Familial hyperhomocysteinemia, age and peripheral vascular diseases - an Italian study

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Abstract

Hyperhomocysteinemia is a widely recognized, although not yet entirely understood, risk factor for cardiovascular disease. Particularly, the complex relationships between age, hyperhomocysteinemia, predisposing genetic factors and peripheral vascular diseases have not been fully evaluated. Our contribution to this issue is a retrospective analysis of a large series of patients with peripheral arterial, venous and lymphatic disease, and of their blood relatives, with special reference to homocysteine plasma levels, age and methylenetetrahydrofolate reductase (MTHFR) polymorphisms. Serum homocysteine was measured in 477 patients (286 males, 191 females, age range 19-78 years) with various vascular clinical conditions: post-phlebitic syndrome (46) recurrent venous ulcers (78), arterial diseases (101) primary lymphoedema (87), secondary lymphoedema (161), arterial diseases (101) primary lymphoedema (87), secondary lymphoedema (161), post-phlebitic syndrome and venous ulcers, respectively. A MTHFR study for polymorphisms and outlet thoracic syndrome (4), and in 50 normol controls. A MTHFR study for polymorphisms in the studied population and therefore claim for the need to study both C677T and A1298C mutations in hyperhomocysteinemic patients.

Introduction

The possible role of homocysteine high levels as a risk factor for vascular disease was described for the first time more than forty years ago,1,2 and in the following decades a large amount of evidence has demonstrated that even mild increases in homocysteina are associated with an increased risk of cardiovascular diseases2-6 including venous thromboembolic disease.5 Hyperhomocysteinemia is frequently associated with MTHFR polymorphisms C677T and A1298C. MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. Figure 1 summarizes homocysteine metabolism.3,13 Figure 2 shows the pathophysiological mechanism(s) of hyperhomocysteinemia-induced vascular damage.14-18

In the last few years, however, the causative role of hyperhomocysteinemia in cardiovascular disease has been questioned mainly based on the reports that failed to demonstrate a clinical benefit after lowering homocysteinaemia levels with vitamins B6, B12 and folate.19-21 Particularly, it has been pointed out that treatment with homocysteine lowering agents, i.e. folic acid and B6 and B12 vitamins, over prolonged times, does not reduce the incidence of cardiovascular adverse effects.19 although it has been reported that folate, vitamin B12 intake is associated with a reduction of the risk of ischemic stroke.20,22 Therefore further studies have been advocated to address this issue.24

In order to understand the relationships between homocysteinaemia and vascular disease we made a retrospective analysis of the patients who were referred to vascular disease rehabilitation program of our institute for peripheral arterial, venous and lymphatic disorders, paying special attention to homocysteinaemia blood levels, MTHFR polymorphisms and age. The influence of MTHFR polymorphisms on homocysteinaemia blood levels was also studied in the available healthy blood related relatives of hyperhomocysteinemic subjects with MTHFR polymorphisms.

Materials and Methods

A general description of the study is reported in Figure 3. Briefly, serum concentration of homocysteine was measured in 477 consecutive patients (286 males, 191 females, mean age 56.5 years, age range 19-78 years) with various vascular clinical conditions: post-phlebitic syndrome (46) recurrent venous ulcers (78), peripheral arterial diseases (101) primary lymphoedema (87), secondary lymphoedema (161), post-phlebitic syndrome and venous ulcers, respectively. A MTHFR study for polymorphisms in the studied population and therefore claim for the need to study both C677T and A1298C mutations in hyperhomocysteinemic patients.

1.6% in the age range <40 years up to 54.9% in the age range >60 years. The present study demonstrate that patients with peripheral arterial disease, post-phlebitic syndrome, venous ulcers and primary lymphoedema show a significantly higher incidence of hyperhomocysteinemia compared to controls, and adds further evidence to the causative role of hyperhomocysteinemia in the development of both arterial and venous disease. Moreover our data indicate a possible causative role of hyperhomocysteinemia in primary lymphoedema. In more than 50% of our hyperhomocysteinemic patients a polymorphism of MTHFR (C677T and/or A1298C) was detected. In subjects with these polymorphisms the frequency of hyperhomocysteinemia increases with age. We observed a quite similar frequency of the two polymorphisms in the studied population and therefore claim for the need to study both C677T and A1298C mutations in hyperhomocysteinemic patients.
homocysteine serum levels was performed with an automated latex enhanced immunoas-
say (HemosIL, Homocysteine - 002007800, Instrumental Laboratory SpA, Milano, Italy). When the plasma homocysteine values exceeded 15 μmol/L patients underwent MTHFR study for C677T and/or A1298C polymorphisms. MTHFR polymorphism analysis was performed after genetic amplification on venous blood EDTA treated samples by molecu-
lar genetic real time techniques in an associat-
ed laboratory (BIOS, Rome, Italy).

Homocysteine plasma determination and MTHFR polymorphism studies were performed also on 1430 healthy blood related relatives (mainly, siblings, descendents and sibling descendents) of the subjects with hyperhomo-
cysteinemia and MTHFR polymorphisms.

Results

Patients

Hyperhomocysteinemia was found in 20% of controls and in 69.3%, 69.5% and 53.8 % of sub-
jects with arterial diseases, postphlebitic syn-
drome and venous ulcers, respectively (Table 1). As expected, the percentage of hyperhomocys-
teinemia in patients with secondary lymphoede-
ma and with thoracic outlet syndrome did not show significant differences compared to the control group (Table 1). At least one MTHFR polymorphism was found in 116 out of the 214 hyperhomocysteinemic patients i.e. in the 54% of the overall patient population with hyperho-
mocysteinemia (Table 2).

Blood related relatives of hyperhomocysteinemic patients bearing a methylenetetrahydrofolate reductase polymorphism

In 750 (52%) out of the 1430 blood relatives of the 116 patients with hyperhomocysteinemia and MTHFR polymorphisms at least one poly-
morphism in MTHFR gene was found. In this group of 750 asymptomatic subjects bearing a MTHFR polymorphism the frequency of the find-
ing of hyperhomocysteinemia increased accord-
ing to the age class from 1.6% in the age range <40 years up to 54.9% in the age range >60 years (Table 3). C677T polymorphism-associated hyperhomocysteinemia did not significantly differ from A1298C-induced hyperhomocysteinie-
ma either in frequency or in hyperhomocys-

Figure 1. Homocysteine metabolism. Homocysteine is a sulphydryl amino acid derived from the intracellular demethylation of methio-
nine. Homocysteine, when activated, yields a methyl group to different receivers (including creatine, steroid hormones, purine bases of DNA and RNA) and then it can be converted into homocysteine. Homocysteine may be, in turn, transformed irreversibly into cystathio-
nine and then cysteine, or, in the absence of dietary methionine, remethylated to methionine. A series of enzymes and cofactors regu-
late these pathways. Homocysteine is produced through two possible pathways: remethylation or trans-sulphuration. The remethyla-
tion process converts back homocysteine to methionine (utilizing folate, vitamin B12 or trimethylglycine). The trans-sulphuration process utilizes vitamin B6, pyridoxal-5-phosphate, and catabolizes the homocysteine excess into metabolites that can be excreted from the organism. A mildly failure of the remethylation pathway (often due to reduced levels of folate, vitamin B12 or genetic defects) can increase significantly the homocysteine plasma levels. A mild failure in the trans-sulphuration pathway (caused by genetic defect or inadequate levels of vitamin B6) can only increase slightly the homocysteine plasma concentration. There are several causes of hyper-
homocysteinemia, some create a deficiency of the enzyme co-factors, and others reduce the activity of enzymes, involved in its metabo-
lism. The deficiencies of vitamin B12, B6 or folic acid may be due to an inadequate diet, intake of drugs like methotrexate, nitoxide and levodopa or conditions involving hormonal changes like pregnancy and hypothyroidism.
Figure 2. Hyperhomocysteinemia mechanisms of endothelial damage and thrombosis. Hyperhomocysteinemia induces an oxidative stress through both direct and indirect effects. Particularly important are the inhibition of glutathione peroxidase, the reduction of cysteine blood concentration, which results in depressed glutathione levels, the overexpression of NADPH and the reduced availability of nitric oxide. In its turn the depression of nitric oxide activity activates MMP-2 and MMP-9 with consequent increased aggregation of platelets and increased interaction between platelets and endothelium. Moreover MMP activation increases the production of endostatin from collagen and of angiostatin from plasminogen with consequent vascular damage. Thrombophilia is also sustained by the hyperhomocysteinemia-enhanced activation of TF, of TAFI and of factor XIII. All these data explain why abnormally elevated homocysteine blood levels result in endothelial damage and in a consequent increase of the risk of both arterial and venous thrombosis. TF, tissue factor; TAFI, thrombin activable fibrinolysis inhibitor; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9.
tein induced levels (Table 4). Moreover, the association of the two polymorphisms in the same subject did not result either in an earlier appearance of hyperhomocysteinemia or in higher homocysteinemia levels. From a clinical point of view our findings indicate a quite similar frequency of the two polymorphisms in the studied population and therefore the need to study both C677T and A1298C mutations in hyperhomocysteinemic patients (Table 4).

**Discussion and Conclusions**

Despite some uncertainty due to the limited number of control subjects the present study shows a far higher incidence of hyperhomocysteinemia in patients with peripheral artery disease, post-phlebitic syndrome, venous ulcers and primary lymphoedema compared to controls, therefore adding further evidence to the causative role of hyperhomocysteinemia in the development of both peripheral thrombotic arterial and venous disease and of primary lymphoedema. The patients with primary lymphoedema recruited in the present study had no evidence of other vascular disease.

In more than 50% of our hyperhomocysteinemic patients a polymorphism of MTHFR (C677T and/or A1298C) was detected. It has been also observed that in subjects with MTHFR C677T and/or A1298C polymorphism the frequency of hyperhomocysteinemia increases with age. This latter finding is especially unexpected taking into account that genetic disorders usually, although not always, produce clinically evident disorders in the first decades of life. This could possibly be explained by an age related reduction of the defences against oxidative stress.

Another interesting observation is that C677T polymorphism-associated hyperhomocysteinemia did not significantly differ from A1298C-induced hyperhomocysteinemia either in frequency or in severity. Moreover, surprisingly, the association of the two polymorphisms in the same subject is not associated either with an earlier appearance of hyperhomocysteinemia or with higher homocysteinemia levels. The very similar frequency of the C677T and A1298C polymorphisms in the patient population implies the need to study both C677T and A1298C mutations in hyperhomocysteinemic patients. Particularly, it is known that in southern Europe, and especially in Italy, the prevalence of C677T homozygous polymorphism is quite higher compared with northern and central Europe.25

Finally, at authors’ knowledge this is the first report indicating a possible causative role of hyperhomocysteinemia in primary lymphoedema.

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**Table 1. Homocysteine plasmatic values according to the underlying disease (data on 477 patients) and in the control group (50 subjects).**

<table>
<thead>
<tr>
<th>Test results</th>
<th>Arterial diseases</th>
<th>Post-phlebitic syndrome</th>
<th>Venous leg ulcers</th>
<th>Primary lymphoedema</th>
<th>Secondary lymphoedema</th>
<th>Thoracic outlet syndrome</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 to 15 μmol/L</td>
<td>Normal levels</td>
<td>31</td>
<td>14</td>
<td>35</td>
<td>61</td>
<td>117</td>
<td>4</td>
</tr>
<tr>
<td>15 to 30 μmol/L</td>
<td>Moderate hyperhomocysteinemia</td>
<td>44</td>
<td>17</td>
<td>30</td>
<td>19</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>30 to 100 μmol/L</td>
<td>Intermediate hyperhomocysteinemia</td>
<td>17</td>
<td>10</td>
<td>9</td>
<td>7</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>&gt;100 μmol/L</td>
<td>Severe hyperhomocysteinemia</td>
<td>-</td>
<td>69.3</td>
<td>69.5</td>
<td>53.8</td>
<td>29.9</td>
<td>27.3</td>
</tr>
</tbody>
</table>

Hyperhomocysteinemic subjects were significantly (P<0.001 at Chi-square test) more frequent in the groups of subjects with arterial diseases, postphlebitic syndrome, venous ulcers and primary lymphoedema compared to controls.

**Table 2. Patients bearing A1298C and/or C677T polymorphisms (n=116), methylenetetrahydrofolate reductase polymorphism, homocysteinemia level and associated clinical disorders.**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Moderate hyperhomocysteinemia</th>
<th>Intermediate hyperhomocysteinemia</th>
<th>Severe hyperhomocysteinemia</th>
<th>Arteriopathy</th>
<th>Thrombo-Phlebitis</th>
<th>Venous leg ulcers</th>
</tr>
</thead>
<tbody>
<tr>
<td>C677T heterozygous 18 subjects</td>
<td>15</td>
<td>3</td>
<td>1</td>
<td>11</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>C677T homozygous 16 subjects</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>A1298C homozygous 28 subjects</td>
<td>17</td>
<td>10</td>
<td>1</td>
<td>19</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>A1298C homozygous 16 subjects</td>
<td>6</td>
<td>11</td>
<td>2</td>
<td>7</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>C677T heterozygous A1298C heterozygous 5 subjects</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>C677T heterozygous+A1298C homozygous 19 subjects</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>C677T homozygous+A1298C heterozygous 12 subjects</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>C677T homozygous+A1298C homozygous 2 subjects</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

The first three columns refer to the degree of hyperhomocysteinemia. The last three columns illustrate the associated vascular disease.
Table 3. Age related finding of hyperhomocysteinemia in asymptomatic methylenetetrahydrofolate reductase polymorphism bearers.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. subjects</th>
<th>C677T eterozygot ic mutation</th>
<th>C677T homozygot ic mutation</th>
<th>A1298C eterozygot ic mutation</th>
<th>A1298C homozygot ic mutation</th>
<th>No. subjects with hyperhomocysteinemia</th>
<th>% subjects with hyperhomocysteinemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤39</td>
<td>83</td>
<td>24</td>
<td>16</td>
<td>28</td>
<td>19</td>
<td>2</td>
<td>1.66</td>
</tr>
<tr>
<td>40-49</td>
<td>254</td>
<td>66</td>
<td>48</td>
<td>89</td>
<td>55</td>
<td>34</td>
<td>13.4</td>
</tr>
<tr>
<td>50-59</td>
<td>211</td>
<td>56</td>
<td>45</td>
<td>61</td>
<td>56</td>
<td>79</td>
<td>37.4</td>
</tr>
<tr>
<td>≥60</td>
<td>202</td>
<td>53</td>
<td>42</td>
<td>63</td>
<td>55</td>
<td>111</td>
<td>54.9</td>
</tr>
</tbody>
</table>

The frequency of hyperhomocysteinemic subjects among age groups resulted always highly significant (P<0.001 at Chi-square test). Notice that in 38 cases a double polymorphism was found.

Table 4. Incidence of methylenetetrahydrofolate reductase (MTHFR) polymorphisms in the 750 blood relatives of the patients with MTHFR mutation and relative incidence and level of related hyperhomocysteinemia.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Moderate hyperhomocysteinemia</th>
<th>Intermediate hyperhomocysteinemia</th>
<th>Severe hyperhomocysteinemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>C677T eterozygot ic 199 subjects</td>
<td>20</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td>C677T homozygot ic 151 subjects</td>
<td>22</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>A1298C eterozygot ic 241 subjects</td>
<td>23</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>A1298C homozygot ic 185 subjects</td>
<td>28</td>
<td>26</td>
<td>6</td>
</tr>
</tbody>
</table>

Notice that 95 out of 226 (42%) bearers of a MTHFR polymorphism presented, at a carefully made clinical interview, a history for peripheral vascular disease.
References