Pediatric rheumatology

Increased concentration of plasma homocysteine in children with systemic lupus erythematosus

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Abstract

Objective

Studies in adults with SLE have evidenced increase of homocysteine related, mainly, to thromboembolic events. The aim of our study was to evaluate plasma homocysteine concentration in children with systemic lupus erythematosus (SLE) and its correlation with renal involvement, serum and erythrocyte folate, vitamin B12, antiphospholipid antibodies, estimated creatinine clearance and dyslipidemia.

Methods

Thirty-two children (29 females) with SLE and 32 healthy controls (29 females) matched for age and sex were included in the study. The mean age of patients and controls was 14.2 years (range from 10 to 18 years). Only one patient presented one thrombotic event. Plasma homocysteine, erythrocyte and serum folate, vitamin B12, lipid profile, antiphospholipid antibodies and estimated creatinine clearance were evaluated. Raised homocysteine concentration was defined as equal or more than 12.9 mol/L.

Results

Raised homocysteine concentration was detected in 15 (46.9%) children with SLE with an important statistical difference in relation to control group ($p < 0.001$). A positive correlation was found between plasma homocysteine concentration and renal involvement (odds ratio 11.1 [95% CI 1.50–82.24], $p = 0.01$) based on the presence of renal biopsy, abnormalities of urine sediment and/or serum creatinine. However, when we performed the estimated creatinine clearance the correlation with homocysteine concentration was not positive. We did not observe abnormalities in serum and erythrocyte folate and vitamin B12 in our patients. However, they presented significant higher concentrations of TC total cholesterol ($p = 0.005$) and of LDL low-density lipoprotein ($p = 0.02$) than controls.

Conclusions

Elevated plasma homocysteine concentration is frequent in children with SLE. We believe that these results may signalize to the possibility of complications in our patients later in life. Further long-term and prospective studies are needed in order to determine the real role of the homocysteine concentration as a risk factor in children.

Key words

Systemic lupus erythematosus, homocysteine, children, dyslipidemia.

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Introduction

Coronary heart disease (CHD) is an important cause of morbidity and mortality in adults with SLE; its frequency ranges from 6.1% to 8.9% (1-4). However, in children's population it is unknown (5-8). There are only case reports of myocardial infarction in children with SLE (6-8). The presence of risk factors such as vascular injury associated with inflammatory processes, vasculitis, immune complex-mediated cell damage, the use of corticosteroids inducing high blood pressure and obesity, hypertension, hyperlipidemia and smoking, can lead to atherosclerosis, and cause CHD in adults as well as in children (4, 9, 10). Falaschi *et al.* (11) in a study with 26 children with SLE, determined the thickness between the media and the intima layers of carotid artery. This thickness was increased in relation to the control group, suggesting that it may be the most sensitive marker for the premature atherosclerosis (11). Asymptomatic abnormalities of the myocardial perfusion have also been observed in children with SLE (12).

Homocysteine is a sulphur containing amino acid derived from the metabolic conversion of methionine, with direct and indirect toxic effects on the vascular endothelium (13, 14). The mechanism involved may comprise an inhibitory effect on endothelial cell growth, promotion of vascular smooth-muscle proliferation, induction of a vascular-endothelial-cell activator, and a direct toxic effect on endothelium (2). Studies in adults with SLE have evidenced increase of homocysteine related, mainly, to the thromboembolic events (2, 4, 13). However, we did not find in the English literature any study about the homocysteine concentrations in children with SLE. In individuals with familial hypercholesterolemia, the elevation of homocysteine seems to be another determinant factor to the cardiovascular disease, but few studies have evaluated the role of homocysteine in dyslipidemia (15-17).

The objectives of the present study were to evaluate the plasma homocysteine concentration in children and adolescents with SLE and the possible correlation with clinical and laboratory features.

Patients and methods

Patients

A cross-sectional study with 32 children and adolescents who met 4 or more classification criteria for SLE was performed (18). All patients were recruited from a tertiary referral center. Twenty nine were females and 20 were caucasians. The mean-age was 14.2 years (range from 10 to 18 years). Children younger than 10 years were excluded due to the great variability of the plasma concentration of homocysteine in this period (19, 20). None of the patients was smoker or alcohol user. SLEDAI was performed in all patients at the day of blood collection. We considered headache as a CNS manifestation when it was resistant to analgesic treatment and responsive to corticosteroid or immunosupressive therapy. All these patients underwent magnetic resonance investigation. Renal involvement was considered if the patient presented WHO class III, IV or V on renal biopsy, raised serum creatinine (above 1.1) or abnormalities on urine sediment (hematuria and/or proteinuria); estimated creatinine clearance was also calculated (21). All patients were taking corticosteroid, 30 cloroquine, 13 cyclophosphamide, 12 azathioprine, 7 methotrexate, and 5 cyclosporine.

Controls

Thirty-two healthy children and adolescents selected from a public school, sex and age-matched to the patients, without acute or chronic diseases, genetic syndromes or familial history of autoimmune disease constituted the control group. None of the controls was a smoker or alcohol user.

Methods

Patients and controls samples were identified by a code unknown by the laboratory staff. Thirty mL of blood were collected from fasting participants and centrifuged at 3000 rpm for 5 minutes. The plasma was separated in aliquots and immediately frozen and stored at –80ºC. The time between the

beginning and the end of this process did not exceed one hour.

One portion of each blood sample was used to determine the total homocysteine concentration and the concentration of vitamins (B12 and erythrocyte and serum folate). The erythrocyte folate was also performed because it reflects the concentration of folate in the last 3 months. The left blood sample was used for the determinations of anticardiolipin, lupus anticoagulant, serum creatinine, total cholesterol (TC) and its fractions, and triglycerides (TG).

Total homocysteine concentration in plasma was measured according to the method of Pfeiffer *et al.*, which uses the High Performance Liquid Chromatography (HPLC), with fluorimetric detection and isocratic elution (22). Raised homocysteine concentrations were defined as a plasma homocysteine concentration of equal or more than 12.9 μmol/L (90 percentile of the control group).

Vitamin B12 concentration was performed by an enzyme immunoassay method with microparticles (Imx B12® of Abbott Laboratories). Serum and erythrocyte folate concentrations were measured by ionic capture (Imx Folate® of Abbott Laboratories).

The lupus anticoagulant was measured in platelet-poor plasma by the modified Russell viper venom time (Viperquik La, BioMérieux®). Anticardiolipin antibodies were measured by enzymatic conjugate anti-IgG, anti-IgM and solution-cromogen, evaluated by spectrophotometric reading (Cardiolipin from Bovine Heart Sodium, Sigma – Aldrich®).

Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), serum creatinine as well as TG were measured by the enzymatic colorimetric method.

Statistical analysis

To establish a reference value (cutting point) in order to make comparisons between the groups of patients and controls, it was made the ROC curve analysis and after that, the categorization by the 90 percentile of the control group.

Some variables like homocysteine concentration, TC and its fractions, TG, vitamin B12, serum and erythrocyte folate concentrations and serum creatinine were transformed in logarithm (Log_{10}) due to the variability and asymmetry of values, in order to obtain a normal distribution of probability.

For the comparison of categoric variables of homocysteine, it was used the chi-square test or, when necessary (expected values lower than 5), the Fisher's exact test. For the comparison of continuous variables, it was used the Student's t-test. The relation between homocysteine concentration and other continuous variables was analyzed by Spearman's correlation coefficient.

To evaluate the relation between homocysteine concentration in groups of normal and high values (normal 12.9 and high ≥12.9) and the other variables, in each group, it was used the Logistic Regression Analysis, with logit model, to estimate odds ratio. Univariate and multivariate analysis were made by the Stepwise variables selection criteria.

The level of significance adopted for the statistical tests was 5%, that is, $p < 0.05$.

The study protocol was approved by the Ethical Committee of the Universidade Federal de São Paulo. Informed consent was obtained from parents or responsible for each patient and control before inclusion in the study.

Results

Demographic characteristics, laboratory findings and risk factors of participants are shown in Table I.

Twenty-nine patients were females and the mean-age at the onset of illness was 11.9 years. The duration of the disease ranged from 3 months to 11 years and 7 months (mean of 2 years and 10 months). There were no differences in sex or age between the patients and the controls.

In relation to the disease activity, according to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), 22/32 (68.75%) patients showed active disease, 5 with important activity (SLEDAI > 12) and 16 with moderate activity (SLEDAI: 5 to 12).

Fourteen patients (43.7%) presented involvement of the central nervous system (CNS), characterized by past history of convulsion (9/14) and headache (5/14).

Table I. Demographic characteristics, laboratory findings and risk factors of children with SLE (32) and controls (32).

	Patients	Controls	p
Demographic characteristics			
Male/ female	03/29	03/29	
Caucasian/not caucasian	20/12	15/17	0.3152
Age in years (mean)	14.2	14.2	
Age at onset in years (mean)	11.9	-	
Familial history of cardiac disease	0/32	0/32	
Laboratory findings (mean)			
Erythrocyte folate (ng/mL)	266	264	0.8114
Serum folate (ng/mL)	7.1	6.3	0.4537
Vitamin B12 (pg/mL)	398	462	0.2932
TC (mg/dL)	181.30	155.03	0.0052*
LDL – cholesterol (mg/dL)	102.53	88.41	0.0221*
HDL- cholesterol (mg/dL)	45.69	44.66	0.7543
Triglycerides (mg/dL)	117.72	100.25	0.269
Serum creatinine (mg/dL)	0.87	0.76	0.0015*
Risk factors			
Total homocysteine (μmol/L)(mean)	14.44	8.94	< 0.001*
aCL (present/total)	16/32	01/32	
LAC (present/total)	08/32	0/32	
aCL and/or LAC (present/total)	20/32	01/32	

TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; aCL: anticardiolipin antibody; LAC: lupus anticoagulant.
*P < 0,05.

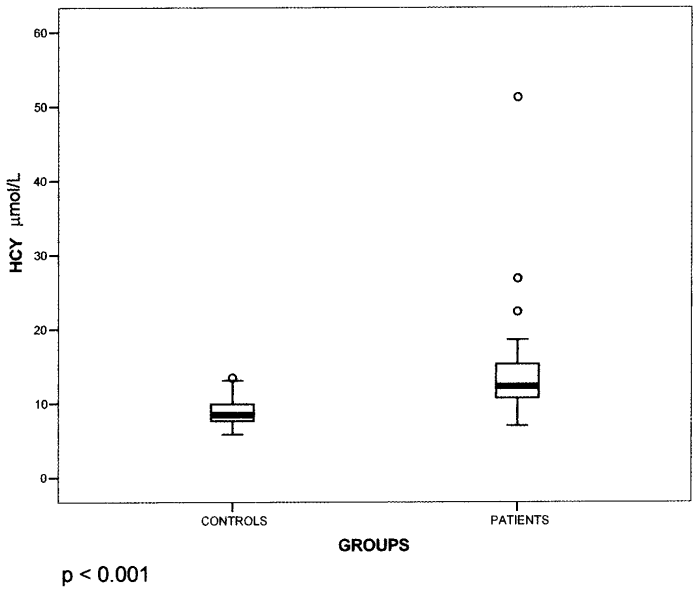

Fig. 1. Median and percentiles of homocysteine concentration in patients (N = 32) and controls (N = 32).

Seventeen (53.1%) patients presented renal involvement at the time of the study, characterized by class III, IV or V on renal biopsy, altered urinary sediment and/or raised serum creatinine. including: 11 (34.4%) with class IV or V on renal biopsy; 5 (15.6%) with hematuria and/or proteinuria, and 2 patients with raised creatinine levels. Nineteen (59.3%) patients had presented raised creatinine during the follow-up, however most of them improved with treatment. Abnormal estimated creatinine clearance at the time of the study was observed in 11 (34.4%) out of the 32 patients. Three of our patients with renal involvement presented nephrotic syndrome; 2 of them had high plasma homocysteine concentration; only 1 patient presented thrombotic event.

Total homocysteine concentration equal or above the cut-off of 12.9 mol/L was found in 15/32 (46.9%) patients and in only 4 controls, with a statistical difference between the groups ($p < 0.001$, chi-square test) (Fig. 1). In multivariate logistic regression analysis the log-transformed concentration of homocysteine was significantly associated with renal involvement (odds ratio 11.1 [95% CI 1.50–82.24], $p = 0.01$). However, no association was found between total homocysteine concentration and actual age of patients, disease duration, renal function estimated creatinine clearance, TC and its fractions and TG, as well as with serum and erythrocyte folates or vitamin B12 concentrations (univariate logistic regression analysis). Lupus patients presented higher TC, LDL and serum creatinine levels than controls ($p = 0.005$, $p = 0.02$, $p = 0.0015$, respectively, Student's t-test) (Table I).

Anticardiolipin and lupus anticoagulant antibodies were present in 16/32 (50%) and 8/32 (25%) patients, respectively. However, no association with high homocysteine was found (Fisher's exact test).

All of our patients were taking corticosteroids with a cumulative dose of 33.41mg/kg. No correlation between disease activity (SLEDAI) and cumulative corticosteroids dose with homocysteine concentration was found (Spearman's correlation). Seven patients had been taking methotrexate associated with folic acid for at least one year; however only 2 of them presented elevated concentration of homocysteine. We also did not observe a correlation between homocysteine concentration and the azathioprine or cyclosporine treatment.

Discussion

Over the last years, due to the increase of survival of lupus patients, we have observed a higher concern about complications resulting from the disease itself and from the medications used for its control. Studies with adult patients have shown a correlation between the homocysteine concentration and the presence of thromboembolic events (2). These findings are of great importance, once the alterations in the homocysteine concentration can be changed, leading, consequently, to a higher survival and to a better quality of life of the patients. The implications of the association among the plasma concentration of homocysteine, vitamin B12 and folate, with vascular disease in children are not yet established.

In the present study we observed elevated concentration of homocysteine in a significant proportion of children with SLE, when compared to controls. The homocysteine concentration can vary according to the age, especially before the age of 10, to the gender (is higher in males due to increase in muscle mass) and in patients with dyslipidemia (15, 20, 23, 24). The endothelial lesion due to the lupus activity and to the use of corticosteroids is another factor that can determine the increase of homocysteine concentration (2, 14). There are only a few studies in children and we did not find, up to the moment, works in the literature about the homocysteine concentration in children with lupus (20, 23).

There were no significant differences of folate and vitamin B12 concentrations among our patients and controls, which may indicate an adequate nutritional support for these vitamins, and no interference by the use of drugs.

Regarding the lipid profile, the HDL and TG concentrations were normal in our patients; however, the TC and the LDL fraction were elevated. Dyslipidemia in lupus can be attributed to the disease activity as well as to the chronic use of corticosteroids (24, 25). In relation to corticosteroids, they increase the hepatic production of VLDL resulting in the elevation of TC, TG, VLDL and LDL. The decrease of the receptor mediated removal of LDL, the increase of lipoprotein lipase and the decrease of hepatic triglyceride lipase activity can be other ways of dyslipidemia related to the use of corticosteroids (26).

In the present study, we observed a

positive correlation between total homocysteine concentration and renal involvement (odds ratio 11.1 [95% CI 1.50-82.24], p = 0.01). Even though it is unknown the exact mechanism by which the renal involvement alters the homocysteine rate, it is believed that there might be an indirect correlation with the diminished glomerular filtration rate (27, 28). Possible mechanisms are decreased renal homocysteine excretion, impaired renal metabolism or inhibition of extrarenal homocysteine metabolism by uremic toxins, or generally reduced B vitamin status in renal failure (29). The creatinine concentration, used in practice as a renal function marker, is insensitive to the detection of minor or moderate reductions of glomerular filtration. The estimated creatinine clearance is more sensitive and although it was altered in 34.4% of our patients we did not find positive correlation with the homocysteine concentration. The size of our sample may have been responsible for the lack of statistical significance (4, 20, 23). Three of our patients with renal involvement presented nephrotic syndrome; 2 of them had high plasma homocysteine concentration. It is worthwhile to emphasize that our patient with the highest homocysteine (51.24 μmol/L) concentration presented the most severe proteinuria (187 mg/kg/day).

We believe that more sensitive methods to detect impairment of glomerular filtration could have shown more significant involvement of renal function in our patients and probably a positive correlation with the plasma homocysteine concentration.

Homocysteine promotes many alterations that can explain the atherosclerosis mechanism that occurs in patients, especially with SLE. This includes oxidative processes, activation of proteolytic enzymes, hyperplasia of vascular smooth muscle, endothelial cell damage, platelets activation and thrombosis (2, 13).

We believe that our results may signalize to the possibility of complications in our patients later in life. The benefit of folate supplementation in order to prevent premature cardiovascular disease remains unclear. Further long-term and prospective studies are needed in order to determine the real role of the homocysteine concentration as a risk factor in children.

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