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Frequency of thrombotic thrombocytopenic purpura diagnosed on the basis of ADMTS13 assay in patients with thrombotic microangiopathy

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Abstract

Thrombotic thrombocytopenic purpura (TTP) is a rare disease with an incidence of less than 10 per million per year in the West. In our hospital, clinical diagnosis of TTP is based on detection of thrombotic microangiopathy (TMA) without any evidence of disseminated intravascular coagulation, history of preceding diarrhoeal illness and acute renal failure. Assay of the enzyme ADAMTS13 (a disintegrin and metalloprotease with thrombo-spondin- type 1 motif, member-13), has been described as a sensitive method to diagnose TTP. We carried out a study to see the frequency of TTP in patients with TMA employing ELISA based ADAMTS13 assay. The study was conducted at haematology department, Army Medical College, in collaboration with Military Hospital, Rawalpindi from November 2014 to April 2016. ADAMTS13 assay was carried on serum samples of all the patients by quantitative sandwich enzyme immunoassay. Frequency and percentage of patients showing normal, reduced and severely reduced levels (levels < 10% of the mean of the normal reference range) of ADAMTS13 were calculated. Only one (3.3%) of 30 patients included in the study had severe deficiency of ADAMTS13, falling within the diagnostic range of TTP. Five (16.6%) patients had ADAMTS13 within normal range while 24 (80%) patients had moderately reduced levels of ADAMTS13. TTP, if diagnosed on the basis of ADAMTS13 assay, is a rarer cause of TMA as compared to clinically diagnosed TTP.

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Introduction

Thrombotic microangiopathy (TMA) is characterized by microangiopathic hemolytic anemia (MAHA), thrombocytopenia and formation of microthrombi which may lead to ischemic tissue injury. Laboratory investigations typically show haemolytic anaemia evidenced by the presence of fragmented red cells in the peripheral blood smear and thrombocytopenia (Arnold *et al.*, 2017). TMAs are fortunately not very common, but are life-threatening and require urgent management. Thrombotic thrombocytopenic purpura (TTP) and haemolytic uraemic syndrome (HUS) constitute the prime examples of TMA. Clinically, TTP is classically described as comprising of fever, MAHA, thrombocytopenia, fluctuating neurological symptoms and renal damage. Renal and neurological damage is due to ischemia caused by microvascular platelet rich thrombi. Full pentad of signs and symptoms is however present only in a minority (<10%) of patients (Joly *et al.*, 2017).

TTP is caused by deficiency of ADAMTS13 (a disintegrin and metalloprotease with thrombo-spondin- type 1 motif, member-13), an enzyme that normally cleaves von Willebrand factor (vWF) and hence inhibits vWF-dependent excessive platelet aggregation. Severe deficiency of ADAMTS13 in TTP is either as a result of a congenitally absent ADAMTS13 due to recessively inherited mutations present in the ADAMTS13 gene (Upshaw-Schulman syndrome), or more commonly as an acquired form of TTP, because of an autoantibody against ADAMTS13.

TTP is a rare disease with incidence less than 10 per million per year (Kremer *et al.*, 2017; Mariotte *et al.*, 2016). However its incidence has not been estimated in Pakistan. In our hospital, clinical diagnosis of TTP is based on detection of TMA without any evidence of disseminated intravascular coagulation (DIC), history of preceding diarrhoeal illness or acute renal failure (ARF). Assay of ADAMTS13 has been described as a sensitive method to diagnose TTP in various studies and if it is found to be less than 10% of the mean of the reference range values, confirms the diagnosis of TTP (Barrows and Teruya, 2014; Kremer *et al.*, 2017; Masias and Cataland, 2018).

The aim of the study was to determine frequency of TTP diagnosed by means of ELISA based ADAMTS13 assay in patients who were provisionally diagnosed as suffering from TTP on the basis of clinical and laboratory evidence TMA.

Material and methods

It was a descriptive study conducted in haematology department of Army Medical College, Rawalpindi in collaboration with Military Hospital, Rawalpindi after obtaining permission from institutional ethical review committee. Duration of study was one and a half year, from November 2014 to April 2016. A total of 30 samples, collected by non-probability purposive sampling, were included in this study.

Inclusion and exclusion criteria

Patients of all age groups, both male and female diagnosed provisionally as suffering from TTP on clinical and laboratory features were included. The features forming inclusion criteria were (a) anaemia, (b) thrombocytopenia and (c) more than 1% schistocytes in the peripheral blood. Patients with evidence of DIC or with history of preceding diarrhoeal illness were excluded. MAHA was diagnosed on the basis of decreased haemoglobin below 13.5g/dl and 12g/dl in males and females respectively, presence of schistocytes >1% in the Leishman stained peripheral blood film viewed at 100x magnification, as per recommendations of ICSH schistocytes working group (Zini *et al.*, 2012). After obtaining history, clinical examination was carried out and findings were recorded. History was obtained to rule out causes of TMA such as chemotherapy, recent cardiac surgery, stem cell transplantation and intake of medicines such as quinine, quinidine, ticlopidine, clopidogrel, ciclosporin, interferon-alfa, statins, and chemotherapy.

Laboratory investigations

Blood counts were carried out by means of Sysmex KX-21 haematology analyzer on ethylene-diamine-tetra-acetic acid anticoagulated blood sample.

Leishman stained peripheral blood smears and New-methylene blue stained reticulocytes smears were examined by trained haematologist. Coomb's test was done to rule out autoimmune haemolysis. Prothrombin time activated partial thrombin time serum fibrinogen and D-dimer assay were done to exclude disseminated intravascular coagulation (DIC). Investigations were done, where ever required, to diagnose other conditions associated with TMA. These includes pregnancy test, anti-nuclear antibody, serum lactate dehydrogenase (LDH), serology for hepatitis B and C and HIV by ELISA. Renal functions were assessed by urine examination, serum urea, creatinine, Na+, K+ assays. Liver function tests included direct and indirect bilirubin, alanine aminotransferase and alkaline phosphatase assays.

ADAMTS13 assay

ADAMTS13 assay on serum samples was done by quantitative sandwich enzyme immunoassay technique. Quantikine ELISA kit (R&D Systems Inc, USA) was used and assays of serum samples from patients and controls were done according the manufacturer's instructions (R&D Systems Inc, 2019).

Statistical analysis

Twelve samples, from healthy subjects (seven male and 5 female) were run to establish the reference range. It was calculated as 2x standard deviation (+2SD) on either side of the mean value. Mean value was 1033.6 ng/ml. Range within +2SD was 816.2 to 1251 ng/ml. Patients were considered to have severe deficiency of ADAMTS13 if they had ADAMTS13 less than 10% of the mean of the normal range.

This cut- off value was found to be 103.3 ng/ml and values <103.3 ng/ml were considered diagnostic of TTP. Patients having values of ADAMTS13 level less than the reference range but >103.3 ng/ml were considered to have moderate deficiency of ADAMTS13. Frequency and percentage of patients with normal, moderately reduced and severely reduced levels of ADAMTS13 were calculated.

Results

A total of 30 provisionally diagnosed TTP patients on the basis of clinical features and laboratory features of TMA were included in the study. Their ages ranged from 15-79 years with a median age of 38.5 and the mean age 42.9 (+16.61) years. There were 13 (43.3%) male and 17(53.3%) female patients. Only one out of 30 (3.3%) patients had severe ADAMTS13 deficiency, within the diagnostic range i.e. <103.3 ng/ml. ADAMTS13 assay showed a value of 92 ng/ml. In 24 (80%) of 30 patients, ADAMTS13 levels were below the normal reference range but above the diagnostic range i.e. <816.2 ng/ml and >103.3 ng/ml.

Table 1. Summary of mean blood counts of TMA patients with normal, moderately reduced and severely reduced levels of ADAMTS 13 (n=30).

Groups of TMA patients based on ADAMTS 13 levels	Mean Hb (g/dl)	Mean WBC Count (x109/l)	Mean platelet count (x109/l)	Mean ADAMTS 13 level
1. Within normal range, 816.2-1251 ng/ml. (n=5)	12.3+1.1	10.42+1.4	118+27.5	982.4+97.5
2. Moderately reduced, <816.2 ng/ml >103.3 ng/ml. (n=24)	10.5+1.4	11. 9+2.7	84.2+10.7	692.4+144.3
3. Severely reduced, < 103.3 ng/ml.(n=1)	7.2+0	3+0	57+0	92+0

Key: ADAMTS 13= a disintegrin and metalloprotease with thrombospondin-1 repeats, Hb=Haemoglobin, TMA= thrombotic microangiopathy, WBC=White blood cells.

In 5 (16.6%) of 30, ADAMTS13 levels were normal. Various laboratory findings of patients with normal, moderately reduced and severely reduced levels of ADAMTS13 levels are summarized in table-1. List of conditions found associated with TMA in our patients are given in Table-2.

Discussion

According to British journal of haematology guidelines, diagnosis of TTP must be considered even

in the presence of thrombocytopenia and MAHA alone (Scully *et al.*, 2012). This formed the core of our criteria for patient selection. It is important to distinguish TTP from other causes of TMA because patients with severe ADAMTS13 deficiency are likely to respond to plasma exchange therapy. On the other hand other causes of TMA with the exception of HELLP syndrome do not respond to plasma exchange. We detected severe deficiency of ADAMTS13 in only 1 (3.3%) out of 30 TMA patients. The patient was a 17-year-old young girl with history of fever, anaemia, confusion and deranged renal function tests. ADAMTS13 assay showed a value of 92 ng/ml. Detection of antibodies against ADAMTS13 and genetic testing were not performed on the patient due to non-availability of these facilities. Severe ADAMTS13 deficiency is generally considered to be specific for congenital and acquired autoimmune varieties of TTP (Joly *et al.*, 2017; Scully *et al.*, 2012). Deficiency is most severe in congenital TTP in which values of ADAMTS13 are found to be <5% of the normal (Scully *et al.*, 2012; Bianchi *et al.*, 2002). In acquired TTP, severely reduced levels (<10%) have been reported in various proportions of patients ranging from 13-100% in different studies (Mannucci *et al.*, 2016).

The variation in the sensitivity of tests in various studies appears to be due to difference in approaches to select the patients and use of different methods of ADAMTS13 assay such as fluorescent resonance energy transfer (FRET) assay, chromogenic endpoint detection method and ELISA.

Reduced levels of ADAMTS13 may also be seen in many other acute illnesses, such as sepsis, malignant hypertension, and preeclampsia (Masias and Cataland, 2018). Two of our patients with TMA and moderately reduced ADMATS13 levels had pregnancy and a final diagnosis of HELLP syndrome was made. Since ADAMTS13 is predominantly synthesized in the hepatic stellate cells in addition to the endothelial cells, involvement of liver may result in moderate reduction of the enzyme (Zheng, 2015).

Table 2. Diseases associated with reduced ADAMTS13 levels in our patients (n=25).

	Disease	Number of patients (%)	Mean ADAMTS13 value (ng/ml)
1	TTP	1 (4%)	92+0
2	Adenocarcinoma stomach	1 (4%)	800+0
3	HELLP syndrome	2 (8%)	250+151.4
4	Presumptive diagnosis of sepsis	21 (84%)	729.4+37.03

Key: TTP= Thrombotic thrombocytopenic purpura, HELLP= Haemolytic anaemia, elevated liver enzymes, low platelets.

Although we could not get any positive results from the blood culture of any of our patients, a presumptive diagnosis of sepsis was made in majority of our patients. Sepsis was diagnosed on the basis of variable presence of features such as fever, cough, flushed skin, tachycardia, tachypnoea and neutrophil leukocytosis.

Infections still constitute a big chunk of our health care burden and appear to be a very common cause of TMA in our setting. Moderate reduction of ADAMTS13 is seen in sepsis, which could be due to degradation of ADAMTS13 by enzymes such as calpains, elastases, thrombin, or plasmin (Mannucci *et al.*, 2016; Peigne *et al.*, 2013). Moreover inflammatory cytokines such as IL-6 may affect VWF proteolysis by ADAMTS13 and the high levels of vWF may result reduced activity of ADAMTS13 activity due to consumption. TMA resulting from infections may respond to antimicrobial or antiviral therapy but generally not to plasma exchange. One of our patients with TMA in our study had adenocarcinoma stomach.

Malignancies are also known to cause microangiopathy and secondary TTP (Pirrotta *et al.*, 2005).

TTP is a rare disease with estimated incidence of 3 per million in adults (Arnold *et al.* 2017). Our study, although consisted of a very small number of patients, suggests that merely on the basis of TMA, TTP is likely to be over-diagnosed in our set up and many of the patients may be unnecessarily subjected to plasma exchange.

High mortality associated with TTP seems to put the physicians under obligation to initiate plasmapharesis at the slightest suspicion. Although reduction in the levels of ADAMTS13 below 10% confirms the diagnosis of TTP, the debate on the role of ADAMTS13 assay kits in the routine diagnosis TTP is still going on.

In the absence of some accurate diagnostic criteria and availability of robust laboratory investigative tools, exclusion of TTP in many patients will remain elusive. This will keep on resulting in unnecessary plasma exchange procedures in many patients because of the tendency of physicians to err on the side of caution.

Conclusion

As compared to clinical diagnosis, TTP is a rarer cause of TMA when diagnosed on the basis of ADAMTS13 assay. Most of the patients with TMA in our set up are secondary to sepsis.

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