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Original Research Article

Relationship among serum TIMP-1, sCD40L and peripheral neuropathy in type 2 diabetes

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Abstract

Purpose: *To determine the correlation of serum TIMP-1 and sCD40L with peripheral neuropathy in type 2 diabetes (T2DM), and potential targets for pharmacological intervention.*
Methods: *The study enrolled 97 subjects with T2DM. The 41 subjects with simple diabetes were classified as T2DM group, while 56 subjects with diabetic peripheral neuropathy (DPN) were included in the DPN group. A control group consisting of 50 healthy volunteers who conducted health checks during a similar time-period was also included. Clinical data and parameters were compared among the three groups, and TIMP-1 and sCD40L levels were determined. Univariate and multivariate logistic regression analyses were conducted to identify factors affecting DPN. The diagnostic value of TIMP-1 and sCD40L in DPN was also determined.*
Results: *Body mass index (BMI) varied among the three groups ($p < 0.05$), but age or gender were not significantly different ($p < 0.05$). Duration of diabetes was longer in DPN group than in T2DM group ($p < 0.05$). The study also revealed statistically significant differences in fasting blood glucose (FBS), postprandial blood glucose, and glycosylated hemoglobin among the three groups. Tissue inhibitor of metalloproteinase-1 (TIMP-1) and soluble CD40 ligand (sCD40L) were important factors that affected the occurrence of diabetic peripheral neuropathy ($p < 0.05$).*
Conclusion: *TIMP-1 and sCD40L levels reflect neurovascular injury in patients with diabetes, with potential as targets for pharmacological intervention for peripheral neuropathy. These findings espouse crucial clinical implications for early identification and treatment in type 2 diabetes.*

Keywords: *Serum, Tissue inhibitor of metalloproteinase-1, Soluble CD40 ligand, Type 2 diabetes*

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INTRODUCTION

Diabetic peripheral neuropathy (DPN) is a usual complication in patients with diabetes, with the pathogenesis not completely clarified. Generally, diabetic peripheral neuropathy is considered to be a series of metabolic disorders caused by hyperglycemia-induced oxidative stress. Microangiopathy and tissue ischemia play a key role in this process [1,2]. Early diagnosis is difficult due to slow onset and progression of the disease. In recent years, many studies sought molecular markers to enable early diagnosis and monitoring of disease progression as well as identify reliable intervention targets [3,4].

Tissue inhibitor of metalloproteinase-1 (TIMP-1) is a specific inhibitor of MMP-9, which binds to MMP-9 through covalent bonds to form a complex. Specifically, TIMP-1 inhibits activity of MMP-9 and is an important regulator of protein degradation. In addition, activation of TIMP-1 is critical to regulate remodeling after nerve injury [5,6]. Soluble CD40 ligand (sCD40L) are novel inflammatory markers discovered in recent years and has become a significant signal transduction factor in inflammation and immune response [7]. In this study, the correlation of TIMP-1, sCD40L, and peripheral neuropathy in type 2 diabetes was investigated.

METHODS

Clinical data

Subjects comprised of 97 patients with type 2 diabetes hospitalized in Huai'an Hospital Affiliated with Xuzhou Medical University from July 2017 to February 2020. The patients included 41 with simple type 2 diabetes, classified as T2DM group; and 56 with combined diabetic peripheral neuropathy (DPH) classified as DPN group. A total of 50 healthy volunteers who underwent physical examination within same period were chosen as the control group. This study had the approval of the Ethics Committee of Huai'an Hospital Affiliated with Xuzhou Medical University (approval no. XYFY2021-K01), and followed international guidelines for human studies.

Inclusion criteria

Diagnostic criteria for type 2 diabetes in this study refer to the WHO diagnostic criteria in 1999, and the diagnostic criteria for DPN in this study refer to the 2013 diagnosis and treatment guidelines. Inclusion criteria include patients with abnormal temperature perception, decreased or disappearing foot sensation via nylon filament examination, abnormal vibration perception, whose ankle reflex has disappeared, whose nerve conduction velocity (NCV) tests show 2 or more slowing of patients. All patients and healthy volunteers voluntarily signed the informed consent.

Exclusion criteria

Patients with Wagner score 3-5 in the diabetes group, other types of diabetes, mental or psychological disorders, skin lesions, central or peripheral neuropathy via other causes, patients in acute inflammatory phase, presence of malignancy or immune system diseases, severe heart/liver dysfunction, and long-term use of immunosuppressants or hormones.

Collection of clinical data

Clinical data and parameters of the three groups, including gender, age, body mass index (BMI), course of disease, fasting blood glucose, postprandial blood glucose, glycosylated hemoglobin, triglyceride, cholesterol, high-density lipoprotein, and low-density lipoprotein, were collected and recorded.

Evaluation of parameters/indicators

Fasting venous blood was collected when patients were admitted to the hospital or during a health check, and centrifuged for 5 mins (1000 rpm) after which serum was collected and stored at -80 °C for later use. Double antibody sandwich enzyme-linked immunosorbent assay was adopted for TIMP-1, and enzyme-linked immunosorbent assay (ELISA) was used for determination of sCD40L according to manufacturer's instructions.

Statistical analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS) 25.0 (IBM, Armonk, NY, USA). Measurement data were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was employed for 3-sample analysis while the student t-test was employed for 2 group analysis. Enumeration data were expressed as percentages; rank sum test was used for comparison of three groups of enumeration data, while chi-square was used for comparing two groups. Risk factors of DPN were analyzed using univariate and multivariate logistic regression. The diagnostic value of TIMP-1 and sCD40L in diabetic peripheral neuropathy was analyzed with a receiver operator characteristic (ROC) curve. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical data

There was no statistical difference in gender or age among the three groups ($p < 0.05$) but the BMI for DPN and T2DM groups were significantly higher than control group ($p < 0.05$; Table 1).

Clinical parameters

The duration of diabetes in the DPN group was longer than in the T2DM group ($p < 0.05$). There was significant differences in fasting blood

Table 1: Comparison of clinical data among groups

Clinical data	DPN group (n = 56)	T2DM group (n = 41)	Control group (n = 50)	F/χ^2	*P*-value
Gender (n, %)					
Male	26(46.43)	19(46.34)	24(48.00)	0.034	0.983
Female	30(53.57)	22(53.66)	26(52.00)		
Age (years), mean ± SD)	56.48±9.30	55.37±8.56	55.9±9.15	0.181	0.835
BMI (kg/m^2, mean ± SD)	26.52±2.07*	26.83±2.52*	22.95±2.15	45.593	0.000

Note: Compared with control group, *$p < 0.05$

Table 2: Comparison of clinical parameters among the groups (mean ± standard deviation

Clinical data	DPN group (n = 56)	T2DM group (n = 41)	Control group (n = 50)	t/F/χ^2	*P-value*
Duration of diabetes (years)	7.25±2.51	5.18±1.54	-	4.665	0.000
Fasting blood glucose (mmol/L)	9.68±3.17	8.19±2.08*	5.10±0.89*#	53.168	0.000
Postprandial blood glucose (mmol/L)	15.98±2.73	13.27±2.93*	7.49±1.53*#	161.822	0.000
Glycosylated hemoglobin (%)	8.64±1.91	7.51±1.49*	4.19±0.61*#	127.659	0.000
Triglycerides (mmol/L)	2.06±0.57	2.11±0.53	1.97±0.53	0.751	0.474
Cholesterol (mmol/L)	4.69±1.21	4.64±1.19	4.39±1.55	0.795	0.454
High-density lipoprotein (mmol/L)	1.40±0.61	1.37±0.52	1.50±0.44	0.826	0.440
Low-density lipoprotein (mmol/L)	2.44±0.78	2.36±0.85	2.49±0.81	0.245	0.873

#*P* < 0.05 compared with DPN group, **p* < 0.05; #*p* < 0.05 compared with T2DM group.

Table 3: Comparison of TIMP-1 and sCD40L (mean ± standard deviation)

Group	Number of cases	TIMP-1 (μg/L)	sCD40L (ng/mL)
DPN	56	201.85±25.64	4.39±1.41
T2DM	41	227.84±17.98*	2.01±0.69*
Control	50	240.87±20.11*#	1.24±0.30*#
F	-	43.814	154.236
P-value	-	0.000	0.000

#*P* < 0.05 compared with DPN group, #*p* < 0.05 compared with T2DM group

Table 4: Multivariate logistic regression analysis of risk factors for DPN

Factor	B	SE	Z	Wald χ^2	*P--value*	OR	95%CI
Fasting blood-glucose (mg/dL)	0.037	0.251	0.148	0.022	0.882	1.038	0.634-1.699
Postprandial blood glucose (mg/dL)	-0.324	0.203	-1.593	2.538	0.111	0.7223	0.485-1.077
Glycosylated hemoglobin (%)	-0.469	0.413	-1.137	1.292	0.256	0.625	0.278-1.405
TIMP-1 (μg/L)	0.114	0.044	2.602	6.770	0.009	1.121	1.028-1.221
sCD40L (ng/mL)	-3.284	1.088	-3.108	9.106	0.003	0.037	0.004-0.316
Course of disease	-0.345	0.284	-1.215	1.476	0.224	0.708	0.406-1.236

glucose, postprandial blood glucose, and glycosylated hemoglobin among three groups ($p < 0.05$), while there was no significant difference in levels of cholesterol, high-density lipoprotein and low-density lipoprotein ($p > 0.05$; Table 2).

TIMP-1 and sCD40L

There was statistically significant differences in TIMP-1 and sCD40L among the three groups ($p < 0.05$). Tissue inhibitor of metalloproteinase-1 (TIMP-1) in DPN group was lower than those in T2DM and control groups ($p < 0.05$). Also, sCD40L in the DPN group was higher than those in T2DM and control groups ($p < 0.05$) (Table 3).

Multivariate logistic regression analysis of risk factors

Multiple logistic regression analysis was performed on the factors that were different in the univariate analysis. The results showed that TIMP-1 and sCD40L entered the regression model ($p < 0.05$), and were important factors affecting the occurrence of diabetic peripheral neuropathy (Table 4).

Diagnostic values of TIMP-1 and sCD40L in diabetic peripheral neuropathy

In this research, ROC curve was adopted for diagnostic value of TIMP-1 and sCD40L in DPN. The results revealed that AUC for TIMP-1 was 0.207 % (p < 0.05, 95 % CI: 0.120-0.294), the optimal cut-off value was 196.14 µg/L, the sensitivity was 51.80 % and the specificity was 97.69 %. The area under the curve for sCD40L was 0.930 % (p < 0.05, 95 % CI: 0.879 - 0.981), and supreme cut-off value was 3.12 ng/ml, with a sensitivity of 83.90 % and specificity of 100.00 % (Figure 1).

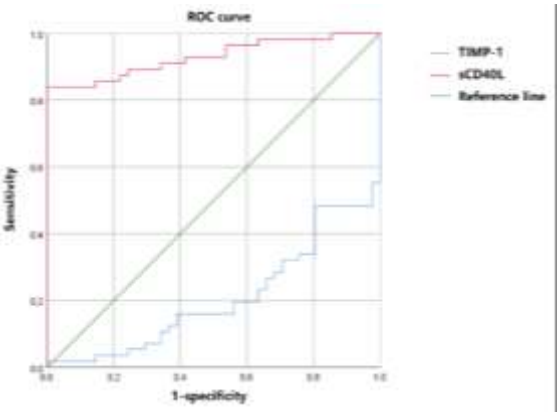

Figure 1: ROC curve analysis of diagnostic value of TIMP-1 and sCD40L in DPN

DISCUSSION

Diabetic peripheral neuropathy (DPN) is a frequent complication in Type 2 diabetes mellitus (T2DM). About 50 % of patients develop into DPN, and clinical symptoms caused by nerve damage brings great pain to them [8,9]. However, due to lack of attention to the disease, most DPN patients fail to receive early diagnosis and timely disease control [10,11]. Mechanism of DPN has not been fully understood. It is currently believed that DPN mainly causes mitochondrial dysfunction, oxidative stress, inflammation and related gene expression changes through the polyol pathway and protein kinase C (PKC) pathway, which eventually leads to nerve damage [11]. Main treatments for DPN involves nourishing nerves, controlling blood sugar, improving blood circulation, and oxidative stress [12,13]. Due to irreversibility of the disease process, early diagnosis, timely and effective intervention are of great value to control progress of the disease.

Matrix metalloproteinase 9 (MMP-9) is a zinc-dependent protease that proteolytically degrades a variety of extracellular matrix protein components, regulates and activates neurotrophic system [14,15]. Tissue inhibitor of metalloproteinase-1 (TIMP-1) is a specific inhibitor of MMP-9, which combines with MMP-9 to form a complex through a covalent bond. It also specifically inhibits activity of MMP-9 as an important protein degradation regulator [16,17]. Activation of TIMP-1 is a key factor regulating remodeling after nerve injury. This study revealed that TIMP-1 in the three groups was statistically significant. Level of TIMP-1 in DPN group was significantly lower in T2DM group and control groups. Results of this study are consistent with earlier studies [18,19], suggesting that decrease in TIMP-1 may provide an early warning effect on emergence of DPN. As patients develop DPN, decreased expression of TIMP-1 may lead to insufficient inhibition of MMP-9, which changes balance of MMP-9/TIMP-1, and further aggravates nerve injury.

Soluble CD40 ligand (sCD40L) is a novel inflammatory factor of TNF Superfamily CE, mainly produced after activation of platelets and T lymphocytes [20,21]. Studies have shown that sCD40L has biological activity, which reduces survival and proliferation of endothelial cells, leading to endothelial dysfunction [22]. In addition, sCD40L promotes activation of platelets and activates vascular smooth muscle cells, thereby causing cell proliferation and further vascular lesions [23]. This study has revealed that sCD40L of three groups were statistically significant, and DPN group had significantly higher sCD40L than T2DM and control groups. Similar to the results of previous studies [24,25], sCD40L expression in patients with DPN significantly increased, and activation of sCD40L further caused nerve and vascular injury, thereby aggravating the disease. This may also be one of the mechanisms involved in DPN disease progression.

Multivariate logistic regression analysis and ROC curve analysis were utilized in this study. There was statistical significance in TIMP-1 and sCD40L levels (p < 0.05). The AUC of TIMP-1 and sCD40L were 0.207 and 0.930 respectively. Furthermore, TIMP-1 and sCD40L were found to be important factors for peripheral neuropathy in diabetes, and thus are of great value in clinical diagnosis of DPN.

CONCLUSION

Peripheral neurocytopathy in type 2 diabetes mellitus is a cytokine mediated inflammatory response, and inflammation contributes to disease onset. Levels of TIMP-1 and sCD40L reflects neurovascular injury in diabetes, and

have valuable clinical reference for early identification in DPN.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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