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Original Article

Association of homocysteine with type 1 diabetes mellitus: a meta-analysis

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Abstract: Purpose: To figure out the association between plasma Hcy status and type 1 diabetes mellitus (T1DM). Methods: We searched the PubMed Web of Science, and The Cochrane Library to identify eligible studies. The Newcastle-Ottawa Quality Assessment Scale was used to assess the quality of selected studies. All analyses were performed using the STATA, version 12 software. Results: 15 studies were included in this investigation. Our meta-analysis indicated that plasma Hcy concentrations in T1DM patients without any complications were normal compared with healthy people [13 studies, SMD: -0.08, 95% confidence interval (CI): -0.44 to 0.28, P=0.67]. However, a significant elevation of plasma Hcy concentrations was observed in T1DM patients with only diabetic retinopathy (DR) (5 studies, SMD: 0.34, 95% CI: 0.13 to 0.55, P=0.002), only diabetic nephropathy (DN) (4 studies, SMD: 0.76, 95% CI: 0.18 to 1.33, P=0.01) and both the two complications (3 studies, SMD: 1.05, 95% CI: 0.03 to 2.07, P=0.043) compared with T1DM patients without any complications. Conclusions: Homocysteine levels elevate in T1DM patients with DR and DN, but don't elevate in T1DM without any complications.

Keywords: Homocysteine, type 1 diabetes mellitus, diabetic nephropathy, diabetic retinopathy, meta-analysis

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease manifesting as the destruction of pancreatic β-cells and the onset of hyperglycemia [1], resulting from a complex interaction between host genetics, immune system and environmental factors [2]. Diabetes-related microvascular complications, such as nephropathy, retinopathy are life-threatening complications in patients with T1DM [3]. Diabetic nephropathy (DN) is characterized by persistent albuminuria, elevation of arterial blood pressure and a decline in glomerular filtration rate (GFR) [4]. Diabetic retinopathy (DR) is the fourth most common cause of vision loss in adults [5], the main changes encompass thickening of the basement membrane, loss of pericytes and proliferation of mesangium [6].

Homocysteine (Hcy), a sulfhydryl-containing amino acid, has been reported to be elevated in patients with type 2 diabetes mellitus (T2DM) and its vascular complication [7, 8]. However, plasma Hcy levels have been found to be lower [9, 10], normal [11, 12] or higher [13, 14] in patients with T1DM compared with healthy subjects in conflicting studies. Moreover, most previous cross-sectional studies in patients with T1DM indicated positive associations between Hcy and DN [15, 16], but not for DR [17, 18], although some did [15, 19].

Considering all those conflicting reports, meta-analysis may be an appropriate way to summarize current available data to provide more robust evidences than the individual study.

Materials and methods

Search strategy

All the studies that investigated plasma Hcy status and the association between Hcy and the risk of vascular complications in patients with T1DM were considered in this meta-analysis. A comprehensive literature search was performed for original studies published up to

Table 1. Characteristics of studies included in this meta-analysis

			Control			Case 1			Case 2			Case 3			Case 4			Quality
Study	Year	Country	n	Age (Y)	Hcy (μM)	n	Age (Y)	Hcy (μM)	n	Age (Y)	Hcy (μM)	n	Age (Y)	Hcy (μM)	n	Age (Y)	Hcy (μM)	score
Hultberg et al	1991	Sweden	46	52	11.0±3.4	25	45	10.7±4.3	18	40.6	12.1±5.5	No	No	No	12	No	20.1±4.5	5
Agardh et al	1994	Sweden	No	No	No	9	34.6	7.6±2.0	No	No	No	10	37	8.0±1.6	15	No	9.8±3.9	5
Targher et al	2000	Italy	30	33	10.3±2.2	60	32	12.5±4.8	No	No	No	No	No	No	No	No	No	6
Vaccaro et al	2000	Italy	44	44.2	9.3±3.6	27	43.3	6.9±3.1	5	40.7	8.8±2.6	9	47	8.9±2.7	No	No	No	6
Mutus et al	2001	Italy	34	46	9.4±2.0	28	38	9.9±1.8	No	No	No	No	No	No	No	No	No	5
Abdel et al	2001	Egypt	15	13.21	11.10±2.56	15	13.7	20.10±3.24	No	No	No	15	15.7	30.16±8.05	No	No	No	6
Saeed et al	2003	England	28	11.9	6.6±1.7	16	13.4	5.7±2.1	No	No	No	No	No	No	No	No	No	5
García-Unzueta et al	2005	Spain	64	32	6.9±2.4	117	No	5.7±2.1	24	No	5.7±1.4	No	No	No	14	No	6.7±3.4	6
Atabek et al	2006	Turkey	27	10.9	5.7±2.2	27	11.3	5.6±2.9	No	No	No	No	No	No	No	No	No	5
Janickova et al	2007	Czech	13	30.8	7.61±3.71	13	25.8	8.29±3.71	No	No	No	20	34.2	10.39±4.43	No	No	No	6
Jehlicka et al	2009	Czech	30	15.1	8.6±3.86	30	14.6	5.42±1.9	No	No	No	No	No	No	No	No	No	6
Harrington et al	2010	Australia	32	14.2	9.0±2.6	66	14.1	7.0±2.4	No	No	No	No	No	No	No	No	No	6
Giannattasio et al	2010	Italy	123	14.2	8.3±2.5	41	20	7.3±2.7	No	No	No	No	No	No	No	No	No	5
Babar et al	2011	American	15	7.6	3.9±1.55	21	8.3	4.3±1.37	No	No	No	No	No	No	No	No	No	6
Bulum et al	2014	Croatia	No	No	No	85	42	9.3±2.3	78	49	10.4±2.9	No	No	No	No	No	No	7

No = undescribed, Control = healthy people, Case 1 = type 1 diabetes without any complications, Case 2 = type 1 diabetic retinopathy, Case 3 = type 1 diabetic nephropathy with microabuminuria, Case 4 = type 1 diabetic retinopathy and nephropathy, Hcy = homocysteine mean ± SD (μM).

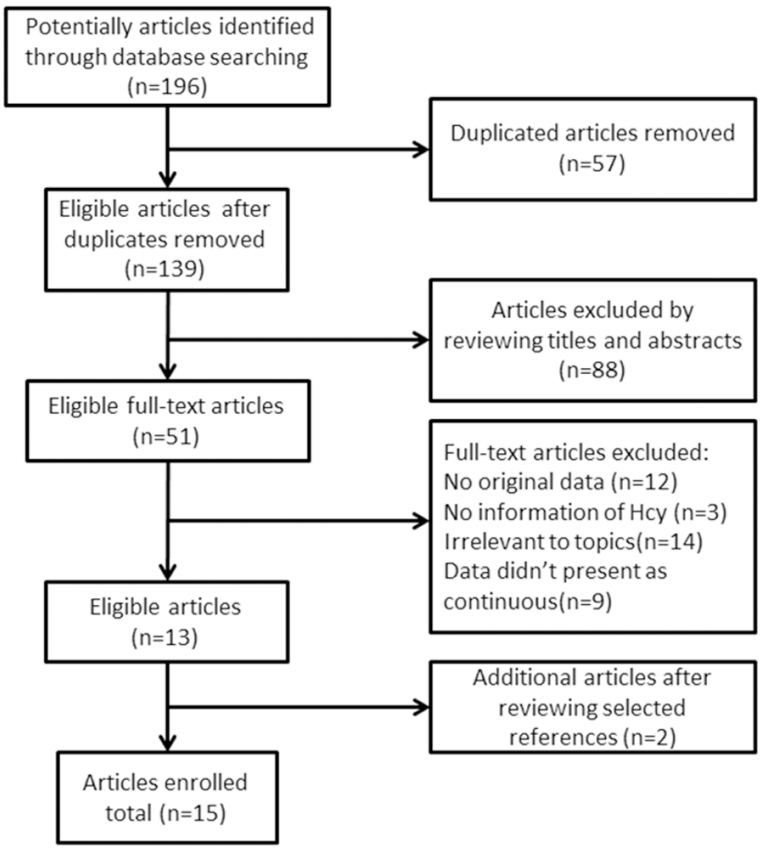

Figure 1. Search strategy for meta-analysis.

June, 2015 using PubMed, Web of Science databases and The Cochrane Library. No restriction was imposed on search language. The search terms used were as follows: "type 1 diabetes", "T1DM", "diabetic retinopathy", "diabetic nephropathy", "homocysteine" and "Hcy". References of retrieved articles were also reviewed for fear of neglecting additional published reports not included in PubMed, Web of Science databases and The Cochrane Library.

Study selection

First screening was based on titles and abstracts in searching studies, any studies lacking information regarding plasma Hcy levels in patients with T1DM was rejected. Editorials, abstracts, and review articles were also excluded. Then, second screening was based on the full texts of interested studies. Inclusion criteria for study selection were as follows: 1) cross-sectional, case-control, prospective or cohort study; 2) case: T1DM without any complications, T1DM with DR or DN, T1DM with both DR and DN; 3) control: healthy people; 4) data of interest (plasma Hcy concentration) in both controls and cases presented as continuous (mean value and SD).

Quality scale

The results of quality assessment using the Newcastle-Ottawa Quality Assessment Scale were in **Table 1**. The quality scores of included studies ranged from 5 to 7 (low quality: 1-3, median quality: 4-6, high quality: 7-9).

Data extraction

The data elements of interest were extracted by two investigators from each study independently and another senior researcher reviewed all items for completeness and accuracy. Information was recorded as follows: first author's surname, year of publication, subjects' country, participant number, definition and characteristics of cases and controls, plasma Hcy concentration in all groups.

Statistical analyses

We used standard mean deviation (SMD) as effect measure to assess the differences in Hcy status among healthy people, T1DM patients without vascular complications, T1DM patients with DR/DN and T1DM patients with both DR and DN. Heterogeneity of SMDs was quantified using the I-square (I^2) value. I^2>50% was considered to represent significant heterogeneity [20]. Given with heterogeneity, SMDs were calculated using random-effects model. If there was no heterogeneity, fixed-effects model was applied. Potential publication bias was assessed by Begg's and Egger's test. All analyses were performed using STATA 12.0 (Stata Corp, College Station, TX, USA).

Results

Literature search

We initially retrieved 196 articles from PubMed, Web of Science databases and The Cochrane Library. After duplicates removed, 78 articles were excluded by reviewing titles and abstracts,

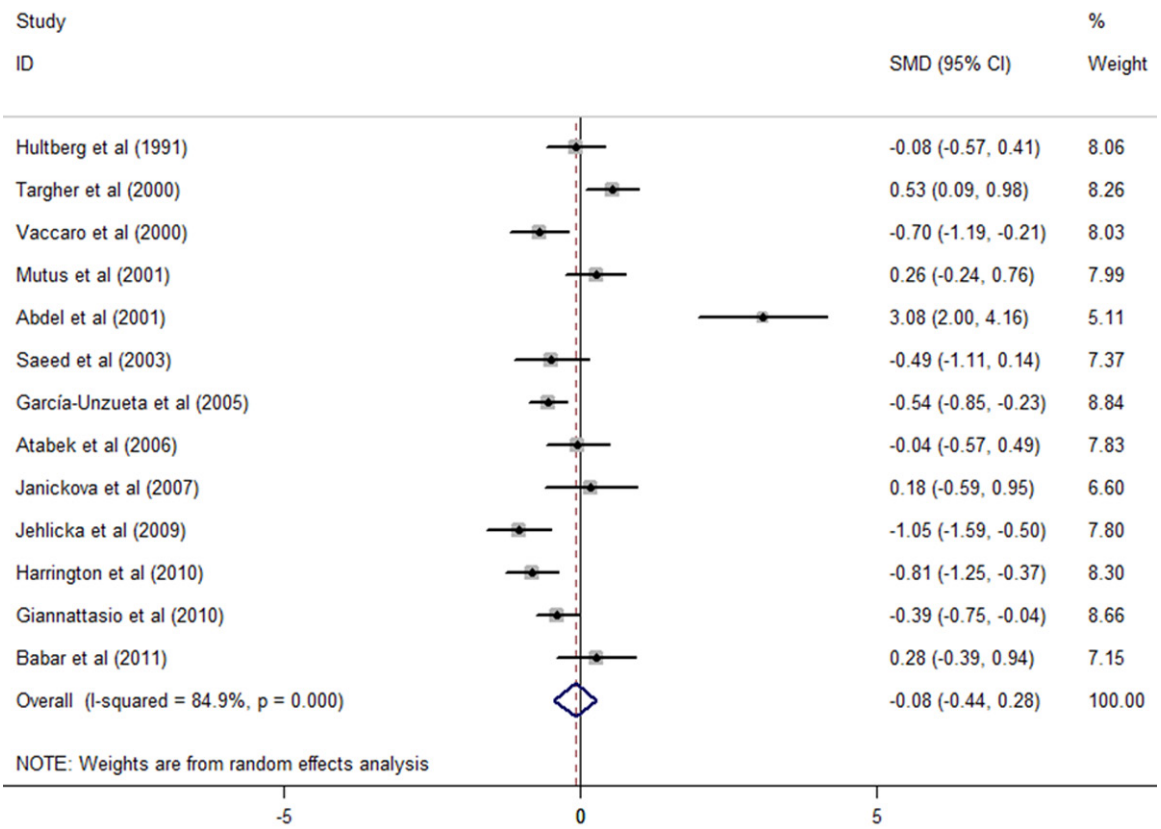

Figure 2. Comparasion of plasma homocysteine level between T1DM patients without any complications and healthy people.

mainly because they were reviews/editorials or irrelevant to topics. Then 39 full-text articles were excluded because of some detail reasons showed in **Figure 1**. Finally, 15 studies [15, 21-34] were included in our meta-analysis.

Study characteristics

The characteristics of the 15 enrolled studies are shown in **Table 1**. There were 12 case-control studies [15, 21-23, 26-30, 32-34], 2 prospective studies [24, 29] and 1 *post hoc* analysis study [31]. The mean age of T1DM patients ranged from 8.3 to 45 years which were generally matched in healthy controls and other cases. The sizes of studies ranged from 34 to 219.

Meta-analysis of plasma Hcy levels

Firstly, we compared plasma Hcy concentrations between healthy controls and T1DM patients without any complications and found that plasma Hcy levels in T1DM patients without any complications were similar to that in healthy controls [13 studies, SMD: -0.08, 95% confidence interval (CI): -0.44 to 0.28, P=0.67, as shown in **Figure 2**]. Significant heterogeneity was observed among studies (I^2=84.9%, P=0). Begg's test (P=0.127) and Egger's test (P=0.045) indicated the existence of publication bias.

The sources of heterogeneity were then investigated by meta-regression, which showed that the significant heterogeneity could not be explained by the factors such as publication year, studies' region, mean age of healthy controls and T1DM patients without any complications, study designs and Hcy detection method-high performance liquid chromatography (HPLC) or not. Duration of T1DM was not included in meta-regression due to lack of information in some studies.

6 studies [23, 24, 27, 29-31] were excluded by using sensitivity analysis because they were appearing to be outliers with others. Among these, there were one small-sample studies [29], one study [30] with T1DM patients signifi-

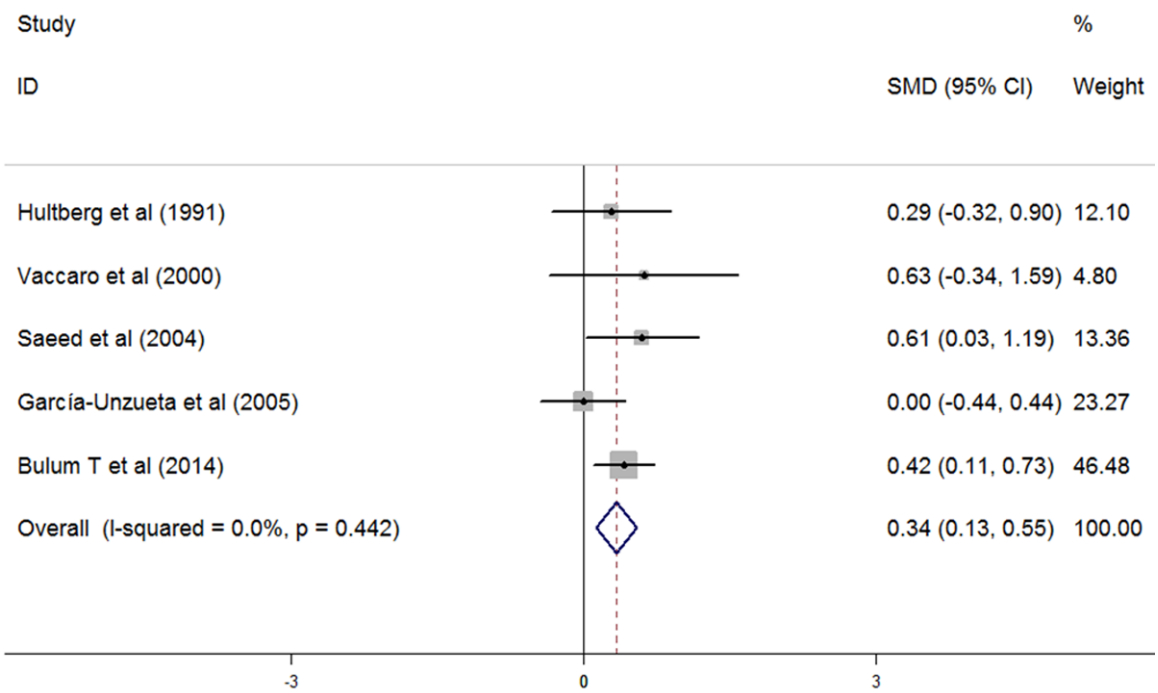

Figure 3. Comparasion of plasma homocysteine level between T1DM patients with only DR and T1DM patients without any complications.

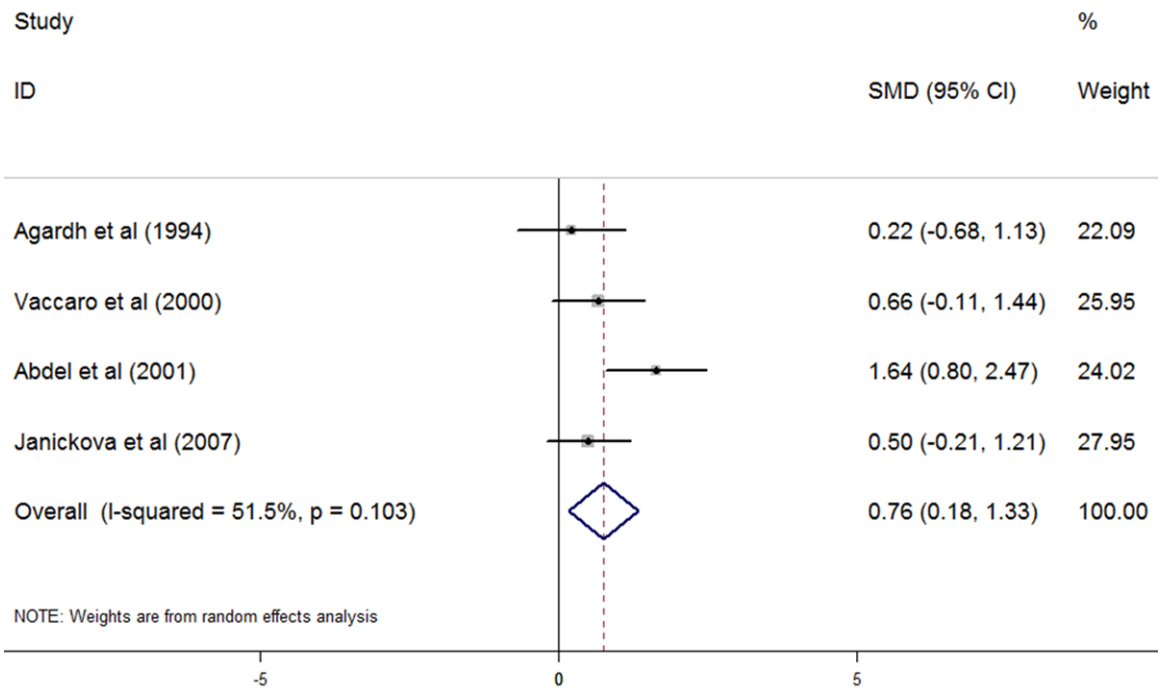

Figure 4. Comparasion of plasma homocysteine level between T1DM patients with only DN and T1DM patients without any complications.

cantly younger than healthy controls. After exclusion, a meta-analysis of other 7 studies indicated that the main results remained unchanged, a significant elevation or reduction

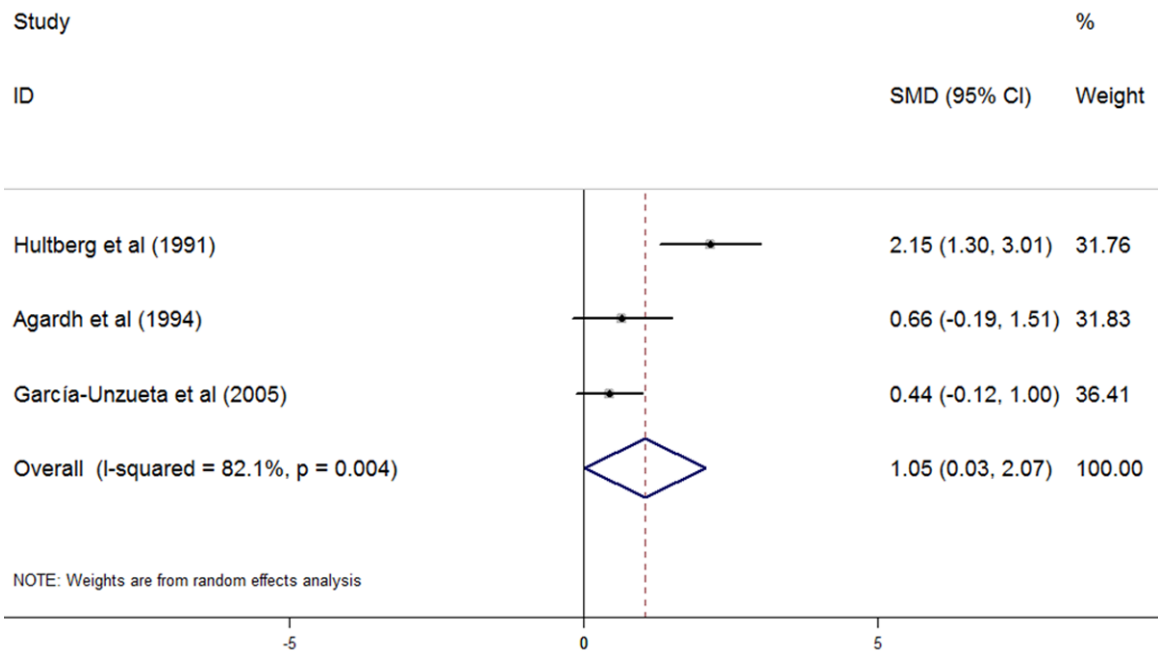

Figure 5. Comparasion of plasma homocysteine level between T1DM patients with both DR and DN and T1DM patients without any complications.

of plasma Hcy levels was not observed in T1DM patients without any complication compared with healthy controls (SMD: -0.23, CI: -0.48 to 0.02, P=0.066). There was no significant heterogeneity among studies (I^2=33.8%, P=0.17). No evidence of publication bias was noted (Begg, P=0.133; Egger, P=0.222).

Plasma Hcy levels were higher in T1DM patients with only DR than in T1DM patients without any complications (5 studies, SMD: 0.34, 95% CI: 0.13 to 0.55, P=0.002, as shown in **Figure 3**). No significant heterogeneity was observed among studies (I^2=0%, P=0.442). No evidence of publication bias was noted (Begg, P=1.000; Egger, P=0.770). T1DM patients with only DN demonstrated significantly higher levels of plasma Hcy than T1DM patients without any complications (4 studies, SMD: 0.76, 95% CI: 0.18 to 1.33, P=0.01, as shown in **Figure 4**). Significant heterogeneity was observed among studies (I^2=51.5%, P=0.103). Omission of any single study didn't significantly influence the overall SMD. No evidence of publication bias was noted (Begg, P=1.000; Egger, P=0.887). Also, plasma Hcy levels were significantly higher in T1DM patients with both DR and DN than in T1DM patients without any complications (3 studies, SMD: 1.05, 95% CI: 0.03 to 2.07, P=0.043, as shown in **Figure 5**). Significant heterogeneity was observed among studies (I^2=82.1%, P=0.004). Omission of any single study didn't significantly influence the overall SMD. No evidence of publication bias was noted (Begg, P=0.296; Egger, P=0.512).

Discussion

The association between Hcy and T1DM has been paid increasing attention. Our meta-analysis indicated that plasma Hcy concentrations in T1DM patients without any complications were normal compared with healthy people. Nevertheless, significant elevations of plasma Hcy concentrations were observed in T1DM patients with only DR, only DN and both the two complications compared with T1DM patients without any complications.

In vivo, the main source for synthetizing Hcy is methionine, obtained from diet. As key intermediaries in Hcy synthesis, S-adenosyl-methionine and S-adenosyl-homocysteine generate in the process called demethylation [35]. Kidney is a major issue for removal and metabolism of Hcy [36], which is independently associated with GFR and albuminuria [37]. The deteriorating renal function may reduce the renal clearance of Hcy resulting in elevated plasma Hcy concentration. Thus, proper renal function is crucial to Hcy metabolism [38]. Our meta-analysis indicated that there were no significant differences

in Hcy concentration between healthy people and T1DM patients without any complications, implying that Hcy may not an independently risk factor in the progression of T1DM. However, in the early stages of T1DM and in T1DM patients with proper renal function, hyperfiltration may cause lower plasma Hcy [39]. We may suggest that it's too early to negate the effect of Hcy on the progression of T1DM, since hyperfiltration may cover the changes of plasma Hcy status in T1DM patients without any complications. Therefore, more studies excluded the effect of hyperfiltration in patients with T1DM should be carried out in the future.

The toxic effect of Hcy has been identified by a number of studies. Hcy is a pro-inflammatory amino acid and actives inflammatory transcriptional signal pathways [40]. Hyperhomocysteinemia induces endoplasmic reticulum stress through activating mitogen-activated protein kinase, promotes proinflammatory cytokine production via activating JNK and facilitates macrophage infiltration [41, 42]. *In vitro* studies have demonstrated that Hcy auto-oxidation formed reactive oxygen species (ROS), and reduced the production of glutathione peroxidase [43]. Elevated plasma Hcy represents an oxidative stress, elicits an increase in glutathione peroxidase activity [44]. Hcy can also induce endothelial dysfunction via increased ROS production and impaired nitric oxide's bioactivity [45]. Hcy involves in the initiation and progression of vascular diseases via inducing the expression and secretion of inflammatory cytokines, such as interleukin-8 and monocyte chemoattractant protein-1 (MCP-1) [46].

Impaired renal function leads to hyperhomocysteinemia, elevated hcy status can also produce toxic effect on kidney [47]. Diet-induced chronic hyperhomocysteinemia could promote vascular remodeling and induce tubulointerstitial injury in the kidney [48]. Elevated Hcy concentration induces glomerular injury via ceramide-redox signaling pathway [49]. Another study has confirmed that Hcy induced MCP-1 expression in the kidney by activating NF-κB, which contributed to renal injury in hyperhomocysteinemic rat [50]. The results of our meta-analysis also showed that a significant higher plasma Hcy concentration was observed in T1DM patients with DN, compared with T1DM patients without any complications, suggesting a close association between Hcy and renal function.

In recent years, increasing studies have identified the toxic effect of hyperhomocysteinemia on retina. Hyperhomocysteinemia is not only an independent risk factor for macular edema [51] and retinal venous occlusion [52], but also a catalyst in retinopathy of prematurity [53] and age-related macular degeneration [54]. Moreover, elevated Hcy concentration has been measured in the blood plasma, vitreous body and aqueous humor of patients with proliferative diabetic retinopathy [55]. Hcy produces superoxide from NADPH oxidase resulting in impaired endothelium-dependent NO-mediated dilation in the retinal arterioles, which facilitates the development of retinal vascular diseases [56]. Our meta-analysis showed that plasma Hcy concentration is higher in T1DM patients with only DR than in T1DM patients without any complications, implying that pathological retina could cause elevated Hcy status without the involvement of impaired renal function. Nevertheless, the specific mechanisms relating Hcy status and retinopathy in T1DM are not clear. One study included in this meta-analysis demonstrated that compared with T1DM patients without any complications, T1DM patients with only DR accompanied with slightly increased (non-significant and normal) urinary albumin excretion (UAE) and significantly reduced, but normal estimated GFR, which may provide an explanation to elevated Hcy [34].

Although our investigation enrolled relatively high-quality studies which shared similar baseline characteristics, there were several limitations existing in this meta-analysis. First, detection methods of Hcy concentration varied among enrolled studies, which may influence the accuracy of Hcy concentration. Second, substantial heterogeneity was observed across studies in several meta-analyses, which might affect the outcomes, although a random effects model was used. Finally, we needed more studies included in our meta-analysis.

Based on our results, we may conclude that in T1DM patients without any complications, plasma Hcy concentrations keep in normal range. While as the disease progresses, T1DM microvascular complications, such as DR and DN, may accompany with elevated plasma Hcy concentrations.

Disclosure of conflict of interest

None.

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