Homocysteine is Related to Aortic Mineralization in Patients with Ischemic Heart Disease

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Introduction

Homocysteine is an amino acid, a metabolic byproduct of methionine and an independent risk factor for atherosclerotic vascular disease. In addition, high levels of homocysteine are associated with an increased mortality rate in patients with ongoing coronary artery disease (CAD). High levels of homocysteine are caused by genetic defects in the enzymes that are implicated in amino acid metabolism, such as the thermolabile variant of methylenetetrahydrofolate reductase (MTHFR), which confers reduced enzymatic activity. High levels of homocysteine are also induced by a deficiency of folate, vitamin B6 and vitamin B12 or by the presence of fibrates, nicotinic acid, cigarette smoking and chronic kidney failure.

Homocysteine is implicated as an early atherosclerotic promoter and a hazardous prothrombotic trigger. Homocysteine may initiate intimal thickening, elastic lamina disruption, vascular smooth muscle cell proliferation and produces free radicals that induce cellular damage. These factors must have a role in the progression of atherosclerosis that subsequently leads to vascular mineralization.

Aim: Identify a correlation between the plasma concentration of total homocysteine and the amount of minerals that accumulate in the aorta of patients with atherosclerosis.

Methods: We performed a cross-sectional study in 13 patients with three-vessel coronary artery disease, undergoing coronary artery bypass surgery. Aortic and mammary artery specimens were analyzed using a scanning electron microscope with an energy dispersive X-ray spectrometer. The homocysteine was determined using an immunonephelometry method.

Results: The amount of minerals in the aorta was greater (300 ± 181.6 particles per 500 μm²) than that in the mammary artery (64 ± 45 particles per 500 μm²) (p < 0.01). The average tHcy was 9.5 ± 2.3 μmol/L. The Spearman’s rank correlation coefficient was positive between tHcy, and aortic iron (p < 0.05).

Conclusions: Our study demonstrates that the aorta is dramatically affected by mineralization compared to the mammary artery. In addition, a direct correlation was identified between the levels of tHcy and the iron particles in the aortic wall.


Key words: Homocysteine, Vascular mineralization, Iron, Atherosclerosis, Ischemic heart disease
(VSMCs) proliferation with enhanced collagen production and also accelerate osteoblast-like differentiation and subsequent calcification in VSMCs. Moreover, the thiolactone metabolite of homocysteine combines with LDL-cholesterol to produce foam cells that discharge the lipids into atherosclerotic plaques and directly produces free radicals that form during oxidation of the homocysteine to induce cellular damage.

All of these factors have a role in the progression of atherosclerosis that subsequently leads to vascular mineralization mainly by calcium and phosphorus particles, although other particles have been identified, such as magnesium, zinc, and iron. Mineralization initially occurs in necrotic zones of atherosclerotic plaque that are promoted by the presence of oxidized lipids in the subendothelial space. Mineralization is a tightly regulated process, which regulates the apoptosis of vascular smooth muscle cells, cell-cell-interactions between oxidized-lipids and the presence of plasma inorganic minerals.

The clinical association between homocysteine and vascular calcification has been assessed previously by computed tomography, but this technique does not offer the possibility to detect other minerals in the vessel, which is important because other minerals may be associated with homocysteine, such as iron, for which several studies have indicated synergistic interaction between the iron plasma concentration and the total homocysteine level, which promotes blood vessel injury.

Several methods have been developed to visualize and measure artery calcifications; these methods have helped both to identify other factors that are associated with mineralization and, ultimately, to elucidate the mechanisms of mineralization. A novel method is the electron microprobe (EMP), a hybrid instrument combining the capabilities of both a scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDS). EDS is an analytical technique used for the analysis of elements in a sample, analyzing the characteristic energy of X-rays emitted from a specimen in response to a high-energy beam of electrons. EMP may identify the entire range of elements that are present in the tissue and provides excellent image quality to measure mineral particles.

**Aim**

The current study aims to identify a novel correlation between the plasma concentration of total homocysteine and the amount and type of minerals that accumulate in the arteries of patients with atherosclerosis.

### Table 1. Demographic and clinical characteristics of the patients

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>PATIENTS (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (SD)</td>
<td>60.97 (9.34)</td>
</tr>
<tr>
<td>Gender M/F</td>
<td>13/0</td>
</tr>
<tr>
<td>Diabetes mellitus type 2, n (%)</td>
<td>5 (35.5)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>8 (61.5)</td>
</tr>
<tr>
<td>Previous smokers, n (%)</td>
<td>4 (30.8)</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>8 (61.5)</td>
</tr>
<tr>
<td>Total cholesterol (SD) mg/dL</td>
<td>139.86 (38.65)</td>
</tr>
<tr>
<td>LDL-C (SD) mg/dL</td>
<td>86.58 (37.65)</td>
</tr>
<tr>
<td>HDL-C (SD) mg/dL</td>
<td>33.75 (17.51)</td>
</tr>
<tr>
<td>TG (SD) mg/dL</td>
<td>130.89 (66.11)</td>
</tr>
<tr>
<td>BMI (SD) kg/m²</td>
<td>25.65 (2.38)</td>
</tr>
<tr>
<td>Systolic BP (SD) mmHg</td>
<td>118.61 (16.02)</td>
</tr>
<tr>
<td>Diastolic BP (SD) mmHg</td>
<td>75.46 (8.95)</td>
</tr>
<tr>
<td>Fibrates, n (%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>11 (84.6)</td>
</tr>
<tr>
<td>Beta-blockers, n (%)</td>
<td>10 (76.9)</td>
</tr>
<tr>
<td>ACE inhibitors, n (%)</td>
<td>8 (61.5)</td>
</tr>
<tr>
<td>Aspirin, n (%)</td>
<td>10 (76.9%)</td>
</tr>
</tbody>
</table>

The variables are expressed as the mean ± standard deviation (SD). BMI = body mass index, M/F = male/female, TG = triglycerides, BP = blood pressure, ACE = angiotensin-converting enzyme.

### Methods

We performed a cross-sectional study and included patients with stable coronary artery disease of two or three vessels, which was documented by coronary angiography, who were undergoing coronary artery bypass surgery.

We excluded patients who had had recent myocardial infarction, had been diagnosed with unstable angina for less than one month, thyroid disease, kidney or liver failure or who had undergone coronary bypass surgery or another type of coronary intervention.

We recorded anthropometric measurements, traditional risk factors and clinical variables of patients that met the inclusion criteria. Table 1 shows the demographic and clinical characteristics of patients. For each patient, we obtained a blood sample after a fast of at least eight hours before the surgery. Venous blood (10 mL) was extracted by puncture and placed in a tube with EDTA as an anticoagulant. The sample was then centrifuged at 2500 rpm for 15 minutes. The plasma was distributed into aliquots and fro-
zen at \(-70^\circ C\) for less than six months. The plasma concentration of total homocysteine (tHcy) was determined using a commercially available immunonephelometry kit (Dade Behring, in a BN-ProSpec Nephelometer). The values are expressed as micromoles per liter (\(\mu\)mol/L).

During the patient’s bypass surgery, we obtained samples from the aorta and mammary arteries. As previously described\(^{18}\), 0.5-cm sections of the vessels were dissected and immediately preserved in formaldehyde, dried at room temperature for 24 hours, placed on a glass cover slip, and covered with graphite. The samples were studied under a scanning electron microscope (Japanese Electronic and Optical Laboratory, Model JEOL JXA8900-R) with an energy dispersive X-ray spectrometer at an acceleration voltage of 20 kiloelectron volts, an acquisition time of 30 to 60 seconds and a 20-nanoampere current. The digital images were obtained at a resolution of 1024 \(\times\) 1024 pixels. The images of this mapping were processed using IMAGE-PRO PLUS 4.1 software; mineral deposits were contrasted with the image background. Elemental quantitative analysis of each sample was performed using automatic background subtraction and a ZAF (atomic number, absorption and fluorescence) correction matrix to calculate the elemental composition in weight percent ratios of each mineral per studied field. To avoid bias, the same dimension of 500 \(\mu\)m\(^2\) was always analyzed.

The protocol was approved by the Institutional Ethics Committee, and informed consent was obtained from each participant.

**Statistics**

Descriptive statistics using the mean \(\pm\) SD and the median with minimum and maximum values was used in accordance with their distribution. The Mann-Whitney test was performed to compare the differences between continuous variables. Spearman’s rank correlation coefficient was used to evaluate the relationship between the level of plasma tHcy and the amount of mineralization in the arteries.

\(P \leq 0.05\) was considered significant. Statistical calculations were performed using SPSS software, version 15.

**Results**

The mean level of tHcy was 9.5 \(\pm\) 2.3 \(\mu\)mol/L. The aorta displayed 4.7 times more mineralization than the mammary (300 \(\pm\) 181.6 particles per 500 \(\mu\)m\(^2\) and 64 \(\pm\) 45 particles per 500 \(\mu\)m\(^2\), respectively, \(p<\)

Fig. 1. Minerals in the aorta and mammary artery
Scanning electron micrograph of A. Aorta and B. Mammary artery. Images are obtained by backscattered electrons from arteries. The minerals give off high emission intensity of backscattered electrons; this could be seen as white spots that contrast with the grayscale background of the artery. The aorta clearly has more minerals than the mammary artery.

0.01\(^{\text{st}}\) (Fig. 1).

Table 2 shows the average of silicon, sulfur, calcium, zinc, phosphorus and iron particles per studied field, which were different between both arteries. We did not identify any association between the number
Table 2. Mineralization patterns according to chemical elements in the aorta and mammary artery

<table>
<thead>
<tr>
<th>Element</th>
<th>Aorta</th>
<th>Mammary</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Silicon</td>
<td>20.85</td>
<td>24.13</td>
<td>2.45</td>
</tr>
<tr>
<td>Sulfur</td>
<td>60.25</td>
<td>59.49</td>
<td>13.38</td>
</tr>
<tr>
<td>Chlorine</td>
<td>43.98</td>
<td>66.48</td>
<td>8.21</td>
</tr>
<tr>
<td>Calcium</td>
<td>30.28</td>
<td>23.79</td>
<td>9.12</td>
</tr>
<tr>
<td>Zinc</td>
<td>8.29</td>
<td>14.93</td>
<td>2.04</td>
</tr>
<tr>
<td>Aluminum</td>
<td>9.08</td>
<td>15.10</td>
<td>2.73</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>31.76</td>
<td>25.83</td>
<td>7.37</td>
</tr>
<tr>
<td>Iron</td>
<td>33.75</td>
<td>42.93</td>
<td>4.17</td>
</tr>
<tr>
<td>Potassium</td>
<td>16.79</td>
<td>25.33</td>
<td>1.86</td>
</tr>
<tr>
<td>Magnesium</td>
<td>11.48</td>
<td>19.82</td>
<td>2.24</td>
</tr>
<tr>
<td>Manganese</td>
<td>6.81</td>
<td>10.65</td>
<td>2.41</td>
</tr>
<tr>
<td>Sodium</td>
<td>19.73</td>
<td>34.79</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Results are expressed as the percentage of the weight ratio of each mineral per studied field.

Fig. 2. Correlation between the level of total homocysteine (tHcy) and the amount of mineralization in the aorta.

Discussion

The aorta is a well-recognized territory in the vascular tree that is susceptible to the arteriosclerotic process; however, the mammary artery is free of atherosclerotic plaque development because this vessel does not experience shear stress. In the aortic wall, the differences in shear stress may contribute to endothelial dysfunction and, eventually, to the deposition of minerals in the aorta.

The aortic and mammary arteries are susceptible to mineralization. Silicon, sulfur, calcium, zinc, phosphorus and iron particles were distributed throughout the vascular segment in the aortic and mammary arteries; however, the amount of mineralization in the aorta was 4.7 times greater than that in the mammary artery. In our current study, we did not analyze coronary mineralization using a microscope but it was documented by angiography. Previous results showed that coronary artery calcification correlates with aortic wall calcification.

Iron catalyzes a variety of free-radical oxidative reactions and exhibits a high degree of correlation with the depth of the lesion in the artery wall. Iron depletion using chelators protects against ischemic heart disease and may improve endothelial dysfunction.

Homocysteine has been largely implicated in the aortic mineralization.
terial stiffening\textsuperscript{7} by several mechanisms, which may act cooperatively to promote aortic lesions. Baggott \textit{et al.} postulated a mechanism in which free iron is exposed to thioethers to form homocysteine and concluded that plasma homocysteine levels are directly affected by the amount of free iron\textsuperscript{15}.

The role of iron in homocysteine formation is a matter of controversy. Some studies have suggested a positive association between plasma homocysteine and serum iron in acute myocardial infarction\textsuperscript{26,27}, whereas other studies have reported a negative association between homocysteine and iron intake through an iron-fortified diet\textsuperscript{28}.

Our current study is the first to demonstrate the localization of iron particles in aortic tissue in the presence of other mineral particles and to correlate this finding with the concentration of plasma tHcy in patients with severe atherosclerosis.

The current study has some limitations, such as the inclusion of only male patients. These studies must be repeated in female patients and must consider the fluctuation of iron levels during menstruation.

**Conclusion**

Our study identified a significant amount of mineralization in the aortic artery, which is more affected by atherosclerosis than the mammary artery. In addition, we demonstrated a direct correlation between the level of homocysteine and iron particles in the aorta wall.

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**References**

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