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HYPERHOMOCYSTEINEMIA IN PATIENTS WITH PULMONARY EMBOLISM

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Abstract - Investigation of hyperhomocysteinemia (HHcy) as an important risk factor for pulmonary thromboembolism (PTE), which represents a most dangerous consequence of a unique phenomenon of venous thromboembolism which still suffers from sometimes conflicting or inadequately clarified results. The role of homocysteine in the clinical manifestation of this life-threatening disease and its treatment (in which any further information may be decisive) requires detailed examination.

The purpose of this study is to determine the differences in HHcy incidence and homocysteinemia levels between patients with PTE and healthy persons. The study enrolled 70 patients with PTE and 50 healthy persons. Homocysteine was measured using the HPLC method with fluorescent detection and HHcy was defined as homocysteinemia above 12 μmol/L. Statistical analyses included chi-square and Mann Whitney U tests. The median homocysteinemia value was significantly higher (p=0.017) in the patients (12.10 μmol/L) than in the controls (10.35 μmol/L). The comparison of HHcy incidence between the patients (51.5%) and controls (30%) revealed a significant difference (p=0.021). In patients, homocysteinemia was significantly higher (p=0.002) in men (14.05μmol/L) than in women (10.01 μmol/L). HHcy was present in 67.6% of men with PTE, which was significantly higher (p=0.006) than the incidence in women with PTE (33.3%). Healthy males had significantly higher (p=0.001) homocysteinemia (12.54 μmol/L) than healthy females (9.4 μmol/L). A significant difference (p=0.031) was observed between the incidences of HHcy in healthy males (44.0%) and healthy females (16.0%).

We conclude that the incidence of hyperhomocysteinemia and homocysteinemia are significantly higher in all the patients compared with the healthy persons, as well as in both healthy males and males with PTE compared with healthy females and female patients. This indicates that HHcy findings in PE are likely to have a clinical importance.

Key words: Homocysteine, pulmonary embolism (PE)

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INTRODUCTION

As a rather common cardiovascular emergency, pulmonary embolism (PE) resulting from occlusion of the pulmonary arterial bed may lead to acute life-threatening complications (Torbicki et al., 2008). Regional registry data show that about 18-208 per 100000 persons have been diagnosed with pulmonary embolism annually (Torbicki et al., 2008). The data collected between 1979 and 1999, found a 0.4% prevalence of pulmonary embolism among hospitalized patients (Torbicki et al., 2008). PE as a potentially fatal disease has a higher overall mortality than that in patients with acute myocardial infarction, exceeding 10% at 30 days and 16% at 3 months (Goldhaber et al., 2003, Goldhaber et al., 1999, Kucher et al., 2006.).

Identifying the risk factors for the occurrence and recurrence of venous thromboembolism (VTE), manifested as pulmonary embolism or/and deep vein thrombosis, and defining the place and role of each respective parameter, plays a crucial role in risk stratification and determination of prevention measure against these serious diseases (Zhu et al., 2009).

Homocysteine is a sulfhydryl amino acid formed from the demethylation of dietary methionine (Konkle et al., 2008). The studies examining a more explicit relationship between elevated plasma homocysteine concentration and coronary artery diseases are much more numerous than those examining the sometimes conflicting but now more appreciated relationship between hyperhomocysteinemia (HHcy) and venous thrombosis. (Bauer et al., 2005). Homocysteine is considered to be a risk factor for venous thrombosis as well as for arterial thrombosis (Hirsh et al., 2001, Goldhaber et al., 2008, Kucher et al., 2004).

The purpose of this study is to determine the differences in HHcy incidence and homocysteinemia levels between patients with PE and healthy persons.

MATERIALS AND METHODS

Our study enrolled 70 patients (37 men and 33 women) with PE (mean age was 42.5 ± 17.08 years). The pulmonary embolism diagnosis was made on the basis of well-established clinical criteria (Torbicki et al., 2008). Our study detected the following acquired risk factors for PE in the participants as the most frequent: long immobilization patients (pts) (30%), pregnancy/puerperium (22.8%), surgery (18.6%), trauma (11.4 %), obesity (6.8%) and hormone replacement therapy/oral contraceptive usage (4.5%).

The family medical history for VTE was positive in 23 (33%) patients, while an analysis of the personal medical histories revealed that 18 (26%) patients had already experienced certain episodes of VTE. Exclusion criteria were the presence of the following comorbidities affecting the Hcy blood level: renal insufficiency, thyroid dysfunction, diabetes mellitus, malignancies as well as drugs interfering in homocysteine metabolism. The control group comprised 50 healthy persons (25 men and 25 women) of equivalent age. All the participants received no supplementation with vitamins B_6, B_{12} and folate.

Blood samples were collected from all the participants after 12 h overnight fasting. Following venipuncture, samples were kept on ice prior to centrifugation in order to lower the increase in the Hcy blood level via synthesis in erythrocytes. Sera were separated within 45 min and kept at -20°C until analysis. The total Hcy concentration was determined by high-performance liquid chromatography (HPLC) with fluorescent detection (Mirkovic et al., 2004). According to recent recommendations, Hcy concentrations above 12 μmol/L were considered as HHcy (Herrmann et al., 2006).

The HHcy incidence between the patients and controls was compared by the Chi-square test. Gender-related differences in HHcy incidence between groups of patients formed according to stratification based on the presence and type of the most frequent acquired risk factors for PTE, were evaluated by the same test. The distribution of Hcy levels in our study did not follow the Gaussian mode. Therefore the Mann-Whitney U test was employed to compare the median Hcy levels between patients and controls, to evaluate gender related variations in Hcy concentrations and to test the difference in Hcy levels related to the presence of the most frequent acquired risk factors for PTE. The Kruskal-Wallis test was used to estimate differences in Hcy levels among groups of patients formed on the basis of the type of most frequent acquired risk factors for PTE. A p-value of less than 0.05 was considered to be significant. Statistical analyses were performed using the program Statgraphics vers. 4.2 (Aslan et al., 2007)

RESULTS

HHcy was present in 51.5% of the patients and 30% of the controls, revealing a significant difference (P=0.021), as illustrated in Fig. 1. The comparison

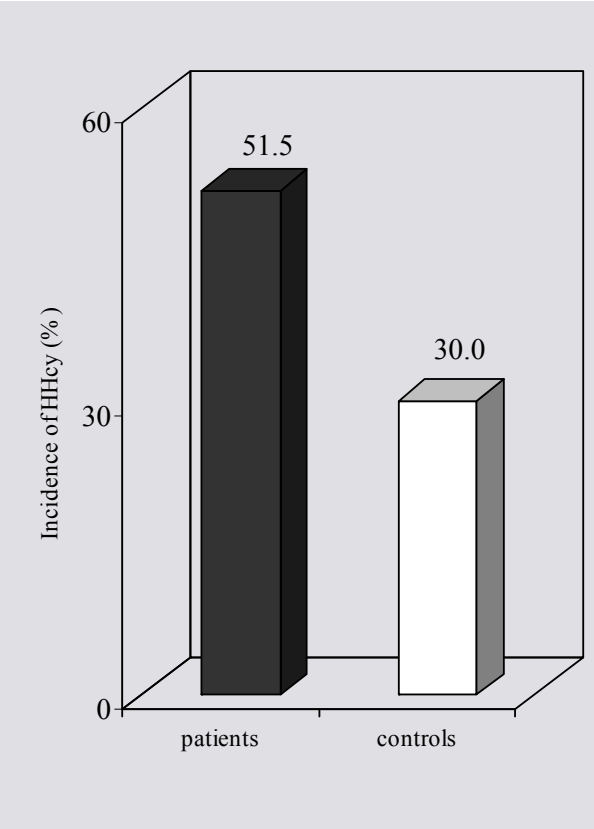

Fig. 1. Comparison of HHcy incidence among patients and controls (statistically significant difference; P=0.021).

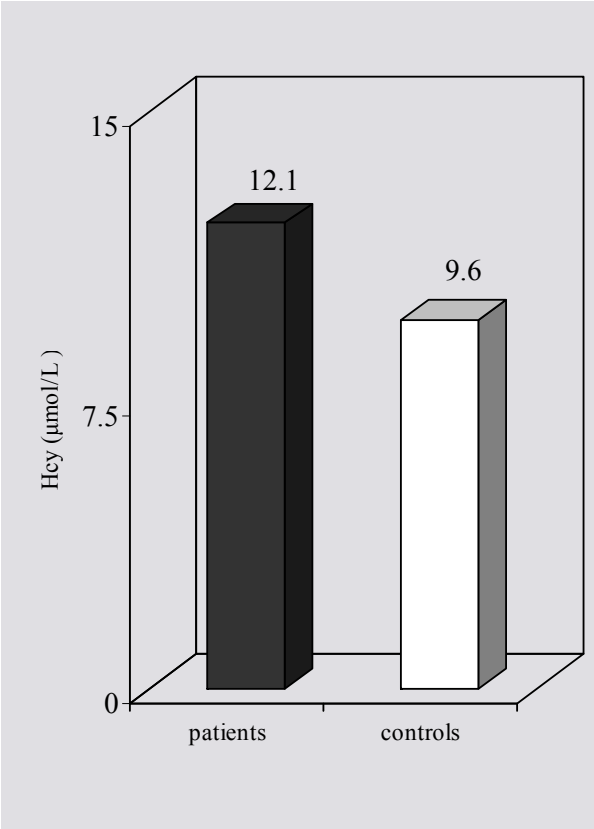

Fig. 2. Comparison of Hcy concentrations among patients and controls (statistically significant difference; P=0.017).

of median Hcy values in patients (12.1 μmol/L) and controls (9.6 μmol/L), represented in Fig. 2, confirmed a significant difference (P=0.017).

Fig. 3 illustrates gender related differences in HHcy incidence, both in patients and controls. HHcy incidence was present in 67.6% of the male PE pts, which was significantly higher (p=0.006) than the incidence in female PE pts (33.3%). Similarly, in the control groups a significant difference (p=0.031) was found between the incidence of HHcy in healthy males (44.0%) and healthy females (16.0%). The analogous relationship between gender and Hcy level is presented in Fig. 4. In the PE group, homocysteinemia was significantly higher (p=0.002) in male patients (14.1 μmol/L) than in female pts (10.1 μmol/L). In the control group, males had significantly higher (p=0.001) homocysteinemia (12.5 μmol/L) than females (9.4 μmol/L).

Comparing the groups of patients with the positive and negative history of previous VTE events, this parameter did not prove significant with regard to HHcy incidence (60.0% vs. 52.5%) as well as Hcy concentrations (15.3 μmol/Lvs. 12.2 μmol/L). Though a family history of VTE comparison showed higher HHcy incidence and Hcy levels in the group with positive family history (54.5% and 13.7μmol/L) than in the group with a negative one (46.1% and 11.6μmol/L), the level of statistical significance was not exceeded. Also, the comparison of HHcy incidence and Hcy levels between groups regarding the most frequent acquired risk factor associated with PTE revealed no significant differences.

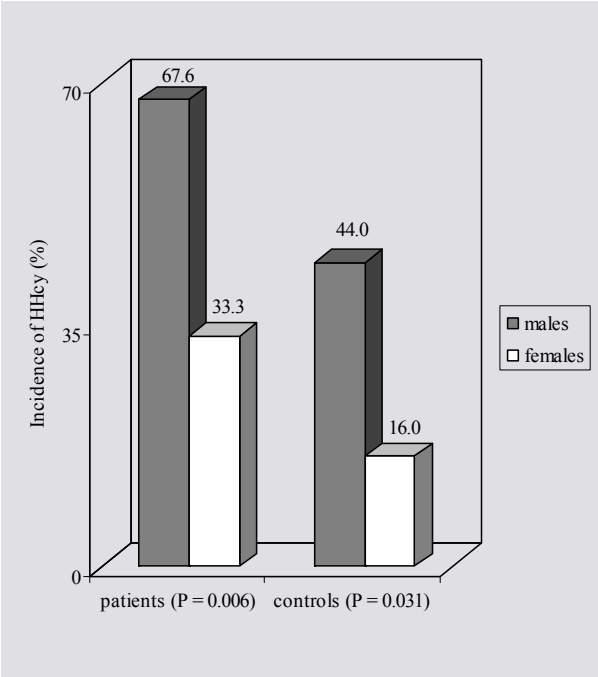

Fig. 3. Gender related differences in HHcy incidence between patients and controls.

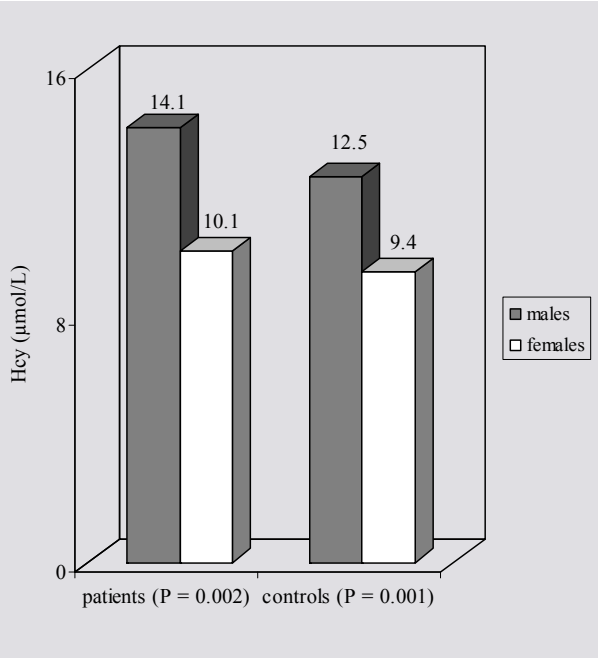

Fig. 4. Gender related differences in Hcy levels between patients and controls.

DISCUSSION

Plasma homocysteine levels (homocysteinemia) is determined by genetic mutations and/or acquired disruption in the homocysteine metabolism pathways. The most frequent genetic causes of hyperhomocysteinemia are defects in the gene encoding for enzymes of homocysteine metabolism (the gene encoding for cystatione beta synthase and defects of gene encoding for methylenetetrahydrofolate reductase – MTHFR – the most common being MTHFR C677T polymorphism). The major acquired factors leading to hyperhomocysteinemia are nutritional deficiencies of folate, vitamin B12, and/or vitamin B6, folate antagonist administration (metotrexate, phenytoin), vitamin B6 antagonists (estrogen, theophylline), disturbed renal function, as well as hypothyroidism (Joffe et al., 2002, Mirkovic et al., 2003, Castro et al., 2006, Fonseca et al., 1999, Colleran et al., 2005, Herrmann et al., 2001). Elevated homocysteine concentrations having been reported in association with several types of carcinoma, including breast, ovarian and pancreatic cancer, as well as acute lymphoblastic leukemia. Our study excluded participants ever having had any type of malignancy or other comorbidity affecting homocysteine metabolism (Welch et al., 1998).

Homocysteine association with atherothrombosis has received much more attention than its association with venous thromboembolism, though homocysteine is known to induce VTE by means of its multiple mechanisms (Welch et al., 1998). Along with a toxic effect on the clotting cascade, homocysteine has thrombogenic and toxic effects on the vascular endothelium, including injury to the vascular endothelium and antagonism of the synthesis and function nitric oxide (den Heijer M et al., 1996, Kucher et al., 2004, Hirsh et al., 2001).

Homocysteine is known to enhance upregulation of IL 8, which leads to enhanced leukocyte recruitment, inhibits prostacyclin synthesis, downregulates thrombomodulin expression, blocks tissue plasminogen activator (t-PA), inhibits plasminogen activation, increases platelet adhesion, induces tissue factor and factor V activation (Melhem et al., 2009, D'Angelo et al., 1997). It also suppresses he-

paran sulfate expression, reduces protein C levels (in homocystinuria, homozygous CBS deficiency) (D'Angelo et al., 1997) and rapidly incorporates into factor Va by activated protein C due to homocysteinylation of the cofactor by modification free cysteine (Undas et al., 2001).

As VTE (deep vein thrombosis and pulmonary embolism) is considered to be a unique entity sharing the same risk factors, the results of our study on PE are comparable with those of other studies of VTE. Similarly to Bienvenu et al., our results demonstrated a significant association between fasting plasma homocysteine and VTE. While Falcon et al. (1994) reported a high prevalence of hyperhomocysteinemia in patients with VTE less than 40 years of age who had VTE, our study confirmed a high prevalence in all age groups. Our results would be even more plausible if we, like Falcon et al. (1994), had used post methionine load increments of homocysteine (standard methionine loading test) along with fasting plasma levels of homocysteine. Our results are also comparable with the data reported by den Heijer (1996) who confirmed mild hyperhomocysteinemia as a risk factor for deep vein thrombosis (den Heijer et al., 1996).

On the other hand, some other studies on homocysteine and venous thrombosis have conflicting results compared with ours. Brattstrom et al. found no significant difference in plasma homocysteine concentrations between 42 patients with VTE and healthy control subjects, although male patients showed a tendency toward higher plasma homocysteine than the male control subjects. Though showing negative association with homocysteine and VTE, the data on post-methionine found a 3-fold elevated risk of hyperhomocysteinemia for thrombosis, which remained non-significant, probably due to small sample size (den Heijer et al., 1998, Brattström et al., 1991). Another small-sample-size study on deep venous thrombosis (DVT) stated no difference in preload and postload homocysteine (Amundsen et al., 1995). An exaggerated homocysteine rise following *methionine loading* may identify individuals at increased risk of vascular disease, as well as individuals with impaired homocysteine metabolism not detected by fasting homocysteine concentration, and is often considered primarily a test of transsulfuration pathway (Apeland et al., 2003). Fasting homocysteine levels are associated more often with remethylation defects, while a postload increase of homocysteine is more associated with sulfuration defects (den Heijer et al., 1998). Though providing strong evidence that hyperhomocysteinemia is an independent risk factor for initial episodes of venous thrombosis in the general population, this finding by den Heijer M et al. (1996) underestimated the disorder prevalence, failing to perform a homocysteine analysis after methionine loading (den Heijer et al., 1996, Bauer, 2005). The relative risk of patients with post-methionine load homocysteine concentration exceeding the 90th percentile was similar to that of patients with fasting hyperhomocysteinemia (den Haijer et al., 1996), which implies that post-methionine load homocysteine should be determined in patients. Determining only fasting homocysteine levels enables detection of only a portion of patients exposed to a higher risk.

Only a modest association of VTE risk has been found by several meta-analyses in individuals with elevated fasting homocysteine levels (Konkle et al., 2008). While prospective studies associate each increase of 5 μmol/L in homocysteine levels with a 27% higher *odds risk* for venous thromboses, retrospective studies report 60% higher *odds risk* for the same homocysteine increment (Den Heijer et al., 2005).

A weak association between total plasma homocysteine and VTE was confirmed by the Physicians' Health Study when participants were confined to persons with so-called idiopathic events without acquired risk factors in the older male population, (Ridker et al., 1997, Bauer, 2005). Likely due to the small number of participants, our study could not prove the association between hyperhomocysteinemia and PE types defined related to the absence or presence of most frequent acquired risk factors.

About 10-20% patients with venous thromboembolism are reported to have hyperhomocysteinemia (Bauer, 2005). Though using recent

Herrmann W. (2006) recommendations stating that homocysteine concentrations above 12 μmol/L are considered as hyperhomocysteinemia, the number of patients with hyperhomocysteinemia in our study was considerably higher (51.3%) compared with other studies, which might be ascribed to a disagreement in defining the upper limits of homocysteine levels applied in different studies.

Moderate hyperhomocysteinemia is registered in 13-18% patients with an initial episode of venous thrombosis before the age of 45, demonstrated by homocysteine measurement obtained with patient fasting and following oral methionine load (Falcon et al., 1994, Bauer et al., 2005). The Leiden Thrombophilia Study reports an elevated homocysteine level exceeding the 95th percentile of the control group was found in 10% of patients and conferred a two-fold increased risk of thrombosis. (den Heijer et al. 1996, Bauer, 2005).

Various studies, like the study of den Heijer (1995) pointed out the role of hyperhomocysteinemia as an established risk factor for recurrent venous thrombosis in patients between 20-70 years of age when compared to the general population (den Heijer et al., 1995). An increased recurrence rate in patients with hyperhomocysteinemia was also reported by Fermo et al, (1995).

A Dutch study showed that 25% of patients with recurrent venous thrombosis had homocysteine levels above the 90th percentile of those in healthy controls, and the presence of this conferred a two-fold increase in risk of recurrence. (den Heijer M et al 1996, Bauer, 2005).

The results of our study in finding statistically significant homocysteine levels and a higher percentage of hyperhomocysteinemia in men than in women, both in the PE and control group, comply with literature data that give 21-25% higher homocysteine concentrations in men than in premenopausal women (Fonseca et al., 1999, Cesari et al., 2005).

Assessing the homocysteinemia contribution to a potential occurrence of VTE requires the determination of specific genetic background in each patient along with the exclusion of the aforementioned acquired risk factors for hyperhomocysteinemia (Cesari et al., 2005, Mirkovic et al., 2003, Diekman et al., 2001, de Lorgeril et al., 1999, Castro et al., 2006, Maron et al., 2004, elevated homocysteine levels further increase the risk of thrombosis in patients with other causes of thrombophylia (Konkle et al., 2008, Ridker et al., 1997, Kucher et al., 2004). The risk of thrombosis is manifoldly increased in patients with idiopathic thromboses who were hyperhomocysteinemic and had the Factor V Leiden mutation (Bauer, 2005, Kucher et al., 2004, den Heijer et al., 1996).

CONCLUSION

Our results confirm that hyperhomocysteinemia represents a risk factor for the occurrence of pulmonary embolism. In order to clarify the hyperhomocysteinemia role in specific VTE categories, such as unprovoked thromboses (without acquired risk factors for VTE), especially those occurring in younger age groups, multiple and recurrent thromboses or combined arterial and venous thromboses, further studies with a larger group of patients are required. More comprehensive conclusions would be contributed by additional investigation that also encompass the methionine postloading homocysteine test which enables identification of high-risk patients that cannot be determined by means of the fasting homocysteine test. More plausible results may be yielded by further research into the interreaction between genetic factors controlling homocysteine metabolism and other genetic markers of thrombophylia, nutritional factors (folate, vitamin B6, vitamin B12 deficiencies), comorbidities affecting homocysteine metabolism and acquired risk factors for VTE.

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