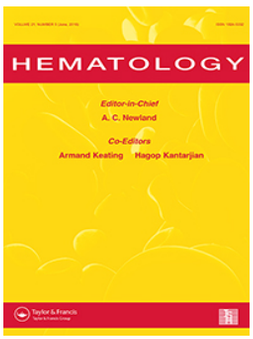

Hematology

ISSN: 1024-5332 (Print) 1607-8454 (Online) Journal homepage: http://www.tandfonline.com/loi/yhem20

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To cite this article: Bulent Karakaya, Anil Tombak, Mehmet Sami Serin & Naci Tiftik (2016) Change in plasma a disintegrin and metalloprotease with thrombospondin type-1 repeats-13 and von Willebrand factor levels in venous thromboembolic patients, Hematology, 21:5, 295-299, DOI: 10.1080/10245332.2015.1125079

To link to this article: http://dx.doi.org/10.1080/10245332.2015.1125079

Accepted author version posted online: 13 Jan 2016.
Published online: 16 Feb 2016.

Change in plasma a disintegrin and metalloprotease with thrombospondin type-1 repeats-13 and von Willebrand factor levels in venous thromboembolic patients

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Objectives: Venous thromboembolism (VTE) is an important cause of morbidity and mortality. A disintegrin and metalloprotease with thrombospondin type-1 repeats-13 (ADAMTS-13) is a metalloprotease that cleaves plasma von Willebrand factor (VWF) multimers. The presence of large VWF multimers in the plasma due to ADAMTS-13 deficiency is the main factor in the pathogenesis of thrombotic thrombocytopenic purpura. The present study aimed to investigate the relation of plasma levels of ADAMTS-13 and VWF antigen with VTE.

Methods: The present study included 30 patients with VTE and age- and gender-matched 30 healthy subjects. Patients with any condition (diabetes, icterus, hyperlipidemia, physical, or surgical trauma, acute coronary syndrome, pregnancy, renal insufficiency, liver disease, malignancy, collagen tissue disease, chronic or acute inflammation, drug use affecting thrombocyte function) that could affect plasma VWF antigen or ADAMTS-13 levels were excluded. Plasma ADAMTS-13 and VWF antigen levels in the VTE and control groups were quantitatively determined by enzyme-linked immunosorbent assay method.

Results: The median ADAMTS-13 level was 280 ng/ml (minimum–maximum, 70–1120 ng/ml) in the VTE group and 665 ng/ml (minimum–maximum, 350–2500 ng/ml) in the control group; the difference between the groups was significant ($P < 0.0001$). The mean VWF antigen level was 1750 ± 616 mU/ml in the patient group, which was significantly higher than that of the control group (950 ± 496 mU/ml) ($P < 0.0001$).

Conclusion: Significantly lower ADAMTS-13 levels and significantly higher VWF antigen levels were concluded to be the result of a pathological process rather than an etiological factor for VTE.

Keywords: ADAMTS-13 protein, Blood coagulation, Thrombotic thrombocytopenic purpura, Venous thromboembolism, Von Willebrand factor

Introduction

Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism, is an important cause of morbidity and mortality.[1] The annual incidence of VTE in the developed countries is 0.75–2.69 per 1000 individuals in the population, whereas the incidence rate has been reported to increase up to 2–7 per 1000 individuals in advanced ages (≥70 years).[2]

The normal clotting process, which is called hemostasis, is activated when a vascular injury occurs. The coagulation system is rapidly activated to stop bleeding, and simultaneously, anticoagulant and fibrinolytic mechanisms become active to prevent growth of clot. The imbalance in hemostasis results in a bleeding or thrombotic events.[3] Thrombosis is a multifactorial disease and usually occurs with the combination of hereditary and acquired risk factors. Acquired risk factors include immobilization, surgery, trauma, female hormonal therapies, pregnancy, puerperium, advanced age, malignancy, myeloproliferative disorders, antiphospholipid syndrome, and inflammatory bowel disease.[4] Etiopathogenesis of thrombosis is still explained by Virchow's triad [changes in blood flow (stasis), vascular wall damage, and hypercoagulability].[4] While arterial thrombus usually arises from alterations in the vessel wall, development of venous thrombus mainly occurs due to stasis and hypercoagulability.[4]

A disintegrin and metalloprotease with thrombospondin type-1 repeats-13 (ADAMTS-13), which has

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DOI 10.1080/10245332.2015.1125079

been recently identified and begun to be mentioned in association with thrombosis, is a novel member of metalloprotease family.[5] As the result of studies concerning pathogenesis of thrombotic thrombocytopenic purpura (TTP), it has been determined that ADAMTS-13 reduces multimer size by affecting specific peptide bonds in the multimers of the von Willebrand factor (VWF) and thereby prevents thrombocyte aggregation.[6,7] Ultra large (UL)-VWF multimers cannot be cleaved in case of deficiency or dysfunction of ADAMTS-13 and thereby cause intravascular thrombus formation by binding to the thrombocytes.[6]

After its relation with TTP was enlightened, whether a decrease in ADAMTS-13 activity is associated with other thrombotic events has become a current issue. With regard to the association between thrombosis and ADAMTS-13, decreased levels of ADAMTS-13 have been demonstrated in patients with connective tissue disease, those with prostate and brain tumor, and those with hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome.[8–10] In the light of above-mentioned information, the present study aimed to investigate the relation of plasma levels of ADAMTS-13 and VWF antigen with VTE.

Materials and methods

The present study included 30 patients, who were diagnosed with VTE from March 2007 through August 2009 at the Departments of Cardiovascular Surgery and Chest Diseases of Mersin University Medical Faculty. Age- and gender-matched 30 healthy subjects formed the control group. All patients and healthy controls in the present study had blood type O. Patients with any condition (diabetes, icterus, hyperlipidemia, physical or surgical trauma, acute coronary syndrome, pregnancy, renal insufficiency, liver disease, malignancy, collagen tissue disease, active infection, acute or chronic inflammation, drug use affecting thrombocyte aggregation) that could affect plasma VWF antigen and ADAMTS-13 levels were excluded. The study was approved by the Medical Faculty Ethics Committee and informed consents of all participants were obtained.

Venous blood samples of the patients were collected between the 1st and 2nd months after the diagnosis of VTE. Venous blood samples were drawn into citrate containing two separate tubes for each participant in the VTE and control group. Plasma was separated and stored at −20°C for 1 month. Thereafter, the plasma levels of VWF and ADAMTS13 were measured from these samples using IMUBIND® vWF ELISA kits (Product Nos. 828 and 813, American Diagnostica Inc., Stamford, Conn) in accordance with the manufacturer's instructions. All samples were thawed at 37°C for 15 minutes on the day of analysis and studied within 4 hours.

Statistical analysis

The MedCalc program version 11.0.0 (Medcalc. Software, Mariakerke, Belgium) was used for statistical analyses. Comparisons between study groups were performed by Mann–Whitney *U* test for ADAMTS-13 levels as these variables were nonnormally distributed and by independent sample *t*-test for VWF levels as these variables were normally distributed. A P value of <0.05 was considered statistically significant.

Results

Each of VTE and control groups consisted of 15 females and 15 males. The mean age was 54.1 ± 12.86 years in the VTE group and 52.96 ± 13.8 years in the control group; there was no significant difference between the groups ($P = 0.424$). The median ADAMTS-13 level was 280 ng/ml (minimum–maximum, 70–1120 ng/ml) in the VTE group and 665 ng/ml (minimum–maximum, 350–2500 ng/ml) in the control group; the difference between the groups was significant ($P < 0.0001$). The ADAMTS-13 levels were below the normal range in 92% of the VTE group and in 40% of the control group and the level of ADAMTS-13 level at the 25th percentile in the control group was higher than that at the 75th percentile in the VTE group (Fig. 1).

The mean VWF antigen level was 1750 ± 616 mU/ml in the VTE group and 950 ± 496 mU/ml in the control group; the difference between the groups was significant ($P < 0.0001$). The VWF levels were above the normal range in 86% of the VTE group and in 35% of the control group. The level of VWF at the 75th percentile in the control group was equal to that at the 25th percentile in the VTE group (Fig. 2).

No significant differences were determined between males and females in terms of ADAMTS-13 and VWF antigen levels both in the VTE group and in the control group (Table 1).

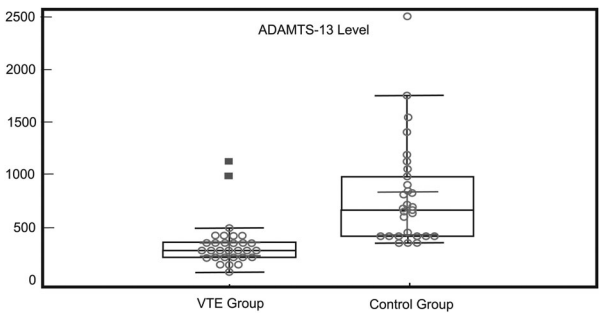

Figure 1 Distribution of a disintegrin and metalloprotease with thrombospondin type-1 repeats-13 (ADAMTS-13) levels in the venous thromboembolism (VTE) and control groups.

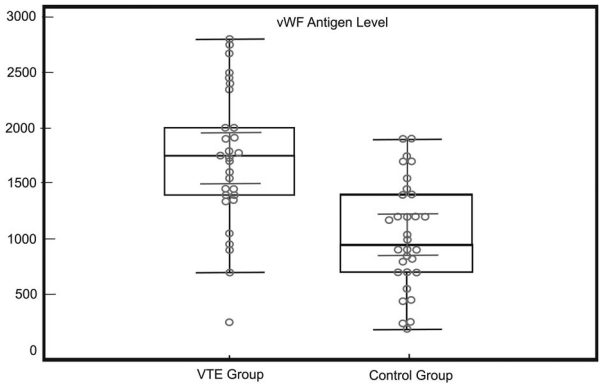

Figure 2 Distribution of von Willebrand factor (vWF) antigen levels in the venous thromboembolism (VTE) and control groups.

Discussion

High VWF levels are associated with increased risk for thrombosis. Plasma VWF level may increase with genetic or transient events. Individuals with types A, B, or AB blood, females, and individuals of black race have higher VWF levels. While advanced age, obesity, diabetes, chronic inflammation, cancer, liver, and renal diseases lead to chronic increase in VWF levels, pregnancy, surgery, exercise, and some agents such as epinephrine, vasopressin, and desmopressin cause transient increase in VWF level.[11]

VWF is a glycoprotein that circulates in the plasma as multimers in different sizes (500–20 000 kDa) and plays a critical role in hemostasis. VWF is synthesized and stored in endothelial cells and megakaryocytes and released in response to various stimulants.[12,13] Released VWF multimers are rapidly cleaved into dimers by ADAMTS-13 and thereby become active. Presence of large VWF multimers in the circulation due to acquired or congenital ADAMTS-13 deficiency and the fact that these alterations initiate pathogenesis of TTP have attracted the interests on ADAMTS-13.[14–16]

The relation of VWF and ADAMTS-13 levels with thrombosis has been investigated in the studies. Franchini and Lippi[17] reviewed the studies on this subject and concluded that VWF was associated with occlusive arterial thrombosis; however, limited information was available on its relation with venous thrombosis. Nossent *et al.*[18] conducted a study in 474 consecutive DVT patients without an underlying malignancy and in 474 controls. They found that VWF antigen levels was higher in DVT patients than in controls and concluded that high plasma VWF levels were associated with thrombosis. Bittar *et al.*[19] carried out a study in 435 patients with DVT and in 580 controls and found VWF antigen levels to be significantly higher in the patient group as compared to the control group. In another study on 97 patients with clinically suspicious disseminated intravascular coagulation, Hyun *et al.*[20] concluded that ADAMTS-13 activity is associated with severity of coagulopathy. Sonneveld *et al.*[21] performed a meta-analysis of studies evaluating the relation of VWF and ADAMTS-13 with arterial thrombosis and reported a relation between high VWF levels and arterial thrombosis in most studies. They also concluded that ADAMTS-13 being an independent causative risk factor remained unclear since there were only case–control studies reporting the relation of low ADAMTS-13 with arterial thrombosis and they emphasized the need for prospective studies.

The relation and balance between ADAMTS-13 and VWF levels have also been investigated in several studies. Reiter *et al.*[22] reported an inverse correlation between VWF and ADAMTS-13 levels by observing an increase in VWF level and a decrease in ADAMTS-13 level after desmopressin infusion as compared to baseline values in healthy volunteers ($n = 10$) and patients with type 1 von Willebrand disease ($n = 3$). Lattuada *et al.*[8] found a lower ADAMTS-13 level and a higher VWF level in pregnant women with HELLP syndrome as compared to healthy pregnant and non-pregnant women. Although Crawley *et al.*[23] reported no correlation between ADAMTS-13 and VWF antigen levels in patients with myocardial infarction and controls, they revealed that the risk of myocardial infarction was negatively correlated with ADAMTS-13 level but positively correlated with VWF level. In a recent

Table 1 Levels of a disintegrin and metalloprotease with thrombospondin type-1 repeats-13 and von Willebrand factor antigen according to gender in the venous thromboembolism and control groups

	n	Female	*n*	Male	*P*
ADAMTS-13 level, ng/ml					
VTE group	15	280 (1400–1120)	15	280 (70–980)	0.561
Control group	15	630 (350–2500)	15	805 (350–1400)	0.575
VWF antigen level, mU/ml					
VTE group	15	1600 (700–2750)	15	1900 (250–2800)	0.577
Control group	15	1170 (190–1900)	15	850 (240–1750)	0.577

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 repeats-13; VTE, venous thromboembolism; VWF, von Willebrand factor.
Data are presented as median (minimum–maximum).

study, Mazetto *et al.*[24] reported that they observed no imbalance between VWF and ADAMTS-13 in VTE patients and that ADAMTS-13 activity was increased despite the increase in VWF levels. They postulated that increased levels of ADAMTS-13 could represent a compensatory mechanism against persistently increased levels of VWF. However, based on the results of their study, Mazetto *et al.*[24] emphasized the particular need for prospective epidemiological studies investigating the pathogenesis of VTE considering the relation among inflammation, coagulation, and thrombosis.

In the present study, conditions likely affecting VWF and ADAMTS-13 levels such as diabetes, icterus, hyperlipidemia, physical or surgical trauma, acute coronary syndrome, pregnancy, renal insufficiency, liver disease, malignancy, connective tissue disease, active infection, chronic or acute inflammation, and drug use affecting thrombocyte function were excluded. Moreover, an age- and gender-matched control group was included and thereby the effects of confounding factors were minimized. In addition, the lack of a significant difference between males and females in terms of ADAMTS-13 levels and VWF antigen levels both in the VTE group and in the control group eliminated the confounding effect of gender.

In the present study, plasma ADAMTS-13 level was found to be significantly lower and VWF level was found to be significantly higher in the VTE group as compared to the control group. In the light of literature information, these findings suggested that increased VWF antigen levels and low ADAMTS-13 levels in VTE patients were the consequence of VTE rather than being a risk factor for VTE. During VTE, thrombin and fibrin are released with the activation of coagulation system. We are in the opinion that these lead to VWF release from the endothelium, megakaryocytes, and thrombocytes. The relationship between decreased ADAMTS-13 level and TTP is known. None of our patients had a history of TTP. This also supported the idea that low ADAMTS-13 level in VTE patients was secondary.

It is important to re-analyze ADAMTS-13 and VWF levels after resolution of thrombus and discontinuation of anticoagulant therapy in VTE patients. Accordingly, it would be understood whether changes in ADAMTS-13 and VWF continue or it is a consequence specific only to thrombosis period. If ADAMTS-13 and VWF levels return to the normal levels after resolution of thrombosis, this strengthens our hypothesis. Moreover, factor VIII levels should also be analyzed in studies performed in similar contexts. As is known, factor VIII is found in the circulation as bound to VWF and this is directly associated with half-life of factor VIII in the plasma. An increase in factor VIII may also appear as the result of an increase in VWF. Further prospective studies are needed to enlighten these issues.

Disclaimer statements

Contributors B.K. conceived and designed the study, obtained ethics approval, collected the data, and wrote the article in whole part. A.T. wrote the article in part and revised the study. M.S.S. analyzed and interpreted the data. N.T. analyzed the data and revised the article.

Funding Authors have no sources of funding and support.

Conflict of interest The authors state that they have no conflict of interest.

Ethics approval The study was approved by the Medical Faculty Ethics Committee.

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