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Original Article

Serum homocysteine levels and diabetic neuropathy in patients with type 2 diabetes mellitus: a systematic review and meta-analysis

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Abstract: Aims/Introduction: Homocysteine (Hcys) is a thiol-containing amino acid formed by a demethylation methionine. Several studies showed a possible association between homocysteine and diabetic neuropathy in patients with type 2 diabetes. We performed a meta-analysis to evaluate the relationship between homocysteine and diabetic neuropathy. Materials and Methods: A systematic review and meta-analysis was performed to comprehensively evaluated the association between serum homocysteine levels and diabetic neuropathy in patients with type 2 diabetes mellitus. Literature updated to October 3, 2015 from Cochrane Library, MEDLINE, Web of Science, China Biology Medicine, and reference lists from relevant articles were identified. Data from eligible studies were pooled using meta-analysis. Results: A total of 784 individuals retrieved from five studies were finally included into the meta-analysis. Meta-analysis showed that there were obviously increased serum homocysteine levels in patients with diabetic neuropathy (WMD = 4.14 μmol/L; 95% CI = 3.32 to 4.95, P < 0.00001). Sensitivity analysis showed that there was no obvious change in the pooled estimated. Conclusion: Homocysteine is involved in the development of diabetic neuropathy in type 2 diabetic patients and hyperhomocysteinemia is very likely to be associated with diabetic neuropathy. Further studies are needed to validate the relationships between hyperhomocysteinemia and diabetic neuropathy.

Keywords: Diabetic neuropathy, homocysteine, meta-analysis

Introduction

Diabetic neuropathy has been defined as the presence of symptoms and/or signs of nerve dysfunction in diabetics after exclusion of other causes [1, 2]. It is a common and complex complication in patients with diabetes. Approximately 50% patients with diabetes suffer from diabetic neuropathy [3]. However, the underlying pathogenesis has not been well defined [4, 5], and there is lack of effective disease modifying treatments for diabetic neuropathy other than strict glycemic control [3, 5]. Identification of diabetic neuropathy risk factor is essential for a better understanding of the pathogenesis and a more effective treatment of diabetic neuropathy. Homocysteine (Hcys) is a thiol-containing amino acid formed by a demethylation methionine. Hyperhomocysteinemia is usually defined as serum homocysteine concentration more than 15 μmol/L. Previously studies have suggested that homocysteine may damaged endothelium by excessive sulphation of connective tissues [6, 7]. While vascular risk factors have been linked to diabetic neuropathy [8]. Therefore, people hypothesized that the status of homocysteine was associated with the risk and progression of neuropathy in type 2 diabetes. The past few decades have witnessed some studies testing this hypothesis. Some of them showed a possible association between increased serum homocysteine levels and diabetic neuropathy risk, but there was no definite conclusion [9-13]. There were also several studies showing that hyperhomocysteinemia can result in increased risk of diabetic neuropathy [9, 12]. Due to the fact that the lack of data from different ethnicities and the number of patients enrolled in every study is insufficient. Meta-analysis is a

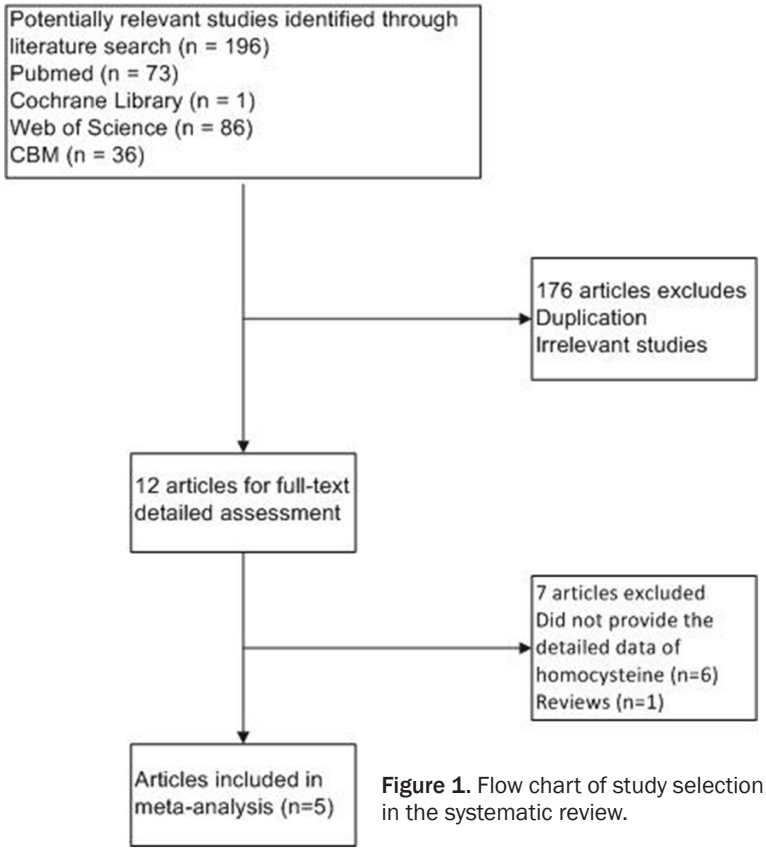

Figure 1. Flow chart of study selection in the systematic review.

good way to summarize the available data to provide more robust results than the individual study.

Methods

Literature search

According to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines [14], we searched Cochrane Library, MEDLINE, Web of Science and China Biology Medicine databases for relevant published articles. No restriction was imposed on search language, and the last search was performed on October 3, 2015. The search terms used as follows: (1) homocysteine, hyperhomocysteinemia; (2) diabetic neuropathy, diabetic autonomic neuropathy, diabetic peripheral neuropathy, diabetic asymmetric polyneuropathy, painful diabetic neuropathy, diabetic asymmetric polyneuropathy, diabetic mononeuropathy, diabetic amyotrophy, diabetic polyneuropathy. The references in relevant articles and reviews were also checked to identify other possible studies.

Inclusion criteria

Studies were considered for inclusion only when they met all the following criteria: (1) case-control, cross-sectional, prospective or cohort study; (2) assessing the association between serum homocysteine levels and diabetic neuropathy in patients with type 2 diabetes or comparing serum homocysteine levels in diabetic neuropathy patients with controls without diabetic neuropathy; (3) mean values of serum homocysteine levels, events of diabetic neuropathy, or odds ratio (OR) with 95% confidence interval (95% CI) were available. Case-only studies, case reports, or reviews were all excluded.

Data extraction and quality assessment

We extracted any reported mean, median and standard deviation. The data from each study were recorded as follows: first author's name; year of publication; origin of region; sample size; mean values of serum homocysteine levels. We evaluated the study quality according to Newcastle-Ottawa quality assessment scale: (1) selection; (2) comparability; (3) exposure [15] Two reviewers performed the literature search in dependently, study selection, quality assessment and data extraction with any disagreements resolved by discussion.

Statistical analysis

To compare serum homocysteine levels in diabetic neuropathy patients with control without diabetic neuropathy, weighted mean difference (WMD) with 95% CI was used in the meta-analysis. To assess the association between hyperuricemia and DPN risk in patients with type 2 diabetes, the odd ratio (OR) with 95% CI was used. The heterogeneity was assessed using the Cochran Q test and a *P* value greater than 0.05 suggested no obvious heterogeneity among the included studies [16]. A *P*-level of less than 0.05 was considered as statistical

Table 1. Characteristics of five studies included into the meta-analysis

Study	Ethnicity	Study design	Case/Control	Outcomes	Adjusted factors	Quality	Disease duration (year) Case/Control
A. Ambrosch, 2001	White	Cross-sectional	43/22	Mean difference; OR	Duration of diabetes, HbA1c, creatinine, RBC folate and vitamin B12	7	6.5/7.0
Li Jianbo, 2011	Asian	Cross-sectional	80/147	Mean difference	HbA1c, duration of diabetes, age, sex, vitamin B12	6	13.3/7.1
Ricardo Gonza' lez 2012	White	Cross-sectional	159/19	Mean difference	None	4	12.6/9.3
Wang Hongli 2007	Asian	Cross-sectional	101/87	Mean difference; OR	None	5	9/9
Wu Qingqin 2012	Asian	Cross-section	56/70	Mean difference	Age, disease duration,	6	8.2/4.2

significance unless otherwise specified. The heterogeneity was also assessed by I^2 which was quantitative analysis of heterogeneity and an I^2 less than 50 % suggested no obvious heterogeneity in the included studies [17]. A fixed-effect model was used when no significant heterogeneity was detected [18]. Otherwise, a random-effect model was used [19]. The heterogeneity across studies was tested by Q statistic which based on the χ^2 test, and its quantitative measure was calculated by I^2 statistic [20]. When statistical heterogeneity occurred, we analyzed its source first, and then performed subgroup analysis or a sensitivity analysis by omitting low-quality studies from the dataset. All statistical analyses were performed using Review Manager (version 5.2.1 for Windows Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration).

Results

Literature selection

We initially retrieved 196 relevant publications from the Pubmed, Cochrane Library, Web of Scienece and China Biology Medicine databases. The majority of these were excluded after first screening in terms of titles or abstracts, mainly because they were duplication or irrelevant studies. 7 articles were excluded because they did not provide the data of homocysteine. 1 study was excluded because it was a review. Finally, 5 studies were included in our meta-analysis. A flow chart showing the study selection is presented in **Figure 1**.

Study characteristics

The characteristics of the 5 enrolled studies are shown in **Table 1**. A total of 5 cross-sectional studies were published between 2000 and 2012. Three studies [9-11] were conducted in the Asia, two [12, 13] in Europe. The size of studies ranged from 65 to 227.

Quality scale

The number of award scores of enrolled studies ranged from 4 to 7. (lowquality: 1-3, median quality: 4-6, high quality: 7-9). Of the studies included, 1 study was awared for 7 scores, 2 for 6 scores, 1 for 5 scores and another for 4 scores. As shown in **Table 1**.

Serum homocysetine levels in diabetic neuropathy patients

There was 5 studies comparing the serum homocysetine levels in diabetic neuropathy patients with diabetic controls without diabetic neuropathy [9-13] (**Figure 2**). There was between-study heterogeneity in those five studies (I^2 = 38%); so the fixed-effect model was used to pool the data. As shown in **Figure 2**, there were increased serum homocysteine levels in diabetic patients with neuropathy compared with those without diabetic neuropathy (WMD = 4.14 μmol/L; 95% CI = 3.32 to 4.95, P < 0.00001) Sensitivity analysis showed that there was no obvious change in the pooled estimates.

Hyperhomocysteinemia and diabetic neuropathy risk

There was a total of 253 type 2 diabetic patients from two studies assessing the association between hyperhomocysteinemia and diabetic neuropathy risk [8, 11] (**Figure 3**). There was no between-study heterogeneity in the two studies (I^2 = 0); so the fixed-effect model was used to pool the data. As shown in **Figure 3**, hyperhomocysteinemia was significantly associated with increased risk of diabetic neuropathy in patients with type 2 diabetes (OR 6.45, 95% CI 1.82-22.80, P = 0.004) (**Figure 3**).

Discussion

There is no comprehensive assessment of the association between homocysteine and diabetic neuropathy. This present study is the first meta-analysis aiming to estimate the association between serum homocysteine and diabetic neuropathy in patients with type 2 diabetes. Five studies involving a total 439 type 2 diabetic patients with diabetic neuropathy and 335 patients without diabetic neuropathy were finally included into the meta-analysis. Our meta-analysis showed that patients with diabetic neuropathy had increased serum homocysteine levels compared without diabetic neuropathy. Moreover, hyperhomocysteinemia was significantly associated with increased risk of DPN in patients with type 2 diabetes. Thus homocysteine is a biomarker of diabetic neuropathy and it has some indications for the prevention or treatment of diabetic neuropathy.

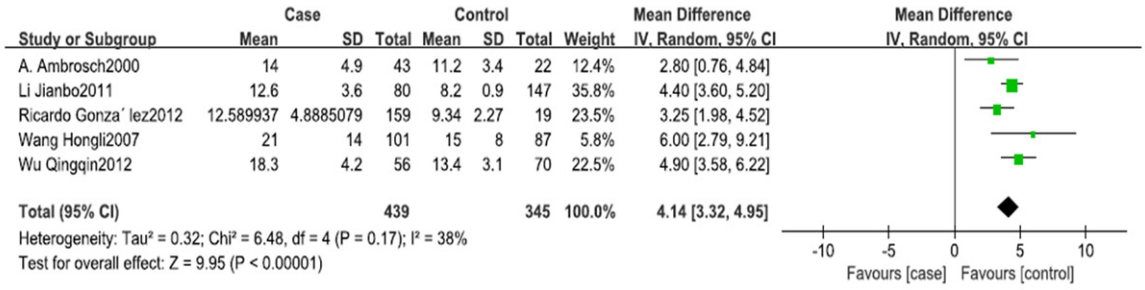

Figure 2. Increased homocysteine levels in diabetic patients with diabetic neuropathy compared with those without diabetic neuropathy.

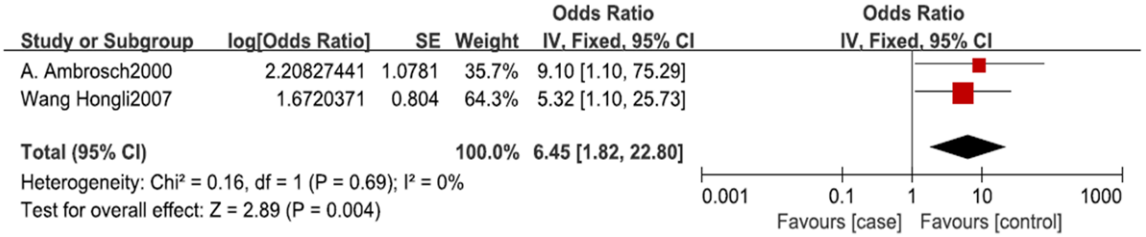

Figure 3. Hyperhomocysteinemia was associated with increased risk of diabetic neuropathy in patients with type 2 diabetes.

Diabetic neuropathy is a significant complication and accounts for significant morbidity and mortality. There are multiple possible etiologies proposed for diabetic neuropathy, including hyperglycemia, oxidative and inflammation [21]. However, the risk factors associated with diabetic neuropathy have not been identified completely, and the mechanism of diabetic neuropathy is still unclear [4, 5]. In addition, there is lack of effective treatments for diabetic neuropathy except glycemic control [22]. Identification of risk factors associated with diabetic neuropathy can provide us some new ways to manage diabetic neuropathy.

Homocysteine (Hcys) is a thiol-containing amino acid formed by a demethylation methionine. Previous studies have shown that hyperhomocysteinemia may be a cause of diabetic nephropathy and diabetic retinopathy [23, 24]. Homocysteine stimulates ceramide-mediated redox signaling [25]. A clinical study of patients with inherited defects of homocysteine metabolism found a significant increase in plasma glutathione peroxidase activity in those with hyperhomocysteinaemia [26]. Many risk factors of diabetic neuropathy are believed to contribute to micro-vascular damage and nerve dysfunction [27]. Hence, homocysteine could promote oxidative stress, which could induce nerve injury. And studies have shown that homocysteine may damaged endothelium by excessive sulphation of connective tissues [28, 29]. Homocysteine may also participate in the progression of diabetic neuropathy by injuring vascular endothelial cells. The results of our meta-analysis indicate that homocysteine is involved in the development of diabetic neuropathy in type 2 diabetic patients. In addition, hyperhomocysteinemia is associated with increased risk of diabetic neuropathy. Since serum homocysteine level is modifiable risk factor for diabetic neuropathy, drugs of lowering homocysteine may have some possible preventive or treatment effects for diabetic neuropathy in type 2 diabetic patients. However, there are no trails assessing the effects of homocysteine-lowering therapies in the prevention and treatment of diabetic neuropathy. Further studies should validate preventive or treatment effects of lowering homocysteine.

The present meta-analysis had several limitations. Firstly, the limited number of studies included in the meta-analysis was a major concern of the study. Moreover the sample size of case and control group from some individual studies was not equal, which could reduce sta-

tistical power and lead to unstable estimate. Secondly, our study only assessed the association between serum homocysteine levels and diabetic neuropathy risk in type 2 diabetes because there was no relevant study from type 1 diabetic patient. Thus, further studies are needed to assess the association between homocysteine and diabetic neuropathy risk in type 1 diabetic patients. Thirdly, publication bias was assessed using funnel plot in the meta-analysis, and we were unable to exclude the possibility of publication bias. The funnel plot may have limited power to detect the exact risk of publication bias because there were only 5 included studies.

Taken together, this meta-analysis indicate that the status of plasma homocysteine is involved in the development of diabetic neuropathy in type 2 diabetic patients and, hyperhomocysteinemia is associated with the increased risk of diabetic neuropathy. To get a more precise assessment of impact of hyperhomocysteinemia, further prospective cohort studies are necessary.

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Disclosure of conflict of interest

None.

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