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Elevated plasma homocysteine concentrations after pediatric heart transplantations

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Abstract Graft coronary artery vasculopathy is the main cause of late morbidity and mortality in cardiac allograft recipients. A high plasma homocysteine (hcy) concentration is now generally accepted as a risk factor for coronary arteriosclerosis, but little information exists for the pediatric age group. We therefore explored the potential role of hcy and antioxidants in 31 pediatric allograft recipients. We found hcy concentrations to be significantly higher in recipients than in control. Hcy continued to rise within the first 2 postoperative years. Vitamin A and E concentrations were significantly lower in transplant patients. Hyperhomocysteinemia was significantly more common in patients with complications than in those without. Our findings suggest that pediatric allograft recipients experience oxidant stress, as highlighted by the high plasma levels of Hcy and the low concentrations of vitamins A and E. Nutritional supplementation may be indicated to lower plasma hcy and to reduce oxidant stress.

Key words Heart transplantation · Coronary arteriosclerosis · Homocysteine · Pediatric · Vitamin

Introduction

Accelerated allograft coronary artery vasculopathy (CAV) is the main cause of late morbidity and mortality in heart transplant patients [5], including those in the pediatric age group. Elevated plasma homocysteine (hcy) concentrations are now generally accepted as an independent risk factor for coronary arteriosclerosis, peripheral vascular disease, and thrombosis [10, 17]. Within a few months after transplant surgery abnormally high hcy values are observed in the majority of adult recipients [4, 13]. It is not clear whether hcy contributes to the development of allograft CAV. Hyperhomocysteinemia was found to be more likely in recipients with vascular complications [2, 13]. The precise mechanism for the vascular damage is unknown. Increased oxidant stress caused by an elevated hcy level has been suggested as one factor [15]. Several studies suggest that antioxidants, especially vitamin E, might lower the risk of coronary heart disease [22, 23, 26]. As very little information concerning hyc and its risks is available for pediatric heart transplant recipients, we examined the potential role of plasma hcy and of vitamins A and E in pediatric allograft recipients with and without CAV. The results were compared with the corresponding values recorded in a normal reference group.

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Table 1 Reference values of total plasma homocysteine [mean (SD)] by age and sex

Age (years)	Male		Female	
		n		*n*
	8.6 (2.1)	92	7.8 (1.9)	114
0–1	5.0 (1.6)	10	4.7 (1.2)	15
1–3	10.0 (2.6)	15	9.3 (2.3)	16
3–6	8.0 (2.0)	11	10.0 (1.3)	17
6–10	10.4 (2.3)	13	8.4 (3.0)	20
10–15	9.1 (2.2)	23	7.7 (3.0)	22
Adults	9.2 (2.0)	20	6.7 (0.5)	24

Patients and methods

Patients

The study population consisted of 31 (16 boys and 15 girls) pediatric allograft recipients (28 heart, two heart–lung, 1 heart–renal transplants). The patients, with a mean age of 12.3 ± 4.9 (range 1–22 years), were seen at routine follow-up clinic visits between November 1994 and October 1996. Informed consent was obtained in each case, and the local ethics committee at Ospedale Bambino Gesù approved the study. Plasma hcy concentrations, lipid profile, and plasma creatinine were measured at 3–6-and at 12 months and then at yearly intervals after the transplant. Hcy concentrations were assessed at a mean of 34.3 (median 24, 3–96) months postoperatively. Thirteen recipients had more than one (up to four) plasma hcy evaluations over the course of the study. Twelve of the patients had survived for more than 5 years when plasma hcy concentrations were assessed for the first time. Vitamin A and E concentrations were evaluated in 10 patients.

At the time of the study only limited information was available concerning plasma hcy concentrations in children. Therefore we obtained hcy, lipid profiles, and creatinine measurements in 206 healthy pediatric and adult controls (92 male, 114 female) who were matched for age and sex (Table 1).

Laboratory evaluations

Samples for total fasting plasma homocysteine were collected in EDTA-containing tubes, immediately placed on ice, and centrifuged within 1–2 h. The plasma was stored at –70 °C until analysis by high-performance liquid chromatography. All forms of plasma homocysteine were analyzed, including oxidized and reduced forms. We defined hyperhomocysteinemia as a concentration greater than the 90th percentile of the age- and sex-appropriate control.

Vitamins A and E were obtained, stored and analyzed in a similar fashion. The normal range for these vitamins was determined from the measurement of vitamin concentrations in healthy volunteers who constitute a normal-value database for our laboratory.

Fasting plasma cholesterol, triglycerides, and creatinine concentrations were obtained at the same times as hcy and measured using standard laboratory techniques.

Table 2 Recipients' plasma homocysteine [mean (SD)] by age and sex

Age (years)	All	Male	Female
	12.2 (3.7)	10.7 (2.3)	12.7 (4.7)
1–3	10.5 (4.9)		10.5 (4.9)
3–6	11.8 (2.3)	11.8 (2.3)	
6–10	11.9 (4.3)	10.7 (1.7)	12.9 (5.6)
10–15	11.0 (2.7)	10.4 (2.7)	11.4 (2.6)
Adults	12.7 (5.6)	10.0 (2.4)	16.1 (6.7)

Table 3 Plasma homocysteine concentration after transplantation

Time (months)	*n*	Mean (SD)	*P*-value
3	8	9.0 (2.2)	
6	8	11.3 (1.7)	0.05[a] *
12	6	11.2 (2.0)	0.99[a]
24	7	15.2 (5.3)	0.14[a]
			0.016[b]*

* $P < 0.05$
[a] Between each interval
[b] Between 3- and 24-month values

CAV/acute and chronic rejection

After transplantation all patients underwent an annual cardiac catheterization procedure with angiography and endomyocardial biopsies. Biopsy results of at least 3A were considered diagnostic of acute rejection [6].

Statistical analysis

Results were expressed as mean (SD). The significance of differences between mean values was determined using a paired *t*-test. Percentages were compared using a Chi-square test. Findings were considered statistically significant if the *P*-value was <0.05 for a two-sided hypothesis.

Results

The allograft recipients had a mean plasma hcy concentration of 12.2 (3.7) μmol/l, which was significantly higher than that of healthy controls [8.2 (2.0) μmol/l, $P < 0.001$; Table 2]. Hyperhomocysteinemia was seen in 16 patients (52%). The plasma hcy concentrations rose in 7 of 8 patients (87.5%) with repeated assessments within the first 2 years after the transplant. Mean increase was 3.3 (3.1) (range –2.9 to 7.0) μmol/l. There was a statistically significant difference between values at 3 and 6 months ($P = 0.05$), and between those at 3 and 24 months ($P < 0.02$; Table 3). Five years after the transplantation no significant differences were found. The individual changes in hcy concentration were variable [mean 0.2 (3.4), range –4.5 to 4.0 μmol/l].

Lipid profile and creatinine were not significantly higher in recipients than in controls, although 4 patients

Table 4 Morbidity and mortality in pediatric heart transplant recipients and risk factors

	Homocysteine	Cholesterol	Triglycerides
Mortality			
1	x	–	–
2	–	–	x
3	x	–	–
4	x	–	–
5	–	x	x
6	x	x	x
Retransplantation			
1	x	–	–
2	x	–	–
3[a]	x	x	x
Rejection			
1	–	–	–
2	x	–	–
3	x	–	–
4	–	–	x

[a] Double-lung transplant after heart-lung allograft

had both elevated triglycerides (> 150 mg/dl) and elevated cholesterol (> 220 mg/dl) concentrations on repeated measurements. Two recipients had isolated high triglycerides. The only patient with an elevated serum creatinine (1.4 mg/dl) was also found to have hyperhomocysteinemia.

Vitamin A and vitamin E concentrations were significantly lower in transplant recipients than in controls [299.8 (85.4) vs 610 (97.6) ng/l and 457.0 (11.5) vs 897.4 (212.4) ng/l, respectively, $P < 0.001$]. All allograft recipients had vitamin concentrations below the tenth percentile of controls; 9 had vitamin A values below the third percentile. Seven of the 10 patients tested had increased plasma hcy concentrations.

By May 1999, 6 (19.4%) of the 31 patients had died, 3 had been retransplanted (1 with a double lung transplant after a heart-lung allograft for bronchiolitis obliterans), and 4 (plus 1 patient after the second allograft) had had chronic rejection or at least two episodes of acute rejection. Hyperhomocysteinemia was observed in 69.2% of the patients who suffered morbidities or mortalities versus 38.9% of those who did not have complications ($P < 0.002$). Four of the patients who died, all retransplanted patients, and 2 of the 4 children who had experienced rejections were diagnosed with hyperhomocysteinemia (Table 4).

Discussion

Over the last 30 years, elevated plasma hcy concentrations have been recognized as an independent risk factor for coronary artery disease [10, 17, 21, 25, 28]. Studies in transplant recipients have shown that plasma hcy concentrations rise within a few months after the procedure, for reasons that remain unclear [4, 13]. With this study we demonstrated that pediatric cardiac allograft recipients, like adults recipients, have higher plasma hcy levels than age-appropriate controls. Slightly more than half the pediatric patients presented with hyperhomocysteinemia. Hcy concentrations rose over the first 2 years after transplant. Possible mechanisms for increased hcy values include renal impairment, interference with the folate-dependent remethylation of hcy, and vitamin B6 deficiencies. Several recent studies have indicated that renal function or creatinine levels might be the most important determinants of plasma hcy concentrations after transplantation [2, 3, 9, 11, 12]. Our patients' creatinine levels were not significantly different from control levels. Only 1 of our patients with hyperhomocysteinemia had an abnormally elevated creatinine concentration. Cyclosporine plasma levels have been implicated as an independent positive predictor of plasma hcy concentrations [2, 8], although recently a negative correlation between hcy and 2-h peak levels of cyclosporine was demonstrated in cardiac transplant patients [12]. All our patients received cyclosporine for immunosuppressive therapy.

A large study in healthy children showed that serum hcy was significantly inversely correlated with serum levels of folic acid and vitamins B6 and B12 [20]. Post-transplantation levels of folate and vitamin B6 in adults have been found to fall and often to reach abnormally low ranges [4, 13]. Our evaluation suggests that deficiencies in allograft recipients might not be limited to folate and B vitamins. Although we assessed only a subset of patients, we found vitamin A and E levels to be significantly lower than in a reference population. Vitamin A was especially affected, being two standard deviations below the norm in the majority of patients. Vitamin A is necessary for the growth and differentiation of epithelial tissues [24]. Tocopherol is thought to be one of the main antioxidants in the defense against oxygen-free radical lipid peroxidation [15, 22]. Hcy is cytotoxic to endothelial cells in vitro and can promote low-density lipoprotein (LDL) oxidation [24]. Foam cells are formed in the arterial wall when macrophage scavenger receptors recognize the oxidized LDL [14], a process that eventually leads to the formation of atheromatous lesions [18]. A shortage in antioxidant vitamins might lead to accelerate CAV. The reasons for the vitamin deficiencies are unclear. Poor nutrition is unlikely, as many pediatric patients demonstrate catch-up growth after their transplant. Low vitamin A and E concentrations in the blood indicate that the hepatic storage of these vitamins may be exhausted. A person keeping to a retinol-free diet will use hepatic reserves and maintain stable blood concentrations for many months [27].

This suggests that unknown factors that interfere with the absorption or the metabolism of the vitamins might be present for an extended period of time in transplant recipients.

We found that patients who suffered rejection, retransplantation, or death had hyperhomocysteinemia more frequently than did recipients without complications. In adult cardiac and renal allograft recipients with elevated hcy values, atherosclerotic complications in general have been shown to be more common [2, 13]. A direct correlation between transplant coronary artery disease and hyperhomocysteinemia was recently questioned, although in the study the diagnosis was made by angiogram and not by intravascular ultrasound [11]. In nontransplant patients with angiographically confirmed coronary artery disease plasma hcy levels can be predictive of mortality [19].

Emerging evidence suggests that nutritional supplementation and pharmacological interventions might reduce the risk of atherosclerosis. Children who use multivitamins have lower mean hcy concentrations than nonusers [20]. Breakfast cereal fortified with folic acid can increase plasma folic acid levels and reduce plasma hcy concentrations in patients with coronary vascular disease [16]. In cardiac and renal transplant patients folic acid supplementation can significantly decrease hcy levels [1, 9]. Vitamin E supplements seem to limit the risk of coronary artery disease in adults [23, 26]. Except for 1 patient who was taking B vitamins for pernicious anemia, our patients did not receive any nutritional supplements.

There are several limitations to this study, including the relatively small population base and the nonuniform methodology. Baseline hcy concentrations were not available, and later measurements were obtained at varying intervals after the transplant. Creatinine clearance would have been a better measure of renal function. Vitamin concentrations were only assessed in a subgroup of patients, which prevented an analysis of a possible association between hyperhomocysteinemia and vitamin A and E deficiencies. Finally, we did not assess the patients in detail for vascular complications. No autopsy results relating to coronary arteriosclerosis were available. No intravascular ultrasound evaluations were performed during angiographies.

In summary, we found that pediatric allograft recipients had higher than normal plasma hcy levels. Slightly more than half had hyperhomocysteinemia. In a subgroup of recipients we demonstrated vitamin A and E deficiencies. At this point investigations avoiding the limitations of the current study are necessary to delineate the mechanism of allograft vasculopathy further. If deficiencies of antioxidants can be confirmed in larger patient populations we will have to study the etiologies and consequences. We also have to evaluate whether therapeutic interventions, such as vitamin supplementation, can reduce hcy concentration and prevent complications in pediatric transplant recipients.

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