ABSTRACT

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Title of diploma thesis: Comparison of platelet aggregation results in patients with heterozygous factor V Leiden or prothrombin G20210A who have undergone thrombosis versus those who have not undergone thrombosis.

Background: Heterozygous mutations of coagulation factor V Leiden and prothrombin G20210A are thrombophilic states that increase the risk of venous thromboembolism. However, not all carriers of these mutations will develop thrombosis. The significance of increased platelet aggregability as a risk factor for venous thromboembolism is being discussed.

Study objectives:

Primary objectives: 1. To determine whether individuals with proven heterozygous mutations of factor V Leiden or prothrombin G20210A who have experienced thrombosis have increased platelet aggregability with low concentrations of ADP and epinephrine compared to individuals with these mutations who have not experienced thrombosis. 2. To determine whether increased aggregability, defined as: a) maximum amplitude ≥ 60% b) maximum amplitude corresponding to the highest quartile of values obtained from healthy volunteers, occurs more frequently in individuals with heterozygous mutations of factor V Leiden or prothrombin G20210A who have experienced thrombosis compared to those who have not.

Secondary objectives: 1. To determine the correlations between the results of samples obtained using a closed vacuum system and those obtained using an open system. 2. To determine whether there are differences between the results obtained using a closed vacuum system and those obtained using an open system.

Participants: 55 persons who have experienced thrombosis and 99 persons who have not. Among those who have experienced thrombosis, 46 were carriers of heterozygous mutation of factor V Leiden and 9 were carriers of heterozygous mutation of prothrombin G20210A. Among those who have not experienced thrombosis, 83 were carriers of heterozygous mutation of factor V Leiden and 16 were carriers of heterozygous mutation of prothrombin G20210A.

Methods: Individuals with proven heterozygous mutations of factor V Leiden or prothrombin G20210A who have not experienced thrombosis (group A) were selected from the registry of thrombophilic states. A group of matched controls (group B) was selected from individuals with these mutations who have not experienced thrombosis, ensuring that there were no statistically significant differences in basic demographic data (age and sex) between the two groups. These individuals were invited to participate in the study and were instructed on how to be prepared for platelet aggregation testing. After signing informed consent and checking for adherence to instructions and after exclusion of medications affecting platelet function, blood samples were collected using: 1 tube with EDTA for blood count, 5 tubes with 0,109 mol/l sodium citrate; the first 3 tubes were vacuum-sealed (Vacutainer system) and the last 2 tubes were not vacuum-sealed (open system).

Preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) was performed by centrifugation starting 30 minutes after blood collection, and platelet aggregation testing was performed within 1 hour of centrifugation completion using optical aggregometry with concentrations of epinephrine 10 µM, 1 µM, and 0,5 µM, and concentrations of ADP 2,5 µM, 1 µM, and 0,5 µM. Maximum amplitude values were evaluated.

Results: Contrary to our original hypothesis, higher values were measured in patients who have not experienced thrombosis compared to patients with a history of thrombosis. Statistically significant differences were found when the samples obtained using a vacuum-sealed system (Vacutainer) were examined at concentrations of ADP 1 µM ($p=0.00708$) and ADP 0,5 µM ($p=0.00077$), and when the samples obtained using an open system were examined at a concentration of ADP 0,5 µM ($p=0.00708$). No statistically significant differences were found with epinephrine and other concentrations of ADP.

A statistically significant relation was found between the occurrence of increased aggregability (defined as maximum amplitude within the range of the highest quartile of values obtained

by investigation of healthy volunteers) and the absence of thrombosis in the history when the samples obtained using an open system were examined with ADP at a concentration of 2,5 μM ($p=0.026$). In all other cases, no statistically significant differences of the proportions of persons with increased platelet aggregability were found between persons with a history of thrombosis and persons without a history of thrombosis.

A strong and statistically significant correlations were found between the results obtained using a vacuum-sealed system and those obtained using an open system (Spearman's coefficients in the range of 0,767 to 0,840, $p<0.00001$). However, statistically significant differences were found between the results obtained using a vacuum-sealed and open system.

Conclusion: Our initial hypothesis was not confirmed: that patients with a heterozygous mutation in factor V Leiden or prothrombin G20210A who have experienced thrombosis (Group A) would exhibit a higher frequency of increased platelet aggregability in response to low concentrations of ADP and epinephrine, compared to individuals with these mutations who have not experienced thrombosis (Group B). The results obtained from samples collected using a vacuum system strongly correlate with those obtained from samples collected using a non-vacuum system, but they are not comparable; there are statistically significant differences between them. Therefore, in clinical practice, only one of the collection methods should be used.

Keywords: Venous Thromboembolism (VTE); Factor V Leiden; Prothrombin G20210A Mutation; Platelet Aggregation; Light Transmission Aggregometry (LTA)