HOMOCYSTEINE IS NOT AN INDICATOR OF RESTENOSIS RISK AFTER PERCUTANEOUS TRANSLUMINAL CORONARY ANGIOPLASTY

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ABSTRACT

Objective: Elevated homocysteine levels have been associated with increased risk of atherosclerotic vascular disease. The possible association of homocysteine with restenosis after percutaneous transluminal coronary angioplasty (PTCA) has not been widely investigated.

Methods: In order to determine if a relationship exists between serum homocysteine levels and restenosis after PTCA, serum homocysteine level were determined in 204 patients who underwent a successful PTCA procedure and stant implantation. The patients were followed with clinical examinations and exercise tests at 1, 3, and 6 months, and a control coronary angiography was performed after 6 months to evaluate restenosis. Homocysteine levels were determined with fluorescence polarization immunoassay.

Results: Of the 146 patients who underwent angiographic evaluation, 57 (39 %) had restenosis, whereas 89 (61 %) did not. The homocysteine distributions were compared in these two groups of patients. Although the average homocysteine levels were higher in the restenosed group, the difference was not statistically significant.

Conclusion: Because of its wide distribution, serum homocysteine values do not seem to be a useful indicator for the risk of restenosis after PTCA.

Key Words: Homocysteine, Restenosis, PTCA

INTRODUCTION

Homocysteine is a naturally occurring sulphur containing amino acid produced during methionine metabolism. Methionine is converted to homocysteine through intermediates S-adenosyl methionine and S-adenosyl homocysteine. Homocysteine can be remethylated to methionine by methionine synthase which requires methylene tetrahydrofolate (MTHF) as a cofactor, but the majority of homocysteine goes through a pyridoxal-5'-phosphate (vitamin B_6) dependent

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condensation with serine to form cystathionine in a reaction catalyzed by cystathionine beta-synthase (CβS)(1). The homocysteine in blood is either found as covalently bound to plasma proteins, or bound to other thiols -including itself. Only a very small fraction, (~1%) circulates in the form of free thiol.

The homocysteine levels may be elevated because of either genetic causes, such as homozygous deficiency of CβS, or homozygous deficiency of MTHF reductase, diet (methionine intake, nutritional deficiencies of folic acid, vitamin B_{12} and vitamin B_6), age and estrogen status. Other causes such as renal impairment also effect homocysteine levels by impairing the metabolism of homocysteine by the kidneys.

Elevated homocysteine levels has been widely associated with atherosclerotic vascular disease. Although the mechanism by which homocysteine causes vascular damage and contributes to atherosclerosis is not yet known, mechanisms relating to direct (by directly reacting with cellular constituents) and indirect (by auto-oxidation of homocysteine to homocystine and hydrogen peroxide) cytotoxicity of homocysteine to endothelial and vascular smooth muscle cells have been proposed. Various studies have suggested that homocysteine facilitates oxidative arterial injury, augments the proliferation of smooth muscle cells, alters the coagulant properties of blood and impairs endothelium dependent vasomotor regulation. In response to homocysteine-induced injury damaged endothelial cells become prothrombotic with decreased thrombomodulin and heparan sulfate synthesis and increased expression of tissue factor. Increased levels of homocysteine can also increase vascular smooth muscle cell proliferation via activation of protein kinase C, cyclin dependent kinase, and induction of c-fos and c-myb. These hypotheses and related studies have been reviewed by Berwanger (2), Welch (3) and Hankey (4). The possible interaction of homocysteine with endothelial functions, vascular smooth muscle cell proliferation and thrombotic properties of blood are also closely related to restenosis after PTCA. The roie of homocysteine in the development of restenosis after PTCA has not been investigated widely. In a rat carotid endarterectomy model, hyperhomocysteinemia was found to increase neointimal hyperplasia (5), but in a prospective case-control study plasma homocysteine was not a predictive factor for restenosis after coronary angioplasty (6). Therefore we aimed to observe any possible association between homocysteine levels and restenosis after PTCA with or without stent implantation.

MATERIALS AND METHODS

Patients

From January to December 1997, 204 patients were enrolled in this prospective clinical trial who underwent a coronary intervention because of stable or unstable angina pectoris. The criteria for coronary intervention was the angiographically documented stenosis of≥70%in at least one of the major branches of the coronary tree, and accompanying ischemic changes in electrocardiograms (ECG) at rest or with provocative tests. All patients' whole blood counts, blood glucose levels, liver and renal function tests, ECG and chest X-rays were evaluated before the intervention. Overnight fasting blood samples were obtained from the patients before PTCA for homocysteine determinations.

An informed consent was obtained from all patients and this study was approved by the Ethical Committee of the Marmara University School of Medicine.

Procedure and follow-up

PTCA procedures and stent implantations of the scheduled patients were performed in the Catherization Laboratory of Florence Nightingale Hospital (Kadir Has University School of Medicine). The angioplasty procedures were performed via the femoral approach with an 8 French guiding catheter according to the standard PTCA technique as originally described by Grüntzig et al (7). The angiographic criteria of a successful angioplasty was defined as an increase of greater than 50% in luminal diameter with a final stenosis of less than 30% in luminal diameter and no major complications (death, myocardial infarction, or emergency by-pass surgery). A stent was implanted in bail-out situations and in cases where a suboptimal result was obtained with conventional PTCA. All

patients who did not have a history of previous gastrointestinal bleeding were on aspirin therapy (100-300 mg/day) before and after the intervention, intravenous bolus heparin (10,000 U in PTCA and 15,000 U in stent patients) with aPTT control, and ticlopidine for 6 weeks (stent patients), calcium channel blockers and nitroglycerin for six months as adjunctive medical therapies. Any of the present risk factors such as hypercholesterolemia, hypertension and diabetes were intensively treated with appropriate therapies. The patients were followed with clinical examinations and exercise tests at 1,3 and 6 months after the intervention. A coronary angiogram was scheduled after 6 months' control to evaluate restenosis, in which, patients with greater than 50% stenosis in luminal diameter at the angioplasty site were accepted to have restenosis. Both angioplasty and control angiograpies were recorded with cineangiography for documentation.

Determination of homocysteine

Serum homocysteine levels were determined with fluorescence polarization immunoassay (Abbott Diagnostics) (8,9). The homocysteine adducts in samples were reduced to free homocysteine with the addition of dithiothreitol and the total free homocysteine was enzymatically converted to SAH. The amount of SAH were determined with a fluoresceinated tracer labeled anti-SAH antibody. The results were expressed as total homocysteine in µmol/L.

Data analysis

The difference in the distribution of dichotomous characteristics in outcome groups were explored with the chi-square test. The presence of a statistically significant difference in homocysteine distributions among patients grouped according to outcome or various characteristics were explored with the t-test, using one-tailed p-values. The statistical tests were done using MS-Excel 97 software.

RESULTS

Of the 204 patients included in the study, 146 patients (72%) had a control angiography performed at the end of the follow-up period. The mean time interval for control angiography was 6.9±3.6 months. The mean age of these 111 male (76%) and 35 female (24%) was 56±10 years. According to the angiographic evaluation, 57 of the patients (39%) were decided to have restenosis, whereas 89 patients (61%) did not.

The homogeneity of the restonosis and non-stenosis groups were analyzed for the distribution of gender, lesion number, or type of intervention (PTCA vs. stent) applied, and the number of diabetic and hypertensive patients with the chi-square test. The distribution of these characteristics were not significantly different (Table I) in restenosis and non-stenosis groups. The application of PTCA with or without stent implantation to patients having one, two or three lesions also was not different significantly (p=0.622). The age, and blood lipid parameters of the restenosis and non-stenosis groups are presented in Table II. The mean age (p=0.133), total cholesterol (p=0.280), LDL-cholesterol (p=0.438), HDL-cholesterol (p=0.377), and triacylglycerol (p=0.660) levels of patients prior to PTCA were not significantly different in the restenosis and non-stenosis groups with the t-test. None of these risk factors were found to be associated with restenosis outcome with univariate analyses.

Although the average homocysteine level in the restenosis group was higher than the average homocysteine level of non-stenosis group, the difference was not significant with the t-test (12.06 vs. 10.60 µmol/L; p=0.12) (Table III). In order to see if the elevation in the mean homocysteine level in the restenosis group could

Table I. The distribution of gender, type of intervention used, number of patients with single or multiple lesions and number of patients with diabetes and hypertension in the restenosis and non-stenosis groups.

	Restenosis	Non-stenosis	Total
Male	47	64	111
Female	10	25	35
PTCA	37	53	90
Stent	20	36	56
Single lesion	37	61	98
Multiple lesion	20	28	48
DM	5	6	11
Hypertension	10	24	34

Table II. The age and blood lipid parameters of the restenosis and non-stenosis groups.

	restenosis, (n=57)					non-stenosis, (n=89)				
	mean	median	min	max	SD	mean	median	min	max	SD
Age	57.2	58	38	76	9.8	54.8	55	32	78	9.5
Total cholesterol	212.5	213	122	313	45.4	221.1	220	123	399	45.6
Triacylglycerols	200	171	60	690	130.3	208.6	184	61	573	100.1
HDL-cholesterol	46.4	48	30	56	5.5	45.5	47	24	58	6.1
LDL-cholesterol	127.9	125.9	51.2	238	43.3	133.9	134	42.6	313.2	44.7

Table III. The average homocysteine levels (± 1 standard deviations) in restenosis and non-stenosis groups.

	Homocysteine, µmol/L	
	restenosis	Non-stenosis
Whole group	12.06±4.51	10.60±6.43
Male	12.92±4.31	11.34±7.20
Female	7.36±2.17	8.79±3.56
PTCA	12.10±4.29	9.26±2.73*
Stent	11.97±5.12	12.27±8.95
Single lesion	11.78±5.34	10.57±7.57
Multiple lesion	12.51±2.80	10.81±3.02

* p=0.002

have been masked with the wide distribution of homocysteine levels, the 95th percentile value of the non-stenosis group was regarded as a cut-off value for this cohort, and the percentage of values exceeding this cut-off value were compared and found to be very similar in the restenosis and non-stenosis groups (6.1 % and 6.2 % respectively).

When the homocysteine levels in outcome groups were compared according to gender, men had a non-significant elevation in the restenosis group (12.92 vs. 11.34 µmol/L, p=0.152), whereas women presented a non-significant elevation in the non-stenosis group (7.36 vs 8.79 µmol/L; p=0.202). The homocysteine values of restenosis and non-stenosis groups were also compared among subsets according to the type of intervention applied (PTCA vs stent), and according to patients having single or multiple lesions. The homocysteine levels of patients with conventional PTCA were significantly elevated in the restenosis group (12.10 vs. 9.26, p=0.002), but this pattern was not observed in patients to whom a stent was implanted (11.97 vs. 12.27, p=0.449). Patients who had a single lesion intervention (11.78 vs. 10.57 p=0.259) and an intervention to more than one lesion (12.51 vs. 10.81, p=0.062) both had non-significant elevations in their respective restenosis groups.

DISCUSSION

After its discovery in 1960's, elevated levels of homocysteine were regarded as a risk factor for atherosclerosis. More than 12,000 patients were investigated in more than 100 cross-sectional, case-control, and prospective cohort studies to determine the role of elevated homocysteine levels as an atherosclerotic risk factor (4). The Physicians' Health Study (10), the British United Provident Association study (11), the Tromso study (12), the British Regional Heart Study (13), and the large European Collaborative Study (14) were among the most prominent studies where elevated homocysteine levels were found to be associated with coronary heart disease. In a meta-analysis of 27 observational studies which included 4000 patients, an elevated homocysteine level was associated with an increased risk of fatal and non-fatal atherosclerotic vascular disease in the coronary, cerebral, and peripheral circulations (15). But other prospective studies, -including further reports from the Physicians' Health Study-, have failed to demonstrate a significant association between elevated homocysteine levels and new angina, non-fatal myocardial infarction (MI), death from coronary heart disease (CHD), and ischemic stroke (16-20).

In order to determine if homocysteine levels at the time of angioplasty would be a predictor of restenosis after PTCA, we evaluated the homocysteine levels of 146 patients undergoing PTCA with or without stent implantation. In our prospective cohort, the overall homocysteine distribution obtained is similar to the distributions obtained in large-scale epidemiologic studies (10,13,14,20). Although the homocysteine level was elevated in the restenosis group, the difference did not reach significance. The percentage of values which exceed an arbitrary cut-off value (the 95th percentile value of the non-stenosis group) were also similar in the restenosis and non-stenosis groups (6.1% and 6.2% respectively), which indirectly showed that elevated values were equally distributed. When

the analyses were performed according to gender the same pattern was observed in men whereas women exhibited a non-significant elevation in the non-stenosis group; but the smaller size of the female patient group (n=10 in the restenosis group, 25 in the non-stenosis group) and the non-significance of the difference leads us to question the significance of the change in pattern. When the patients were grouped according to the type of intervention applied (PTCA vs stent), the homocysteine levels of patients with conventional PTCA were significantly elevated in the restenosis group (12.10 vs. 9.26, p=0.002), but this pattern was not observed in patients to whom a stent was implanted (11.97 vs. 12.27, p=0.449). Patients who had a single lesion intervention (11.78 vs. 10.57 p=0.259) and an intervention to more than one lesion (12.51 vs. 10.81, p=0.062) both had non-significant elevations in their respective restenosis groups.

The interpretation of the conflicting results of epidemiologic and other clinical studies regarding the role of homocysteine in the development of atherosclerotic vascular disease is complicated by the differences in study design, the selection criteria and number of cases and controls, the difference in follow-up method and duration, the analytical method used in the determination of homocysteine, the difference in the type and selection of outcome (MI, CHD, fatal MI, stroke etc.), and the statistical analyses used (2,4). Although the atherosclerotic process and the formation of neointimal hyperplasia during restenosis share many similar features,the role of homocysteine as an indicator of restenosis risk, and its possible contribution to the formation of neointimal hyperplasia has not been extensively studied. In our cohort, serum homocysteine levels at the time of angioplasty does not seem to be an indicator of restenosis risk after PTCA. It is possible that serum homocysteine level is not a good indicator of homocysteine's probable cytotoxic role at the cellular level. The role of homocysteine as an indicator of restenosis risk, and the extent of its contribution to the formation of neointimal hyperplasia needs to be further evaluated.

In conclusion, although there is in vitro evidence for the atherogenic and thrombotic properties of homocysteine, serum homocysteine level does not seem to be a useful indicator of restenosis risk after PTCA in our patient cohort.

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