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HYPERHOMOCYSTEINEMIA AND SMOKING IN PRIMARY ANTIPHOSPHOLIPID SYNDROME

HIPERHOMOCISTEINEMIJA I PUŠENJE U PRIMARNOM ANTIFOSFOLIPIDNOM SINDROMU

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Summary: The thrombotic tendency in antiphospholipid syndrome (APS) shares several pathways with atherosclerosis. Atherothrombosis (atherosclerosis superimposed with thromboses) is influenced by nonmodifiable and some modifiable risk factors (smoking, obesity, physical inactivity, alcohol abuse, hyperhomocysteinemia). Therefore, we investigated the association among clinical and serological features of patients with primary APS and potentially modifiable risk factors for the development of atherothrombosis. Also, we compared the analyzed parameters with those in control subjects. Homocysteine concentrations were detected by HPLC (high performance liquid chromatography), while antiphospholipid antibodies were detected by ELISA. Smokers had elevated levels of homocysteine ($\chi^2 = 6.22$, $p < 0.05$). Independently of patients' age, the association between increased levels of homocysteine and history of myocardial infarctions was found ($\chi^2 = 4.61$, $p < 0.05$). Hyperhomocysteinemia and smoking are the most important modifiable risk factors for atherothrombosis in primary APS.

Keywords: hyperhomocysteinemia, smoking, primary antiphospholipid syndrome, myocardial infarction

Kratak sadržaj: Trombotična tendencija u antifosfolipidnom sindromu (APS) deli nekoliko zajedničkih putanja sa aterosklerozom. Na aterotrombozu (ateroskleroza sa superimponiranim trombozama) utiču nepromenljivi i promenljivi faktori rizika (pušenje, gojaznost, fizička neaktivnost, zloupotreba alkohola, hiperhomocisteinemija). Stoga smo ispitali vezu između kliničkih i seroloških osobina pacijenata sa primarnim APS-om i potencijalno promenljivih faktora rizika za razvoj aterotromboze. Takođe, uporedili smo analizirane parametre sa kontrolom. Koncentracije homocisteina utvrđene su metodom HPLC (tečna hromatografija visoke rezolucije) a antifosfolipidna antitela metodom ELISA. Pušači su imali povišene nivoe homocisteina ($\chi^2 = 6,22$, $p<0,05$). Nezavisno od godina pacijenata, otkrivena je veza između povišenih nivoa homocisteina i istorije infarkta miokarda ($\chi^2 = 4,61$, $p<0,05$). Hiperhomocisteinemija i pušenje su najvažniji promenljivi faktori rizika za aterotrombozu u primarnom antifosfolipidnom sindromu.

Ključne reči: hiperhomocisteinemija, pušenje, primarni antifosfolipidni sindrom, infarkt miokarda

Introduction

The thrombotic tendency in antiphospholipid syndrome (APS) shares several pathways with atherosclerosis (1). The antiphospholipid syndrome is an acquired thrombotic disorder characterized by venous and arterial thromboses and/or spontaneous abortions and repeated detection of antiphospholipid antibodies. The APS may be associated with another autoimmune disease (secondary APS = SAPS), or unrelated to an underlying disease (primary APS = PAPS) (2).

Antiphospholipid antibodies are the main serological finding in patients with antiphospholipid syndrome (APS), but patients with atherosclerosis also have elevated levels of the above-mentioned antibodies (3).

Atherothrombosis (atherosclerosis superimposed by thrombosis) is influenced by nonmodifiable risk factors (age, gender, race/ethnicity, family history), well-documented modifiable risk factors (hypertension, diabetes, hiperlipidemia, smoking). Less well-documented or potentially modifiable risk factors are obesity (Body Mass Index, BMI $\geq$ 30), physical inactivity, alcohol abuse, hyperhomocysteinemia.

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The aim of this study was to investigate the association among the clinical and serological features of patients with primary antiphospholipid syndrome (PAPS) and potentially modifiable risk factors for the development of atherothrombosis. Also, we compared the analyzed parameters with those in healthy control subjects.

Patients and Methods

This study included 33 patients (24 women and nine men) with primary antiphospholipid syndrome, according to the updated and revised Sapporo criteria (4, 5). The mean age of the analyzed patients was 41 ± 14, the age ranging from 21 to 78 years. Also, this study included 28 healthy control subjects (21 women and seven men). The mean age of the control subjects was 37 ± 12, ranging from 18 to 72 years.

The criterion for the selection of sera for this study was similar age of all participants. Exclusion criteria for control subjects were the presence of acute or chronic diseases and taking medications, because we wanted to avoid any possible interference with the analyzed parameters. Regarding the fact that APS is a chronic, autoimmune disease, investigated patients were on antithrombotic drugs and/or oral anticoagulants for which no interaction with the analyzed parameters has been reported. Last dose of the previously mentioned medications was taken 12h before venipuncture.

Our study was approved by the local Ethical Committee and all participants provided signed informed consent.

The weight status of the patients is defined by the body mass index (BMI) (weight (kilograms) divided by the square of height in meters). Persons with a BMI of 25 to 29.9 kg/m^2 are classified as being overweight, and those with a BMI of ≥ 30 are classified as being obese (6).

Features of the investigated subjects are shown in *Table I*.

Table I Features of investigated subjects.

		Patients (n = 33)	Control subjects (n = 28)
Smoking	Smokers	10/33	9/28
	Nonsmokers	23/33	19/28
BMI*	< 25	14/33	27/28
	25–29.9	16/33	1/28
	≥ 30	3/33	0/28
Physical activity	Training	0/33	6/28
	Recreative	3/33	3/28
	Passive	30/33	19/28
Alcohol	Abstention	32/33	23/28
	Ocassionally	1/33	5/28

* BMI (Body Mass Index), calculated on the basis of data for weight and height of the analyzed subjects, according to formula m(kg)/h^2(m)

Methods

After overnight fasting (12 hours) and after 24 hours without intensive physical activity, blood samples were collected for serum and plasma from an arm vein (antecubital vein), and then centrifuged for 10 minutes at 3000 revolutions per minute. For the purpose of avoiding falsely positive higher levels of homocysteine, serum was separated from coagulum in 45 minutes (7).

Concentrations of homocysteine were detected by HPLC (high performance liquid chromatography) using commercial reagents from BIORAD, München, Germany and the fluorescent detector Hewlett-Packard 1046A.

Anticardiolipin antibodies of the IgG and of the IgM isotype were determined using commercial kits of Varelisa Pharmacia Deutschland GmbH, Diagnostics Division, Freiburg, Germany. Commercial kits from IMTEC Immunodiagnostica, GmbH, Berlin, Germany, were used for the determination of anti-β2glycoprotein I antibodies of the IgG and the IgM isotype (8, 9).

The presence of lupus anticoagulant was estimated according to the recommendations of the International Society of Thrombosis and Haemostasis (10).

Statistical analysis

Continous variables were expressed as mean ± SD. Descriptive statistics were used to summarize the clinical characteristics of patients with PAPS. The association between the clinical features of APS and analyzed parameters was examined by χ^2-test, Mann-Whitney or t-test, when appropriate. The correlation between two quantitative variables was determined with the Spearman's correlation test. Logistic regression was performed with the APS-related clinical events as the dependent variable and those investigated parameters whose association with the clinical event was statistically significant in the preceding analysis as the independent variables. Comparison among the groups of investigated subjects was done by Mann-Whitney or t-test, when appropriate. In all of the above-mentioned tests, $p < 0.05$ was considered statistically significant (11). Analyses were conducted in SPSS 10 (SPSS, Inc, Chicago, IL, USA).

Results

Arterial events were present in 60.61% (20/33) of the investigated patients while non-arterial events were present in 39.39% (13/33) of patients. Myocardial infarctions were present in 30% (6/20), while cerebrovascular insults and peripheral arterial thromboses were present in 70% (14/20) of patients with arterial events. Deep venous thromboses complicated by pulmonary emboli were present in 53.85% (7/13) and recurrent abortions were present in 46.15% (6/13) of patients with non-arterial events.

In the analyzed patients, independently of their age, we found an association between increased levels of homocysteine and history of myocardial infarction ($\chi^2 = 4.61$, $p < 0.05$), but logistic regression failed to confirm the strength of the association.

Homocysteine concentrations did not correlate with the concentrations of antiphospholipid antibodies.

Patients with PAPS in comparison to control subjects had significantly elevated concentrations (U/mL) of: anticardiolipin antibodies of the IgG isotype [(193.10 ± 173.47) vs. (14.98 ± 9.12), $p < 0.001$], anticardiolipin antibodies of the IgM isotype ((184.76 ± 190.92) vs. (11.22 ± 6.69), $p< 0.001$), anti-β2gpI antibodies of the IgG isotype ((32.57 ± 37.70) vs. (3.13 ± 1.82), $p < 0.001$), anti-β2gpI antibodies of the IgM isotype ((20.63 ± 35.41) vs. (1.97 ± 1.19), $p < 0.001$).

No statistically significant difference was found between the mean concentrations (μmol/L) of homocysteine in PAPS patients and in healthy control subjects [(10.86 ± 3.69) vs. (9.39 ± 2.01), t-test, p = not significant].

Only 9.09% (3/33) of the investigated patients and none of the control subjects were obese, which is shown in *Table I*.

Alcohol abusers were not among the analyzed subjects. Only 3.03% (1/33) of the analyzed patients and 17.85% (5/28) of the control subjects were occasionally consuming alcohol.

Among the analyzed subjects, 30.30% (10/33) of patients and 32.14% (9/28) of control subjects were smokers. Compared to nonsmokers, smokers had elevated levels of homocysteine ($\chi^2 = 6.22$, $p < 0.05$), although the logistic regression could not confirm the strength of this finding.

Discussion

Previously it was reported that elevated plasma homocysteine is an independent risk factor for peripheral vascular, cerebrovascular and coronary heart disease. The increased risk of CHD associated with homocysteine is additive to that of conventional vascular risk factors (12). According to Avivi (13), elevated levels of homocysteine were associated with thromboembolic complications and Stauffenberger et al. (14) showed that levels of homocysteine are in correlation with the rate of progression of coronary arteries stenosis in women. In the analyzed patients, independently of their age, we found an association between the increased levels of homocysteine and history of myocardial infarctions. We did not find any difference in the mean concentrations of homocysteine between the PAPS patients and control subjects.

Smoking has both acute effects on the risk of thrombus formation in narrowed arteries and chronic effects related to an increased burden of atherosclerosis (15), and the significant association between homocysteine and thromboses was reported (16). Thromboses are the main clinical finding in PAPS and the patients who were smokers had increased concentrations of homocysteine.

According to Sofi et al. (17) leisure time physical activity is inversely related to BMI obesity is associated with passive life and generally it is a predisposition for cardiovascular diseases. Among the analyzed patients with PAPS, only 9.09% had BMI ≥ 30 and no association between obesity and the analyzed parameters was found.

Our results suggest that among the investigated modifiable risk factors for the development of atherothrombosis, hyperhomocysteinemia and smoking are the most important in patients with primary antiphospholipid syndrome. Although the results could be considered as preliminary because of the relatively small number of subjects involved, we strongly recommend that smoking cessation, as well as determination of homocysteine levels and their normalization to recommended values, should be advised to all patients with the primary antiphospholipid syndrome.

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