No Evidence for Hyperhomocysteinemia or Increased Prevalence of Genetic Polymorphisms in the Homocysteine Pathway in Patients with Moderate Juvenile Idiopathic Arthritis

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ABSTRACT. Objective. Elevated plasma total homocysteine (tHcy) concentrations are associated with premature cardiovascular disease. We assessed tHcy, folate, vitamin B12 (Vit B12), vitamin B6 (Vit B6), and genetic polymorphisms potentially enhancing tHcy in patients with juvenile idiopathic arthritis (JIA) and healthy controls.

Methods. Open study of 56 consecutive patients with JIA and 62 controls.

Results. tHcy concentrations were normal in JIA patients (mean 6.5 ± 2 µmol/l) and controls (mean 7.5 ± 2.2 µmol/l). Folate concentrations were significantly higher in JIA patients (40.2 ± 67.9 ng/ml) compared to controls (13.6 ± 8.2 ng/ml). The prevalence of genetic polymorphisms coding for key enzymes in the homocysteine pathway did not differ between patients and controls. Erythrocyte sedimentation rate (ESR) showed significant inverse correlations with circulating Vit B6 and tHcy concentrations.

Conclusion. No evidence for hyperhomocysteinemia or evidence for a specific genetic predisposition for hyperhomocysteinemia was present in patients with JIA. Elevated ESR is not associated with hyperhomocysteinemia. (J Rheumatol 2005;32:170–4)

Key Indexing Terms: PREMATURE CARDIOVASCULAR DISEASE JUVENILE RHEUMATOID ARTHRITIS

Homocysteine (Hcy), a sulfur-containing amino acid derived from the essential amino acid methionine, is either irreversibly metabolized to cysteine (transsulfuration pathway) or remethylated to methionine. The transsulfuration pathway is vitamin B6 (Vit B6)-dependent. The remethylation pathway is folate and vitamin B12 (Vit B12)-dependent and involves the enzymes methionine synthase (MS, gene symbol: MTR), methionine synthase reductase (MSR, gene symbol: MTRR) and methylenetetrahydrofolate reductase (MTHFR, gene symbol: MTHFR).1,2 Hyperhomocysteinemia results from the interaction of genetic and exogenous factors. Genetic polymorphisms such as the TT genotype of the MTHFR 677C→T polymorphism, compound heterozygosity for MTHFR 677C→T and MTHFR 1298A→C3,4,10 are associated with increased tHcy. Important exogenous factors are insufficient supply of folate, Vit B6 or Vit B12, immobility, and chronic inflammation.11,12 Total Hcy (tHcy) concentrations increase with age, are higher in males, and vary between ethnic groups.12 In adults, increased tHcy plasma concentrations (> 10 µmol/l) are primarily asymptomatic, but elevated tHcy concentrations are associated with coronary artery disease, premature stroke, and venous thrombosis.10,12-16 In children and adolescents, increased plasma tHcy correlates with stroke,17,18 cardiovascular disease,19 and premature cardiovascular deaths in male relatives.20 Moderate hyperhomocysteinemia can be treated successfully with folate or B vitamin supplements.11 Patients with rheumatoid arthritis (RA) have elevated tHcy concentrations and carry a significantly higher risk for cardiovascular disease.21 Early signs of atherosclerosis have been observed in young patients with chronic arthritis and low disease activity.22 Hyperhomocysteinemia may contribute to this pathology. It is not known whether children with juvenile idiopathic arthritis (JIA), like children with Crohn’s colitis,23,24 have elevated tHcy concentrations and may be at risk of development of early arteriosclerosis. We designed a study to investigate whether hyperhomocysteinemia is present in children and adolescents with JIA and to assess genetic risk factors for...
hyperhomocysteinemia in this population. In this study (1) tHcy, Vit B6, Vit B12, and folate concentrations were measured in patients with JIA and healthy controls; (2) these measures were related to disease activity; (3) the prevalence of relevant genetic polymorphisms in patients with JIA and controls was assessed.

MATERIALS AND METHODS

Subjects. All JIA patients fulfilled the revised classification criteria for JIA. No patient was receiving methotrexate (MTX) treatment at time of blood sample collection, but 11 patients were supposed to start MTX treatment after collection of blood. All patients were treated with nonsteroidal antirheumatic drugs (NSARD) such as naproxen, piroxicam, or indomethacin. Control subjects (children scheduled for elective surgery, allergy tests, ultrasound, or brain imaging) were recruited from the outpatient clinic of the Department of Pediatrics, University of Vienna. No control subject was taking medication or had evidence for conditions known to be associated with an increase or decrease in tHcy. Informed consent was obtained from patients and controls older than 8 years and their parents/guardians.

In 35 JIA patients (26 girls, 9 boys, mean age 11.3 yrs; oligoarthritis, n = 16; seronegative polyarthritis, n = 11; seropositive polyarthritis, n = 2; systemic arthritis, n = 2; psoriatic arthritis, n = 2; arthritis and enthesis, n = 2) and 62 controls (44 girls, 18 boys, mean age 11.3 yrs) all variables of interest were measured: Hcy, Vit B6, Vit B12, and folate concentrations, genetic polymorphisms, and, for patients, the number of affected joints and erythrocyte sedimentation rate (ESR).

In an additional sample of 21 JIA patients (20 girls, one boy; oligoarthritis, n = 7; extended oligoarthritis, n = 2; seronegative polyarthritis, n = 9; seropositive polyarthritis, n = 1; systemic arthritis, n = 1; psoriatic arthritis, n = 1), blood samples could not be obtained in a state of fasting adequate to measure tHcy. In these patients, only genetic polymorphisms were assessed.

The ethics committee of the University of Vienna, Austria approved the study.

Methods. tHcy plasma concentrations were determined using an automated fluorescence polarization immunoassay (FPIA; Abbott IMx® analyzer; Abbott Laboratories, Abbott Park, IL, USA). Vit B12 and folate were measured with the microparticle enzyme immunoassay (Abbott IMx® analyzer). Vit B6 (pyridoxal-5’-phosphate) was measured using high performance liquid chromatography (HPLC) as described.

Restriction fragment length polymorphism analyses. Genomic DNA was isolated from citrated blood samples according to standard procedures. Identification of MTHFR 677C→T29, MTHFR 1298A→C30, and MTR 2756A→G31 was investigated in a multiplex polymerase chain reaction (PCR). Digests of PCR amplification products were analyzed by electrophoresis through 6% polyacrylamide gels (Novex, San Diego, CA, USA) followed by SYBR Green I nucleid acid gel stain (Molecular Probes, Eugene, OR, USA).

Disease activity. Affected joints were defined as joints with swelling or tenderness or pain while moving, or joints with a limited range of movement. Joints were examined by a pediatric rheumatologist. ESR after 1 h was measured according to standard procedures.

Statistical analysis. Continuous variables are reported as means ± standard deviations (SD). Mann-Whitney and chi-square tests were used to evaluate differences between JIA patients and controls, as appropriate. Associations between the variables (tHcy, folate, Vit B6, Vit B12) joint count, ESR) were assessed by correlation analyses using Spearman’s correlation coefficient. SAS® (release 8.01) was used for statistical analyses. A p value < 0.05 was considered to indicate statistical significance.

RESULTS

Disease activity. Mean joint count was 6.9 ± 3.4 joints. Mean ESR was 16.9 ± 11.6 mm.

Homocysteine, folate, Vit B12, and folate concentrations were significantly lower in JIA patients (6.5 ± 2.0 µmol/l; n = 35) compared to healthy controls (7.5 ± 2.2 µmol/l; n = 62; Mann-Whitney U-test p = 0.017). Mean folate concentrations were significantly higher in patients (40 ± 68 ng/ml; n = 35) compared to controls (14 ± 8 ng/ml; n = 62; Mann-Whitney U-test p = 0.02). No significant differences between groups were found for Vit B6 and Vit B12 concentrations (Table 1). No significant difference concerning tHcy was found between boys and girls.

Correlations with tHcy concentrations. Significant negative correlations were found between the concentrations of all cofactors and plasma tHcy concentrations. Correlations were significant between Vit B6, Vit B12, and folate concentrations. Significant correlation was also found between the number of affected joints and ESR in patients and Vit B6, Vit B12, folate, and tHcy (Table 2).

Age correlated inversely with concentrations of Vit B12 (–0.29; p = 0.005), (folate 0.29; p = 0.004), and tHcy (0.56; p = 0.001), but not with Vit B6 concentrations (–0.13; not significant).

Genetic polymorphisms. No differences were found between patients (n = 56) and controls (n = 62) concerning prevalence of the genetic polymorphisms (Table 3).

DISCUSSION

It is known that patients with RA carry a higher risk for the development of arteriosclerosis and cardiovascular disease. Recent data describe endothelial dysfunction, an

| Table 1. Total homocysteine (tHcy), folate, Vit B6, and Vit B12 concentrations in JIA patients and controls. |
|-----------------|-----------------|-----------------|-----------------|
|                  | JIA Patients, n = 35 | Controls, n = 62 | p               |
| tHcy, µmol/l     |                  |                  |                |
| Mean             | 6.5 ± 2.0        | 7.5 ± 2.2        | 0.017           |
| Median           | 6.0              | 7.1              |                |
| Range            | 3.7–13.5         | 3.8–12.5         |                |
| Folate, ng/ml    |                  |                  |                |
| Mean             | 40 ± 68          | 14 ± 8           | 0.02            |
| Median           | 16               | 12               |                |
| Range            | 5–225            | 3–45             |                |
| Vit B6, nmol/l   |                  |                  |                |
| Mean             | 284 ± 1256       | 918 ± 2390       | NS              |
| Median           | 57               | 64               |                |
| Range            | 8–7500           | 6–7500           |                |
| Vit B12, pg/ml   |                  |                  |                |
| Mean             | 382.2 ± 199      | 347 ± 193        | NS              |
| Median           | 364              | 293              |                |
| Range            | 163–1280         | 144–1290         |                |

NS: nonsignificant.
early marker for arteriosclerosis, in young patients with chronic arthritis and low disease activity. Increased plasma tHcy concentrations have been observed in RA patients. Hcy directly affects endothelial cells by induction of oxidative stress. Consequently, endothelium-dependent vasodilatation is impaired. Additionally, Hcy induces proliferation of vascular smooth muscle cells. Recent hypotheses focus on DNA hypomethylation caused by hyperhomocysteinemia. DNA hypomethylation might result in atherogenesis by induction of growth factors. We hypothesized that hyperhomocysteinemia might be present in JIA patients, mediating early atherogenesis in them. Nevertheless, in the patients we investigated, tHcy concentrations were within normal ranges (mean tHcy 6.5 µmol/l) compared to mean tHcy concentrations in 3524 adolescents in the US (tHcy 5.29 µmol/l) and in healthy Belgian (tHcy 7.09 µmol/l) and German children (tHcy 5.6 µmol/l). Folate status is a most significant factor determining tHcy concentrations. High levels of plasma folate are protective for hyperhomocysteinemia and we clearly observed this effect in our study population: folate concentrations correlated inversely with tHcy. We hypothesize that the high folate concentrations measured in patients with JIA resulted in the lower tHcy concentration in this group. Folate concentrations in JIA patients exceeded normal values by far (with a relatively high standard deviation), even though the patients reportedly did not use vitamin supplements. We speculate that at least some of the JIA patients might follow a diet rich in folate.

No significant differences in the prevalence of genetic polymorphisms known to be associated with hyperhomocysteinemia have been found between JIA patients and controls from the same regional population. Therefore no specific genetic predisposition for hyperhomocysteinemia seems to be present in these patients.

The significant inverse correlations between Vit B6 and joint count and ESR correspond with data obtained from RA patients and from participants in the Framingham Heart Study. The underlying mechanisms are less than clear; it is hypothesized that Vit B6 coenzymes might be exported from the liver and peripheral tissues to the sites of inflammation.

In 37 patients with RA, Chiang, et al report, in addition to the inverse correlation of Vit B6 with ESR, a positive correlation of ESR with tHcy concentrations after methionine load. Interestingly, without the stimulus of a methionine loading test, tHcy concentrations in 891 probands were not increased by low plasma Vit B6, and no significant correla-

Table 2. Spearman correlations between homocysteine (Hcy) and vitamin status in JIA patients and healthy controls (n = 97) and correlation of number of affected joints and ESR with Hcy and vitamin status in JIA patients (n = 35).

<table>
<thead>
<tr>
<th>No. of affected joints (n = 35)</th>
<th>Vit B12</th>
<th>Vit B6</th>
<th>Folate</th>
<th>tHcy</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.18 NS</td>
<td>-0.49**</td>
<td>0.12</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>ESR -0.14 NS</td>
<td>-0.27**</td>
<td>-0.31**</td>
<td>-0.31**</td>
<td></td>
</tr>
<tr>
<td>Vit B12, n = 97</td>
<td>0.23*</td>
<td>0.24*</td>
<td>-0.35**</td>
<td></td>
</tr>
<tr>
<td>Vit B6, n = 97</td>
<td>0.25*</td>
<td>0.016</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Folate, n = 97</td>
<td>0.23*</td>
<td>0.013</td>
<td>-0.6**</td>
<td></td>
</tr>
<tr>
<td>tHcy, n = 97</td>
<td></td>
<td>0.001</td>
<td></td>
<td></td>
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</tbody>
</table>

* 2-tailed p < 0.05; ** 2-tailed p < 0.01.

Table 3. Prevalence of genetic polymorphisms associated with mild to moderate hyperhomocysteinemia in 56 JIA patients and 62 controls.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MTHFR 677 C→T</th>
<th>MTHFR 1298 A→C</th>
<th>MTR 2756 A→G</th>
<th>MTRR 997 C→G</th>
<th>MTRR 66 A→G</th>
</tr>
</thead>
<tbody>
<tr>
<td>JIA Patients, %</td>
<td>25 41 46 13 46 50 4</td>
<td>68 23 9 100</td>
<td>—</td>
<td>—</td>
<td>30 48 21</td>
</tr>
<tr>
<td>Controls, %</td>
<td>21 43 48 9 45 47 8</td>
<td>69 29 2 100</td>
<td>—</td>
<td>—</td>
<td>17 67 16</td>
</tr>
<tr>
<td>Chi-square test</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Het: heterozygous; Hom: homozygous; WT: wil-type.
tion was observed between tHcy and C-reactive protein (CRP) as a marker of inflammation. Our data are in part consistent with this finding: for tHcy there is no correlation with joint count as a marker of disease activity and, more important, tHcy concentrations are normal. On the other hand, in the JIA group tHcy correlates inversely with ESR. Further studies are warranted to investigate this relation in children with chronic inflammatory disease. In light of the conflicting results concerning ESR, CRP, and tHcy, we suggest that in future studies longterm accumulated disease activity should be investigated in relation to tHcy concentration.

Concerning the influence of Vit B₆ metabolism on tHcy, it seems important that the intracellular availability of active metabolites of Vit B₆ is probably not precisely reflected by levels of circulating Vit B₆. Impaired Vit B₆ availability may result in a borderline impairment of Hcy metabolism indicated by elevated tHcy concentrations only in a situation of nonphysiological overload of the Hcy pathway. This assumption is supported in part by normal erythrocyte pyridoxal 5' phosphate in systemic inflammatory response with decreased circulating Vit B₆, as reported by Talwar, et al. It is known that individuals heterozygous for the Vit B₆-dependent cystathionin-β-synthase deficiency show abnormal responses to methionine load but are probably not at higher risk for premature cardiovascular disease. Thus the clinical effect of Vit B₆ on cardiovascular disease mediated by the Hcy pathway remains to be elucidated in patients with chronic inflammatory disease. We hypothesize that arteriosclerosis and endothelial dysfunction in arthritis patients might be mediated by other mechanisms such as direct effects of markers of inflammation (e.g., interleukin 6 and CRP) on endothelial cells.

In summary, there is no evidence for hyperhomocysteinemia in patients with JIA with moderate disease activity. The frequency of genetic polymorphisms associated with hyperhomocysteinemia does not differ significantly between patients and healthy controls. The role of Vit B₆ deficiency, its relation to markers of disease activity, and its impact on Hcy metabolism in chronic inflammatory disease remains to be elucidated.

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REFERENCES