Original Article

Comparison of ADAMTS13 Levels in Patients of Acquired Thrombotic Thrombocytopenic Purpura and Disseminated Intravascular Coagulation

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ABSTRACT

Objective: To compare ADAMTS13 levels in patients of acquired thrombotic thrombocytopenic purpura (TTP) and disseminated intravascular coagulation (DIC).

Methodology: This cross-sectional comparative study was carried out in the Haematology Department of Shaikh Zayed Hospital, Lahore after approval from the ethical review board. A total of 64 diagnosed cases of acquired TTP and DIC of both genders and age ≥15 years were included in the study by consecutive sampling technique. The included patients were allocated into two groups. Group A comprised of 32 diagnosed patients of TTP and group B included 32 diagnosed patients of DIC. After taking written informed consent and using aseptic measures, 5 ml of venous blood was drawn from each study participant. The ADAMTS13 levels were estimated by enzyme-linked immunosorbent assay technique (ELISA). The data was entered and analyzed by using the Statistical Package for the Social Sciences (SPSS) version 23.0.

Results: In group A, there were 5(15.6%) males and 27(84.4%) females. In group B, 14(43.8%) patients were males and 18(56.3%) were females. The levels of ADAMTS13 were low in 17(53.12%) patients in group A and 6(18.75%) patients in group B. This difference was statistically significant ($p<0.004$).

Conclusion: The ADAMTS13 levels were low in 53.12% patients of TTP and 18.75% patients of DIC. There exists a remarkable difference in the levels of ADAMTS13 in patients of acquired TTP and DIC.

Keywords: *Thrombotic thrombocytopenic purpura. Disseminated intravascular coagulation. ADAMTS13.*

INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) is characterized by the generation of platelet-rich thrombi in the microcirculation of many organs of the body.[1] The annual incidence of all TTP syndromes in the general population is about 11 cases per million. It is a fatal condition with a high mortality rate. The diagnosis of TTP relies on clinical features such as thrombocytopenia, hemolytic anemia & routine laboratory findings. i.e. elevated serum LDH. Early recognition and prompt treatment allows the patient not only to recover but survive without long term sequelae.[2]

In disseminated intravascular coagulation (DIC), the mechanisms of coagulation and fibrinolysis become abnormally triggered within the vasculature leading to ongoing coagulation and fibrinolysis.[3] It can present as an acute, life-threatening emergency or may follow a chronic, subclinical course.[4] It is encountered in about 1 percent of patients admitted to tertiary care hospitals. Findings consistent with acute DIC include recent history of sepsis, trauma, malignancy or obstetric complications, bleeding (especially from sites of trauma, drains or catheters), low platelet count, prolonged prothrombin time (PT), thrombin time & activated partial thromboplastin time (aPTT), low levels of plasma fibrinogen, raised plasma D-dimer levels and microangiopathic changes on peripheral blood smear.[5]

Von Willibrand factor (vWF) is an adhesive glycoprotein found on endothelial cells, platelets and plasma.[6] Raised levels of very high molecular weight ultra-large multimers of vWF were found in the plasma of a patient suffering from recurrent TTP. The ultra-large multimers of vWF are found in the endothelium but not in the plasma under physiological conditions. When there is abnormal activation of endothelial cells, this ultra-large vWF promotes aggregation of intravascular platelets and subsequent thrombosis.[7]

A Disintegrin And Metalloprotease with a ThromboSpondin type 1 motif, member 13 (ADAMTS13) is a zinc metalloprotease composed of 1427 amino acid residues. The stellate cells of the liver are the main source of production of plasma ADAMTS13.[8,9] It is also generated by other cells such as tubular cells of the kidney, vascular endothelium and platelets, though in low levels.[10,11] It cleaves ultra-large von Willebrand factor (vWF) multimers on the endothelial surface. The usual concentration of ADAMTS13 found in plasma is approximately 1 μg/ml and its half-life is 1-2 days. Its concentration remains stable at room temperature for many hours.[12]

Thrombotic thrombocytopenic purpura is thrombotic microangiopathy which is caused by severely

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decreased activity of ADAMTS13.[13] The etiology of acquired TTP is autoimmune inhibition of ADAMTS13 protease thereby causing deficiency of ADAMTS13.[14,15] Although the diagnosis of TTP is primarily clinical, however, nowadays laboratory assays are used for the evaluation of ADAMTS13 in the plasma.[16,17]

A severe secondary deficiency of ADAMTS13 is also seen in the patients of DIC. Kim et al. observed in their study that one of the factors related to poor outcome in sepsis-induced by DIC is the deficiency of ADAMTS13.[18]

This study aimed to compare the levels of ADAMTS13 in patients of acquired TTP and DIC. There is a potential utility of measuring ADAMTS13 level in the diagnosis, management & prognosis of TTP and DIC. The patients with severe ADAMTS13 deficiency have a good clinical response to therapeutic plasma exchange.

METHODOLOGY

It was a cross-sectional comparative study conducted in the Haematology Department of Shaikh Zayed Hospital, Lahore over a period of one year after the approval of study protocol by the ethical review board. A total of 64 diagnosed cases of acquired TTP and DIC of both genders and age ≥15 years were included in the study by consecutive sampling technique. Patients were divided into two groups. Group A comprised of 32 diagnosed patients of TTP and group B included 32 diagnosed patients of DIC. The objectives of the study were explained to the participants and written informed consent was taken. The study proforma gathered their complete information regarding age, gender and type of disease. Patients with acquired/secondary TTP who have undergone plasmapheresis, DIC patients who have received plasma and patients with hepatitis B and C associated liver disease were excluded. Using aseptic measures, 5 ml of venous blood was drawn from each study participant and put in EDTA vial. The sample was mixed and then centrifuged at 2000-3000 rpm for approximately 20 minutes. The obtained plasma was used for the estimation of ADAMTS13 levels by enzyme-linked immunosorbent assay (ELISA) kit by Shanghai Korain Biotech Company. Reference range of ADAMTS13 level was 0.05 ng/ml-15.0 ng/ml.

STATISTICAL ANALYSIS

The data was entered and analyzed by using the Statistical Package for the Social Sciences (SPSS) version 23.0. The data for age and ADAMTS13 levels were expressed by using mean and standard deviation (SD). The ADAMTS13 levels between the two groups were compared by using the independent t-test. A p-value of ≤0.05 was considered significant.

RESULTS

In group A, there were 5(15.6%) males and 27(84.4%) females. In group B, 14(43.8%) patients were males and 18(56.2%) were females. The mean age of patients in group A was 43.1±13.9 years and in group B was 49.9±14.1 years.

In group A, there were 15(46.9%) patients of age 20-40 years while 11(34.4%) and 6(18.7%) patients were of age 41-60 years and >60 years, respectively. In group B, there were 11(34.4%) patients of age 20-40 years, while 11(34.4%) patients were of age 4-60 years and 10(31.2%) patients were >60 years old. The levels of ADAMTS13 were low in 17(53.12%) patients in group A and 6(18.75%) patients in group B. This difference was statistically significant (p<0.004) (Figure 1).

The mean ADAMTS13 levels in patients of group A and B were 0.83±0.8 ng/ml and 1.2±0.5 ng/ml, respectively. A statistically significant difference (p-value=0.016) was found when means levels of ADAMTS13 in both groups were compared. When stratified the ADAMTS13 levels in both groups with respect to age, it was reported that there was a significant difference of ADAMTS13 levels in group A and group B among patients with age >60 years (p<0.008) (Table 1).

The ADAMTS13 levels in both groups were compared with respect to gender, it was reported that there was a significant difference of ADAMTS13 levels in group A and group B among females (p<0.031) (Table 1).

DISCUSSION

Diagnosing and distinguishing the thrombotic microangiopathy from other closely related diseases and their variants become a challenge due to significant variability among certain clinical and biological criteria as well as the methods of von Willebrand disease assay analysis particularly in the early stage of the disease. The understanding of the pathogenicity of TTP has been increased due to recent advances in the description of ADAMTS13. Severe ADAMTS13

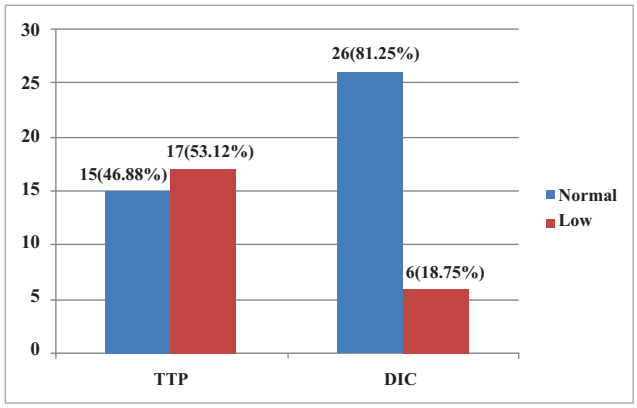

Figure 1: ADAMTS13 Levels in Patients of TTP and DIC

Table 1: Comparison of ADAMTS13 Levels in Both Groups with Respect to Demographic Variables

Demographic Variables		Groups	Frequency	Mean±SD	p-value
Age	20-40 years	TTP	15	0.77±0.86	0.106
		DIC	11	1.24±0.40	
	41-60 years	TTP	11	1.01±1.01	0.941
		DIC	11	1.04±0.62	
	>60 years	TTP	6	0.64±0.66	0.008*
		DIC	10	1.60±0.57	
Gender	Male	TTP	5	0.98±0.94	0.491
		DIC	14	1.32±0.67	
	Female	TTP	27	0.80±0.87	0.031*
		DIC	18	1.26±0.50	

***p-value significant ≤0.05**

deficiency is now accepted as an abnormality specified for TTP.[19-21] The level of ADAMTS13 was found to be deficient, surprisingly, not in all thrombotic microangiopathy but exclusively in TTP.[20,21]

In our study, most of the patients with TTP were females. Five (15.6%) patients were males and 27(84.4%) patients were females. Similarly, the female-to-male ratio was more than 4:1 in a study by Zheng et al.[22] Vesely et al. reported that 69% of the patients were females.[23] In another study by Veyradier et al., out of 111 TTP patients, 64(57.7%) were females and 47(42.3%) were males.[24]

In our study, the mean age of the TTP patients was 43.1±13.9 years. Zheng et al. reported that the median age of patients was 47 years.[22] The mean age of the patients was 40 years in a study by Veyradier et al.[24]

We found a deficiency of von Willebrand factor-cleaving protease in plasma samples of 17(53.12%) patients of TTP. This is in concordance with other studies. Matsumoto et al. and Peyvandi et al. found that 56(52%) of 108 patients and 48(48%) of 100 patients of TTP had low ADAMTS13 levels, respectively.[14,25] Veyradier et al. conducted a prospective study on 66 patients of TTP and found that 47(71%) patients were deficient in ADAMTS13 levels.[24] Zheng et al. and Kremer-Hovinga et al. also reported that 60% and 80% of patients had low ADAMTS13 levels, respectively.[22,26] In contrast, Vesely et al. found low levels of ADAMTS13 in 33% TTP patients.[23]

In our study, out of 32 DIC patients, 14(43.8%) were males & 18(56.3%) were females and the mean age of the patients was 49.9±14.1 years. This is in contrast to the study by Ono et al. According to them, the majority of the DIC patients 65(59.6%) were males and the mean age of DIC patients was 56.9±21.3 years.[27]

Our study showed that only 6(18.75%) patients with DIC had low ADAMTS13 levels suggesting that the deficiency did not result from consumption coagulopathy. This is in concordance with the study conducted by Ono et al. in which decreased levels of ADAMTS13 were found only in few patients with sepsis-induced DIC. They included 109 patients with sepsis-induced DIC and deficiency in ADAMTS13 levels was found in 17(15.6%) patients. Clinical and laboratory data showed that the DIC patients with low ADAMTS13 levels had decreased serum albumin levels, suggesting that decreased ADAMTS13 activity in these patients was partially caused by the reduced synthetic activity of liver reflected by reduced synthesis of albumin in the liver.[27] Further studies should be done to detect the level of ADAMTS13 autoantibodies in patients of TTP & DIC to solidify the diagnosis.

CONCLUSION

The ADAMTS13 levels were low in 53.12% patients of TTP and 18.75% patients of DIC. There exists a significant difference in the ADAMTS13 levels in patients of acquired TTP and DIC.

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