Original Article
Association between plasma homocysteine status and hypothyroidism: a meta-analysis

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Abstract: Purpose: To figure out plasma homocysteine (Hcy) status in patients with subclinical hypothyroidism (SH) and overt hypothyroidism (OH) compared with healthy subjects, and the effect of levothyroxine (L-T4) on plasma homocysteine status in patients with hypothyroidism. Methods: PubMed Web of Science, and The Cochrane Library were used 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: Our meta-analysis indicated that plasma Hcy concentrations elevated in OH patients without L-T4 treatment compared with healthy subjects. However, this elevation was not observed in the comparison between patients with SH without L-T4 treatment and healthy subjects. Moreover, plasma Hcy levels were found to be higher in patients with OH without L-T4 treatment than in patients with SH without L-T4 treatment. Finally, plasma Hcy concentrations decreased after L-T4 treatment in patients with SH or OH. Conclusions: Plasma Hcy status is associated with the severity of hypothyroidism and L-T4 treatment is helpful for patients with hypothyroidism to reduce the plasma Hcy levels.

Keywords: Homocysteine, subclinical hypothyroidism, overt hypothyroidism, levothyroxine, meta-analysis

Introduction

Hypothyroidism is divided into two types, subclinical hypothyroidism (SH) and overt hypothyroidism (OH), according to the decrease extent of thyroid function. SH is a condition defined as a persistently raised serum thyroid stimulating hormone (TSH) level in the presence of normal free thyroxine (fT4) [1]. Subclinical thyroid failure is often asymptomatic; 30% patients with SH may have symptoms indicating the deficiency of thyroid hormone [2]. Recently, SH has been reported to be closely associated with atherosclerosis and myocardial infarction in elderly women [3]. OH, defined by high TSH levels with low levels of fT4 and/or free triiodothyronine (fT3), is a risk factor for cardiovascular diseases, especially coronary heart diseases [4].

Increasing attention is paid to the risk of cardiovascular diseases in patients with hypothyroidism recently. Homocysteine (Hcy), a sulfhydryl-containing amino acid synthesized during the conversion of methionine to cysteine [5], has been identified as an independently risk factor for the progression of vascular diseases [6]. The association between homocysteine and hypothyroidism has been demonstrated in several studies, however, the conclusion is controversial [7-9]. As the major treatment of hypothyroidism, levothyroxine (L-T4) replacement has been used for a long time. The effect of L-T4 treatment on plasma homocysteine status in patients with hypothyroidism has not yet reached a consensus [10-12].

Considering all those conflicting studies, meta-analysis may be an appropriate way to summarize available data to provide more strong evidences than the individual study. This meta-analysis was to elucidate plasma Hcy levels in patients with hypothyroidism, and to evaluate the effect of L-T4 therapy on plasma Hcy levels in patients with hypothyroidism.

Materials and methods

Search strategy

All the studies that investigated the association between plasma Hcy status and hypothyroid-
Table 1. Characteristics of studies included in this meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Control N (F/M)</th>
<th>Case 1 N (F/M)</th>
<th>Case 2 N (F/M)</th>
<th>Case 3 N (F/M)</th>
<th>Case 4 N (F/M)</th>
<th>Quality score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bičíková et al</td>
<td>2001</td>
<td>Czech</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>14 (12/2)</td>
</tr>
<tr>
<td>Deicher et al</td>
<td>2002</td>
<td>Austria</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Bičíková et al</td>
<td>2002</td>
<td>Czech</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>16 (16/0)</td>
</tr>
<tr>
<td>Christ-Crain et al</td>
<td>2003</td>
<td>Switzerland</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>2003 Switzerland</td>
</tr>
<tr>
<td>Atabek et al</td>
<td>2003</td>
<td>Turkey</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Luboshitzky et al</td>
<td>2004</td>
<td>Spain</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Pe’rez et al</td>
<td>2004</td>
<td>Turkey</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ozcan et al</td>
<td>2005</td>
<td>Turkey</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Beyhan et al</td>
<td>2006</td>
<td>Turkey</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cakal et al</td>
<td>2007</td>
<td>Turkey</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Turhan et al</td>
<td>2008</td>
<td>Turkey</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Erdal et al</td>
<td>2008</td>
<td>Turkey</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Adrees et al</td>
<td>2009</td>
<td>Ireland</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ma et al</td>
<td>2012</td>
<td>China</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Bamashmoss et al</td>
<td>2013</td>
<td>Yemen</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Kutluturk et al</td>
<td>2013</td>
<td>Turkey</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Anagnostis et al</td>
<td>2014</td>
<td>Greece</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

No = undescribed, Control = healthy people, Case 1 = subclinical hypothyroidism without L-T4 treatment, Case 2 = subclinical hypothyroidism after L-T4 treatment, Case 3 = overt hypothyroidism without L-T4 treatment, Case 4 = overt hypothyroidism after L-T4 treatment, Hcy = homocysteine mean ± SD (μM).
Homocysteine and hypothyroidism

Entire page is relevant to the topic of homocysteine and hypothyroidism. The text delves into statistical analyses and result details in a structured manner, with a focus on the methodology, data extraction, and findings of the study.
Homocysteine and hypothyroidism

26 full-text studies were excluded due to some detail reasons showed in Figure 1. Finally, 17 studies [4, 7-12, 14-23] were enrolled in our meta-analysis.

Study characteristics

The characteristics of the 17 enrolled studies are shown in Table 1. There were 5 case-control studies [4, 8, 9, 18, 21], 12 prospective studies [7, 10-12, 14-17, 19, 20, 22, 23]. The mean age of each group ranged from 14.9 to 57.5 years which were generally matched in healthy controls and other cases. The duration of L-T4 treatment ranged from 2 months to 18 months. The sizes of studies ranged from 14 to 164.

Meta-analysis of plasma Hcy levels

Firstly, we compared plasma Hcy concentrations between patients with SH without L-T4 treatment and healthy subjects, and found that plasma Hcy levels in patients with SH without L-T4 treatment were similar to that in healthy subjects [8 studies, SMD: 0.19, 95% confidence interval (CI): -0.07 to 0.45, p=0.159, as shown in Figure 2]. Significant heterogeneity was observed among studies (I²=62.9%, p=0.009). No evidence of publication bias was noted (Begg, p=0.902; Egger, p=0.858). We then turn to sensitivity analysis, 1 studies [1] was excluded because it was appearing to be outliers with other studies (Figure 3).
Exclusion, a meta-analysis of other 7 studies indicated that the main results remained unchanged, a significant elevation or reduction of plasma Hcy levels was not observed in patients with SH without L-T4 treatment compared with healthy subjects (SMD: 0.07, CI: -0.10 to 0.24, p=0.425). There was no significant heterogeneity among studies (I²=0.0%, p=0.578). No evidence of publication bias was noted (Begg, p=0.548; Egger, p=0.600).

Next, we compared plasma Hcy concentrations between patients with OH without L-T4 treatment and healthy subjects, and found that plasma Hcy levels were significantly higher in patients with OH without L-T4 treatment than in healthy subjects (5 studies, SMD: 1.10, CI: 0.39 to 1.81, p=0.003, as shown in Figure 4). Significant heterogeneity was observed among studies (I²=85.3%, p=0). No evidence of publication bias was noted (Begg, p=0.462; Egger, p=0.283). We also use sensitivity analysis to exclude those studies that were appearing to be different from others. After excluded 1 study [4], a meta-analysis of other 4 studies indicated that the main results remained unchanged, a significant elevation of plasma Hcy levels was observed in patients with OH without L-T4 treatment compared with healthy subjects (SMD: 0.67, CI: 0.40 to 0.94, p=0). There was no significant heterogeneity among studies (I²=1.3%, p=0.385). No evidence of publication bias was noted (Begg, p=1; Egger, p=0.562).

Plasma Hcy concentrations were then compared between patients with SH without L-T4 treatment and patients with OH without L-T4 treatment. Results showed that plasma Hcy levels were higher in patients with OH without L-T4 treatment than in patients with SH without L-T4 treatment (3 studies, SMD: 0.56, CI: 0.27 to 0.84, p=0, as shown in Figure 5). There was no significant heterogeneity among studies (I²=0.0%, p=0.383). No evidence of publication bias was noted (Begg, p=1; Egger, p=0.773).

Plasma Hcy levels were lower in patients with SH with L-T4 treatment than patients with SH without L-T4 treatment (9 studies, SMD: -0.18, CI: -0.32 to -0.05, p=0.006, as shown in Figure 6). There was no significant heterogeneity among studies (I²=0.0%, p=0.678). No evidence of publication bias was noted (Begg, p=0.466; Egger, p=0.757).

Finally, we compared plasma Hcy concentrations between patients with OH with L-T4 treatment and patients with OH without L-T4 treatment, and found that plasma Hcy levels were significantly lower in patients with OH with L-T4 treatment than in patients with OH without L-T4 treatment (5 studies, SMD: -1.22, CI: -1.96 to
Homocysteine and hypothyroidism

<table>
<thead>
<tr>
<th>Study ID</th>
<th>SMD (95% CI)</th>
<th>% Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Christ-Crain et al (2003)</td>
<td>0.51 (0.15, 0.87)</td>
<td>65.19</td>
</tr>
<tr>
<td>Lubosphitzky et al (2004)</td>
<td>0.99 (0.28, 1.70)</td>
<td>16.45</td>
</tr>
<tr>
<td>Cakal et al (2007)</td>
<td>0.33 (-0.35, 1.00)</td>
<td>18.37</td>
</tr>
<tr>
<td>Overall (I-squared = 0.0%, p = 0.383)</td>
<td>0.56 (0.27, 0.84)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Figure 5. Comparison of plasma homocysteine level between overt hypothyroidism patients without levothyroxine treatment and subclinical hypothyroidism patients without levothyroxine treatment.

<table>
<thead>
<tr>
<th>Study ID</th>
<th>SMD (95% CI)</th>
<th>% Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delcher et al (2002)</td>
<td>-0.09 (-0.55, 0.36)</td>
<td>8.46</td>
</tr>
<tr>
<td>Christ-Crain et al (2003)</td>
<td>0.03 (-0.40, 0.46)</td>
<td>9.50</td>
</tr>
<tr>
<td>Pe'irez et al (2004)</td>
<td>-0.03 (-0.46, 0.40)</td>
<td>9.61</td>
</tr>
<tr>
<td>Ozcan et al (2005)</td>
<td>-0.25 (-0.56, 0.05)</td>
<td>19.06</td>
</tr>
<tr>
<td>Beyhan et al (2006)</td>
<td>-0.16 (-0.48, 0.16)</td>
<td>17.10</td>
</tr>
<tr>
<td>Cakal et al (2007)</td>
<td>-0.51 (-1.23, 0.22)</td>
<td>3.32</td>
</tr>
<tr>
<td>Erdal et al (2008)</td>
<td>-0.27 (-0.63, 0.09)</td>
<td>13.60</td>
</tr>
<tr>
<td>Adrees et al (2009)</td>
<td>-0.44 (-0.82, -0.06)</td>
<td>12.04</td>
</tr>
<tr>
<td>Anagnostis et al (2014)</td>
<td>0.08 (-0.41, 0.57)</td>
<td>7.31</td>
</tr>
<tr>
<td>Overall (I-squared = 0.0%, p = 0.678)</td>
<td>-0.18 (-0.32, -0.05)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Figure 6. Comparison of plasma homocysteine level between subclinical hypothyroidism patients with levothyroxine treatment and subclinical hypothyroidism patients without levothyroxine treatment.

-0.47, p=0.001, as shown in Figure 7). Significant heterogeneity was observed among studies (I²=86.4%, p=0). Begg’s test (p=0.027) and Egger’s test (p=0.007) indicated the existence of publication bias. We then used the trim-and-fill method to adjust for funnel plot asymmetry, however, results showed no trimming performed and data unchanged. After excluded 2 studies [12, 20] by using sensitivity analysis, a meta-analysis of other 3 studies
indicated that the main results remained unchanged, a significant reduction of plasma Hcy levels was observed in patients with OH with L-T4 treatment compared with patients with OH without L-T4 treatment (SMD: -0.50, CI: -0.77 to -0.23, p=0). There was no significant heterogeneity among studies ($I^2=4.6\%$, $p=0.351$). Nevertheless, Begg's test ($p=0.296$) and Egger's test ($p=0.010$) indicated the existence of publication bias.

Discussion

Plasma Hcy level is affected by several genetic, physiological and life-style factors [24, 25]. The reasons for hyperhomocysteinemia are excess of dietary methionine, deficit of folate and vitamins taking part in Hcy metabolism, and deficit of kidney function [26, 27]. Kidney is a major issue for removal and metabolism of Hcy, which is closely associated with glomerular filtration rate (GFR) and albuminuria [28]. Hyperhomocysteinemia induces endothelial injury, oxidative stress, oxidation of LDL-cholesterol and smooth muscle hypertrophy [29, 30]. Toxic effect of Hcy and its spontaneous oxidation product, homocysteic acid, have the ability to activate N-methyl-D-aspartic acid (NMDA) receptors, then increase intracellular levels of calcium ion and reactive oxygen species [31, 32]. Moreover, platelet aggregation, vasomotor function and plasma anticoagulant function are altered in the presence of elevated plasma Hcy concentrations [33]. Hyperhomocysteinemia is also one of the pathogenic factors for neuropathy, such as brain stroke and Alzheimer's disease [34]. Severe hyperhomocysteinemia can lead to convulsions and dementia [35].

Thyroid hormones strongly affect the heart and the vascular system [36]. Hypothyroidism is a common condition that is related to premature atherosclerosis and its clinical consequences, such as myocardial infarction [37]. Autopsy findings support an increase in atherosclerosis events in patients displaying hypothyroidism [38]. Several studies reported that plasma Hcy concentrations elevated in patients with OH compared with those healthy subjects [7, 39]. Consistent with these studies, the results of our meta-analysis indicated a higher plasma Hcy levels in patients with OH than in healthy subjects who have euthyroidism. The elevation of plasma Hcy level can be explained by impaired renal clearance or reduced urinary excretion in hypothyroidism [7]. The haemodynamic effects of hypothyroidism may be the reason of reduced renal blood flow and GFR [40]. Experimental studies have also implied that methylenetetrahydrofolate reductase, a key enzyme in folate metabolism, decreased in patients with hypothyroidism [39, 41]. As the

![Figure 7. Comparison of plasma homocysteine level between overt hypothyroidism patients with levothyroxine treatment and overt hypothyroidism patients without levothyroxine treatment.](image)
Homocysteine and hypothyroidism

A major determinant of plasma Hcy status, folate level decreases in patients with hypothyroidism, leading to elevated plasma Hcy status. Auer J et al demonstrated that variation of thyroid function within the normal range might affect the presence and severity of coronary atherosclerosis [42]. Taddei and his co-workers also identified a great prevalence of endothelial dysfunction in patients with SH, resulting from a reduction in NO availability [43]. However, the evidences are still judged as insufficient [44, 45]. Our meta-analysis indicated that plasma Hcy concentrations in patients with SH were similar to that in healthy subjects. This phenomenon can be interpreted as a hypothesis that slight reduction in renal function and methylenetetrahydrofolate reductase may be insufficient to influence plasma Hcy status. We also observed an elevation of plasma Hcy levels in patients with OH compared with patients with SH, which indicated that with the progression of hypothyroidism, the alteration of plasma Hcy concentrations became more and more obvious.

A randomized controlled prospective study, assessing the effect of L-T4 treatment on patients with hypothyroidism, showed a reduction in body weight, although slight, after 3 months [46]. It is also reported that L-T4 replacement decreased blood pressure and central arterial stiffness and improved endothelium-dependent vasodilatation in patients with SH [15, 47]. Moreover, a significant increase in plasma high-density lipoprotein cholesterol levels was found in patients with SH after 3 months of L-T4 treatment [48]. Another study indicated that L-T4 therapy might cause a reduction in lipoprotein (a) status in patients with OH [49]. However, a conflicting study claimed that L-T4 substitution therapy had no effect on cardiovascular risk profile in patients with subclinical hypothyroidism [10]. Besides the dose treatment duration of L-T4, hypothyroidism severity and duration may also contribute to the elevation of plasma Hcy levels. In our meta-analysis, plasma Hcy concentrations decrease after L-T4 treatment in patients with SH or OH.

Even though our meta-analysis enroll relatively high-quality articles which shared similar characteristics, there were some limitations existing in this investigation. First, detection methods of plasma Hcy concentration varies among enrolled studies, which may affect the accuracy of plasma Hcy concentration. Second, Clinical diversity among the studies enrolled in this meta-analysis will result in statistical heterogeneity, which may influence the outcomes, although a random effects model is used. Third, calculated Begg's and Egger's tests indicated the existence of publication bias. We are unable to obtain the original data from corresponding authors. Fourth, we need more high-quality and large-sample studies included in our meta-analysis.

Taken together, this meta-analysis suggests that the status of plasma Hcy is associated with the severity of hypothyroidism and L-T4 treatment is good for patients with hypothyroidism to reduce the plasma Hcy levels.

Disclosure of conflict of interest

None.

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