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Plasma glycosylphosphatidylinositol phospholipase D (GPI-PLD) and abdominal aortic aneurysm

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Abstract: Recent reviews state that a circulating biomarker predicting aortic rupture risk would be a powerful tool to stratify patients with small screen-detected abdominal aortic aneurysm (AAA). In a current proteomic pilot-study elevated levels of the enzyme Glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) was shown in patients with small AAA compared with controls without aneurysm. In the present study we investigated the impact of plasma GPI-PLD as a biomarker in patients with AAA in relation to aneurysm size, and rupture. Plasma GPI-PLD was measured in patients with AAA (nonruptured, n=78 and ruptured, n=55) and controls without aneurysm (n=41) matched by age, sex and smoking habit. The plasma GPI-PLD levels were significantly lower in patients with ruptured compared nonruptured AAA which we interpreted as a result of hemodilution due to hemorrhage in patients with ruptured AAA. The plasma GPI-PLD levels were similar in patients with nonruptured AAA compared to the controls without aneurysm. Furthermore, there was no correlation between plasma GPI-PLD and aneurysm size in the group of patients with nonruptured AAA. In conclusion, the present study fails to show a connection between GPI-PLD and AAA. However, the definite role of GPI-PLD as a predictive marker needs to be further clarified in a follow-up cohort study.

Keywords: Glycosylphosphatidylinositol phospholipase D, biomarker, aortic aneurysm

Introduction

Abdominal aortic aneurysm (AAA) is a common condition that is often asymptomatic until sudden death due to rupture [1]. AAA screening programs have been introduced in an attempt to reduce mortality due to ruptured AAA in the general population [2]. Little is known about the biological processes causing aortic aneurysm rupture. Rupture risk stratification relies mostly on the size of the aneurysm [1]. In a review by Choke et al [3] it was stated that AAAs do not conform to the law of Laplace and there is growing evidence that aneurysm rupture involves a complex series of biological changes in the aortic wall. In this way, studies analyzing the association between possible risk factors and AAA rupture are important since we need valuable predictive tools for selection between surveillance and surgery. Furthermore, recent reviews state that a circulating biomarker predicting aortic rupture risk would be a powerful tool to stratify patients with small screen-detected aneurysms [4-6]. Identification of such circulating biomarkers has to date been unsuccessful. In a current proteomic pilot-study we found elevated levels of the enzyme Glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) in patients with small AAA compared with controls without aneurysm [7]. GPI-PLD is an enzyme that cleaves the GPI anchor of the urokinase plasminogen activator receptor (uPAR), thus forming a free soluble receptor (suPAR) [8]. The cell surface bound uPAR localize and intensify the action of urokinase plasminogen activator (uPA) [9]. uPA catalyses the formation of the proteolytic enzyme plasmin, which in addition to fibrinolytic function is involved in matrix degeneration in the processes of tissue remodeling [10,11]. Matrix degeneration is the main hypothesis in AAA pathogenesis. The enzyme GPI-PLD was isolated and characterized twenty years ago by Hoener et al [12]. The function of this enzyme is poorly understood. Inflammation seems to participate in both arteriosclerosis and AAA disease. However, it has been suggested
that GPI-PLD may also participate in regulating inflammation in atherosclerosis [13]. Furthermore, a recent study shows that GPI-PLD improves glucose tolerance; an interesting fact since the connection between arteriosclerosis and AAA has been questioned since arteriosclerosis is associated with diabetes in contrast to AAA [14-16]. Finally, GPI-PLD has recently become available as a commercial assay for scientific investigations.

Hence, based on the above discussion, investigation is justified to assess the role of GPI-PLD as a potential circulating biomarker associated with aneurysm enlargement or predictive of rupture. For this purpose 133 patients with ruptured and nonruptured AAA and a control group of 41 volunteers matched by sex, age and smoking habit were investigated by plasma GPI-PLD, with special regard to the relation of AAA diameter and rupture.

**Material and Methods**

One hundred thirty-three patients with infrarenal AAA treated at Sundsvall County Hospital were studied prospectively. The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the regional ethics committee. Patients and control subjects gave their approval by written informed consent. The case-control cohort has been described in detail elsewhere [17,18].

**Ruptured AAA patients**

Fifty-five patients with ruptured AAA were included. All patients had a retroperitoneal hematoma confirmed by operation.

**Nonruptured AAA patients**

Forty patients with elective surgery for nonruptured AAA, with an aneurysm diameter of at least 5.0 cm (large AAA), were included. Thirty-eight surveillance patients with asymptomatic AAA with aneurysm diameter smaller than 5.0 cm (small AAA) were also included.

**Controls**

The control group was selected in accordance with the guidelines given by Grimes and Schulz [19]. A control group of forty-one volunteers with normal infrarenal aortic diameter were matched to the AAA patients according to age, sex and smoking habit. Smoking was defined as having a smoking habit at the time of inclusion.

**Imaging**

The aortic size was confirmed in all patients and controls by ultrasonography and the largest aortic diameter was recorded. Normal diameter was defined as maximum infrarenal aortic diameter < 3.0 cm.

**Blood sampling and assays**

Peripheral venous blood samples were taken from controls and patients. Samples were centrifuged within 30 minutes at 2000 g for 20 minutes and aliquots of citrated plasma were frozen and stored at −70°C until analysis.

For quantitative determination of GPI-PLD, an enzyme-linked immunosorbent assay (ELISA), (Human GPLD1 ELISA, Uscn Life Science Inc) was used according to manufacturers’ instructions. The detection range of the assay is 0.78-50 μg/L with an intraassay coefficient of variation (CV) <10% and an interassay CV <12%.

**Statistical analysis**

All analyses were carried out using SPSS® statistical software 16.0 for Windows™ (SPSS, Chicago, Illinois, USA). Median (interquartile range) values were calculated for continuous variables and categorical data was expressed as absolute numbers with percentages. Differences in findings between study groups were assessed by Chi-square tests (two-tailed without Yates correction) for categorical variables and by Mann-Whitney tests for continuous variables. Correlation was assessed by Spearman’s method between GPI-PLD and the maximum diameter of the AAA. Results were considered statistically significant when p-values were < 0.05.

**Results**

The matching procedure gave similar age, sex and current smoking habits in the controls and AAA patients (Table 1). Furthermore, there were no significant differences between patients with ruptured AAA and nonruptured AAA according to age, gender and current smoking habit. The levels of Hemoglobin and GPI-PLD are shown in Table 1. There was no significant correlation (r=
GPI-PLD and abdominal aortic aneurysm

Discussion

Several studies have established male, age, smoking, and a family history of abdominal aortic aneurysm as independent risk factors for abdominal aortic aneurysm development [1]. Abdominal aortic aneurysm is traditionally regarded as a consequence of atherosclerosis. However, little is known about the biological processes causing aortic aneurysm rupture. In recent reviews it has been stated that a serum/plasma biomarker predicting aortic rupture risk would be a powerful tool to stratify patients with small screen-detected aneurysms [4-6]. Identification of such circulating biomarkers has so far been unsuccessful. In a current proteomic pilot-study, we found elevated levels of the enzyme GPI-PLD in patients with small AAA compared with controls without aneurysm [7]. Little is known about the role of GPI-PLD in vascular disease. However, GPI-PLD has been suggested to participate in regulating inflammation in atherosclerosis and also improving glucose tolerance [13,14]. In the present study we explored whether plasma GPI-PLD is a biomarker predicting aortic rupture risk and in this way a tool to stratify patients with small screen-detected aneurysms. Since male, age, and smoking are the dominant risk factors for AAA [1] we used a control group matched by age, sex and smoking habit to the AAA patient group in the present study to eliminate possible bias in accordance with the guidelines given by Grimes and Schulz [19].

In the present study we found that patients with ruptured AAA have significantly lower levels of plasma GPI-PLD compared to patients with nonruptured AAA. However, it is well-known that concentration of blood cells and plasma proteins are decreased by massive bleeding due to hemodilution. In this way, the decreased levels of hemoglobin and GPI-PLD in ruptured compared to nonruptured AAA reflect the influence of the hemodilution. The plasma GPI-PLD levels were similar in patients with nonruptured AAA compared to the controls without aneurysm. Moreover, the study shows no significant correlation between GPI-PLD levels and maximum diameter of the nonruptured AAA. The present study has some limitations. First, the function of GPI-PLD is to date poorly understood. Second, the used GPI-PLD assay is new and poorly documented with poorly defined antibodies and gives a high CV in our study. Third, the current study design does not show individual changes in GPI-PLD levels as in the case of a follow-up cohort.

In summary, the present study fails to demonstrate a relationship between GPI-PLD levels and AAA. However, the suggested role of GPI-PLD as a biomarker in AAA disease must be further investigated with an evaluated GPI-PLD assay and in a follow-up cohort study.

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