

Elevated serum homocysteine levels in paediatric patients with primary Raynaud's phenomenon

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ABSTRACT

Introduction: Raynaud's phenomenon (RP) is a paroxysmal and recurrent acral ischemia resulting from an abnormal arterial vasospastic response to cold or emotional stress. Homocysteine, a sulphured amino acid, has been linked to cardiovascular and neurodegenerative diseases, diabetes, thrombosis, and bone fragility. Homocysteine has been also linked to the pathogenesis of RP, as increased serum homocysteine (S-homocysteine) levels were observed in patients with RP. **Objective:** As all publications concerning S-homocysteine in RP involved only adult patients, our aim was to evaluate S-homocysteine in children and adolescents with RP. **Methods:** Nineteen patients (two boys and 17 girls; mean age 16.1 ± 2.2 SD) with primary RP were enrolled. The controls were 51 children and adolescents (21 boys and 30 girls; mean age 15.1 ± 1.8 SD). **Results:** The S-homocysteine level was significantly higher in the RP group in comparison with controls (11.2 ± 2.4 vs. 8.0 ± 2.0 $\mu\text{mol/L}$; $P = 0.00001$). S-homocysteine levels in RP were not age-dependent. **Conclusion:** Paediatric patients with RP have increased S-homocysteine levels, suggesting that homocysteine plays an important role in the development of vascular dysfunction, even at an early age.

Keywords: Raynaud's phenomenon, homocysteine, children.

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INTRODUCTION

Raynaud's phenomenon (RP) is a paroxysmal and recurrent acral ischemia resulting from an abnormal arterial vasospastic response to cold or emotional stress.^{1,2} RP is categorized as primary or secondary, the latter in association with an underlying disease, such as scleroderma or lupus erythematosus, respectively. The RP probably originates from dysregulation in the vascular motility mechanisms, resulting in an imbalance between vasodilatation and vasoconstriction.^{1,2} Progressive deficiency in vasodilatory capacity of the vessels is proposed as a mechanism of RP, particularly in systemic sclerosis. In addition, decreased fibrinolysis and enhanced coagulation pathways undoubtedly contribute to vascular dysfunction.¹⁻³ Homocysteine, a sulphured amino acid, has been linked to cardiovascular and neurodegenerative diseases, diabetes, thrombosis, and bone fragility.⁴ Homocysteine has been also linked to the RP pathogenesis,

as increased serum homocysteine (S-homocysteine) levels were observed in patients with RP.²⁻¹⁰ So far, all publications concerning S-homocysteine in RP involved only adult patients. Our primary aim was to evaluate S-homocysteine in paediatric patients with RP; the secondary aim was to look for correlations between S-homocysteine and patients' age, disease duration, and serum cholesterol levels, respectively.

PATIENTS, MATERIALS, METHODS

Nineteen patients (two boys and 17 girls; aged 12–20 years, mean age 16.1 ± 2.2 SD) with primary RP were enrolled. Disease duration was in the range of 0.5–9 years (mean 1.5 ± 2.0 years). Seven patients (37%) were treated with oral isradipine. Control group consisted of 51 children and adolescents (21 boys and 30 girls) aged 12–20 years (mean 15.1 ± 1.8 SD) who were either healthy, or did not suffer from acute or chronic inflammation, autoimmune disorders including rheumatic diseases,

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musculoskeletal disorders, inflammatory bowel disease, diabetes mellitus, hypercholesterolemia, epilepsy, and chronic renal failure and had a negative personal history of thrombosis and/or thromboembolism. Informed consent, fully in accordance with the Declaration of Helsinki, was obtained from all study patients and/or their legal representatives prior to the presented procedures. All investigated subjects were on a standard central European diet, consisting mostly of meat and carbohydrates. None was on a diet poor in vitamin B, nor was receiving doses of vitamin B exceeding recommended daily allowances. Nailfold capillaroscopy was performed and analysed in all RP patients. Photoplethysmography of the fingers was performed with the use of a pulse oximeter in 18 RP patients – before and after 10 minutes’ immersion in 10°C cold water. The curves were subsequently analysed. Antinuclear antibodies (ANAs) were assessed in 18 RP patients. Fasting S-homocysteine level was evaluated by chemiluminescence (Immulite 2500 immunoassay system, Siemens Healthcare Diagnostics, Germany) and expressed in µmol/L. Serum cholesterol levels in RP patients were analyzed and expressed in mmol/L. Mean values and standard deviations (SD) were calculated. Unpaired *t*-test was used to calculate differences between groups. Correlation analysis was performed to compare the relationship between age, disease duration, and S-cholesterol and S-homocysteine levels, respectively. For all results, *P* < 0.05 was required for statistical significance.

RESULTS

Nailfold capillaroscopy was abnormal in two RP patients (10.5%). Photoplethysmography was initially normal in 18 examined patients and subsequently revealed pathological curves in four patients (21%) after cold water immersion. ANAs were negative in eight RP patients (44%), while in the remaining 10 subjects these were in the range of 1:40 to 1:5120 (mean 1:588). S-homocysteine level was significantly higher in the RP group in comparison with controls (Table 1). In the control group, S-homocysteine levels did not differ between boys and girls (*P* = 0.56).

Table 1
Subjects’ characteristics and S-homocysteine results

Parameter	Control group (n = 51)	RP (n = 19)	P
Age (years)			0.1
range	12–20	12–20	
mean ± SD	15.1 ± 1.8	16.1 ± 2.2	
S-homocysteine (µmol/L)			0.00001
range	2.0–13.6	5.9–14.6	
mean ± SD	8.0 ± 2.0	11.2 ± 2.4	

RP = Raynaud’s phenomenon; S-homocysteine = serum homocysteine. SD, standard deviation.

Serum cholesterol level in the RP group was in the physiological range of 3.2–5.7 mmol/L (mean ± SD, 4.3 ± 0.7).

We did not find a significant correlation between S-homocysteine values and age in neither RP, nor controls (*R* = 0.11 in both groups). There was no correlation between S-homocysteine levels and disease duration, nor between S-homocysteine and serum cholesterol (*r* = 0.12 and *r* = 0.01, respectively).

DISCUSSION

Previous studies mainly focused on S-homocysteine in adult patients with primary and secondary RP. S-homocysteine was elevated in RP patients in comparison with healthy controls.^{5–7} High S-homocysteine levels were also found in patients with secondary RP in lupus erythematosus,⁸ scleroderma,^{3,5} or end stage renal disease.⁹ Patients with primary RP had significantly lower plasma levels of folate in comparison with patients with secondary RP.⁵ Furthermore, increased levels of von Willebrand factor were observed in RP patients with end stage renal disease.⁹ In another study, patients with RP and systemic sclerosis had higher S-homocysteine and von Willebrand factor concentrations than controls.¹⁰ Mean S-homocysteine levels in adult RP patients were mostly above 10 µmol/L, while in the control groups these were in the range of 5–8 µmol/L,^{5,7,8} which is very similar to our results. In the present study, radiological assessment was not done and we did not evaluate the methylene-tetrahydrofolate reductase (MTHFR) genotypes, nor vitamin B or folate levels, and these are the limitations of this paper. In our subjects in the age groups of 12–20 years, S-homocysteine levels were not related to age, regardless of the RP presence. This is in accordance with previous observations, where close relationship between S-homocysteine and age was observed in younger children or adults, but not in adolescents.¹¹ S-cholesterol levels were within the normal range in our RP patients. Furthermore, this study did not find any relationship between S-homocysteine and disease duration or S-cholesterol, respectively. Nailfold capillaroscopy and post-immersion photoplethysmography findings were not homogenous in our patients and we can hypothesize that these are related to the RP severity. The various ANAs titres might illustrate the heterogeneity of primary RP even in the absence of an apparent underlying disease. Homocysteine involvement in the pathogenesis of RP remains unclear; a possible association between hyperhomocysteinemia and endothelial injury has been proposed. Hyperhomocysteinemia has been associated with alterations in vascular morphology, loss of endothelial anti-thrombotic function, and induction of

a procoagulant environment.⁴ Most known forms of damage or injury are due to homocysteine-mediated oxidative stress. It has been hypothesized that homocysteine might influence endothelial injury and modifications in circulating mediators

of vasomotion.² In conclusion, even paediatric patients with RP have increased S-homocysteine levels, suggesting that homocysteine plays an important role in the development of vascular dysfunction even at an early age.

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