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Homocysteine, vitamins B_6, B_{12}, folate, and risk of coronary artery disease in patients undergoing diagnostic coronary angiography

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Summary. Homocysteine and vitamins B were correlated with coronary artery disease in patients undergoing diagnostic coronary angiography. 160 patients having ≥1 stenosis (G1), 55 patients having normal coronary arteries (G2) and 171 healthy volunteers (G3) were prospectively recruited.

Homocysteine levels were significantly higher in patients, particulary in those with normal coronary angiograms, than in healthy subjects ($13.8 \pm 6.3\,\mu$mol/L in G1 ($p < 0.0001$) and $15.2 \pm 8.8\,\mu$mol/L in G2 ($p < 0.0001$) versus $10.1 \pm 3.1\,\mu$mol/L in G3). Homocysteine levels were not related to the extent of coronary artery disease. In patients with normal angiogram, vitamin B12 and folate levels were significantly higher compared with the other groups ($p < 0.05$ and $p < 0.001$, respectively) showing that vitamin B deficiency was not involved in the hyperhomocysteinemia.

In conclusion, homocysteine and vitamins B levels do not contribute to discriminate for the presence of coronary artery disease in patients undergoing diagnostic coronary angiography. Homocysteine levels, however, were higher in patients referred for coronary angiography than in healthy controls.

Keywords: Amino acids – Homocysteine – Vitamin B12 – Vitamin B6 – Folates – Coronary artery disease

Introduction

Among the risk factors for atherosclerosis known to date, an intermediate of the intracellular metabolism of methionine, homocysteine, has been suggested as an important candidate because it could be modified by appropriate nutritional measures (Harker et al., 1974; Wilcken and Wilcken, 1976). In a number of epidemiological studies, elevated levels of homocysteine have been

associated with peripheral vascular (Boers et al., 1985; Malinow et al., 1992), and cerebrovascular disease. High levels of homocysteine have also been observed in patients with coronary artery disease (CAD) (Kang et al., 1986). The association of hyperhomocysteinemia with peripheral or cerebrovascular disease is stronger than that with coronary artery disease (Genest and Malinow, 1992). However, other investigators have failed to find any association between the serum levels of homocysteine and the risk of atherosclerotic disease (Alfthan et al., 1994).

Homocysteine is a sulfur-containing amino acid formed from methionine during a transmethylation process, which can be either condensed with serine to form cystathionine or remethylated to methionine. Plasma homocysteine levels are dependent upon nutritional factors such as folates, vitamin B6 and vitamin B12, and are also likely related to genetic factors such as the methylenetetrahydrofolate reductase (MTHFR), the methionine-synthase (MS), or the cystathionine-β-synthase (C-β-S) genes.

Increased homocysteine concentration may be correlated with low plasma levels of vitamin B6, vitamin B12 or folic acid (Ueland and Refsum, 1989; Dudman et al., 1993; Joosten et al., 1993). Consequently, the status of these nutrients may be associated with a higher CAD risk (Kang et al., 1987; Brattström et al., 1988; Pancharuniti et al., 1994). Moderate, intermediate and severe hyperhomocysteinemia are defined as plasma homocysteine concentrations in the range 16–30, 31–100, and $>100\mu$mol/L respectively (Kang et al., 1992). They are usually attributable to folate and/or cobalamin deficiencies (Kang et al., 1987; Brattström et al., 1988), and also genetically determined (Kang et al., 1988; Genest et al., 1991; Engbersen et al., 1995; Christensen et al., 1997).

The aim of this study was to determine whether levels of homocysteine[4] and coenzymes B were correlated with the presence of coronary artery disease in patients undergoing diagnostic coronary angiography.

Material and methods

1 Study population

The study population consisted of a series of 215 patients undergoing diagnostic coronary angiography at the Cardiology Department of the University Hospital of Nancy, and who were recruited consecutively on the basis of the investigators' availability at the time of admission for angiography. All patients who received vitamins were excluded from this study.

Coronary angiography was performed via the femoral approach, and included at least 5 views of the left coronary artery and 3 views of the right coronary artery. Coronary angiograms were reviewed by two experienced observers who were unaware of the results of serum measurements and classified as normal (no discernible atherosclerosis), mildly atherosclerotic (lumen irregularities or atherosclerotic plaques without >50% diameter stenosis) or definitely atherosclerotic (at least 1 > 50% diameter stenosis). When the observers disagreed on the classification between mildly or definitely atherosclerotic lesions, the segments with intermediate degree stenoses were analyzed using the McIntosh-based Cardiovascular Angiography Analysis System (CAAS) (Pie Medical, Maastricht, the Netherlands), which enables automatic edge detection of the arterial lumen and gives an observer-independent percentage of stenosis (Serruys et al., 1984). Because mild atherosclerosis at coronary angiography may be associated with extensive

Table 1. Baseline clinical characteristics of patients with normal or abnormal coronary angiography and control group

Characteristics	Patients with coronary artery disease G 1 (n = 160) n (%)	Patients with normal coronary angiograms G 2 (n = 55) n (%)	Healthy controls G 3 (n = 171) n (%)
Age (years)	61 ± 12*	57.8 ± 13	59 ± 12
Gender (M/F)	132/28 (82.5/17.5) °°	34/21 (62/38)†	133/38 (78/22)
Smoking	73 (46)	16 (29)	12 (7)
HTA	62 (39) ***	21 (25.6) †	1 (0,6)
Hyperlipidemia	100 (63) *** °°	16 (29) †	0
Diabetes mellitus	36 (22.6) *** °°	3 (5.4)	0
Family history	44 (27.6) ***	9 (16) †	0

p values: Group 1 vs Group 2: °° < 0.05.
Group 1 vs Group 3: * < 0.05, *** < 0.005.
Group 2 vs Group 3: † < 0.05.

deposits in the arterial wall, the 17 patients with mildly atherosclerotic angiograms were excluded. In addition, 4 patients with normal coronary arteries were excluded because of the presence of extra-cardiac atherosclerosis, leaving 160 patients with definite coronary atherosclerosis (Group 1) and 55 patients with normal coronary angiograms and no extra-cardiac atherosclerosis (Group 2) for the final analysis. In addition, a control group of 171 subjects who had no history of myocardial infarction, coronary heart disease, other cardiovascular events or cancer was constituted from a population of volunteers (Group 3). The baseline characteristics of the 3 groups are displayed in Table 1. G2 patients were more frequently women than G1 and G3 subjects (38% versus 17.5% and 22% respectively, $p < 0.05$). In addition, the prevalence of diabetes mellitus and hyperlipidemia was significantly higher in patients with normal or abnormal coronary angiograms compared with the healthy subjects.

2 Chemical determinations

After coronary angiography, blood samples were collected in ethylenediaminetetraacetic acid-containing tubes and kept at +4°C. Plasma was separated within 2 hours. Total plasma homocysteine levels were determined on frozen samples by ion-exchange chromatography (C-IE) (Anderson et al., 1989). Serum vitamin B12 and folates were measured by a quantitative radioassay with purified intrinsic factor and purified folate-binding protein (Kit: Ciba-corning, Medfield, Massachusettes) (Selhub et al., 1993).

Vitamin B6 or pyridoxal-5′-phosphate was measured by C-IE. The dosage of cystathionine and plasma methylmalonic acid were done by capillary gas chromatography and mass spectrometry (Stabler et al., 1986).

3 Statistical analysis

Data were analyzed using the Statview for Windows statistical package. Univariate comparisons between the 3 groups were made using Student's unpaired t tests or Mann and Whitney tests for continuous variables, and Chi-square tests or Fisher's exact tests depending on the sample sizes for categorical variables.

To determine independent predictors of the presence of coronary artery disease, multivariate logistic regression analysis was used with a model including all variables with a p value <0.15 on univariate analyses.

For all results, a p value <0.05 was considered significant.

Results

Univariate analyses (Table 2)

Homocysteine levels were significantly higher in patients undergoing coronary angiography than in healthy subjects (13.8 ± 6.3μmol/L in G1 and 15.2 ± 8.8μmol/L in G2 versus 10.1 ± 3.1μmol/L in G3, $P < 0.0001$). In patients with normal coronary angiograms, vitamin B12 and folate levels were significantly higher compared with those with coronary artery disease and the healthy subjects, respectively ($p < 0.05$). The distribution of homocysteine levels in groups of patients undergoing coronary angiography was similar: 69.8% and 61.5% respectively for Groups 1 and 2 had low (<15μmol/L) homocysteine levels, and 30.2% and 38.5% had elevated levels (>15μmol/L), while all healthy subjects had homocysteine concentrations <15μmol/L. Plasma homocysteine levels in CAD patients were not related to the extent of coronary artery disease: the respective values for patients with one-, two- or three-vessel coronary artery disease were 13.53 ± 6.7, 13.17 ± 4.9 and 15.09 ± 6.5μmol/L (p = NS). Lastly, it has been suggested that homocysteine levels were more discriminant in younger patients with premature arterial occlusive disease (Aronson et al., 1994); when we analyzed the homocysteine levels in patients younger than 60 years of age, there was no significant difference between Group 1 (12.6 ± 6.69μmol/L) and Group 2 (13.4 ± 6.2μmol/L) p = 0.6.

Multivariate analysis

Using multivariate logistic regression analysis, only age, sex, total cholesterol, triglyceride and apolipoprotein B concentrations were independently related to the presence of angiographically visible coronary artery disease (data not shown).

Table 2. Comparison of biological markers in the three groups

	Group 1 (n = 160)	Group 2 (n = 55)	Group 3 (n = 171)
Homocysteine μM (SD)	13.8 ± 6.3 ***	15.2 ± 8.8 ††	10.1 ± 3.2
Folates nM (SD)	15.5 ± 6.5 *** °°	19.9 ± 10.7 ††	10.2 ± 4.3
Vitamin B12 pM (SD)	361.7 ± 239.3 *	409.8 ± 295.6 †	334.8 ± 124.4
Vitamin B6 nM (SD)	53.8 ± 28.8	43.5 ± 19.3	–

p values: Group 1 vs Group 2: °° < 0.001.
Group 1 vs Group 3: * < 0.05, *** < 0.0001.
Group 2 vs Group 3: † < 0.01, †† < 0.0001.
Group 1: patients with coronary artery disease.
Group 2: control patients with normal coronary angiograms.
Group 3: healthy controls.

Discussion

Several epidemiological studies have shown a correlation between homocysteine levels and the presence of cardiovascular disease and homocysteine has therefore been considered an independent risk factor for cardiovascular disease. This sulphur-amino acid has different effects on endothelial and smooth muscle proliferation and may exert its proatherogenic effect through multiple mechanisms. Elevated homocysteine may damage endothelial cells by generation of H_2O_2 which has a deleterious effect on endothelial cell function. Furthermore, homocysteine has been shown to increase the activity of tissue factor in endothelial cells, and to modulate tissue plasminogen activator binding to endothelial cells receptors (Hajjar, 1993). Homocysteine may induce a procoagulant state by enhancing the activity of factor V in endothelial cells (Rodgers and Kane, 1984). In addition, high homocysteine levels may decrease the activity of thrombomodulin and so decrease protein C activation (Rodgers and Conn, 1990). Previous epidemiological observations indicate that moderate hyperhomocysteinemia increases the risk for atherosclerotic plaque formation and seems to be a risk factor for premature vascular complications (Clarke et al., 1991; Selhub et al., 1995; Heijer et al., 1995). Inconsistent results were observed in other studies (Alfthan et al., 1994; Verhoef et al., 1997). In most case-control studies on the relationship between hyperhomocysteinemia and coronary artery disease, the control group was not coronary angiography documented. Few data are available comparing homocysteine levels in patients with coronary angiography. The first one was the study by Kang et al. (1986). Murphy-Chutorian et al. (1994) found a significant difference in plasma homocysteine levels, after a methionine loading test, between the groups with and without documented CAD. Contrary to other studies (Montalescot et al., 1997), we failed to observe any significant relationship between basal homocysteine levels and the presence or extent of coronary artery disease, even in young patients with premature coronary artery disease. It must be stressed, however, that hyperhomocysteinemia is associated more strongly with peripheral or cerebrovascular disease than with coronary artery disease. Similarly, high plasma homocysteine level appears to be more involved in thrombotic than in atherosclerotic events (Genest and Malinow, 1992; Verhoef et al., 1997).

Vitamin B6, B12, and folates deficiency may be responsible for an increase in plasma levels of homocysteine. In addition, vitamin B12 and folates deficiency has been proposed as an independent risk factor for vascular disease (Pancharuniti et al., 1994). In the present study, no abnormality of the vitamin status was observed in patients with compared with those without coronary artery disease. The homocysteine/cystathionine ratio was normal, corresponding to a normal transsulfuration pathway in the homocysteine metabolism. In the Framingham (Daly et al., 1995) and ARIC (Malinow et al., 1993) studies, it was shown that about 67% of subjects with hyperhomocysteinemia had vitamin B6, B12 or folates deficiency.

The homocysteine levels in our patients were high, both for patients with and those without coronary artery disease (29% of Group 1 and 25.5% of Group 2 patients had homocysteine concentrations $>16\mu$mol/L), possibly reflecting geographical specificities in our population from the North-East of France though the technique used to measure homocysteine in our study might yield higher values than others (Murphy-Chutorian and Aldesman, 1994; Verhoef et al., 1997). In previous studies, the percentage of patients with premature CAD who had high homocysteine levels ranged from 9 to 44% (Genest and Malinow, 1992; Frohlich, 1995).

It must also be stressed that our population with angiographically normal coronary arteries was not always free from heart disease. It was composed both of patients with chest pain and suspected coronary artery disease and of patients with cardiac disease, such as dilated cardiomyopathy, or valvular heart disease. However, there is no evidence of an association between homocysteine levels and these cardiac conditions.

In contrast with homocysteine levels, most traditional risk factors for coronary artery disease (e.g. cholesterol and triglycerides levels) were found related to the presence of angiographic disease in our patients, confirming that, even if there was a selection bias in our population, it did not affect these risk factors, which appeared stronger than would have been homocysteine.

In conclusion, in a population of patients referred for diagnostic coronary angiography, homocysteine, contrary to conventional risk factors, cannot help to discriminate between the presence or absence of angiographically visible coronary artery disease. Homocysteine levels, however, appear higher in patients referred for coronary angiography than in presumably healthy controls.

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