ASSOCIATION BETWEEN HYPERHOMOCYSTEINAEMIA AND ISCHAEMIC HEART DISEASE IN SRI LANKANS

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ABSTRACT

The objective of this study was to examine the relation between hyperhomocysteinaemia and ischaemic heart disease in a cohort of Sri Lankan patients with ischaemic heart disease.

Serum homocysteine, cysteine and cysteinylglyceine were measured in 54 patients with a definite diagnosis of ischaemic heart disease and compared with those of an age and sex matched control group.

Patients with coronary ischaemia had significantly higher mean concentrations of homocysteine and its metabolite cysteine ($P < 0.01$). Of the 54 patients with ischaemic heart disease, 14 (35%) had fasting homocysteine concentrations above the 90th percentile of the controls (odds ratio 3.2, 95% CL 1.0-11.3).

Hyperhomocysteinaemia is associated with a three-fold increase in coronary risk.

INTRODUCTION

An elevated plasma homocysteine level has recently received attention as an important predisposing factor in the pathogenesis of ischaemic heart disease (IHD) and other vascular diseases (von ckardstein, Malinow & Upsonb 1994). Pooled results from retrospective studies indicate that fasting homocysteine concentrations in patients with vascular disease are on average 31 percent higher than in normal subjects (Graham, 1994). Many studies have demonstrated associations between carotid artery stenosis (Selhub et al. 1995), cerebrovascular disease (Clarke et al. 1991), venous thrombosis (Heijer et al. 1995), peripheral vascular disease (Clarke et al. 1991), and hyperhomocysteinaemia. In 1992, high homocysteine concentrations were reported to be an independent risk factor for myocardial infarction in male participants prospectively enrolled in the U.S. Physicians Health Study (Stampfer et al. 1992). Later similar studies have been reported from Norway (Arnesen et al. 1995) and Finland (Alfthan et al. 1994).

The exact mechanism for the atherogenic and thrombotic tendencies of homocysteine has not been elucidated. However several in vitro and in-vivo studies have demonstrated the direct cytotoxic effects of homocysteine on endothelial cells

grown in tissue culture (Harker, Harlan and Ross, 1983), (Ueland, Refsum & Brattstrom, 1992), (Harker et al. 1976). In addition to a direct pathologic effect on endothelial cells, a role for the effect of homocysteine on platelets (Harker et al. 1976) and clotting factors (Giannini, Coleman & Innerfield, 1975) has also been suggested.

Preliminary studies also suggest a possible difference in the prevalence and impact of hyperhomocysteinaemia on vascular disease between races (McMartin et al. 1995). The significance of hyperhomocysteinaemia in non-Caucasian groups is relatively understudied. The prevalence of hyperhomocysteinaemia in the Sri Lankan population is not known and its relation if any, to IHD in Sri Lankans has not been studied. The objective of this study was to examine the relation between hyperhomocysteinaemia and IHD in a cohort of Sri Lankan patients with IHD.

MATERIALS AND METHODS

Fasting serum homocysteine and its metabolites (cysteine and cysteinylglyceine) were measured in a cohort of patients with IHD and compared with a control group. The age range of the patients was 35-73 years, and 48 of them were males.

The control group consisted of 51 age and sex matched healthy subjects (48 males). Forty eight percent of IHD patients and 42% of controls were current smokers. None of the subjects in the control group had any clinical evidence of ischemic heart disease, peripheral vascular disease or cerebrovascular disease. Their electrocardiograms were normal.

All patients were attending the Cardiology Clinic at the Peradeniya Teaching Hospital. All of them had a history of angina and definite electrocardiographic evidence of IHD (Minnesota codes 1.1, 1.2.1/2/3/4/5/6, 1.2.7/8 and 1.3) (Mendis & Ekanayake, 1994, Rose et al. 1982). None of them had had a myocardial infarction during the previous 6 months. They were not on any medications that are 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. The serum was separated and stored at -20 C until analysis. Samples from patients and controls were stored for the same amount of time and handled together and identically throughout processing.

HOMOCYSTEINE, CYSTEINE AND CYSTEINYLGLYCEINE 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 (USA). L-cysteine and HPLC-grade acetonitrile for high performance liquid chromatography (HPLC) were purchased from Nakarai (Kyoto, Japan). All other chemicals were of analytical-reagent grade.

Apparatus

High performance liquid chromatography was performed with Shimadzu LC-6A system with two pumps 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-ml sample loop. Separation was carried out at ambient temperature with an analytical column, Shim-pack CLC-ODS (150x6.0 mm I.D., 5-mm particle size). The fluorescence intensities were measured with excitation at 385nm and emission at 515 nm, using a Shimadzu RF-530 fluorescence spectrophotometer, equipped with a 12-ml flow cell. The detector signal was recorded and peak height was quantified.

Chromatographic conditions

We used a 0.1 mol/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. (Araki and Sako, 1987).

Sample preparation

A 1-ml sample of the fresh blood, drawn with a syringe, was poured into a disposable tube. The tube was centrifuged at 1000g for 10 min at 4°C. Serum thiols were derivatized with SBD-F essentially according to method of Araki and Sako (Ubbink, Vermaak & Bissbort, 1991). 15ml of a 10% solution of tri-n-butyl-phosphine in dimethylformamide were added to 150 ml of serum or standard. The mixture was incubated at 4°C for 30 minutes to accomplish reduction of homocysteine and the mixed disulphide as well as release of protein-bound homocysteine. Therefore this method measures total serum homocysteine levels. Subsequently 150 ml of 10% trichloroacetic acid, containing 1 mmol/L EDTA, was added. After centrifugation, a 50 ml of the clear supernatant were added to a mixture of 10 ml of 1.55 mol/l of sodium hydroxide, 125 ml of 0.125 mol/l borate buffer (pH 9.5) containing 4 mmol/l EDTA and 50 ml of SBD-F solution (1 mg/ml dissolved in borate buffer). The mixture was incubated for 1h at 60 °C to accomplish complete derivatization of homocysteine and other plasma thiols. A 20-ml aliquot was subsequently used for HPLC analysis.

Comparison of means was by the unpaired t tests. Sample odds ratios were calculated in 2 x 2 tables. A two sided P value of less than 0.05 was considered to indicate statistical significance .

RESULTS

There was no significant correlation between age and homocysteine, cysteine and cysteinylglycine levels and no significant difference in these parameters between males and females.

The mean homocysteine, cysteine and cysteinylglycine levels were significantly higher in patients with IHD compared to control subjects (Table I).

Table I. Comparison of serum homocysteine, cysteine and cysteinylglyceine concentrations (mean, SD) between ischemic heart disease patients and controls.

	Age mean(SD)	Homocysteine (μmol/l)	Cysteine (μmol/l)	Cysteinylglyceine (μmol/l)
Patients (n=54)	57.8(10.1)	30.9 12.9	304.6 107.3	47.4 13.4
Controls (n=51)	55.5(9.5)	11.6	23.8 11.6	267.4 103.9	33.3 18.3

Patients vs Controls P< 0.01 for homocysteine, and cysteine

Among the patients with ischemic heart disease 14 (35%) had fasting homocysteine concentrations above the cut-off point of 36 μmol/l (90th percentile of the controls) versus 5 (9.8%) in the control group (Table II). This yields a crude odds ratio of 3.2 (1.0-11.3), p< 0.05

Table II. Homocysteine concentrations and risk of ischemic heart disease

Fasting homocysteine concentrations (μmol/l)	Cases	Con	Odds R (95% CI)
>90th percentile (>36)	14	05	3.2 (1.0-11.3)
<90th percentile	40		46

p < 0.05

DISCUSSION

High homocysteine concentrations have been reported to be an independent risk factor for myocardial infarction in male participants prospectively enrolled in the U.S. Physicians Health Study (Stampfer et al 1992). Similar studies have been reported from Norway and Finland (Arnesen et al 1995, Alfthan et al 1994).

This study is the first to analyze homocysteine and its metabolites in healthy volunteers and patients with IHD in Sri Lanka. Homocysteine levels and its metabolites were significantly higher in IHD patients compared to healthy controls. We observed that those with homocysteine levels above the 90th percentile (based on the control distribution) had a three fold increased risk of IHD compared with those with values in the bottom 90% of the control distribution. Up to now there is no consensus about reference values for plasma homocysteine concentrations. We analyzed our data at different cut-off points. A cut-off point at the 90th percentile of the control group was 36 umol/l. The cut-off point at the 50th percentile of the control group was 23 umol/l

and this was much higher than the reference value of 14 µmol/l quoted for some Western populations (Heijer et al. 1995). This means that with respect to the reference values given for other Western populations hyperhomocysteinaemia is common in our control group and this is also most probably the case 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. A population study is required to confirm that the prevalence of hyperhomocysteinaemia in our population is high.

The determinants of elevated levels of homocysteine and the mechanism of the atherogenic action of homocysteine is poorly understood. Several metabolic defects involved in the metabolism of homocysteine can lead to elevated levels. In addition vitamins B6, B12 and folate are involved as cofactors in this metabolic process and low levels of these vitamins either through deficient intake or through other conditions can also lead to high levels of homocysteine (Kang et al. 1993). Possible mechanisms by which elevated homocysteine levels lead to the development and progression of IHD include effects on platelets, clotting factors and endothelium (Giannini, Coleman & Innerfield, 1975, Harker et al. 1976, Harker, Harlan, & Ross, 1983, Ueland, Refsum & Brattstrom, 1992).

Homocysteine is an intermediate formed during the metabolism of methionine an essential sulphur containing aminoacid supplied from dietary proteins. Once formed homocysteine either enters the remethylation cycle and is converted back to methionine or it enters the transsulfuration pathway and is metabolized to cysteine. Approximately 50% enters the transsulfuration pathway where it is irreversibly combined with serine by the B6 dependent enzyme cystathionine beta-syntheses to form cystathionine. This then is metabolized to cysteine by gammacystathionase, another B6 dependent enzyme (Finkelstein, 1990).

As far as we know this is the first study in which metabolites of homocysteine have been measured together with homocysteine levels. Since in the present study there was a concomitant elevation of cysteine levels together with homocysteine levels, elevated homocysteine levels in Sri Lankans is unlikely to be due to a metabolic abnormality in the transsulfuration metabolic pathway or due to a deficiency of the enzyme cystathionine beta syntheses. The defect therefore is most likely to be in the remethylation pathway. Methylcobalamine and methyltetrahydrofolate serve as cofactor and cosubstrate in the remethylation pathway. Therefore administration of folic acid would be expected to reduce homocysteine levels.

Preliminary studies indicate that elevated homocysteine levels can be reduced by oral folic acid therapy (Mubbink, et al. 1994). At present we are conducting a randomized, double blind, placebo controlled trial to investigate whether administration of folic acid could reduce homocysteine levels and whether such a reduction would reduce the coronary risk. If the elevated homocysteine levels can be reduced by folic acid and the coronary risk is thereby reduced this would have important therapeutic implications.

Many Sri Lankan patients have precocious atherosclerosis without having any of the standard coronary risk factors such as smoking, hyperlipidemia, hypertension,diabetes and a positive family history (Mendis and Ekanayake, 1994). We

conclude that hyperhomocysteinaemia is associated with a three fold increase in coronary risk in Sri Lankans. If hyperhomocysteinaemia is found to be prevalent in our population it would indeed account for a substantial fraction of the incidence of IHD in our population.

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