Homocysteine, Folate and Cobalamin Levels in Hypothyroid Women before and after Treatment

ANNA ORZECHOWSKA-PAWILOJC, KRZYSZTOF SWORCZAK, ANNA LEWCZUK AND ANNA BABINSKA

Department of Internal Medicine, Endocrinology and Haemostatic Disorders, Medical University of Gdansk, Poland

Abstract. Hypothyroidism may result in accelerated atherosclerosis. Hyperhomocysteinaemia is an independent risk factor for premature atherosclerotic vascular disease. The aim of the present study was to assess plasma total homocysteine (tHcy), folate and cobalamin concentrations in hypothyroid patients before and after treatment. Thirty-one hypothyroid and thirty health young women were studied. The hypothyroid patients were investigated in the untreated state and again after restoration of euthyroidism. The levels of homocysteine, folate, cobalamin and thyroid stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3) and renal function were measured before and after treatment. In hypothyroidism tHcy was higher but not statistically significant than in control group. Serum level of folate was higher and serum cobalamin was lower in the hypothyroid state. Following L-thyroxine therapy tHcy significantly decreased as well as the concentration of cobalamin. Level of folate remained unchanged. Univariate analysis in hypothyroid group indicated that tHcy negative correlated with creatinine clearance, fT3, fT4, cobalamin and positive with TSH. In multivariate analysis tHcy correlated with creatinine clearance, cobalamin and fT4. Thyroid status influences the plasma tHcy. Free triiodothyronine and next free thyroxine have the greatest negative influence. This would account for hyperhomocysteinemia in the hypothyroid state and premature atherogenesis.

Key words: Homocysteine, Hypothyroidism, Atherosclerosis

HYPOTHYROIDISM is associated with an increased risk for atherosclerotic cardiovascular disease and cardiovascular morbidity [1, 2]. Hypothyroidism alters several of the traditional cardiovascular risk factors via increase in total cholesterol, low-density lipoprotein cholesterol (LDL-CH) particles, lipoprotein B, induction of diastolic hypertension, direct effect on vascular smooth muscle and altered coagulability. These conventional risk factors fail to account for part of the cases of the accelerated atherogenesis. In recent years researchers have investigated the role of “new risk factors”: homocysteine, CRP protein and lipoprotein A [3, 4]. A few studies have reported moderately elevated concentrations of plasma total homocysteine (tHcy) in hypothyroid patients compared with euthyroid controls [5].

Numerous studies have demonstrated an independent relationship between mild hyperhomocysteinemia and arterial and venous occlusive disease [6–8]. In epidemiologic studies hyperhomocysteinemia is a frequent cause of dementia, psychiatric disorders, pregnancy complications and birth defects [9–11]. In large metaanalysis, a 25% (3 µmol/l) reduction in tHcy was associated with a 11–16% decrease in the risk of ischemic heart disease, 19–22% decrease in the risk of stroke and 25% decrease of deep venous thrombosis [12].

Starting at a plasma tHcy concentration of 10 µmol/l, the risk increases linearly [13]. Elevated plasma tHcy level (>12 µmol/l) are found in 5–10% of the general population and in up to 40% of patients with vascular disease [14]. Hyperhomocysteinemia induces endothelial injury, oxidative stress, smooth muscle hy-
pertrophy and oxidation of LDL-CH. Platelet aggregation, anticoagulant functions of plasma and vascular vasomotor function are altered in the presence of high plasma levels of tHcy [15].

Homocysteine is a sulfur-containing amino acid biosynthesized from methionine, an essential amino acid. Hcy is an intermediate product in the transfer of activated methyl groups from tetrahydrofolate to S-adenosylmethionine (the reversible remethylation pathway). This reaction is catalyzed by the methionine synthetase and requires vitamin B₁₂ as a cofactor and 5-methyl-THF from folate as a methyl donor.

Hcy can also be metabolized by the irreversible transulfuration pathway. In this pathway Hcy is the substrate of the vitamin B₆-dependent enzyme cystathionine β-synthase, which catalyses the synthesis of cystathionine. This pathway can catabolize effectively the potentially toxic excess of Hcy, which is not required for methyl transfer [16].

Elevated plasma tHcy levels are due to several genetic and acquired factors. Impaired enzyme function, vitamin deficiencies, medicaments and diseases such as renal failure, pernicious anaemia, cancers and age, sex or life style (coffee, alcohol), have an impact on Hcy metabolism [17].

In the present study we measured tHcy, folate and cobalamin in women with hypothyroidism before and after treatment with L-thyroxine.

Material and Methods

1 Participants

Thirty-one female study participants were prospectively recruited from subjects referred to our thyroid out-patient service. These patients were between 20–52 years of age (average age 37.9 ± 10.3). Inclusion criteria were a newly, non-treated hypothyroidism and regular menses. Diagnosis of hypothyroidism was based on clinical and basal serum TSH values >5 mU/l. All hypothyroid women had Hashimoto disease (positive thyroid peroxidase antibodies and characteristic in ultrasonography). The control group consisted of 30 female healthy volunteers between 20–44 years (average age 31.4 ± 7.7).

Exclusion criteria were: diseases and drugs (folate, vitamin B₁₂ and B₆ antagonists, anticonvulsants, thiazides, fibrate) that change plasma Hcy level; pregnancy, lactation and oral contraceptive; clinical or history of arteriosclerotic disease; excess of alcohol and coffee consumption, special restriction diet. The study protocol was approved by the regional ethics committee. All participants gave their informed consent to participate in this study.

At baseline routine blood chemistry tests (including creatinine concentration) were done for each patient. Serum TSH, fT₄, fT₃, vitamin B₁₂, folate and tHcy levels were also measured. Second blood analysis was done after obtaining euthyroid state, over 3-4 months of treatment with L-thyroxine (Euthyrox Merck, Darmstadt, Germany) in individualized doses. Participants were advised not to change their life style.

2 Biochemical methods

After physical examination body mass indexes (BMI) were measured and blood samples were taken at 9:00 AM after an overnight fasting. The plasma was separated within 20 min by centrifugation at 3000 rpm for 5 min and stored at −20°C until analysis.

Serum fT₄, fT₃, and TSH levels were determined by microparticle enzyme immunoassay (MEIA), obtained from Abbott Laboratories (AxSYM analyzer). The normal range for fT₄ was 9–24 pmol/l, for fT₃: 2.2–5.3 pmol/l and for TSH: 0.3–5.0 mU/l. Serum folic acid was determined by MEIA assay (Abbott Laboratories) by IMx analyzer. The reference range is: 2.9–18.7 ng/ml. Vitamin B₁₂ was determined by chemiluminescent microparticle immunoassay (CMIA) (Abbott Laboratories) with Architect system—reference range: 179–1162 pg/ml.

tHcy was determined by fluorescence polarization immunoassay (FPIA). The reference range: <12 µmol/l for healthy persons and <10 µmol/l for individuals with a high cardiovascular risk [18, 19]. The relative coefficient of variation of this assay varies between 1.4% and 5.2% and the correlation with standard high performance liquid chromatography (HPLC) is high (r = 0.989) [20].

Serum creatinine was measured by an automated enzymatic method and creatinine clearance (Clcr) was calculated using the Cockcroft-Gault formula: $C_{cr} \text{ (ml/ min)} = \frac{[(140 - \text{age (year)})/72 \times C_{cr}] \times \text{weight (kg)}}{72 \times \text{weight (kg)}}$; for women this value was multiplied by 0.85. This formula has a significant correlation to GFR in the literature [21].
3 Statistical analysis

The data of the hypothyroid group (before and after treatment) and control group were determined using Student’s paired t-test. In the case of non-Gaussian distribution, the original data were transformed to attain normal distribution. After transformation all of these variables had a normal distribution. Univariate relations between tHcy and other variables are presented as Pearson rank correlations. To assess the simultaneous relation among the various predictors of tHcy, multiple linear regression models were used. The analyses were performed with log-tHcy as the dependent variable. We used statistical package Statistica 6.0 (StatSoft).

Results

Table 1 summarizes clinical and laboratory data of patients and controls. Women with hypothyroidism had statistically significant higher TSH and lower fT4 than the control group. Mean tHcy levels in patients before treatment (12.73 ± 5.58 µmol/l) were higher but in terms of statistics not significantly different from the values in controls (10.81 ± 2.44 µmol/l). In patients group three women had vitamin B12 deficiency, the mean values of which were significantly lower than in control group (329.69 ± 154.37 pg/ml vs 420.83 ± 142.07 pg/ml). No subject had evidence of folate deficiency, albeit folate was significantly higher in women with hypothyroidism in comparison with controls (8.58 ± 2.91 vs 5.88 ± 3.09 ng/ml).

After recovery of euthyroidism in fasting state tHcy significantly decreased from 12.73 ± 5.58 to 11.15 ± 9.50 µmol/l, vitamin B12 also significantly decreased from 329.69 ± 154.37 to 274.9 ± 118.4 pg/ml, although folate were unchanged.

The mean levels of creatinine clearance were significantly lower in hypothyroid patients vs controls and after treatment significantly increased.

In univariate analysis increased tHcy was significantly associated with high TSH levels (r = 0.33; p = 0.013) and with low: fT3 levels (r = –0.37; p = 0.006), fT4 levels (r = –0.34; p = 0.012), creatinine clearance (r = –0.42; p = 0.001) and vitamin B12 (r = –0.31; p = 0.020).

In multivariate analysis, increased fasting tHcy state was associated with low: vitamin B12 levels (β = –0.38; p = 0.001), creatinine clearance (β = –0.38; p = 0.002) and fT4 (β = –0.27; p = 0.044).

Table 1. Characteristics of the patients and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hypothyroid</th>
<th>Control</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>31</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>37.9 ± 10.3</td>
<td>31.4 ± 7.7</td>
<td>0.008</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.91 ± 5.8</td>
<td>23.13 ± 5.54</td>
<td>0.061</td>
</tr>
<tr>
<td>Coffee (cup/d)</td>
<td>0.8 ± 0.7</td>
<td>2.0 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cigarette (piece/d)</td>
<td>0.4 ± 1.8</td>
<td>0.2 ± 0.4</td>
<td>0.654</td>
</tr>
<tr>
<td>Multivitamin (% of person)</td>
<td>9.7%</td>
<td>33.3%</td>
<td>0.024</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>21.88 ± 28.98</td>
<td>1.32 ± 0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FT4 (pmol/l)</td>
<td>11.07 ± 3.98</td>
<td>15.61 ± 3.98</td>
<td>0.002</td>
</tr>
<tr>
<td>FT3 (pmol/l)</td>
<td>3.22 ± 9.91</td>
<td>no date</td>
<td>no date</td>
</tr>
<tr>
<td>tHcy (µmol/l)</td>
<td>12.73 ± 5.58</td>
<td>10.81 ± 2.44</td>
<td>0.166</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>8.58 ± 2.91</td>
<td>5.88 ± 3.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>329.69 ± 154.37</td>
<td>420.83 ± 142.07</td>
<td>0.007</td>
</tr>
<tr>
<td>Clcr (ml/min)</td>
<td>83.2 ± 18.0</td>
<td>113.1 ± 23.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of the hypothyroid group before and after treatment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hypothyroid</th>
<th>Euthyroid</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>25.91 ± 5.8</td>
<td>25.50 ± 5.61</td>
<td>0.009</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>21.88 ± 28.98</td>
<td>1.35 ± 1.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FT4 (pmol/l)</td>
<td>11.07 ± 3.98</td>
<td>15.53 ± 2.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FT3 (pmol/l)</td>
<td>3.22 ± 0.91</td>
<td>3.62 ± 0.85</td>
<td>0.101</td>
</tr>
<tr>
<td>tHcy (µmol/l)</td>
<td>12.73 ± 5.58</td>
<td>11.15 ± 9.50</td>
<td>0.001</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>8.58 ± 2.91</td>
<td>7.51 ± 2.52</td>
<td>0.067</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>329.69 ± 154.37</td>
<td>274.9 ± 118.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Clcr (ml/min)</td>
<td>83.2 ± 18.0</td>
<td>94.7 ± 22.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3. Correlations between plasma tHcy and other parameters in hypothyroid group in univariate analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ln (tHcy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>0.13</td>
</tr>
<tr>
<td>BMI</td>
<td>–0.13</td>
</tr>
<tr>
<td>Ln (TSH)</td>
<td>0.33</td>
</tr>
<tr>
<td>Ln (fT4)</td>
<td>–0.34</td>
</tr>
<tr>
<td>Ln (fT3)</td>
<td>–0.37</td>
</tr>
<tr>
<td>Ln (folic acid)</td>
<td>–0.03</td>
</tr>
<tr>
<td>Ln (vit.B12)</td>
<td>–0.31</td>
</tr>
<tr>
<td>Clcr</td>
<td>–0.42</td>
</tr>
</tbody>
</table>
Discussion

In the present study, we have found that women with hypothyroidism have a mean tHcy 1.92 µmol/l higher compared with the control group, but this difference was not significant. Normalization of thyroid hormone levels with L-thyroxine supplementation was associated with a significant tHcy decrease. In univariate analysis the serum levels of thyroid hormones and TSH were significant determinants of plasma tHcy. These observations are consistent with the results of other authors [5, 22, 23]. Nedrebo et al. demonstrated that tHcy level was significantly higher in patients with hypothyroidism, than in the group with hyperthyroidism. After 12 months of L-thyroxine treatment plasma tHcy in these two groups the same concentrations were observed [24]. Lien et al. [25] investigated patients who had undergone total thyroidectomy for thyroid cancer during short-term iatrogenic hypothyroidism after discontinuing L-thyroxine supplementation before diagnostic scintigraphy. The plasma tHcy gradually increased during development of hypothyroidism and decreased when L-T4 supplementation again returned. Diekman et al. [26] similarly found higher tHcy in hypothyroid patients and decreased tHcy of about mean 4.6 µmol/l after treatment. In our study the mean tHcy value and the decrease after treatment were smaller than found in others, probably because the hypothyroid group was mild hypothyroid (60% of participants with TSH between 5–10 mU/l).

The important elements of our findings are prospective, longitudinal design and homogeneous population. We ruled out the other main factors associated with hyperhomocysteinemia. Our patients were younger women, with regular menses, led healthy styles life and were without other diseases. In adults, tHcy is usually about 2 µmol/l higher in men than in women. After menopause tHcy gradually rises and reaches the level of men. Sex-related differences are explained by the effects of estrogen status [27, 28]. As Tallova et al. concluded that the concentrations of tHcy are different in the follicular and luteal phase of the menstrual cycle [29, 30]. We have investigated the variability of tHcy during physiological menstrual cycle. We have used hormonal tests and USG examinations to select only the proper menstrual cycles for tHcy levels investigation. We found significant correlations of tHcy levels with estradiol in both phases of the menstrual cycle (unpublished data). In recent investigation we did not take into consideration the phase of the cycle because hypothyroidism can be a cause of the anovulation. Therefore, the differences in menstrual status may affect tHcy levels and complicate the results. It could be worth to consider the level of estradiol in multivariate analysis in subsequent investigations.

In our study we found the association between tHcy and vitamin B12 in hypothyroid patients, but not between tHcy and folate. The mechanism of this significantly low serum vitamin B12 in hypothyroid state and the further decrease during treatment is uncertain. The participants have no symptoms of anaemia. Others have demonstrated that vitamin B12 levels are reduced [31] or unchanged [24, 32] during hypothyroidism. Low cobalamin level in hypothyroid state might be related to a disturbance in intestinal absorption of this vitamin. The decrease of vitamin B12 levels after treatment of hypothyroidism might be related to a depletion of hepatic vitamin B12 (as found in hypothyroid rats [33]) or accelerated rate of metabolism. Diekman et al. [26] found that cobalamin slightly decreased during hypothyroid patients, and did not found any correlation between vitamin B12 and tHcy. We found that B12 was negatively correlated with tHcy in hypothyroid patients. However, cobalamin levels further decreased after treatment with L-T4 in hypothyroid patients despite the fact that tHcy significantly decreased. The same was observed by Lien et al. [25].

In our study folate status was not a determinant of tHcy and remained unchanged during treatment. This observation is not in agreement with other authors [23, 26]. Catargi et al. found lower levels of folate in hypothyroid patients and in univariate analysis tHcy was significantly negatively associated with folate levels [22]. Recently, Ozmen et al. reported low folate levels in hypothyroidism and concluded that hyperhomocysteinemia in hypothyroidism is associated with an altered folate status [34].

Thyroid status has a profound influence on a variety of biochemical processes, which may have an effect on homocysteine metabolism. Several experimental studies have shown that hypothyroidism affects the enzymes involved in the remethylation of homocysteine to methionine, particularly the flavoprotein methylene-tetrahydrofolate reductase (MTHFR). Hepatic activity of MTHFR is reduced in hypothyroidism and is increased in hyperthyroidism [35]. In hypothyroid state the riboflavin conversion to the active coenzyme flavin-adenine dinucleotide is defective and thus
MTHFR has a lower activity [36].

The following mechanism for alterations of tHcy levels is a renal function. In hypothyroidism the reduction in glomerular filtration rate (GFR) has been well documented [37], some patients have creatinine levels greater than normal. Even mild reduction in GFR leads to increased levels of homocysteine. Normalization of elevated TSH levels after treatment with L-thyroxine causes normalization of GFR and renal filtration of homocysteine. The clearance of homocysteine plays a major role in kidney metabolism of this amino acid and renal excretion of homocysteine is negligible [38]. We have demonstrated in women with hypothyroid state that creatinine clearance decreased compared to control group and after treatment was significantly increased. In univariate and multivariate analysis this parameter was the strongest predictor of tHcy. In the literature there are similar reports on a positive correlation between tHcy and serum creatinine concentrations [39].

In conclusion, our study confirms previous observations, showing that thyroid status profoundly influenced tHcy, both through effects on tHcy formation and its elimination from organism. The mild hyperhomocysteinemia in hypothyroidism with altered lipid profile and diastolic hypertension may contribute to accelerated atherogenic state.

References


