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Original Article

Correlation between elevated serum homocysteine level and the development of diabetic peripheral neuropathy: a comparative study and meta-analysis

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Abstract: *Background:* This study aims to explore the correlations between serum homocysteine (Hcy) levels and diabetic peripheral neuropathy (DPN), which is further verified by meta-analysis. *Methods:* From June 2012 to June 2014, 188 type 2 diabetes mellitus (T2DM) patients admitted to Department of Endocrinology in Yantai Zhifu Hospital were enrolled in this study. T2DM patients were divided into simple diabetes mellitus group (SDM group) (n = 101) and DPN group (n = 87) based on Electromyography (EMG). Fasting blood glucose (FBG), fasting insulin (FINS), glycosylated hemoglobin (HbAlc), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and serum creatinine (Scr) level were detected. Data analysis was conducted by SPSS 18.0 software. Meta-analysis utilized R 3.1.0 software. *Results:* The diabetic duration, HbAlc and Hcy level of patients in DPN group were significantly higher than those in SDM group; the difference was statistically significant ($P < 0.05$). Pearson correlation analysis showed that serum Hcy level was positively correlated with disease duration, HbAlc, while no significant correlation was found between serum Hcy level with other indexes such as age, gender, BMI, FBG, TG, TC, LDL-C, HDL-C, SBP, DBP, etc. ($P > 0.05$). Logistic regression analysis results indicated that Hcy, course of disease and HbAlc had a positive correlation with DNP, and they were the risk factors of DPN. The result of our meta-analysis showed that serum Hcy level in DPN patients was significantly higher than that in healthy controls. *Conclusion:* Serum Hcy level is significantly related to the occurrence of DPN and the detection of serum Hcy level has important clinical significance in the prevention and treatment of DPN.

Keywords: Homocysteine, diabetic peripheral neuropathy, diabetes mellitus, electromyography, glycosylated hemoglobin, meta-analysis

Introduction

Diabetic peripheral neuropathy (DPN) is known as a debilitating complication of both type 1 and type 2 diabetes, affecting more than half of the total number of diabetes patients [1]. In UK, estimated data showed that the prevalence was 4.5% for diabetic patients worldwide, and the prevalence of painful DPN was 26.4% among those diabetic patients [2] In Korea, it has been documented that the frequency of DPN among diabetic subjects has risen from 14.1% to 54.5% in the last five decades [3]. For the neurological effects of ageing, the differences in the type of patients, and diagnostic methods and criteria, the prevalence of DPN vary to a large extent [4]. Increasing age, poor glycemic control and longer duration of diabetes are well-recognized risk factors for DPN, while obesity, cigarette smoking, hypertension, and hyperlipidemia are also implicated as potential risk markers [5]. Clinical symptoms of DPN include paresthesias, sharp pains or cramps, numbness or insensitivity to pain or temperature, and extreme sensitivity to touch [6]. What's more, about 30% of people with DPN experience decreased balance and coordination, muscle weakness, and loss of ankle reflexes [7]. Previous studies indicated that homocysteine (Hcy) is independently related to

Table 1. Comparison of baseline data in simple diabetes mellitus group and diabetic peripheral neuropathy

Base situation	SDM group (101)	DPN group (87)	*P*
Age (years old)	56.24 ± 7.05	57.76 ± 7.69	0.157
Gender (male/female)	56/45	45/42	0.875
Course of disease	4.49 ± 2.24	8.82 ± 4.75	0.001
BMI	24.89 ± 4.02	24.45 ± 3.52	0.424
FBG	6.97 ± 1.29	7.28 ± 1.69	0.157
FINS	4.79 ± 1.40	4.46 ± 1.67	0.145
HbA1c	6.95 ± 2.06	7.86 ± 2.57	< 0.001
TG	2.27 ± 1.42	2.43 ± 1.33	0.433
TC	5.23 ± 2.30	5.07 ± 1.50	0.597
LDL-C	4.79 ± 0.46	4.84 ± 0.82	0.608
HDL-C	1.13 ± 0.16	1.10 ± 0.30	0.318
Scr	85.27 ± 29.80	89.61 ± 25.42	0.289
SBP	135.89 ± 13.13	138.94 ± 11.37	0.093
DBP	80.34 ± 7.71	79.33 ± 8.81	0.403
Hcy	11.32 ± 2.33	17.84 ± 3.09	< 0.001

SDM, simple diabetes mellitus; DPN, diabetic peripheral neuropathy; BMI, body mass index; FBG, fasting blood glucose; FINS, fasting insulin; HbAlc, glycosylated hemoglobin; TG, triglyceride; TC, total cholesterol; LDL-C, high-density lipoprotein cholesterol; HDL-C, low-density lipoprotein cholesterol; Scr, creatinine; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hcy, homocysteine.

the prevalence of DPN in a collective of type 2 diabetic (T2DM) patients [8, 9].

Hcy, an requisite amino acid derived from diet, is composed of a four-carbon amino acid and a free thiol group, which is formed by demethylation of methionine [10]. There are three major forms for the occurrence of serum Hcy: protein-integrated Hcy, free type Hcy, and di-sulfurated Hcy and Cys-di-sulfurated Hcy, which are commonly known as total Hcy [11]. Elevated serum total Hcy play an important role in a rare hereditary disease because of smoking, aging, climacteric and sedentary lifestyle, diet with high methionine, and the deficiency of B vitamins and cystathionine-β-synthesis enzyme (CβS) in nutrition [12]. The normal Hcy level generally ranges from 5~15 μmol/L while its level exceeds 15 μmol/L is regarded as hyperhomocysteinemia [13]. The elevation in Hcy level mainly due to various reasons like genetic defects, renal insufficiency, certain drugs, and deficiencies of folate, vitamin B6, or vitamin B12 [14]. Hcy was considered as a predictive factor of demential neurodegenerative pathologies (Alzheimer's disease and Parkinson's disease), neurovascular alterations, and cognitive dysfunctions [15]. Additionally, previous studies have shown that the reactivity of the plasmas Hcy levels may be involved in a few of diabetic complications, such as stroke, foot ulceration, coronary artery disease, cerebrovascular disease, thrombosis and atherosclerosis [16, 17]. Of note, several studies have proposed that elevated Hcy levels might predict the risk of death or coronary events in patients with DPN and plasma Hcy levels tended to be increased, unchanged or decreased in DPN patients [18, 19]. Since the correlation of plasma Hcy level with the development and progress of DPN still remains unknown, we carried out this study to investigate the potential role of plasma Hcy level in pathogenesis and treatment of DPN to prevent further complications.

Materials and methods

Ethic statement

All experiments in the study were carried out with the approval of the Institutional Review Board of the Yantai Zhifu Hospital. Prior to the study design, each eligible patient had provided the written informed consent. The whole study was performed based on the Declaration of Helsinki [20].

Study participants

From June 2012 to June 2014, 188 T2DM patients (98 male and 90 female with mean age of 56.95 ± 7.37 years) admitted to Department of Endocrinology in Yantai Zhifu Hospital were enrolled in this study. T2DM patients were divided into simple diabetes mellitus group (SDM group) (n = 101) and DPN group (n = 87) based on Electromyography (EMG). T2DM was diagnosed according to the WHO criteria in 1999; DPN was diagnosed according to limb electromyogram: slowed conduction velocity of sensory and motor nerve, decreased evoked potential amplitude, prolonged latency and other specific screening standards [21]. Exclusion criteria: (1) patients that suffered lumbar spinal diseases and cerebrovascular disease sequela; (2) patients that underwent medication or operation, or they had other metabolic disorders; (3) patients that had serious liver and kidney dysfunction.

Table 2. Correlation between Hcy and other indexes

	Correlation coefficient (r)	*P*
Age (years old)	0.097	0.185
Course of disease	0.505	< 0.001
BMI	0.091	0.215
FBG	0.086	0.240
FINS	-0.088	0.230
HbA1c	0.725	0.002
TG	0.231	0.681
TC	0.131	0.686
LDL-C	0.254	0.507
HDL-C	-0.066	0.367
SCr	0.071	0.330
SBP	0.102	0.164
DBP	0.107	0.280

BMI, body mass index; FBG, fasting blood glucose; FINS, fasting insulin; HbAlc, glycosylated hemoglobin; TG, triglyceride; TC, total cholesterol; LDL-C, high-density lipoprotein cholesterol; HDL-C, low-density lipoprotein cholesterol; Scr, creatinine; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Research method

The general data of all subjects: gender, age, course of disease, body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP). All the patients kept on an empty stomach for 12 h until next morning, for the detection of fasting blood glucose (FBG), fasting insulin (FINS), glycosylated hemoglobin (HbAlc), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and serum creatinine (Scr) level. The detection of Hcy was using Enzyme-Linked Immuno Sorbent Assay (ELISA), which was in strict accordance with manufacturer's instructions of kit. Nicolet Viking IVD type motor evoked potential instrument made in America was used to test nerve conduction velocity, amplitude and incubation time of bilateral median nerve, ulnar nerve, motor branches of the tibial nerve, median nerve and sensory branch of sural nerve in T2DM patients.

Statistical analysis

Data analysis was conducted by SPSS 18.0 software. Measurement data were expressed by means ± standard deviation (SD). The comparison between groups was analyzed using *t* test or chi-square test. Correlation analysis between variables was analyzed using Pearson and multiple logistic regression analysis. *P* value of less than 0.05 denoted statistical significance.

Combining keywords and free words, we searched PubMed, Wanfang database, China National Knowledge Infrastructure (CNKI) and VIP database through their inception to 2014 for identifying relevant articles concerning the correlation between serum Hcy level and DPN. The search terms were: ("homocysteine" or "homocysteine" or "2-amino-4-mercaptobutyric acid" or "Hcy" or "homocystein") and ("diabetic neuropathies" or "diabetic neuropathies" or "diabetic peripheral neuropathy" or "DPN" or "painful diabetic peripheral neuropathy" or "PDPN"). Two investigators independently extracted data based on a standard reporting form. A third investigator was consulted in case of discrepancy. Meta-analysis utilized R 3.1.0 software (Robert Gentleman and Ross Ihaka, Auckland University, New Zealand) for statistical analysis. To evaluate the correlation between serum Hcy level and DPN, the fixed/random effect model was applied to estimate the standardized mean difference (SMDs) with its corresponding 95% confidence interval (95% CI). The overall effect size was examined with the utilization of Z test [21]. *Q*-statistic ($P < 0.05$ was considered significant) and I^2 tests were utilized to quantify heterogeneity among studies [22, 23]. Random effects model was applied for the evidence of significant heterogeneity ($P < 0.05$ or I^2 test exhibited > 50%), otherwise a fixed-effect model was utilized [24]. To further confirm the original result, the funnel plot and Egger's linear regression test were implemented to assess whether publication bias existed [25].

Results

Comparison of baseline data between two groups

As shown in **Table 1**, there was no significant difference of age, gender, BMI, TC, LDL-C, HDL-C, TG, FBG, Scr, FINS, and other indexes of blood pressure in consulting room between SDM group and DPN group ($P > 0.05$). The diabetic duration, HbAlc and Hcy level of patients in DPN group were significantly higher than

Table 3. Logistic regression analysis of multiple risk factors for diabetic peripheral neuropathy in patients with type 2 diabetes mellitus

	Regression coefficient	Standard error	wals	*P*	OR	95% CI
Course of disease	0.082	0.018	21.045	0.020	1.750	1.320-2.680
HbA1c	0.040	0.020	4.855	0.009	1.190	1.025-1.430
Hcy	0.253	1.103	4.748	0.010	2.750	1.100-2.420

HbAlc, glycosylated hemoglobin; Hcy, homocysteine; OR, odds ratio; 95% CI, confident interval.

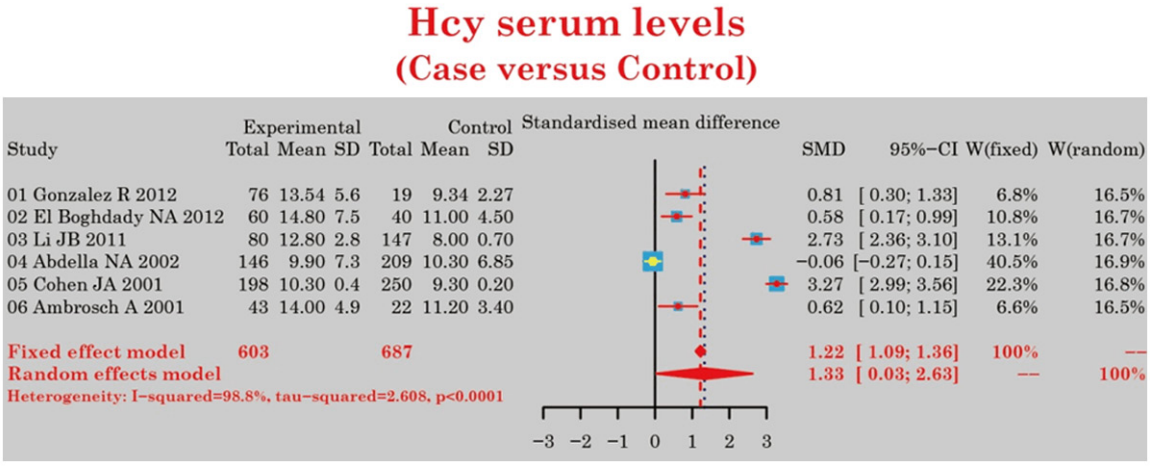

Figure 1. Forest plot for the differences of serum homocysteine level between patients with diabetic peripheral neuropathy and healthy controls.

those in SDM group; the difference was statistically significant ($P < 0.05$).

Pearson correlation analysis

Pearson correlation analysis showed that serum Hcy level was positively correlated with disease duration (r = 0.505, $P < 0.001$), HbAlc (r = 0.725, $P = 0.002$), while no significant correlation was found between serum Hcy level with other indexes such as age, gender, BMI, FBG, TG, TC, LDL-C, HDL-C, SBP, DBP, etc. ($P > 0.05$) (**Table 2**).

Logistic regression analysis

Logistic regression analysis was carried out as follows: with or without DPN (dependent variable), independent variable (indexes of age, gender, course of disease, BMI, TC, TG, FBG, FINS, Hb A1c). The results indicated that Hcy, course of disease and HbAlc had a positive correlation with DNP, and they were the risk factors of DPN (Hcy: OR = 2.750, 95% CI: 1.100~2.420, $P = 0.010$; course of disease: OR = 1.750, 95% CI: 1.320~2.680, $P = 0.020$; HbAlc: OR = 1.190, 95% CI: 1.025~1.430, $P = 0.009$) (**Table 3**).

Pooled results of meta-analysis

Based on our rigorous search strategy, we retrieved 106 studies through electronic database searching and manual searching. The retrieved studies were carefully screened, and 4 articles were excluded for duplicates, 9 for letters, reviews or meta-analyses, 15 for non-human studies, 21 studies not related to the subjects. The fill-texts of the 57 remaining studies were reviewed and additional studies were excluded in that they were not case-control study (n = 7), or not relevant to Hcy (n = 16), or not relevant to DPN (n = 25) or lacked sufficient data (n = 3). Finally, 6 eligible studies were selected for the present meta-analysis [26-31]. The selected studies contained DPN patients (n = 603) and healthy controls (n = 687). Of the 6 studies, a total of 3 studies were performed in Caucasians, and 3 studies were in Asians. All six studies reported the correlation between serum Hcy level and DPN. Random effect model was applied due to heterogeneity among stud-

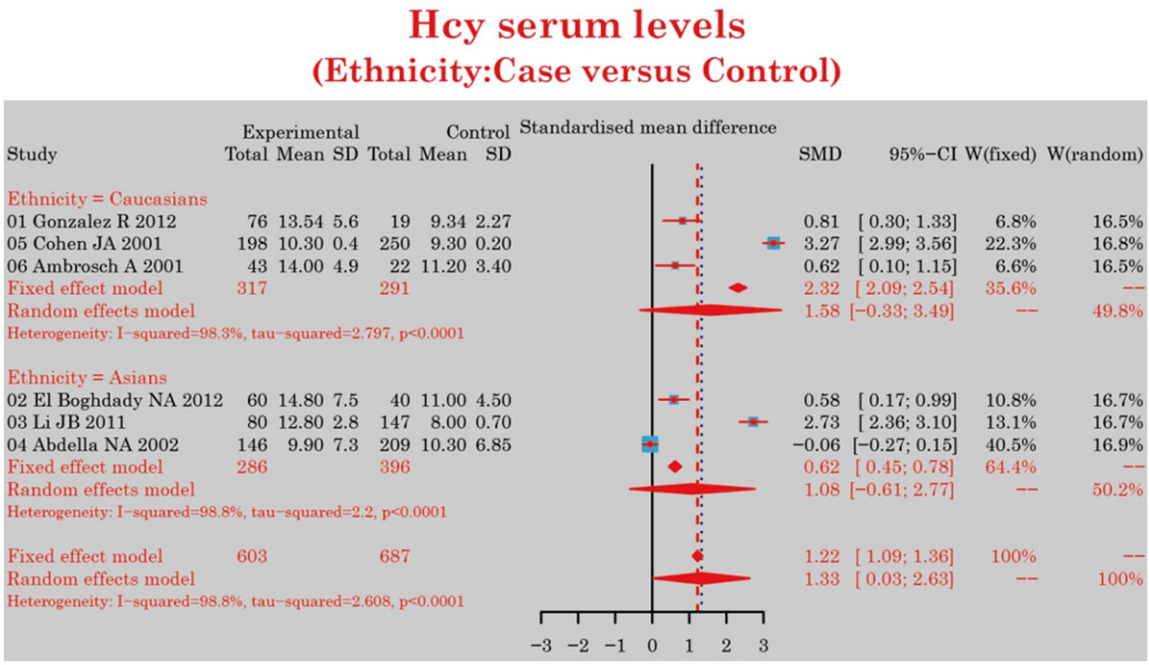

Figure 2. Subgroup analysis based on ethnicity for the differences of serum homocysteine level between patients with diabetic peripheral neuropathy and the healthy controls.

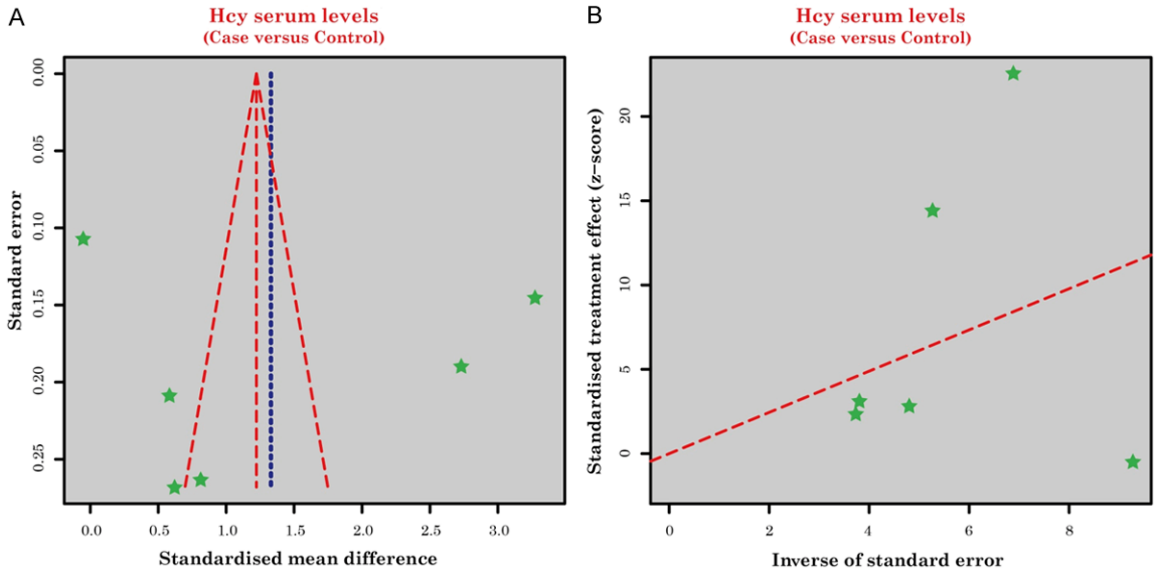

Figure 3. Funnel plot of publication biases for the differences of serum homocysteine level between patients with diabetic peripheral neuropathy and the healthy controls.

ies (I^2 = 98.8%, P_h < 0.001). The result of our meta-analysis showed that serum Hcy level in DPN patients was significantly higher than that in healthy controls (SMD = 1.33, 95% CI: 0.03~2.63, P = 0.045) (**Figure 1**). Subgroup analysis results by ethnicity showed that both among Caucasians and Asians, serum Hcy level in DPN patients was significantly higher when compared to that in healthy controls, indicating that high serum level of Hcy might be one of the most important risk factors for DPN in both Asians and Caucasians (Caucasians: SMD = 2.32, 95% CI: 2.09~2.54, P < 0.001; Asians: SMD = 0.62, 95% CI: 0.45~0.78, P < 0.001)

(**Figure 2**). No publication bias existed, as evidenced by the symmetrical funnel plot (**Figure 3A**). The Egger linear regression analysis further confirmed the lack of publication bias (all $P = 0.699$) (**Figure 3B**).

Discussion

Overall, our study confirmed that plasma Hcy levels significantly increased in DPN patients, suggesting that elevated plasma Hcy level can be considered as an important risk factor for the occurrence and development of DPN. As one of the most common complications in diabetes, the pathogenetic mechanisms of DPN include increased aldose reductase activity, impaired neurotrophic support, advanced glycation/glycoxidation, activation of protein kinase C, poly (ADP-ribose) polymerase, and oxidative-nitrative stress [32]. Hcy, a sulphur-containing amino acid, is an intermediary product in methionine metabolism; and folic acid and vitamin B12 are necessary for participating in the metabolism of Hcy. While metabolic disorders occurred in diabetic patients often lead to the deficiency of folic acid and vitamin B12 in the body, which further causing elevated levels of Hcy in vivo [11]. Hcy is an independent risk factor for peripheral vascular disease in diabetic patients, coronary artery disease, as well as cerebrovascular disease, the potential mechanisms might be that: elevated Hcy serum concentrations can induce the oxidative stress, which has cytotoxic effect and oxidative damage on the endothelial cells, and can inhibit the production of nitric oxide (NO) resulted in arteriosclerosis and thrombosis [33]. In addition, hyperhomocysteinemia can also inhibit the synthesis of heparin and the expression of thrombomodulin, promote platelet aggregation and the expression of plasminogen activator inhibitor, further leads to the formation of vascular sclerosis and microthrombus [34]. Hcy can directly damage proteins, lipids and nucleic acid of nerve cells, and lead to cell necrosis or apoptosis; hyperhomocysteinemia significantly decreases the neurotrophic factor secretion by damaging Schwann cells of peripheral nerve, is not conducive to the survival of neural cells and the repair after damage [35]. Furthermore, blood hypercoagulative state and narrowed or thicken microthrombus caused by high levels of Hcy are likely to slow down the velocity of blood flow, result in the deficiency of nerve perfusion, neuronal ischemia and anoxia, thereby aggravate the occurrence and development of DPN [15]. Study has shown that elevated plasma Hcy values would contribute to the presence of DPN due to the resultant circulation impairment, nerve damage and nutrient deficit, and the risk of DPN in patients with higher Hcy is twice greater than those with lower Hcy values [36]. It has also been indicated that high serum Hcy level makes a person more prone to endothelial injury, disorders of glucose and lipid metabolism and neural lesion, which might play a significant role in the development and progress of DPN [8].

Our study also suggested that except for Hcy level, the diabetic duration and HbAlc of patients in DPN group were significantly higher than those in SDM group, and Hcy, course of disease and HbAlc had a positive correlation with DNP, and they were the risk factors of DPN. The diabetic duration and HbAlc level are known to be important predictors for DPN, but the present data revealed that compared to "classical" predictors for neuropathic complications such as duration of diabetes and glucose steady state (estimated by HbA1c), Hcy adjusted for creatinine and related vitamins is independently associated with DPN and appears to be a stronger discriminator [37]. We also carried out subgroup analysis based on ethnicity to evaluate the correlation between the serum Hcy level and the development of DPN. The results of stratified analyses have shown that in both Caucasians and Asians, the increased serum Hcy level revealed a significant correlation with the development of DPN, indicating that serum Hcy level may be strongly related to the development and progress of DPN and serum Hcy level can be used as potential biomarker for the diagnosis of DPN.

There were limitations in our study. First, only six eligible studies were enrolled into the meta-analysis part, this comparatively small sample size might cause minor bias for the confirmation of our results. Second, for the restriction of the enrolled studies, the different grouping situations in comparative study (SDM group and DPN group) and meta-analysis (DPN patients and healthy controls) might also have an impact on the final outcomes.

In conclusion, the present data show that serum Hcy level is independently linked with the development of DPN. Although this might

reflect a pathophysiological mechanism of serum Hcy level in diabetes, large-scale prospective studies are needed to confirm the role of serum Hcy level in the development of DPN.

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Disclosure of conflict of interest

None.

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