Increased Serum Concentrations of Homocysteine and Lipoprotein (a) in Familial Mediterranean Fever

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Abstract. Serum homocysteine, folic acid, lipoprotein (a) (Lp(a)), fibrinogen, and C-reactive protein (CRP) concentrations and erythrocyte sedimentation rates (ESR) were measured in 52 patients with familial Mediterranean fever (FMF) during attack-free periods and in 30 healthy control subjects. Serum homocysteine levels were significantly higher in the FMF patients (median 17.8 µg/dl; range 5.6-80.8) than in controls (median 11.7; range 5.6-42.2; p = 0.013). Serum homocysteine levels were elevated above the upper reference limit (15 µg/dl) in 56% of the FMF patients compared to 27% of the controls (p = 0.011). Serum Lp(a) levels were significantly higher in the FMF patients (median 39.3 mg/dl; range 6.6-124.5) than in controls (median 27.2; range 11.1-78.1; p = 0.035). Serum Lp(a) levels were elevated above the upper reference limit (30 mg/dl) in 71% of the FMF patients compared to 47% of the controls (p = 0.028). The ESR, fibrinogen, CRP, and folic acid levels were similar in both groups. In conclusion, serum homocysteine and Lp(a) concentrations are often increased in FMF patients during attack-free periods. The elevated homocysteine and Lp(a) levels, which are markers of sub-clinical inflammation, may be mediators of atherosclerotic disease in FMF patients.

Keywords: familial Mediterranean fever, homocysteine, lipoprotein (a), folic acid, atherosclerosis

Introduction

Familial Mediterranean fever (FMF) is an inherited, recurrent, inflammatory disease that is characterized by acute self-limited attacks of fever, peritonitis, pleuritis, and arthritis. It is frequent among Eastern Mediterranean populations, such as Turks, Arabs, Armenians, and non-Ashkenazi Jews [1]. Amyloidosis is the most serious manifestation of FMF disease and leads to renal failure. Colchicine treatment dramatically decreases the frequency and severity of FMF attacks and prevents the development of amyloidosis [2].

Homocysteine, a sulfur-containing amino acid, is an intermediary product in the metabolism of methionine, which is also an amino acid [3]. Hyperhomocysteinemia is an independent risk factor for atherosclerotic vascular disease. Elevated homocysteine can contribute to cardiovascular risk through endothelial dysfunction, platelet activation, increased oxidation of low-density lipoproteins (LDL), and proinflammatory mechanisms [4]. Several inflammatory rheumatic diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis, have been associated with accelerated atherosclerosis and increased cardiovascular morbidity and mortality [5]. Elevated serum homocysteine and lipoprotein (a) (Lp(a)) levels have been found to be common risk factors in the pathogenesis of the atherosclerosis that underlies rheumatic disease [5].
There has been little research on the associations among inflammation, homocysteine, Lp(a), and cardiovascular disease in FMF patients [6-8]. The present study is an investigation of serum homocysteine, folic acid, and Lp(a) levels of FMF patients during attack-free periods.

Materials and Methods

Fifty-two adult patients with FMF (23 men, 29 women; age 20-40 yr) and 30 matched, healthy controls (14 men, 16 women; age 21-40 yr) were enrolled in this study. All participants were informed of the study protocol and their written informed consent was obtained, according to the Declaration of Helsinki.

The diagnosis of FMF was established according to criteria defined by Livneh et al. [9]. All patients with FMF were receiving 1–1.5 mg/day of colchicine. Assessments of FMF patients were performed during attack-free periods. The control participants were of similar age and gender distribution to the FMF patients. They had no known diseases and were not using any medication. A detailed medical history was taken and clinical and laboratory examinations were performed on all participants in both groups. Subjects in both groups with a history of smoking, hypertension, angina pectoris, myocardial infarction, diabetes mellitus, amyloidosis, or chronic renal insufficiency were excluded from the study, as were those taking any medication except colchicine.

Fasting venous blood was collected from all participants in vacutainer tubes and quickly centrifuged to avoid glycolysis. Serum samples were kept at −80°C until assay. Blood ESR was determined according to the Westergren method. The nephelometric method was used to measure CRP levels. Serum homocysteine levels were determined using high-performance liquid chromatography (HPLC, Agillent 1100). Folic acid and Lp(a) levels were measured using commercial kits by spectrophotometric methods (Roche Diagnostics, USA, and Olympus, Japan, respectively). Fibrinogen levels were measured with a commercial kit (Diagnostica Stago, France) by an autoanalyzer (STA Compact, France).

Statistical analysis was performed using the program Social Science 11.0 for Windows. Values of the measured parameters were checked for normal distribution by means of the Kolmogorov-Smirnov test prior to statistical analysis. The Mann-Whitney U test and Chi-square test were used to compare the two groups and the elevation ratios. Correlations among laboratory parameters were analyzed using Spearman’s rank correlation test. Only p values ≤0.05 were considered statistically significant.

Results

The demographic properties and clinical and laboratory characteristics of the FMF patients and the control subjects are shown in Table 1. The serum levels of homocysteine and Lp(a) in patients with FMF were significantly higher than those in the healthy controls (p < 0.05). ESR, fibrinogen, CRP, and folic acid levels were similar between the two groups. There was no significant correlation between serum homocysteine and folic acid concentrations (p >0.05).

Table 2 presents the elevation ratios for homocysteine and Lp(a) in FMF patients and healthy controls, based upon the standard upper limits of the reference ranges for the assays (15 µg/dl for homocysteine; 30 mg/dl for Lp(a)). In the FMF patients, the ratio of elevated/normal homocysteine levels was 3.5-fold higher than in the controls (p = 0.011), and the ratio of elevated/normal Lp(a) levels was 2.8-fold higher than in the controls (p = 0.028). There were no significant correlations among the laboratory parameters.

Table 1. Demographic properties and laboratory characteristics of FMF patients and healthy controls (median, (range)).

<table>
<thead>
<tr>
<th>Parameter (reference range)</th>
<th>FMF group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female) (23/29)</td>
<td>(14/16)</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Age (yr) (20-40)</td>
<td>29 (20-40)</td>
<td>29 (21-40)</td>
<td>ns</td>
</tr>
<tr>
<td>Disease duration (yr) (8.5-27)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Homocysteine (5-15 µg/dl)</td>
<td>17.8 (5.6-80.8)</td>
<td>11.7 (5.6-42.2)</td>
<td>0.013</td>
</tr>
<tr>
<td>Folic acid (3.1-17.5 ng/ml)</td>
<td>8.9 (4.4-20)</td>
<td>9.3 (5.6-20)</td>
<td>ns</td>
</tr>
<tr>
<td>Lp(a) (0-30 mg/dl)</td>
<td>39.3 (6.6-124.5)</td>
<td>27.2 (11.1-78.1)</td>
<td>0.035</td>
</tr>
<tr>
<td>ESR (mm/h) (10 (2-25))</td>
<td>10 (2-25)</td>
<td>10 (3-23)</td>
<td>ns</td>
</tr>
<tr>
<td>CRP (mg/dl) (0.46 (0.23-1.19))</td>
<td>0.48 (0.31-1.14)</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (180-350 mg/dl)</td>
<td>276 (155-441)</td>
<td>257 (150-355)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Table 2. Ratios of elevated/normal values for serum homocysteine and Lp(a) in FMF patients and healthy controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FMF group n = 52</th>
<th>Control group n = 30</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine n (high/normal)</td>
<td>(29/23)</td>
<td>(8/22)</td>
<td>0.011</td>
</tr>
<tr>
<td>% (high/normal)</td>
<td>(55.8/44.2)</td>
<td>(26.7/73.3)</td>
<td></td>
</tr>
<tr>
<td>Lp(a) n (high/normal)</td>
<td>(37/15)</td>
<td>(14/16)</td>
<td>0.028</td>
</tr>
<tr>
<td>% (high/normal)</td>
<td>(71.2/28.8)</td>
<td>(46.7/53.3)</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Elevated serum homocysteine levels can occur for various reasons, including genetic factors, such as methylene-tetrahydrofolate reductase gene polymorphisms, vitamin deficiencies in folic acid, B12, and B6, the use of certain drugs, and renal insufficiency [3,11]. Several studies have shown an inverse relationship between levels of homocysteine and folic acid [3,10,11]. In addition, elevated homocysteine levels have been reduced by administration of folic acid supplements. Doses of 0.2 and 0.4 mg/day folic acid can achieve maximum reductions in homocysteine levels [12]. Therefore, folic acid is the most important vitamin in homocysteine metabolism [11,12]. In this study, we measured serum folic acid concentrations in addition to homocysteine levels. While serum homocysteine concentrations were significantly higher in the FMF patient group, folic acid levels were within the normal range. Therefore, the elevated homocysteine values did not seem to depend on folic acid deficiency.

On the other hand, many studies have found increased homocysteine levels in chronic renal insufficiency patients [11]. Even in subjects with normal serum creatinine levels, the importance of renal functions for homocysteine concentrations has been demonstrated [13,14] Furthermore, amyloidosis has been shown to impair renal function and contribute to end-stage renal disease in FMF patients. Several studies have found renal lesions to occur in conjunction with amyloidosis [15]. In the current study, we excluded patients with amyloidosis or chronic renal insufficiency. However, elevated homocysteine concentrations may also be associated with sub-clinical renal inflammation or sub-clinical impaired renal function during attack-free periods in FMF patients. Thus, homocysteine may not only be an important risk factor for atherosclerotic vascular disease, but also a parameter that affects the development of disease-specific target organ damage.

The effects of colchicine on homocysteine and Lp(a) levels, which are acute phase markers, have not yet been fully determined. Anti-inflammatory drugs used in the treatment of inflammatory rheumatic disorders can be either proatherogenic or anti-atherogenic, as can colchicine treatment [5]. Glucocorticoids may cause obesity, insulin resistance, glucose intolerance, dyslipidaemia, and hypertension, so they are associated with atherosclerosis [16,17]. Anti-malarial drugs, such as chloroquine and hydroxychloroquine, decrease the production of very low-density lipoproteins (VLDL), LDL cholesterol, and triglyceride, while methotrexate (MTX) treatment increases homocysteine levels in RA [16,18]. In this context, possible effects of colchicine on serum Lp(a) and homocysteine concentrations should be taken into account.

In recent years it has been established that inflammation promotes every process of atherosclerosis [19]. In addition, local inflammatory processes can lead to the occurrence of vascular events [20]. Several new candidate risk markers, including CRP, homocysteine, and Lp(a), have been defined as predictors of atherosclerosis and its complications [19]. Chronically elevated levels of homocysteine, Lp(a), and inflammatory cytokines may be important contributors to the development of endothelial dysfunction [21]. A strong relationship has been reported between carotid intima media wall thickness (IMT), a predictor of generalized atherosclerosis, and plasma homocysteine levels [22]. To date, few studies have investigated the IMT of the internal carotid arteries (ICA) or common carotid arteries (CCA) in FMF patients [6-8,23]. The limited research that has been conducted in this regard has demonstrated increased CCA-IMT and ICA-IMT in FMF patients in comparison to healthy controls. In contrast, however, another study has found no difference between the IMT of these arteries in FMF patients and controls [23].

Studies investigating homocysteine levels in FMF are also limited, and the results are controversial [6,7]. The research has, like the present study, investigated homocysteine levels in FMF patients during attack-free periods. Peru et al. [7] reported higher homocysteine and Lp(a) levels in FMF patients than in healthy controls, as well as increased CCA-IMT in FMF patients. In contrast, Bilginer et al. [6] found no elevation of homocysteine levels in FMF patients, but did report significantly increased CCA-IMT and ICA-IMT.
in this patient group. These studies have been performed in FMF patients from various age groups, including pediatric and adult patients. It is possible that the controversy regarding homocysteine levels in FMF patients has arisen due to the inclusion of more vulnerable age groups. However, the present study was performed in adult FMF patients and indicated elevated serum homocysteine and Lp(a) levels in these patients.

FMF is a recurrent inflammatory disease, mainly characterized by acute self-limited attacks of inflammation. Significant inflammatory reactions usually continue even during attack-free periods in FMF patients. Musabak et al. [24] demonstrated that levels of sIL-2R are higher in FMF patients during attack-free periods than they are in controls. It was also shown that proinflammatory cytokines increase during attack-free periods [25-27]. These findings suggest that a sub-clinical immune reaction may be present even during attack-free intervals in FMF patients. Moreover, atherosclerosis can be considered the result of this chronic inflammation in adult FMF patients [6]. However, further controversial results have been reported in studies of acute phase reactants, such as ESR, CRP, fibrinogen, and serum amyloid A protein (SAA), during attack-free periods [28-31]. Some studies found these markers to remain in the normal range, while others found elevated levels between attacks. The present study did not find any elevations in ESR, CRP, or fibrinogen levels, but did show increased homocysteine and Lp(a) concentrations during attack-free periods. In this context, elevated levels of homocysteine and Lp(a) may better reflect subclinical inflammation in attack-free periods than do the other acute phase reactants.

Lp(a) is also an acute phase reactant, and it increases in response to the proinflammatory cytokines [32]. It modulates the chemotaxis of human monocytes, and is a highly atherothrombotic lipoprotein [33,34]. The majority of retrospective case-controlled studies have reported strong association between increased Lp(a) levels and coronary heart disease [34,35]. A recent meta-analysis [36] found that Lp(a) levels are an independent risk factor for cardiovascular disease in both men and women. The PRIME study [37], which included 9,133 subjects, confirmed this finding for men. Elevated Lp(a) levels may also contribute to the development of atherosclerotic vascular disease and the continuation of subclinical inflammation in FMF patients. However, it has also been reported that Lp(a) is largely under genetic control [38]. MEFV gene mutations, which are known to be responsible for the genetic element of FMF, may affect Lp(a) concentrations in FMF patients.

In conclusion, the present study, in agreement with the findings of Peru et al. [7], found serum homocysteine and Lp(a) concentrations to be increased in FMF patients during attack-free periods. These elevated levels may play an important role in the development of atherosclerosis and may contribute to increased cardiovascular morbidity and mortality. However, it is unknown whether elevated homocysteine and Lp(a) levels are a marker of sub-clinical inflammation or a mediator of atherosclerotic disease in FMF patients. Further studies are required that investigate this relationship in FMF patients during attack periods and in those not receiving colchicine therapy.

References


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