Association between hyperhomocysteinemia and primary pulmonary hypertension

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Summary

Study objective: This case-control study was conducted to test the hypothesis that fasting homocysteine levels are higher in PPH patients than in healthy controls.

Design: Levels of plasma total homocysteine, serum folate, vitamin B-12, and serum creatinine in 18 consecutive patients with PPH were compared with data from 36 age- and sex-matched controls.

Results: Eight of the 18 patients (44.4%) and three of the 36 controls (8.3%) had elevated plasma total homocysteine (tHcy) levels (>15 μmol/l, odds ratio 8.8; 95% CI: 2.0–39.6; P = 0.005). There was an inverse correlation between tHcy levels and creatinine clearance in patients with PPH (P = 0.036).

Conclusion: PPH patients are significantly more likely to have hyperhomocysteinemia, and higher mean plasma total homocysteine levels than in controls. Plasma total homocysteine may be an important factor in the pathogenesis of PPH.

Key words
Hyperhomocysteinemia;
Primary pulmonary hypertension

Introduction

Primary pulmonary hypertension (PPH) is a rare disease of unknown etiology characterized by high pulmonary artery pressures and pulmonary vascular resistance.1 The pathogenesis of PPH is unknown, but vasoconstriction, vascular remodeling, and thrombosis of small pulmonary blood vessels are thought to be contributory factors.2

The sulfur-containing amino acid homocysteine and its oxidized derivatives, homocysteine, homocysteine-cysteine-mixed disulfide and protein-bound homocysteine, collectively known as plasma total homocysteine (tHcy) are associated with some types of endothelial and platelet dysfunction that may be implicated in PPH. In healthy subjects, mildly elevated tHcy levels impair vasodilation and platelet function and facilitate coagulation and cell adhesion.3 Thus, tHcy may facilitate platelet deposition and clot formation and impair vasodilation of the pulmonary arterioles, which could contribute to the pathogenesis of PPH or worsen existing cases. Hyperhomocysteinemia has been implicated in the pathogenesis of atherosclerosis4–6 and of venous thromboembolism.7 One study that concluded that PPH was not associated with high tHcy levels8 was limited by the small number of patients.
In vitro studies suggest that homocysteine reacts with nitric oxide (NO) produced by endothelial cells. Homocysteine also impairs flow-mediated vasodilation during transient hyperhomocysteinemia associated with methionine loading. We recently found that levels of nitric oxide (NO) and its biochemical reaction products are low in the lungs of PPH patients. In addition, levels of glutathione (an antioxidant) in the bronchoalveolar fluid of these patients are high suggesting that PPH is associated with a state of oxidative stress. For these reasons, we hypothesized that PPH is associated with elevated tHcy levels. We compared PPH patients with healthy controls to determine whether tHcy levels were higher in PPH patients, and whether increased homocysteine levels were associated with a higher risk of PPH.

**Methods**

**Study design**

In this case-control study, we compared 18 consecutive patients with PPH evaluated for lung transplantation at the Cleveland Clinic Foundation with 36 healthy controls. All of the patients and controls were Caucasian. Controls were selected from a pre-existing database of asymptomatic, healthy individuals attending in the section of Preventive Medicine at the same institution. The control group was matched by age and sex with the patient population. The study protocol was approved by the Institutional Review Board of The Cleveland Clinic Foundation and informed consent was obtained from all individuals participating in the study.

**Study protocol**

Demographic data were collected and a history and physical examination performed by a pulmonary physician for all patients with PPH at the initial assessment. PPH was diagnosed according to standard diagnostic criteria used in the National Registry for PPH. At the time of initial assessment, fasting venous blood was drawn from both PPH and control patients. All blood samples were analyzed for tHcy, serum B-12, serum folate, complete blood count (CBC), prothrombin time (PT), partial thromboplastin time (PTT), and serum creatinine. Control patients with an abnormal CBC, PT, or PTT, and subnormal folate and vitamin B-12 levels were excluded from the study. Laboratory personnel performing the assays were blinded to the patient’s clinical and laboratory data.

**Homocysteine and vitamin assays**

Total plasma homocysteine was measured by the method of Jacobsen et al. Blood samples were collected in tubes containing ethylenediamine tetracetic acid (EDTA) on ice and were centrifuged within 1–2 h of collection in a refrigerated centrifuge. The plasma supernatant was stored at –70 °C until the assays were performed. The assay measured all forms of plasma homocysteine, including reduced and oxidized forms (homocysteine, homocysteine–cysteine-mixed disulfide, and homocysteine-mixed disulfide). Based upon prior experiments, a homocysteine concentration equal to or greater than 15 μmol/l was considered abnormal. This level corresponded to the 90th percentile in the screening database from which we selected our control subjects.

Folic acid and B-12 concentrations in serum were measured using a simultaneous radioligand-binding assay (Becton Dickenson, Simultrac). Vitamin B-12 deficiency was defined as a plasma concentration less than 200 pg/ml (normal range 200–600 pg/ml). Folate deficiency was defined by a concentration less than 3 ng/ml (normal range 3–16 ng/ml). Serum creatinine was measured and the creatinine clearance was estimated by a previously described formula.

**Statistical analysis**

Normally distributed continuous data were summarized with means, standard deviations, and ranges, and skewed data with medians. Nominal data were summarized with frequency and percent. The association between predictor variables and the presence of PPH was assessed with logistic regression, with odds ratios (odds of having PPH in those with a risk factor versus those without) and their 95% confidence intervals. The PPH and non-PPH groups were also compared on continuous variables with two-sided t-tests or Wilcoxon rank sum tests and on nominal variables with likelihood ratio chi-square or Fisher’s exact tests. Correlations among continuous variables were assessed with Spearman or Pearson correlation analysis, as appropriate. A significance level of alpha = 0.05 was used for all hypotheses. All statistical analyses were performed using SAS statistical software (version 6.12, Cary, NC, USA).
Results

There were six males and 12 females with PPH with a median age of 43 years (range: 13–73 years). The median duration of PPH in the patient population was 9 months (range: 3–81 months). Other characteristics are shown in Table 1.

There were 36 subjects in the control group. Controls were matched for sex and age, and they had demographic characteristics similar to the PPH patients. The only variable that differed slightly (although non-significantly) between the cases and controls was the frequency of cigarette smoking; 2/18 patients (11.1%) of the PPH group were smokers versus 2/36 (5.6%) in the control group ($P = 0.47$, odds ratio 2.1, 95% CI: 0.27–16.5).

Table 2 shows that abnormally elevated tHcy levels were much more frequent among PPH patients than among controls, even after adjusting for serum creatinine level ($P = 0.013$). The mean tHcy levels were higher in PPH patients. Among patients with PPH, no significant correlation was found between tHcy and mean pulmonary artery pressure (PAP) (Spearman correlation $-0.064$, $P = 0.80$). All PPH patients had normal levels of serum vitamin B-12 and folate.

Serum creatinine was correlated with tHcy for the combined group of PPH patients and controls (Spearman correlation $0.35$, $P = 0.01$). For PPH patients, the Spearman correlation was $0.45$ ($P = 0.062$). For control patients, the Spearman correlation was $0.32$ ($P = 0.06$). The mean creatinine clearance in patients with PPH was $124 \pm 49$ ml/min. There was an inverse correlation between tHcy levels and the creatinine clearance in patients with PPH (Pearson correlation was $-0.50$; 95% CI: 0.04, $-0.78$; $P = 0.036$).

Discussion

In this study, a high proportion of patients with PPH (44.4%) had elevated levels of tHcy. Mean tHcy levels were also higher in PPH patients than in control subjects. In addition tHcy levels $>15 \mu$mol/l was still predictive of PPH after adjusting for serum creatinine. However, there was an inverse correlation between the creatinine clearance and the tHcy levels in patients with PPH.

The patients with PPH had normal level of creatinine clearance ($124 \pm 49$ ml/min); however, we found an inverse correlation between the creatinine clearance and the tHcy levels. High plasma value of tHcy is a common feature in patients with advanced renal failure and is an independent risk factor for atherothrombotic disease in patients with renal dysfunction. The mechanism(s) that may explain the association between the degree of creatinine clearance and the tHcy levels is(are) unknown.

Serum vitamin B-12 and folate levels did not differ between patients with PPH and controls. We did not examine the effect of folate, vitamin B-12, vitamin B-6, and vitamin C on the tHcy levels.

![Table 1](image1.png)

<table>
<thead>
<tr>
<th>Demographic variable</th>
<th>Value</th>
<th>N = 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, median (range)</td>
<td>43 (13–73)</td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>6 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>Duration of PPH in months, median (range)</td>
<td>9 (3–81)</td>
<td></td>
</tr>
<tr>
<td>PAP (mmHg), median (range)</td>
<td>52 (30–89)</td>
<td></td>
</tr>
<tr>
<td>Cardiac index (l/min/m²), median (range)</td>
<td>2.08 (1.2–3.7)</td>
<td></td>
</tr>
<tr>
<td>Patients on epoprostenol</td>
<td>6 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>Patients on Ca⁺⁺⁺ channel blockers</td>
<td>5 (27.8%)</td>
<td></td>
</tr>
<tr>
<td>Patients on coumadin</td>
<td>13 (72.2%)</td>
<td></td>
</tr>
</tbody>
</table>

PPH = primary pulmonary hypertension, PAP = pulmonary artery pressure, Ca⁺⁺⁺ = calcium.

*Frequency unless stated.

![Table 2](image2.png)

<table>
<thead>
<tr>
<th>Variable</th>
<th>PPH (N = 18)</th>
<th>Controls (N = 36)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy level (mean ± SD) (µmol/l)</td>
<td>14.7 ± 7.2</td>
<td>10.2 ± 5.1</td>
<td>1.14 (1.02-1.3)</td>
<td>0.027*</td>
</tr>
<tr>
<td>Patients with tHcy &gt; 15 µmol/l (%)</td>
<td>8 (44.4%)</td>
<td>3 (8.3%)</td>
<td>8.8 (2.0-39.6)</td>
<td>0.005*</td>
</tr>
<tr>
<td>Serum folate (mean ± SD) (ng/ml)</td>
<td>8.6 ± 3.6</td>
<td>7.9 ± 4.5</td>
<td>1.04 (0.9-1.9)</td>
<td>0.61</td>
</tr>
<tr>
<td>Serum B-12 (mean ± SD) (pg/ml)</td>
<td>441.5 ± 155.9</td>
<td>387.2 ± 157.6</td>
<td>1.02 (0.98-1.06)</td>
<td>0.24</td>
</tr>
<tr>
<td>Serum creatinine (mean ± SD) (mg/dl)</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>1.3 (0.99-1.8)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

PPH = primary pulmonary hypertension. Odds ratio shows relative odds of having PPH with 1-unit increase (in continuous variables) or with positive value (for nominal variable). P values from logistic regression, * statistically significant if $P < 0.05$. tHcy = total homocysteine.
or vitamin B-6 supplementation in patients with hyperhomocysteinemia. The effect of folate, B-12, and B-6 supplementation in patients with hyperhomocysteinemia and PPH needs to be studied further.

We postulate that irrespective of the reasons for the high levels of tHcy, hyperhomocysteinemia may have deleterious effect in patients with PPH. What may be the role of homocysteine in PPH? Homocysteine may exert its effect on the pulmonary vasculature by promoting vasoconstriction, promoting endothelial dysfunction, and enhancing in situ thrombosis by complex mechanisms. Homocysteine is readily oxidized in plasma to homocysteine, homocysteine-mixed disulfides, and homocysteine thiolactone. Generation of superoxide and hydrogen peroxide during oxidation may enhance endothelial toxicity and lipoperoxidation in the pulmonary vasculature. Furthermore, hyperhomocysteinemia is prothrombotic because it increases levels of factors XII and V, decreases activation of protein C, inhibits thrombomodulin, induces expression of tissue factor, suppresses heparin sulfate, and reduces binding of tissue plasminogen activator to endothelial receptors. Nitric oxide produced by endothelial cells may detoxify homocysteine by forming S-nitrosohomocysteine, which can serve as a vasodilator and platelet inhibitor. This protective action of nitric oxide appears to be overwhelmed by chronic hyperhomocysteinemia, which leads to unopposed action of homocysteine on vascular endothelium with resultant oxidative injury. In addition, homocysteine inhibits glutathione peroxidase (endothelial antioxidants), changing cellular redox potential and causing endothelial injury. These deleterious effects of homocysteine may play a significant role in patients with PPH who already have low levels of NO in the lungs and in a state of oxidative stress further amplifying any endothelial injury caused by homocysteine. Finally, hyperhomocysteinemia may promote vascular smooth muscle proliferation by inhibiting NO and by increasing cyclin D1 and cyclin A mRNA expression.

Conclusion

Plasma total homocysteine levels are significantly higher in patients with PPH compared with age- and sex-matched controls. Mean serum folate, vitamin B-12, and serum creatinine do not appear to differ significantly between cases and controls. Based on the results of this study, we postulate that homocysteine may play an important role in the pathogenesis of PPH. Larger studies of patients with PPH could clarify this issue and evaluate the potential benefits of folic acid, vitamin B-12, vitamin B-6, and antioxidant therapy in PPH.

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References