Comparison of Serum Level of Antiphospholipid Antibodies and Homocysteine in Patients with Pemphigus Vulgaris and Healthy Subjects: A Case-Control Study

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Introduction

Pemphigus vulgaris (PV) is an organ specific autoimmune blistering disease. It is characterized by mucous membrane and subsequent skin involvement with blisters and erosions. PV is caused by autoantibody-mediated disruption of cell adhesion through direct inhibition of desmoglein 3 transinteraction as well as cellular signaling events resulting in reduction of desmoglein 3 -binding.

Antiphospholipid antibodies (aPLs) are a heterogeneous group of autoantibodies against phospholipid-binding proteins. They consist of anticardiolipin antibodies and beta-2 glycoprotein I antibodies that are found in patients with autoimmune diseases such as systemic lupus erythematosis (SLE), autoimmune thyroid diseases and Inflammatory Bowel Disease (IBD). Elevated levels of antiphospholipid antibodies (aPLs) are characteristic of antiphospholipid syndrome, an autoimmune disorder with the clinical manifestation of hypercoagulability, that affects any blood vessel and leads to recurrent spontaneous abortions.

According to one study, antiphospholipid antibodies are frequently detected in autoimmune blistering diseases including pemphigus vulgaris. APLs positive subjects have an increased rate of annual arterial/venous thrombosis risk.

Homocysteine (Hcy) is an amino acid which is produced during the metabolism of methionine. Elevated plasma levels of homocysteine are associated with atherothrombosis. Mild hyperhomocysteinemia has been extensively investigated as a putative risk factor for accelerated atherosclerosis and thromboembolism.

Autoimmune disorders are frequently associated with relevant and early signs of atherothrombotic events. The aim of this study was to evaluate the correlation between pemphigus vulgaris as an...
autoimmune disease and predictive elements of atherothrombosis including aPLs and homocysteine.

**Patients and Methods**

**Subjects**
This study was conducted between March 2007 and March 2009 in Lohman and Shohada-e Tajrish Hospitals, affiliated to Shahid Beheshti Medical University. We enrolled 39 new cases of pemphigus vulgaris and 39 age and sex matched healthy controls in this study. The sample size was determined according to the prevalence of PV and previous reports. The clinical diagnosis of PV was confirmed by 1) direct immunofluorescence of lesions revealing intra-epidermal deposition of IgG between cells throughout the epidermis, 2) histopathology (acantholytic blisters in suprabasal layer) or 3) indirect immunofluorescence microscopy to detect serum autoantibodies. The exclusion criteria were 1) history of arterial or venous thrombosis, 2) history of anticoagulant or immunosuppressive therapy.

This study was performed in accordance with the declaration of Helsinki and approved by the Ethics Committee of Shahid Beheshti Medical University. Informed written consents were obtained for participation in the study.

**Detection of aPLs**
Antiphospholipid antibodies, i.e. IgG and IgM anticardiolipin antibodies (aCL), were detected in the sera of study subjects using the standard aCL enzyme-linked immunosorbent assay (ELISA). The IgG b2GPI was also measured using the specific ELISA method according to the manufacturer’s instruction. Serum anticardiolipin level (IgG, IgM) ranges between 5 and 15 U/ml in healthy individuals and the normal human plasma level of β-2GPI is about 200 μg/mL.

**Detection of lupus anticoagulant**
Lupus anticoagulant was reported negative or positive according to the guidelines which are recommended by the Subcommittee on Lupus Anticoagulant/Phospholipid Dependent Antibodies 14.

**Detection of Homocysteine**
Total plasma homocysteine concentration was measured by high-performance liquid chromatography (HPLC) fluorometric method after the samples were reduced with tri-n-butylphosphine, precipitated with trichloroacetic acid (10%) and derivatized with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate. 7-fluorobenzen-2-oxa-1, 3-diazol-4 sulfonamide was used to convert total plasma homocysteine to a fluorescent compound 15. Fasting plasma homocysteine ranges between 5 and 15 μmol/L in healthy individuals and the values more than 15 μmol/L are defined as hyperhomocysteinemia.

Statistical methods were performed using the Wilcoxon–Mann–Whitney nonparametric test for comparing aPLs frequencies. Pearson’s chi-square test was applied for lupus anticoagulant. Student’s t-test was used to compare means between the two groups. P < 0.05 was considered as a statistically acceptable level for difference. All statistical analyses were performed using the program SPSS-17.

**Results**
Study patients consisted of 33 women and 6 men (mean age: 44.77 ± 13.23). Healthy gender and age-matched controls included 32 women and 7 men (mean age 43.82 ± 12.51). Pearson’s chi-square test for comparing sex between two groups revealed no difference (P= 0.76). T-test showed no significant difference in mean age between cases and controls (P= 0.75).

The level of aPLs in the serum of PV patients and controls showed no difference. The serum levels of Anticardiolipin (IgG, IgM) and b2-glycoproteinI (IgG) were analyzed in patients and controls using Wilcoxon- Mann-Whitney test but no difference was observed (Table 1).

Pearson’s chi-square test was applied for compare serum lupus anticoagulant level between cases and controls but the difference was not significant (Table 1).

Homocysteine was elevated in the serum of 5 patients (12.8%) and 3 controls (7.7%) which showed no significant difference (P=0.71). Also, the serum level of homocysteine was compared between patients with skin and mucous membrane involvement using Kruskal Wallis test but no significant difference was observed (P=0.265) (Table2).

**Discussion**
It is well known that antiphospholipid antibodies (aPLs) are frequently detected in some systemic autoimmune diseases 8. Antiphospholipid antibodies may be sufficient to increase the tendency to atherothrombosis regardless of other predisposing factors 16. APLs are also associated with a significantly increased risk of atherothrombotic disease 17.
In the majority of the subjects in our study irrespective of their group, aCLs and anti-b2GPI were negative and lupus anticoagulants were not detected in the sera of the patients. Our findings differ with those reported by Echigo who found higher aPLs in PV group. Thrombotic events were also detected in patients with aPLs who had autoimmune blistering diseases 8.

Pemphigus vulgaris (PV) is one of the rare autoimmune skin disorders characterized by the presence of blisters and erosions. Extensive inflammation and coagulation are demonstrated in these diseases 18. In one study that evaluated the coagulation activity in autoimmune bullous diseases, the absence of tissue factor (TF) expression in lesional skin by eosinophils and the low plasma levels of D-dimer were demonstrated, indicating that blood coagulation was not activated in PV patients 19. Also, we did not detect any atherothrombotic events in PV patients during the study and none of them had a history of atherothrombosis either.

However, a follow-up of patients with PV, particularly aPL-positive ones, could give additional insight into the atherothrombotic process in this group of patients.

Hyperhomocysteinemia, which is potentially a predisposing factor to vascular diseases, is also recognized as a risk factor for venous occlusive disease 20. Mild hyperhomocysteinemia is independently associated with the development of coronary artery disease (CAD), cerebral and peripheral vascular diseases and deep-vein thrombosis in the general population 21. Recent studies have demonstrated the effects of homocysteine in autoimmunity triggering mechanisms, suggesting a possible role for Hcy in the pathogenesis of autoimmune diseases 12. Atherothrombotic cardiovascular involvement is particularly frequent in patients affected with several autoimmune diseases 13. In fact, the relationship between Hcy and autoimmune diseases is reciprocal; immuno-inflammatory activation in autoimmune diseases may contribute to elevated levels of Hcy while homocysteine acts as a pro-inflammatory and immuno-stimulating molecule at least in rheumatoid arthritis and inflammatory bowel diseases 12.

Systemic corticosteroids are often used for the treatment of PV. They may enhance the hypercoagulative state but none of our subjects in both groups received corticosteroid before collecting blood samples.

Although elevated thrombotic factors are observed in patients with autoimmune diseases, we found no difference in two atherothrombotic elements between pemphigus vulgaris patients and healthy controls. However, further studies are recommended to evaluate this relationship more clearly.

References
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