Association between Hyperhomocysteinaemia and Hypertension in Sri Lankans

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This study examined the relationship between homocysteine and its metabolites, and hypertension in a cohort of Sri Lankan patients with essential hypertension. Serum homocysteine, cysteine, cysteinylglycine and glutathione were measured in 86 patients with a diagnosis of essential hypertension and compared with those of an age- and sex-matched control group. Patients with hypertension had significantly higher mean serum concentrations of homocysteine, cysteine and cysteinylglycine. The odds ratio for hypertension for those with a mean serum homocysteine concentration above 18 µmol/l was 2.8. Hyperhomocysteinaemia is a risk factor for hypertension in Sri Lankans and can lead to a threefold increase in risk.

KEY WORDS: HYPERTENSION; SRI LANKANS; HYPERHOMOCYSTEINAEMIA

INTRODUCTION

Homocysteine is a recently recognized important risk factor for the development of atherosclerosis in the coronary, peripheral and cerebrovascular systems.¹⁻⁵ Pooled results from retrospective studies indicate that fasting homocysteine concentrations in patients with vascular disease are on average 31% higher than in normal subjects.² In 1992 high homocysteine concentrations were reported to be an independent risk factor for myocardial infarction in male participants prospectively enrolled in the US Physicians Health Study.⁶ Later similar studies have been reported from Norway⁷ and Finland.⁸ The atherogenic and thrombotic tendencies of homocysteine have been attributed to
direct cytotoxic effects of homocysteine on endothelial cells,9-11 promotion of platelet aggregation11 and clotting.12

Most studies have shown that hypertension is not linked to hyperhomocysteinaemia,13-21 although a few studies have shown such a correlation.22,23 Preliminary studies also suggest a possible difference between races in the prevalence and impact of hyperhomocysteinaemia on vascular disease.24 The significance of hyperhomocysteinaemia in non-Caucasian groups is relatively under-studied. We have reported a high prevalence of hyperhomocysteinaemia in the Sri Lankan population25 and an association with ischaemic heart disease.26

The objective of this study was to examine the relationship between hyperhomocysteinaemia and hypertension in a cohort of Sri Lankan patients with essential hypertension.

**PATIENTS AND METHODS**

**PATIENTS AND CONTROLS**

Fasting serum homocysteine and its metabolites (cysteine and cysteinylglycine) and glutathione were measured in a cohort of patients with hypertension and compared with a control group. The characteristics of patients and controls are shown in Table 1. None of the subjects in the control group had any clinical evidence of ischaemic heart disease, peripheral vascular disease or cerebrovascular disease. All patients were newly diagnosed hypertensives from a community study. All of them had a resting seated diastolic blood pressure of > 100 mmHg measured at the fifth phase. Three measurements were taken on different days before confirming the diagnosis of hypertension. None of the patients had had a myocardial infarction during the previous

<table>
<thead>
<tr>
<th>Characteristics of patients and controls</th>
<th>Patients</th>
<th>Controls</th>
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</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>86</td>
<td>82</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>35-65</td>
<td>35-65</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>46</td>
<td>45.5</td>
</tr>
<tr>
<td>No. of males</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>No. of smokers</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>No. of cigarettes smoked/day*</td>
<td>8 ± 5</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>BMI males*</td>
<td>21.9 ± 3.8</td>
<td>20.5 ± 3.2</td>
</tr>
<tr>
<td>BMI females*</td>
<td>23.8 ± 4.9</td>
<td>20.9 ± 3.7</td>
</tr>
<tr>
<td>Mean SBP (mmHg)*</td>
<td>148 ± 18.1</td>
<td>107.7 ± 10.7</td>
</tr>
<tr>
<td>Mean DBP (mmHg)*</td>
<td>117 ± 10.4</td>
<td>80.0 ± 6.1</td>
</tr>
</tbody>
</table>

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

*Mean ± SD.
6 months. They were not on any medication that is known to interfere with homocysteine metabolism (phenytoin, carbamazepine, oral contraceptives, methotrexate, penicillamine). None of them had psoriasis, blood disorders, cancer or chronic renal failure. Informed written consent was obtained for the study from patients and control subjects.

Fasting blood samples were obtained from the antecubital vein. Samples were placed on ice and centrifuged for 5 min within 2 h after they were obtained. The serum was separated and samples were stored at −20 °C until they were analysed. Samples from patients and controls were stored for the same length of time, and were handled together and identically throughout processing.

ASSAY MATERIALS
Ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (SBD-F) was purchased from Wako (Kyoto, Japan) and d,L-homocysteine was obtained from Sigma Chemical Company (St Louis, Missouri, USA). For high-performance liquid chromatography (HPLC) 1-cysteine and HPLC-grade acetonitrile were purchased from Nakarai (Kyoto, Japan). All other chemicals were of analytical-reagent grade.

CHROMATOGRAPHY
A Shimadzu LC-6A HPLC system with two pumps was used and an SCL-6A system controller for solvent mixing (Shimadzu, Kyoto, Japan). Samples were introduced with a Rheodyne 7125 injection valve fitted with a 20 μl sample loop. Separation was carried out at ambient temperature with an analytical column, Shim-pack CLC-ODS (150 × 6.0 mm internal diameter, 5 μm particle size). The fluorescence intensities were measured with excitation at 385 nm and emission at 515 nm, using a Shimadzu RF-530 fluorescence spectrophotometer equipped with a 12-μl flow cell. The detector signal was recorded and peak height was quantified.

We used a 0.1 ml/l potassium dihydrogenphosphate buffer, pH 2.1 (adjusted with orthophosphoric acid) containing 4% acetonitrile as mobile phase with a flow rate of 2.0 ml/min as described.27 28

SAMPLE PREPARATION
A 1-ml sample of fresh blood, drawn with a syringe, was poured into a disposable tube. The tube was centrifuged at 1000 g for 10 min at 4 °C. Serum thiols were derivatized with SBD-F essentially according to the method of Araki and Sako,27 and 15 μl of a 10% solution of tri-n-butyl-phospine in dimethylformamide were added to 150 μl of serum or standard. The mixture was incubated at 4 °C for 30 min to accomplish reduction of homocysteine and the mixed

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tr>
<td>Comparison of serum homocysteine, cysteine, cysteinylglycine and glutathione concentrations in hypertensive patients and controls</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Homocysteine (μmol/l)</th>
<th>Cysteine (μmol/l)</th>
<th>Cysteinylglycine (μmol/l)</th>
<th>Glutathione (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n=86)</td>
<td>27.8 ± 18.7*</td>
<td>306.5 ± 86.0*</td>
<td>59.9 ± 40.4*</td>
<td>3.1 ± 1.4</td>
</tr>
<tr>
<td>Controls (n=82)</td>
<td>18.1 ± 8.3</td>
<td>261.7 ± 66.7</td>
<td>45.8 ± 24.3</td>
<td>2.8 ± 2.6</td>
</tr>
</tbody>
</table>

Values are means ± SD.

*P < 0.01 compared with the control value.
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TABLE 3

<table>
<thead>
<tr>
<th>Fasting homocysteine concentrations and risk of hypertension</th>
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<tbody>
<tr>
<td>Patients</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>&gt;Mean*</td>
</tr>
<tr>
<td>&gt;Mean* + 1 SD</td>
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<tr>
<td>&gt;Mean* + 2 SD</td>
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<tr>
<td>&gt;Mean* + 3 SD</td>
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</tbody>
</table>

Crude odds ratio
3.0 2.5, 6.1 0.001

*Mean fasting homocysteine concentration 18 μmol/l.

The mean homocysteine, cysteine and cysteinylglycine concentrations were significantly higher in patients with essential hypertension than in control subjects (P < 0.01; Table 2). There was no significant difference between glutathione levels in patients and controls.

The hypertensive risk associated with hyperhomocysteinaemia as determined by the crude odds ratio was 3.0 (2.5 – 6.1; P < 0.001). We calculated the crude odds ratio as an estimate of the relative risk and used Miettinen's test-based 95% confidence limits. The odds ratios for several cut-off points of serum homocysteine are shown in Table 3.

DISCUSSION

In most studies of patients with coronary heart disease no relationship between blood pressure and homocysteine concentration has been reported.13–21 Malinow et al.12 found, however, that 77% of patients with elevated homocysteine concentrations were hypertensive compared with 40% of those with normal homocysteine concentrations. In another study21 total plasma homocysteine was significantly elevated in hypertensive patients compared with that in normotensive subjects.

STATISTICAL ANALYSIS

Comparison of means was by unpaired t-tests. Sample odds ratios were calculated in two by two tables. A two-sided P-value of < 0.05 was considered to indicate statistical significance.

RESULTS

There was no significant correlation between age and homocysteine, cysteine, cysteinylglycine and glutathione concentrations and no significant difference in these measurements between males and females.
This study is the first to analyse homocysteine and its metabolites in patients with hypertension in Sri Lanka. The concentrations of homocysteine and its metabolites were significantly higher in hypertensive patients compared with healthy normotensive controls. We observed that those with elevated homocysteine levels had a three-fold increased risk of hypertension.

We analysed our data at different cut-off points. A cut-off point at the 50th percentile of the control group was 18 μmol/l. Although there is no definite consensus about reference values for plasma homocysteine concentrations, the normal range is quoted as 4 – 18 μmol/l for some Western populations. This means that according to the reference values given for Western populations hyperhomocysteinaemia is common in our control group and this is also probably the case in the general population. In our cohort, 63% of subjects with elevated homocysteine concentrations (above 18 μmol/l) were hypertensive, compared with 33% of those with normal concentrations. The importance of hyperhomocysteinaemia as a risk factor for hypertension is a function of the odds ratio and its prevalence in the general population. Although a modest odds ratio of 3.0 was determined, our observation becomes particularly important if the prevalence of hyperhomocysteinaemia in the general population is high.

The determinants of elevated plasma concentrations of homocysteine and the mechanism of the atherogenic action of homocysteine are poorly understood. Several metabolic defects involved in the metabolism of homocysteine can lead to an increased plasma concentration. In addition vitamins B₆, B₁₂, and folate are involved as co-factors in this metabolic process and low levels of these vitamins, either through deficient intake or through other conditions, can lead to high levels of homocysteine.

Homocysteine is an intermediate formed during the metabolism of methionine, an essential sulphur-containing amino acid supplied from dietary proteins. Once formed, homocysteine either enters the remethylation cycle and is converted back to methionine or it enters the trans-sulphuration pathway and is metabolized to cysteine. Approximately 50% enters the trans-sulphuration pathway, where it is irreversibly combined with serine by the B6-dependent enzyme cystathionine β-synthase to form cystathionine. This then is metabolized to cysteine by γ-cystathionase, another B6-dependent enzyme.

Preliminary studies indicate that elevated homocysteine levels can be reduced by oral folic acid therapy. At present we are conducting a randomized, double-blind, placebo-controlled trial to investigate whether administration of folic acid could reduce hypertension. If the elevated homocysteine levels can be reduced by folic acid and the blood pressure is thereby reduced this would have important therapeutic implications particularly because Sri Lanka has a substantial burden of essential hypertension in the general population.

We conclude that hyperhomocysteinaemia is associated with a three-fold increase in hypertensive risk in Sri Lankans. Preliminary studies indicate that hyperhomocysteinaemia is prevalent in our population. If the present findings were confirmed by a large-scale study this factor would account for a substantial fraction of the incidence of hypertension in our population.

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