Analysis of Plasma Homocysteine Levels in Patients with Unstable Angina

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Objective - To determine the prevalence of hyperhomocystinemia in patients with acute ischemic syndrome of the unstable angina type.

Methods - We prospectively studied 46 patients (24 females) with unstable angina and 46 control patients (19 males), paired by sex and age, blinded to the laboratory data. Details of diets, smoking habits, medication used, body mass index, and the presence of hypertension and diabetes were recorded, as were plasma lipid and glucose levels, C-reactive protein, and lipoperoxidation in all participants. Patients with renal disease were excluded. Plasma homocysteine was estimated using high-pressure liquid chromatography.

Results - Plasma homocysteine levels were significantly higher in the group of patients with unstable angina (12.7±6.7 µmol/L) than in the control group (8.7±4.4 µmol/L) (p<0.05). Among males, homocystinemia was higher in the group with unstable angina than in the control group, but this difference was not statistically significant (14.1±5.9 µmol/L versus 11.9±4.2 µmol/L). Among females, however, a statistically significant difference was observed between the 2 groups: 11.0±7.4 µmol/L versus 6.4±2.9 µmol/L (p<0.05) in the unstable angina and control groups, respectively. Approximately 24% of the patients had unstable angina at homocysteine levels above 15 µmol/L.

Conclusion - High homocysteine levels seem to be a relevant prevalent factor in the population with unstable angina, particularly among females.

Keywords: acute ischemic syndrome, unstable angina, homocysteine

The clinical risk factors for coronary artery disease include hypercholesterolemia, hypertension, smoking, and diabetes mellitus. An increase in the plasma concentration of homocysteine has also been associated with early coronary artery disease 1-8. The relation between hyperhomocystinemia and coronary artery disease was due to the discovery of the deficiency of the cystathionine beta-synthase enzyme. This enzyme is deficient in homocystinuria, a rare genetic disorder characterized by high concentrations of homocysteine, in which the patients usually develop early occlusive arterial disease 9. Alterations in the methylenetetrahydrofolate reductase enzyme, which is necessary for the metabolism of homocysteine, may also play a significant role in hyperhomocystinemia. Since the identification of homocysteine as a risk factor for the development of atherosclerosis in the 1960s 10, the current research has focused on determining the actual importance of and the role played by homocysteine as a risk factor for vascular diseases. However, no similar study has ever been published in Brazil.

High plasma homocysteine levels are associated with coronary artery disease, but the precise level associated with an increased risk is yet controversial. Our study aimed at analyzing plasma homocysteine levels that are associated with the risk of coronary artery disease in our environment, particularly in the acute ischemic syndromes of the unstable angina type.

Methods

We studied 92 patients divided into 2 groups as follows: 46 patients (24 females) with unstable angina diagnosed at the time of recruitment (unstable angina group) and 46 patients (19 males) with no coronary artery disease (control group). The patients were consecutively and prospectively recruited at the coronary unit of the Hospital São Paulo of the Escola Paulista de Medicina of the UNIFESP.

All patients in the unstable angina group met the clinical criteria for the diagnosis of unstable angina and had
liquid chromatography with fluorometric detection and iso-
specificity, and reproducibility). This is high-pressure
combines all the conditions for a good analysis (sensitivity,

cules. When C-reactive protein levels are above 8 mg/L, visi-
ted by a pinkish color, and it is measured with a spectro-
cellular membrane. The presence of this substance is indi-
londialdehyde, which appears after lipoperoxidation of the
tances reactive to thiobarbituric acid (TBARs), mainly ma-

Who classification of obesity.

The 46 patients (19 males) with no cardiovascular di-
ase were randomly recruited in the outpatient clinics of
other specialties with no disease considered risky. These pa-
tients were paired by sex and age.

All participants completed a questionnaire about their
habits, diets, use of medication, presence of risk factors, an-
tecedents, and procedures related to coronary disease. They
also underwent blood withdrawal after formal written

According to the established diagnosis of unstable
angina and to control patients who supposedly had no co-
ronary artery disease, we analyzed differences in age, sex,
race, and body mass index (tab. II).

We also assessed the presence of risk factors, pre-
vious cardiovascular history, percutaneous interventional
procedures related to coronary artery disease, alterations in
the T wave and in the ST segment, and also the presence of
previous stroke (tab. II). We considered as diabetic, hyper-
tensive, and dyslipidemic, the patient being treated for any
of these conditions or who reported a previous diagnosis of
any of these conditions established by a physician. A smo-
ker was defined as someone who had been regularly smok-
ing up to 5 cigarettes a day for at least 1 year, or someone
who had not smoked for more than 10 years; and an ex-smoker
was someone who had never smoked or who

Measurement of C-reactive protein was determined
through an immunochemical reaction that occurs between
that protein and antiprotein C antibodies fixed to latex par-
ticles. When C-reactive protein levels are above 8 mg/L, visi-
able agglutination of the latex particles occurs (RapiTex CRP,
Behring Diagnostics). This technique provides a qualitati-
ve or semiquantitative determination. Another methodolo-
gy used that provided a precise determination of the C-reac-
tive protein levels was nephelometry (NA Latex CRP Rea-
gent, Behring Diagnostics), in which case the results were
also expressed in mg/L, and values up to 8 mg/L were consi-
idered normal.

Other measurements, such as glucose, triglycerides,
total cholesterol, and fractions were quantified using the
standard methodology of the clinical laboratory of the Hos-
pital São Paulo.

Table II - General characteristics of the participants

<table>
<thead>
<tr>
<th></th>
<th>Unstable angina (n=46)</th>
<th>Controls (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.6 ± 12</td>
<td>55.7 ± 11.8</td>
<td>0.46</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>52.1</td>
<td>41.3</td>
<td>0.67</td>
</tr>
<tr>
<td>Caucasian (%)</td>
<td>58.7</td>
<td>60.5</td>
<td>0.99</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8 ± 7.2</td>
<td>27.9 ± 5.7</td>
<td>0.94</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>69.6</td>
<td>17.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>28.2</td>
<td>8.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>23.9</td>
<td>6.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>19.5</td>
<td>17.3</td>
<td>0.98</td>
</tr>
<tr>
<td>Previous angina (%)</td>
<td>34.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Previous myocardial infarction (%)</td>
<td>41.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Typical angina (%)</td>
<td>80.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Previous stroke (%)</td>
<td>8.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ECG alterations (%)</td>
<td>84.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Previous angioplasty (%)</td>
<td>8.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stent placement (%)</td>
<td>2.2</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

BMI - body mass index; ECG - electrocardiographic
despite the sample size, followed a normal distribution curve according to the Kolmogorov-Smirnov test that assesses normality (p=0.17).

Results

Table III shows the mean values found in the laboratory tests. The mean values of total cholesterol, LDL-cholesterol, lipoperoxidation, and triglycerides were similar in both groups. No biochemical examination showed any difference between the proportion of altered values in the 2 groups. The mean glycemia and HDL-cholesterol values, however, were different in both groups. To compare the total plasma homocysteine levels, the means of the 2 groups ± standard deviation were considered, and the values of plasma concentration were expressed in micromoles per liter (µmol/L). The mean total plasma homocysteine levels were significantly more elevated in the unstable angina group (p=0.001).

Measuring the C-reactive protein levels may have practical clinical significance in managing patients hospitalized with acute ischemic syndromes, but this was not our objective. In the unstable angina group, 43.5% of the patients had normal C-reactive protein levels (< 8 mg/L), which was found in 65.2% of the patients in the control group (p=0.07). However, more elevated levels (≥ 8 mg/L) were found in 56.5% of the patients in the unstable angina group and in 34.8% of the patients in the control group (p=0.056). Even though the means for C-reactive protein were similar in both groups, when we classified the patients according to their levels of C-reactive protein as individuals with normal (< 8 mg/L) and elevated (≥ 8 mg/L) values, we observed a tendency towards more elevated levels in the unstable angina group (fig. 1). These results show that more elevated levels of C-reactive protein were found among the patients with higher homocysteine levels, both for the unstable angina and control groups.

The homocysteine values (mean ± standard deviation) found in the total sample divided by the increased or normal C-reactive protein levels were compared using the 1-way ANOVA and followed by the Duncan test. This showed that C-reactive protein levels do not relate to homocysteine levels and unstable angina, but, when increased, these levels relate to higher homocysteine values, regardless of the group the individuals belong to. In the unstable angina group with elevated C-reactive protein levels, we found mean homocysteine levels of 14.4±5.9 µmol/L, and, in the control group, we found mean homocysteine levels of 13.6±4.6 µmol/L. On the other hand, for normal C-reactive protein levels, we found mean homocysteine levels of 12.1±7.3 µmol/L in the unstable angina group, and 7.8±3.5 µmol/L in the control group (fig. 1).

Analyzing the mean plasma homocysteine levels, when we compare the 2 groups (patients and controls) paired by sex, we observed a higher and more significant elevation among the females of the unstable angina subgroup (p=0.005) (tab. IV).

In the international literature, normal fasting homocysteinemia ranges from 5 to 15 µmol/L. Concentrations between 16 and 30 µmol/L are considered slightly increased, between 31 and 100 µmol/L are considered moderately increased, and above 100 µmol/L are considered markedly increased. In our study, analyzing the plasma homocysteine levels above 15 µmol/L in both groups again paired by sex, we observed a significant difference in the female unstable angina subgroup as compared with the female control subgroup. This fact was not observed for the male sex (tab. V). Analyzing the sample with no sex distinction, no significant difference was observed in the 2 groups (tab. V).

Considering the male sex, the mean plasma homocysteine levels in the unstable angina group as compared with those in the control group were not significantly higher (p=0.52). It is worth noting that, in this small sample, the mean homocysteine levels in the control group were lower. In the unstable angina group, approximately 24% of the patients had undoubtedly increased plasma homocysteine levels, which occurred in only 8.6% of the control group.

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Table III - Biochemical analyses

<table>
<thead>
<tr>
<th></th>
<th>Unstable angina (n=46)</th>
<th>Controls (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>144.5 ± 75.1</td>
<td>101.6 ± 15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>214.4 ± 40.5</td>
<td>212.7 ± 45.3</td>
<td>0.85</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>40 ± 16.1</td>
<td>49.5 ± 12.3</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>133.7 ± 42.4</td>
<td>123.9 ± 53</td>
<td>0.26</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>190.9 ± 101.3</td>
<td>180.6 ± 95.5</td>
<td>0.62</td>
</tr>
<tr>
<td>Lipoperoxidation (nmol/mL)</td>
<td>1.8 ± 1</td>
<td>1.7 ± 1.5</td>
<td>0.67</td>
</tr>
<tr>
<td>Homocysteinemia (mmol/L)</td>
<td>12.7 ± 6.7</td>
<td>8.7 ± 4.4</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table IV - Homocysteine level according to sex (mmol/L)

<table>
<thead>
<tr>
<th></th>
<th>Unstable angina (n=46)</th>
<th>Controls (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>14.3 ± 5.8 (24p)</td>
<td>11.6 ± 4.2 (19p)</td>
<td>0.096</td>
</tr>
<tr>
<td>Female</td>
<td>10.9 ± 7.2 (22p)</td>
<td>6.4 ± 2.9 (27p)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Fig. 1 - * Homocysteine levels compared with C-reactive protein levels; * standard error of the mean; elevated levels: > 8 mg/L; normal levels: < 8 mg/L; UA- unstable angina; CG - control group.
Plasma homocysteine levels in patients with unstable angina

In an attempt to answer the question whether homocysteine is an independent risk factor for unstable angina, the logistic regression analysis was performed. When compared with other risk factors, such as diabetes, dyslipidemia, smoking, hypertension, sex, and age, homocysteine proved to be an independent risk factor (p=0.0025, with a confidence interval of 95%) in patients with unstable angina.

**Discussion**

Measuring C-reactive protein levels may have practical clinical significance in managing hospitalized patients with acute ischemic syndromes, but this was not our objective. Even though the means for C-reactive protein were similar in the 2 groups, when the groups were subdivided into individuals with normal (<8 mg/L) and high (>8 mg/L) levels, a tendency towards a difference was observed in the unstable angina group (fig. 1). It is worth emphasizing that the highest levels of C-reactive protein were found in individuals with the highest homocysteine levels both for the unstable angina and control groups.

Some studies have shown that C-reactive protein levels measured on hospital admission in patients suspected of having ischemic heart disease identified patients at high risk for acute myocardial infarction and unstable angina.

Mach et al 14 reported that C-reactive protein levels measured on hospital admission in patients suspected of having ischemic heart disease may be a marker of acute coronary syndromes and may be very useful in identifying patients at high risk for developing myocardial infarction.

In another study, Luigi et al 15 reported that, in unstable angina, C-reactive protein may remain elevated for 3 or more months after the disease symptoms and may be associated with recurring instability.

Ernesto et al 16 concluded that C-reactive protein is a strong independent marker of an increase in risk in 90 days. Comparing C-reactive protein on admission and C-reactive protein on hospital discharge, the latter may be very useful for risk stratification.

An elevated homocysteine level is 1 of many risk factors identified for vascular diseases. Homocysteine is an amino acid that contains a sulfur radical and is an intermediate product of the metabolism of cysteine from methionine (an essential amino acid found in the dietary proteins).

Intriguing observations suggest that homocysteine plays a significant role in the development of vascular diseases. Individuals with homocystinuria 17, a recessive autosomal disorder, have severe hyperhomocysteinemia, early atherosclerosis, and thromboembolic complications 18. Homocysteine may promote the oxidation of low-density lipoproteins, proliferation of smooth muscle cells, activation of platelets and of the coagulation factors, and endothelial dysfunction 19.

Determining the normal homocysteine levels is difficult because of its continuous biochemical variability. The prevalence of homocystinemia in the general population ranges from 5% to 10%, approximately 15 mmol/L in the 90th to the 95th percentile 20. However, it seems to be higher in the elderly population (30% to 40%) 21. If these results are correct, approximately 10% of coronary artery disease events may be attributed to elevated homocysteine levels 22.

Elevated homocysteine levels are associated with sex (higher levels in males), postmenopausal status in females, age increase, nutritional aspects, plasma levels of vitamins B6, B12, and folate, renal function, genetic predisposition, and thyroid function 20. Some medications, such as niacin, methotrexate, and phenytoin, may also increase homocysteine levels 20-23. Ethnic differences have been reported to interfere with the homocysteine levels, which are lower in black individuals as compared with Caucasian and Asian individuals 24-33.

For high-risk patients, some authors recommend the 10-µmol/L level as a therapeutic target 25-26. Approximately 30 studies have compared the homocysteine levels of patients with coronary artery disease and those of control groups with no disease 27-28. Patients with coronary artery disease had significantly higher fasting plasma homocysteine levels in 22 out of 27 studies 29,30 with a risk ratio of 1.2 to 10.9 after adjusting for other risk factors. Two metaanalyses of retrospective studies confirm these findings 22-31. Homocysteine may be considered a risk factor, as may smoking 32-33, hypertension 32-34, dyslipidemia 31,35,36, and hyperglycemia 37; it seems, however, to be an independent factor 38,39.

In a recent study, Evans et al 42 did not show the relation of homocysteine levels, nonfatal myocardial infarction, and coronary artery disease. In that study, the mean homocysteine levels in the patients with nonfatal myocardial infarction (n=93) and in those with death due to coronary artery disease (n=47) were 12.6 µmol/L and 12.8 µmol/L, respectively. In the control group, the homocysteine levels in individuals with nonfatal infarction and in those with death due to coronary artery disease were 13.1 µmol/L.

| Table V - Plasma homocysteine levels above 15 mmol/L |
|-----------------|-----------------|-----------------|-----------------|
| **Sex**         | **Unstable angina** | **Control** | **P** |
| **(n=46)**      | **(n=46)**       | **(n=46)**    | **(n=46)**     |
| Male            | 29.2% (07)*      | 21% (04)*     | 0.52 |
| Female          | 18.2% (04)*      | -             | - |
| **Total (male + female)** | 23.9% (11)*     | 8.6% (04)*    | 0.097 |

* Number of patients with homocysteine levels above 15 mmol/L.
Plasma homocysteine levels in patients with unstable angina

The American Heart Association guidelines consider it reasonable to select for treatment patients with elevated homocysteine levels who have a high risk for vascular disease, such as those with renal failure, or those with a familial or personal history of early atherosclerosis. Several specialists in this area agree and suggest a reduction in the fasting total homocysteine levels to below 10 µmol/L.

In our study, more elevated mean homocysteine levels were strongly associated with the presence of unstable angina, particularly in the female sex. The association of diabetes and dyslipidemia was higher in the group with unstable angina. This is an important study because of the epidemiological connotation of coronary artery disease. The cost-benefit relation of the treatment for hyperhomocysteinemia, as compared with that of the treatment for other risk factors, is good because it comprises the use of B complex vitamins and folic acid.

Our study points to the need for wider studies to assess this significant risk factor and also to clarify the role played by homocysteine, as well as its relation to other risk factors for coronary artery disease.

Even considering the elevated homocysteine levels reported in the literature (> 15 µmol/L) for cut values, we found 24% of our population with unstable angina with values above that level.

As limitations of our study, we can cite the higher number of dyslipemic and diabetic patients in the unstable angina group, and the lack of cardiac catheterization in all patients. Larger samples may be required to exclude variables with bias potential. This is an initial study that may be expanded to encompass a future analysis of young patients with early coronary artery disease and elderly patients.

Briefly, we should be aware that hyperhomocysteinemia is an independent risk factor for coronary artery disease, and that, in addition, it may act synergistically with other properly defined risk factors, such as diabetes, hypercholesterolemia, and hypertension, to induce endothelial dysfunction, early atherosclerosis, and thrombosis.

Acknowledgments

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References


