 levels; (C) Correlation between plasma HbA1c and sTREM1 levels; (D) Correlation between plasma HbA1c and sTREM2 levels; (E) Correlation between plasma HbA1c and total scores of BRIEF-A; (F) Correlation between plasma HbA1c and the scores of behavioral regulation index; (G) Correlation between plasma HbA1c and the scores of metacognition index.

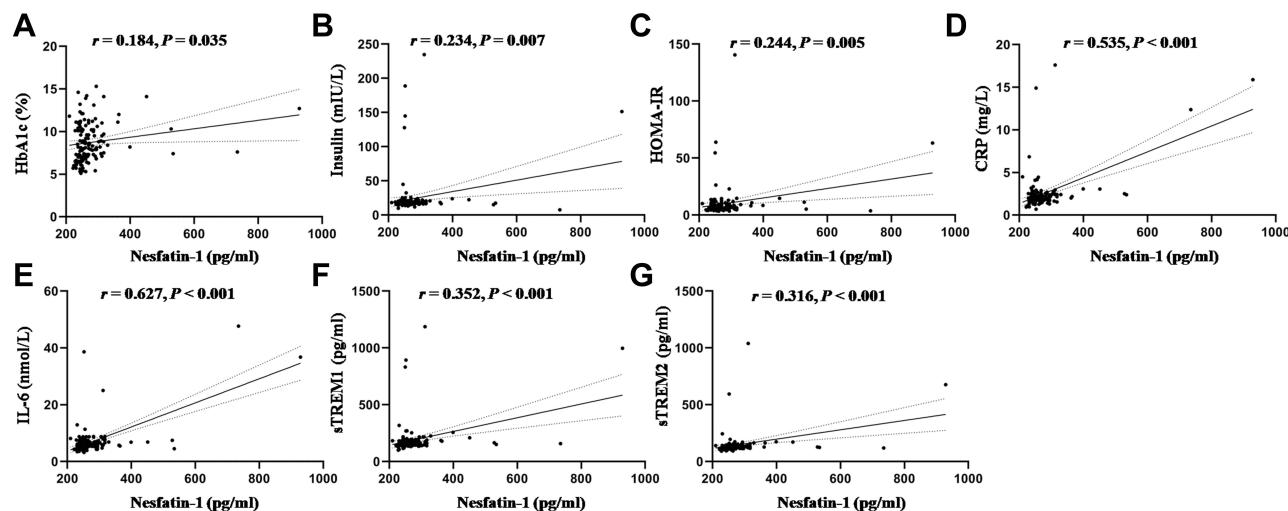


Figure 3 Positive relationship between plasma nesfatin-1 and HbA1c, insulin, HOMA-IR, CRP, IL-6, sTREM1, or sTREM2 in patients with T2DM.

Notes: (A) Correlation between plasma nesfatin-1 and HbA1c levels; (B) Correlation between plasma nesfatin-1 and insulin levels; (C) Correlation between plasma nesfatin-1 and HOMA-IR levels; (D) Correlation between plasma nesfatin-1 and CRP levels; (E) Correlation between plasma nesfatin-1 and IL-6 levels; (F) Correlation between plasma nesfatin-1 and sTREM1 levels; (G) Correlation between plasma nesfatin-1 and sTREM2 levels.

nating T2DM patients with high total scores of BRIEF-A (scores $>$ 50th percentile) from T2DM patients with low total scores of BRIEF-A (scores \leq 50th percentile). The area under the ROC curve (AUC) for nesfatin-1 was 0.663 (95% confidence interval, 0.571–0.756). Furthermore, at a cut-off nesfatin-1 value of 260.81 pg/mL, the sensitivity and specificity for discriminating T2DM patients with high total scores of BRIEF-A (scores $>$ 50th percentile) from T2DM patients

with low total scores of BRIEF-A (scores \leq 50th percentile) were 59.1% and 72.7%, respectively.

Comparison of Scales of BRIEF-A of the Low-Nesfatin-1 Group and the High-Nesfatin-1 Group

Table 3 shows that the total scales of BRIEF-A were significantly higher in the high-nesfatin-1 group than that of the low-

Table 2 Comparison of Demographic Values and Biochemical Indicators Between the Low-Nesfatin-1 Group and the High-Nesfatin-1 Group

Biochemical Indicators	Low-Nesfatin-1 Group	High-Nesfatin-1 Group	Statistics ($t/Z/\chi^2$)	P-value	Power
Age	57.77±11.37	57.95±13.59	-0.08	0.94	
BMI	24.19±3.31	24.15±3.84	0.07	0.95	
Waist-hip ratio	0.90 (0.87, 0.96)	0.92 (0.87, 0.96)	-0.69	0.49	
Year of diabetes	4 (1, 8)	6 (1, 11)	-1.24	0.21	
Gender	42/33	42/33	0.34	0.56	
Smoking	51/24	51/24	0.14	0.71	
HbA1c (%)	8.38±2.27	9.20±2.21	-2.07	0.04	0.54
TC (mmol/L)	4.75±1.52	4.93±1.31	-0.72	0.47	
TG (mmol/L)	1.76±2.16	2.17±2.01	-1.12	0.26	
HDL (mmol/L)	0.97±0.28	0.98±0.31	-0.23	0.82	
LDL (mmol/L)	3.01±1.17	3.12±1.21	-0.52	0.61	
ALT (U/L)	23.24±12.53	26.26±25.75	-0.81	0.42	
AST (U/L)	19.64±9.43	20.86±12.10	-0.65	0.52	
TBIL (umol/L)	16.69±7.68	15.68±6.78	0.78	0.43	
DBIL (umol/L)	4.59±2.61	3.79±2.03	1.91	0.06	
IBIL (umol/L)	12.26±6.09	11.89±5.54	0.35	0.72	
FPG (mmol/L)	8.65±2.90	10.07±3.77	-2.43	0.02	0.66
PPG (mmol/L)	15.72±4.40	16.79±4.61	-1.32	0.19	
SCR (umol/L)	69.26±33.60	71.33±43.13	-0.31	0.76	
BUN (mmol/L)	6.07±4.79	5.76±2.24	0.46	0.65	
Insulin (m IU/L)	23.95±27.52	24.39±33.52	-0.08	0.93	
HOMA-IR	8.80±9.14	11.46±19.36	-1.04	0.30	
CRP (mg/L)	2.34±1.75	2.83±3.04	-1.17	0.24	
IL-6 (nmol/L)	6.34±4.14	7.95±7.20	-1.62	0.11	
sTREM1 (pg/mL)	181.52±118.68	205.30±173.71	-0.93	0.35	
sTREM2 (pg/mL)	138.12±78.95	159.70±139.97	0.24	0.26	

Note: Bold results are statistically significant.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; BUN, blood urea nitrogen; CRP, C-reactive protein; DBIL, direct bilirubin; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; IBIL, indirect bilirubin; IL-6, interleukin-6; LDL, low-density lipoprotein cholesterol; PPG, postprandial glucose; SCR, serum creatinine; sTREM, soluble triggering receptors expressed on myeloid cells; TBIL, total bilirubin; TC, total bilirubin; TG, triglyceride.

nesfatin-1 group ($t = -2.53$, $P = 0.01$), with a power value of 0.71. In terms of the items, the BRI (including inhibit, shift, and self-monitor), and metacognition index (including initiate and plan/organization) were higher in the high-nesfatin-1 group ($P < 0.05$).

Discussion

In the present study, we demonstrated that the plasma HbA1c concentration was positively correlated with the CRP, IL-6, sTREM1, and sTREM2 levels in patients with T2DM, and that plasma nesfatin-1 concentrations were positively associated with diabetes-related biochemical indicators (including HbA1c, insulin, and HOMA-IR), and inflammation-related indicators (including CRP, IL-6, sTREM1, and sTREM2) among patients with T2DM. Moreover, T2DM patients with high nesfatin-1 levels showed higher HbA1c and FPG levels.

Furthermore, T2DM patients with high nesfatin-1 levels also showed higher BRIEF-A scores. Additionally, T2DM patients with high scales of BRIEF-A (scores $>$ 50th percentile) could be identified by plasma nesfatin-1. Therefore, plasma nesfatin-1 level may be considered as a biomarker to identify T2DM patients with cognitive dysfunction.

HbA1c is a convenient and stable indicator of long-term blood glucose concentrations, reflecting blood glucose levels over the last 2 to 3 months.³⁷ Both the American Diabetes Association and World Health Organization (WHO) recommended that HbA1c should be included as a diagnostic test for diabetes in addition to oral glucose tolerance test or fasting blood glucose.^{38,39} Current treatment guidelines recommend an HbA1c level of $< 7.0\%$ as the goal for most people with T2DM.^{40,41} Moreover, accumulating evidence has indicated that HbA1c levels are closely related to the occurrence and

ROC curve: ROC of nesfatin-1

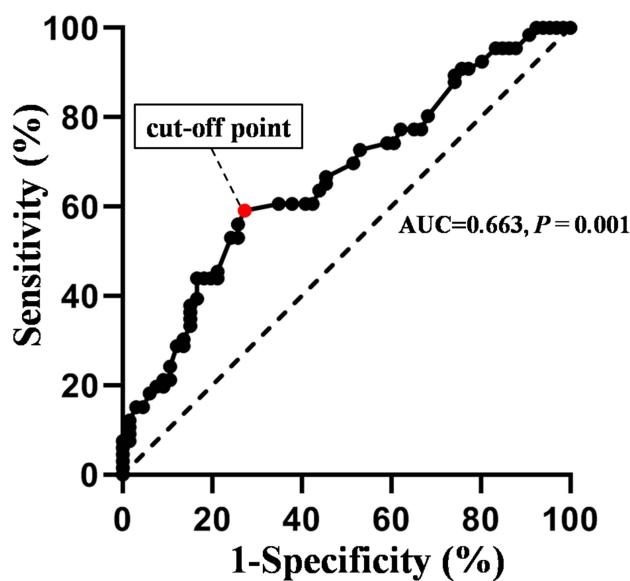


Figure 4 Receiver operating characteristic curve analysis of plasma nesfatin-1 in discriminating T2DM patients with high total scores of BRIEF-A (scores > 50th percentile) from T2DM patients with low total scores of BRIEF-A (scores ≤ 50th percentile).

Notes: Plasma nesfatin-1 cut-off point of 260.81 pg/mL showed 59.1% sensitivity and 72.7% specificity, and with the AUC of 0.663 (95% CI 0.571–0.756) was the optimal cut-off point for identification of T2DM patients with high total scores of BRIEF-A (scores > 50th percentile) from T2DM patients with low total scores of BRIEF-A (scores ≤ 50th percentile). AUC, area under the curve.

development of diabetic complications, and HbA1c levels should not be higher than 7% to avoid these complications.⁴² Therefore, in the present study, 7% was used as the cut-off point for HbA1c in patients.

Previous evidence suggest that HbA1c levels are closely related to the occurrence and development of diabetic

complications, and HbA1c levels should not be higher than 7% to avoid these complications.⁴² Consistently, in the present study, the total scales and all the terms of BRIEF-A were significantly higher in the HbA1c > 7% group than in the HbA1c ≤ 7% group. Furthermore, apart from the positive correlation between plasma HbA1c levels and inflammation-related indicators, our results showed that the plasma HbA1c level was also positively correlated with the total scores of BRIEF-A, the BRI, and metacognition index. Similarly, a positive significant correlation between HbA1c level and CRP and IL-6 has been demonstrated in several diabetes-related diseases.^{43–45} Moreover, HbA1c ≥ 8% has been reported to be an independent factor of cognitive decline and is associated with the severity of cognitive decline in elderly patients with T2DM.⁴⁶ Collectively, the results of the present study reiterates the important role of plasma HbA1c level in diagnosing diabetes and predicting its severity.

Widely distributed in both the central and peripheral tissues, nesfatin-1 is reported to be involved in food intake, glucolipid metabolism, and the regulation of emotion and cognition. The results of our previous study showed that nesfatin-1 is a very important factor linking the dysfunction of glucolipid metabolism and impairment of learning and memory in nonalcoholic fatty liver disease (NAFLD) in rats.⁴⁷ More recently, a significant correlation between HbA1c and serum nesfatin-1 levels was found in patients diagnosed with metabolic syndrome.⁴⁸ Similar to these findings, plasma nesfatin-1 concentrations were positively associated with HbA1c in patients with T2DM in the present study. Considering its close relationship with

Table 3 Comparison of Scales of BRIEF-A of the Low-Nesfatin-1 Group and the High-Nesfatin-1 Group

BRIEF-A	Low-Nesfatin-1 Group	High-Nesfatin-1 Group	Statistics (t)	P-value	Power
Behavioral regulation index	205.49±28.20	220.58±33.73	-2.80	0.01	0.78
Inhibit	49.91±8.20	54.6±10.86	-2.53	0.01	0.79
Shift	53.91±9.45	58.16±10.67	-2.42	0.02	0.66
Emotional control	52.16±10.31	55.77±11.79	-1.87	0.06	
Self-monitor	49.52±9.37	52.39±10.21	-1.67	0.10	
Metacognition index	277.04±54.51	295.37±52.22	-1.95	0.05	0.49
Initiate	55.88±14.32	62.05±14.21	-2.46	0.02	0.69
Working memory	64.32±14.46	67.98±12.90	-1.51	0.13	
Plan/organization	57.47±13.39	62.21±14.56	-1.94	0.05	0.48
Organization of materials	46.27±9.20	47.84±8.31	-1.02	0.31	
Task monitor	53.11±10.68	55.28±11.02	-1.14	0.26	
Total scores	482.53±75.25	515.95±75.39	-2.53	0.01	0.71

Note: Bold results are statistically significant.

insulin and HOMA-IR, nesfatin-1 may be a potential precursor in the diagnosis and treatment of T2DM.

BRIEF-A has been proven to be a tool for identifying executive function deficits in many clinical populations, including mild cognitive impairment,⁴⁹ attention deficit hyperactivity disorder,⁵⁰ and multiple sclerosis.⁵¹ Therefore, cognitive function deficits in T2DM patients were evaluated in the present study. The results showed that the total scores of BRIEF-A were significantly higher in T2DM patients with high nesfatin-1 levels than in T2DM patients with low nesfatin-1 levels. In terms of the subscales, the BRI (including inhibit, shift, and self-monitor) and metacognition index (including initiate and plan/organize) was also increased in the high-nesfatin-1 group. Moreover, T2DM patients with high scales of BRIEF-A (scores > 50th percentile) could be identified by plasma nesfatin-1. These findings suggest that there may be a potential link between plasma nesfatin-1 levels and cognitive decline in T2DM patients.

Inflammation and related diabetic dyslipidemia, oxidative stress, and magnesium deficiency are involved in the pathogenesis of T2DM and cognitive decline.^{52–54} It has been reported that the serum levels of IL-6 and CRP in T2DM patients were significantly higher than those in control participants.⁵⁵ Higher IL-6 concentration was associated with an increased rate of cognitive decline in both executive and memory functions.⁵⁶ In T2DM patients, cognitive impairment demonstrated association with IL-6,⁵⁷ CRP,⁵⁸ and IL-1 β .⁵⁹ Nesfatin-1 has recently been reported to have potent anti-inflammatory effects in several diseases.³⁶ However, the relationship between nesfatin-1 and inflammatory factors in T2DM has not yet been explored. The results of the present study first confirmed that nesfatin-1 levels were positively correlated with IL-6 and CRP levels in patients with T2DM, further suggesting that abnormal inflammation may be one of the possible mechanisms by which nesfatin-1 participates in cognitive impairment in patients with T2DM.

As immunoglobulin superfamily receptors, TREM-1 and TREM-2 are responsible for the activation of the innate immune response and play a vital role in inflammation.^{60,61} Recently, TREM-1 and TREM-2 have been shown to participate in cognitive regulation. It has been reported that peripheral TREM-1 and TREM-2 mRNA levels were significantly higher in AD patients than in controls, indicating that TREM-1 and TREM-2 may be useful as early peripheral biomarkers for the development of AD.^{62,63} In the present study, the results of the Pearson's correlation analysis showed a positive

relationship between nesfatin-1, sTREM-1 and sTREM-2. Combined with the role of sTREM-1 and sTREM-2 in inflammation and cognitive regulation, it is reasonable to assume that nesfatin-1 may be involved in the comorbidity of T2DM and cognitive impairment through the regulation of peripheral STREM-1 and sTREM-2 levels.

Several lines of evidence have indicated a close connection between nesfatin-1 and cognitive dysfunction. First, nesfatin-1 is widely expressed in the learning and memory-related brain regions, including the hippocampus and prefrontal cortex.⁶⁴ Second, nesfatin-1 can cross the blood-brain barrier.^{65,66} The results of our previous study have demonstrated that consecutive IPinjections of nesfatin-1 for 3 weeks could induce a lower preference index of the novel arm in the Y-maze, accompanied by decreased BDNF protein expression in the hippocampus and prefrontal cortex.⁶⁷ Third, the results of Pearson's correlation analysis showed that the plasma nesfatin-1 concentration was negatively correlated with swimming distance in the target quadrant in the Morris water maze task, indicating the important role of nesfatin-1 in the pathogenesis of NAFLD-induced impairment of learning and memory in rats.¹⁶

Due to the cross-sectional study design, no causality between nesfatin-1 and cognitive dysfunction could be determined. Given that T2DM patients with high nesfatin-1 levels showed higher HbA1c levels and higher scales of BRIEF-A, we hypothesized that plasma nesfatin-1 might be involved in the comorbidity of T2DM and cognitive dysfunction. Moreover, considering that the plasma nesfatin-1 concentrations were positively associated with diabetes-related biochemical indicators (HbA1c, insulin, and HOMA-IR) and inflammation-related indicators (CRP, IL-6, sTREM1, and sTREM2) among patients with T2DM, we further speculated that the involvement of nesfatin-1 in the impairment of cognitive function in diabetic patients may be related to these factors. However, these hypotheses derived from clinical data, summarized in Figure 5, need to be verified by multidimensional and in-depth studies.

This study has two limitations. First, the findings of this study should be interpreted with caution until they have been replicated in larger patient groups as the sample size was small. Second, because of the cross-sectional design of the study, no causality can be determined.

Several clinical implications have also been highlighted. First, our study suggests that the level of nesfatin-1 in the peripheral blood may be related to cognitive impairment in patients with T2DM. Second, the level of nesfatin-1 in peripheral blood may be used as a diagnostic marker for screening T2DM patients with high total scores of BRIEF-A (scores

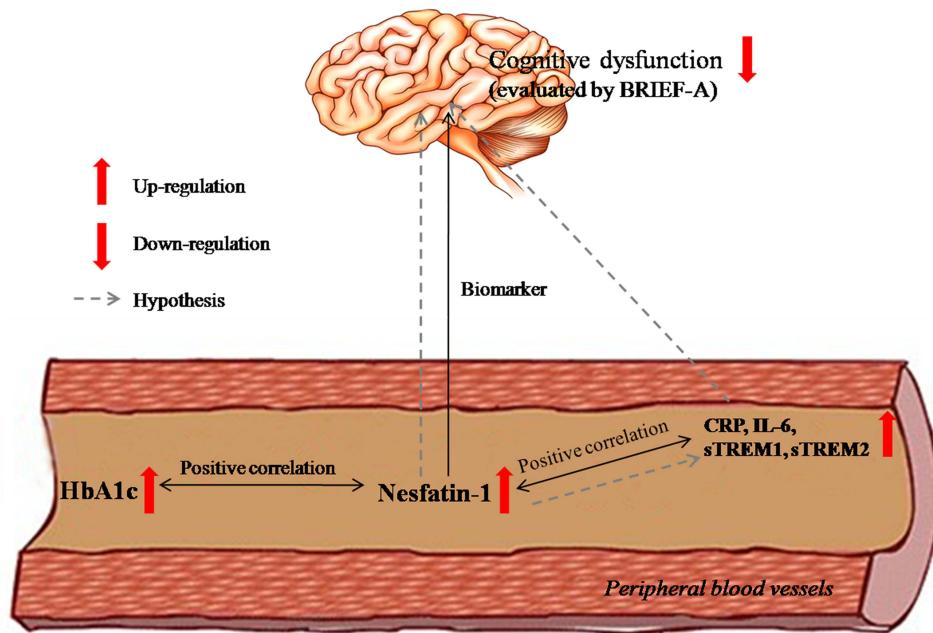


Figure 5 Plasma nesfatin-1 might be involved in the comorbidity of T2DM and cognitive dysfunction, and the mechanism underlying this involvement is related to the imbalance expression of CRP, IL-6, sTREM1, and sTREM2.

Notes: The nesfatin-1 in plasma was positively correlated with the plasma HbA1c levels and the CRP, IL-6, sTREM1, and sTREM2 levels in patients with T2DM. Moreover, T2DM patients with high nesfatin-1 levels showed higher scales of BRIEF-A. Furthermore, the plasma nesfatin-1 level may be considered as a biomarker to identify T2DM patients with high total scores of BRIEF-A. Collectively, these data suggest that plasma nesfatin-1 might be involved in the comorbidity of T2DM and cognitive dysfunction, and the mechanism underlying this involvement is related to the imbalance expression of CRP, IL-6, sTREM1, and sTREM2.

> 50th percentile). Third, nesfatin-1 and its related inflammatory factors may play a role in the pathogenesis of impaired cognitive function in T2DM, which needs to be confirmed by further studies. Additionally, considering that nesfatin-1 is an anorexigenic peptide involved in the regulation of homeostatic feeding⁶⁸ and HbA1c levels, glucose metabolism indices, and inflammation markers could be affected by dietary interventions,^{69–71} dietary intervention may be a possible strategy to improve the cognitive function of T2DM patients as it affects the indicators mentioned above.

Conclusion

In conclusion, this study is the first to reveal that plasma nesfatin-1 might be involved in the comorbidity of T2DM and cognitive dysfunction, which is related to the imbalance in the expression of CRP, IL-6, sTREM1, and sTREM2.

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Disclosure

The authors declare no conflicts of interest.

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