Mild to moderate reduction of a von Willebrand factor cleaving protease (ADAMTS-13) in pregnant women with HELLP microangiopathic syndrome

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Background and Objectives. Among the array of microangiopathies that may occur during pregnancy, HELLP syndrome and thrombotic thrombocytopenic purpura (TTP) produce similar laboratory findings (hemolytic anemia and thrombocytopenia), although neurological symptoms prevail in TTP and abnormal liver function in HELLP syndrome. It is clinically important to distinguish the two entities given that their managements differ (prompt induction of delivery in HELLP syndrome, plasma exchange in TTP). The purpose of this study was to evaluate whether or not ADAMTS-13, the metalloprotease that disposes ultralarge, highly thrombogenic multimers of von Willebrand factor (VWF) and is severely deficient or undetectable in many patients with TTP, is deficient in HELLP syndrome.

Design and Methods. We measured ADAMTS-13 and VWF (antigen, ristocetin cofactor activity, collagen binding, multimeric structure) in 17 pregnant women during HELLP syndrome and after 6 months during clinical remission. Controls were 25 healthy pregnant women and 50 healthy non-pregnant women.

Results. All the women with HELLP syndrome had lower plasma levels of ADAMTS-13 activity (median and range: 31%, 12-43) than did the healthy pregnant (71%, 48-105) and non-pregnant women (101%, 45-152); the reduced levels returned to normal on remission (115%, 90-170). Reduced levels were not due to the presence of inactivating autoantibodies and in no case was the protease undetectable in plasma. Ultralarge VWF multimers were not present in plasma, the levels of VWF were higher than in normal pregnancy.

Interpretation and Conclusions. Because none of the pregnant women diagnosed with HELLP syndrome had undetectable ADAMTS-13 levels in pregnancy-associated thrombotic microangiopathies, the finding of severe ADAMTS-13 deficiency would argue against a diagnosis of HELLP syndrome and for a diagnosis of TTP.

Key words: HELLP syndrome, pregnancy, microangiopathy, ADAMTS-13, von Willebrand factor.

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The HELLP syndrome (*H*emolytic anemia, *E*levated *L*iver enzymes, and *L*ow *P*latelets) is a manifestation of pre-eclampsia that presents as a moderately severe form of thrombotic microangiopathy occurring in approximately 0.6% of all pregnancies, typically during the third trimester or at term.[1,2] Microangiopathic hemolytic anemia (MAHA) is due to the fragmentation of red cells as they pass through arterioles obstructed by platelet-fibrin deposits, with formation of schistocytes. Elevated liver enzymes are secondary to the impairment of hepatic blood flow caused by platelet-fibrin thrombi in the sinusoids, which lead to hepatocellular injury and necrosis. Thrombocytopenia is due to increased consumption of platelets following their adhesion to damaged or activated endothelium and intravascular aggregation. The diagnosis of HELLP syndrome is difficult and is often confused with that of other microangiopathic thrombocytopenic conditions which may occur during pregnancy, such as disseminated intravascular coagulation and thrombotic thrombocytopenic purpura (TTP). HELLP syndrome has many features in common with TTP, such as thrombocytopenia, MAHA and thrombotic occlusions in terminal arterioles that result in ischemic organ damage, the liver being mainly affected in HELLP syndrome and the central nervous system in TTP. The differential diagnosis between TTP and HELLP syndrome is important, given that management strategies differ: on the basis of a randomized clinical trial plasma exchange is the mainstay of treatment in TTP[3,4] whereas this procedure is less often indicated in HELLP syndrome[5,6] and prompt induction of delivery is the treatment of choice.[1]

In the last few years, considerable progress has been made in understanding the pathophysiology and diagnosis of TTP. Furlan *et al.*[7] and Tsai and Lian[8] have independently demonstrated that most cases of TTP are associated with severe plasma deficiency of the metal cation-dependent protease ADAMTS-13[9-12] which physiologically cleaves von Willebrand factor (VWF), a large multimeric adhesive glycoprotein, at the peptide bond between amino acid residues Tyr1605 and Met1606.[13] When the protease is deficient due to gene defects or inactivating autoantibodies[7,8] endothelium-derived ultralarge VWF multimers, which are highly thrombogenic, circulate uncleaved in plasma, aggregate platelets under the conditions of high fluid shear that occur in partially occluded arterioles and cause thrombus formation in the terminal circulation.[14] Surmising that the measurement of plasma ADAMTS-13 might be

of value in the differential diagnosis between TTP and HELLP syndrome, we measured ADAMTS-13 in 17 pregnant women with HELLP syndrome studied sequentially in the acute phase of the syndrome and six months thereafter during clinical remission. Plasma levels of VWF and the multimeric structure of this protein were also investigated.

Design and Methods

Patients

Plasma samples were obtained from 17 women in the third trimester of pregnancy who were consecutively admitted to hospital because they developed moderate to severe HELLP syndrome, diagnosed according to Sibai *et al.*[15] on the basis of at least 4 of the following laboratory abnormalities: serum lactate deydrogenase (LDH) >600 U/L, elevated liver enzymes [aspartate aminotransferase (AST) >70 U/L, and alanine aminotransferase (ALT) >50 U/L], microangiopathic hemolytic anemia (Hb <10g/dL) and low platelet count ($<100\times10^3$/mL). None of the women had proteinuria, infection or was taking any drug when the HELLP syndrome developed. Thirteen of the 17 patients had high blood pressure that required antihypertensive medication. The severity of thrombocytopenia further categorized the HELLP syndrome, according to Martin *et al.*:[16] class 1 (severe), platelet nadir $< 50\times10^3$/mL; class 2 (moderate), platelet nadir 50,000-100,000 and class 3 (mild), platelet nadir >100,000. Blood samples were obtained on admission, at the time of the acute phase, and again after 6 months during remission. As controls 25 age-matched women during the third trimester of normal pregnancies and 50 healthy non-pregnant women not taking oral contraceptives were similarly investigated. Pooled plasma used as reference (100%) for all assays was prepared from 50 women who were healthy, not pregnant and not taking oral contraceptives. These women were different from those used as controls. All patients and controls gave informed consent to blood sampling and assays and the study was approved by the Institutional Review Board.

Blood samples

Nine volumes of venous blood for the measurement of the functional activities of VWF and ADAMTS-13 were collected into one volume of 0.129 M sodium citrate and on EDTA (1.5 mg/mL) for VWF multimeric analysis. Platelet-poor plasma was obtained by double centrifugation at 3,000 × g for 20 min, snap frozen and stored at –80°C until tested.

Laboratory measurements

Platelets were counted electronically. Serum LDH, ALT and AST were determined with standard laboratory methods. The prothrombin time (PT), activated partial thromboplastin time (APTT), antithrombin, fibrinogen and D-dimer levels were determined by standard methods using the automated ACL Futura Plus coagulometer (Instrumentation Laboratory, Milan, Italy).

ADAMTS-13 activity

ADAMTS-13 activity was measured with the collagen binding assay described by Gerritsen *et al.*[17] Within–assay (n=18) coefficient of variation was 8% and between- assay (n = 74) coefficient of variation was 14%; the lower limit of sensitivity of the method was 6% of normal protease levels. To evaluate the presence of antibodies inactivating ADAMTS-13, plasma samples from patients with HELLP syndrome were incubated at 56° C for 30 min to destroy any residual ADAMTS-13. Serial dilutions of heated plasma in phosphate-buffered saline with 1% bovine albumin were mixed with equal volumes of normal pooled plasma and incubated at 37° C for 90 min; the levels of residual ADAMTS-13 activity were then measured. Undiluted reference plasma (taken as 100% activity) and serially diluted reference plasma were also run in the same assay. The dilution of patients' plasma that corresponded to 50% of residual ADAMTS-13 activity was arbitrarily defined as 1 U/mL of inhibitor.

VWF measurements

VWF activity was measured using both the collagen binding (VWF:CB) and ristocetin cofactor activity assays (VWF:RCo).[18,19] VWF antigen (VWF:Ag) was measured by an automated latex immunoturbidimetric assay (IL Instrumentation Laboratory) on an ACL 9000/7000 (IL).

Multimeric analysis of VWF

The VWF multimeric pattern was analyzed by discontinuous SDS-agarose gel electrophoresis[20] using 0.9% low gelling temperature agarose. After electrophoresis, the proteins were transferred to nitrocellulose membranes and stained with peroxidase-conjugated rabbit antibodies against human VWF. VWF multimers were scanned with a densitometer (Scanjet 5200 C, Hewlett Packard), which resolved multimers into a series of peaks. Areas under the peaks were calculated by a computer program (Image J). High molecular weight (HMW) multimers were arbitrarily defined as the peaks comprising 30% of the length of the gel. The corresponding area was computed and expressed as a percentage of the total area for each gel.

Table 1. Main clinical and laboratory findings in 17 patients with HELLP syndrome.

Case n.	*Age*	*Parity*	*Week of pregnancy*	*Platelet count* $\times 10^9$*/L*	*Hb, g/dL*	*LDH, U/L*	*ALT, U/L*	*AST, U/L*	*Perinatal outcome*	*Maternal outcome*	*Treatment*
1	35	P0	31	81	7.7	685	74	86	Live born	CD	AHT
2	39	P0	34	20	8.2	1426	128	142	Live born	CD	AHT
3	30	P0	37	56	8.5	731	157	184	Live born	CD	AHT
4	27	P0	35	71	9.7	683	91	78	Live born	CD	AHT
5	26	P0	27	25	7.2	1247	193	248	Fetal death	CD	NONE
6	30	P0	34	28	9.5	847	171	255	Live born	VD	NONE
7	36	P0	32	35	8.3	691	98	95	Live born	CD	AHT
8	36	P0	39	19	7.8	1392	365	691	Live born	CD	AHT, FFP
9	34	P0	36	24	8.0	910	138	154	Live born	CD	AHT
10	33	P0	40	65	9.5	778	148	108	Live born	VD	NONE
11	24	P1	26	52	7.0	805	178	267	Fetal death	CD	AHT
12	27	P0	40	51	8.4	748	228	191	Live born	CD	AHT
13	25	P0	37	18	7.9	1359	248	314	Live born	CD	AHT,FFP
14	22	P0	28	20	8.8	830	362	415	Fetal death	CD	AHT
15	21	P0	39	27	7.3	1305	228	274	Live born	VD	AHT,FFP
16	21	P0	31	34	8.0	698	235	282	Live born	CD	NONE
17	24	P1	33	30	7.5	812	184	301	Live born	CD	AHT

CD: Cesarean delivery; VD: vaginal delivery; FFP: fresh frozen plasma; AHT: antihypertensive treatment. Normal laboratory values for LDH, ALT and AST were 450, 30 and 35 U/L, respectively.

Thrombophilic mutations

The factor V Leiden and prothrombin mutations were detected by a LightCycler instrument (ROCHE, Molecular Biochemicals, Mannheim, Germany) using specific kits. PCR and hybridization probes for genotyping were analyzed in the same glass capillaries for each sample. The mutation probe had a different melting temperature, thus ensuring that the fluorescent signal generated during analysis of the melting curve was determined only by the mutation probe.

Statistical analysis

Data are expressed as medians and ranges, because the results of the assays were not normally distributed. Analysis of variance according to the Kruskal-Wallis test was used to compare women in the acute phase of HELLP, the same women in remission, healthy pregnant women and healthy non-pregnant women.

Results

Clinical and laboratory features of patients with HELLP syndrome

Table 1 summarizes the clinical features, general laboratory findings and treatment of the 17 women with HELLP syndrome in the acute phase. Among them, 11 had class 1 (severe) syndrome and the remaining 6 had class 2 (moderately severe), according to their degree of thrombocytopenia. Serum LDH, ALT and AST levels were very high in the acute phase, consistent with the diagnosis of HELLP syndrome (Table 1).

The outcome of all women was excellent, as all survived and are currently healthy, but fetal death occurred in three cases. In remission there was a normalization of abnormal laboratory values, which became similar to those found in a normal pregnancy (*data not shown*).

Table 2. Main hemostasis, von Willebrand factor and ADAMTS-13 measurements.

	HELLP syndrome (n=17)	*Post HELLP (n=17)*	*Normal pregnancy (n=25)*	*Normal women (n=50)*	*Normal laboratory range*
Fibrinogen, mg/dL	515 (410-833)	309* (256-403)	471 (328-696)	300* (228-437)	190-398
Antithrombin, per cent	72 (59-95)	108* (95-135)	101* (78-126)	104* (81-126)	79-119
D-Dimer, mg/mL	3500 (1190-4900)	201* (14-250)	463* (105-690)	< 250* (50-280)	50-280
VWF:Ag, per cent	314 (215-422)	108 (60-191)	186* (102-320)	96* (47-175)	48-139
VWF:RCo, per cent	243 (109-336)	93* (54-267)	140* (84-258)	89* (64-154)	46-142
VWF:CB, per cent	208 (120-332)	112* (71-152)	152* (80-228)	94* (43-176)	51-155
ADAMTS-13, per cent	31 (12-43)	115* (90-170)	71* (48-105)	101* (45-152)	47-152
High molecular weight multimers, per cent	21 (19-27)			25 (20-28)	20-28

*Values are given in as medians, with observed ranges between parentheses For each condition and measurement, statistically significant differences in comparison with HELLP syndrome are shown. *p < 0.01.*

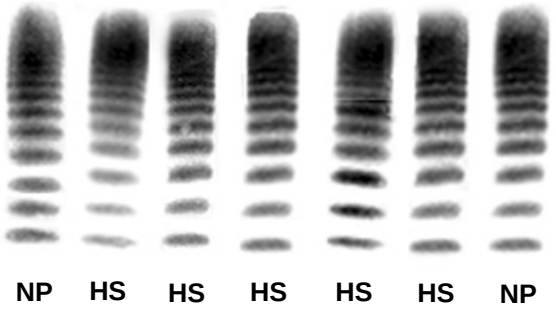

Figure 1. Values of ADAMTS-13 (expressed in percent of average in normal plasma) in 17 women with HELLP syndrome in acute phase (HS), in remission (post-HS), in 25 normal pregnant women (NP) and in 50 normal women (NW). Closed circles indicate cases in Martin's class 1 (platelets <50×10³/μL), open circles those in class 2 (platelets 50-100×10³/μL).

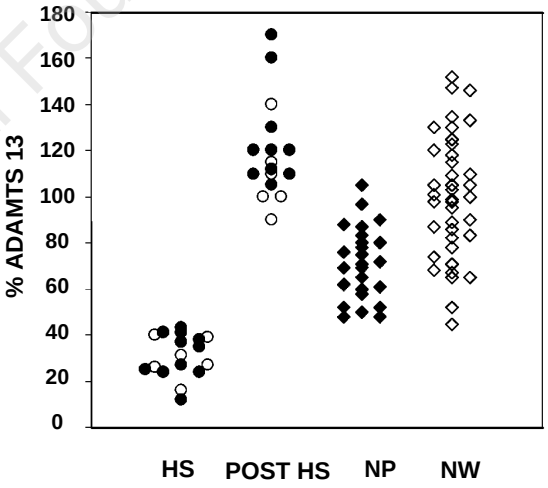

Figure 2. Representative examples of the intact multimeric structure of von Willebrand factor in 5 patients with HELLP syndrome (HS) in the acute phase and in two samples of normal plasma (NP).

Hemostasis, VWF and ADAMTS-13 measurements

Table 2 summarizes the main hemostasis findings in 17 women with HELLP syndrome both in the acute and remission phases, in 25 healthy pregnant women (third trimester) and in 50 healthy, non-pregnant women. During the acute phase, coagulation screening tests (PT, APTT) were normal (*data not shown*) but the levels of antithrombin were significantly lower and D-dimer values higher than in normal pregnancy. Fibrinogen levels were high in HELLP syndrome but not higher than in normal pregnancy. No case had factor V Leiden or the prothrombin mutation. VWF:RCo and VWF:CB were both higher in women with HELLP syndrome than in healthy women in the same period of pregnancy (third trimester) and in healthy, non-pregnant women (Table 2). Elevated levels of plasma VWF:Ag were also found in all patients in the acute phase and were always higher than the levels of VWF:CB and RCo. In remission VWF levels normalized and became similar to those in healthy non-pregnant women.

Reduced but detectable levels of ADAMTS-13 activity were found in all women with HELLP syndrome in the acute phase ($p<0.01$), with no obvious relationship between the severity of the syndrome and the values of ADAMTS-13 (Figure 1). The same women in remission showed a complete recovery of ADAMTS-13, with values similar to or slightly higher than those found in normal, non-pregnant women. In normal pregnant women, protease levels were lower than in healthy non-pregnant women ($p<0.01$) but higher than in women with HELLP syndrome ($p<0.01$). In no women was an inhibitor inactivating ADAMTS-13 found and the multimeric pattern of VWF was normal in all (Figure 2).

Discussion

A pregnancy-associated thrombotic microangiopathy such as HELLP syndrome may have clinical presentations, laboratory and histopathologic findings similar to those of TTP that develops during pregnancy.[21] There are some clinical features that may be used for differential diagnosis, such as the prevalence of central nervous system involvement in TTP and liver involvement in HELLP syndrome and the presence of more severe anemia and thrombocytopenia in TTP. In TTP levels of antithrombin and D-dimer are usually normal while in HELLP syndrome these values are frequently abnormal.[1] The general clinical and laboratory findings of our series of 17 patients are more consistent with the diagnosis of HELLP syndrome than with that of TTP. None had neurological signs, thrombocytopenia was moderate in one third of them and all had a marked increase of serum aminotransferases. There were signs of compensated intravascular coagulation, such as high D-dimer levels with normal or high fibrinogen levels in the absence of significant alterations of the global coagulation screening tests, PT and PTT. Signs of compensated intravascular coagulation are typically absent in TTP. This study shows that pregnant women with HELLP syndrome have lower plasma levels of ADAMTS-13 activity than a group of healthy pregnant women comparable for gestational age, and that low protease levels returned to normal 6 months after remission of the syndrome. It also shows that low levels of ADAMTS-13 are not due to the presence of inactivating autoantibodies[8] and that, at variance with the situation in TTP, the low levels of ADAMTS-13 are not accompanied by the presence of ultralarge VWF multimers in plasma.[14]

Having ruled out the action of inhibitory antibodies, two possible mechanisms can be postulated to explain low levels of ADAMTS-13 activity during the HELLP syndrome: reduced production and increased clearance. Genetic defects are very unlikely, because protease levels returned to normal on remission. The levels of ADAMTS-13, synthesized by the liver,[11] are low or very low in patients with liver cirrhosis.[22] The impairment of liver function was not severe in women with HELLP syndrome, because prothrombin times and plasma antithrombin, sensitive indices of liver synthetic function, were normal or only mildly abnormal. As to the possibility of increased plasma clearance of ADAMTS-13, this hypothetical mechanism cannot be explored at the moment, because the protease is present in small concentrations in plasma (1 μg/mL or less) and cannot be purified in sufficiently large amounts to evaluate plasma half-life *ex vivo*. A significant relation was found between high VWF and low ADAMTS-13 plasma levels, as previously found in patients with long-term increases of VWF (e.g. during the post-operative period, chronic inflammatory states, pregnancy itself)[22] or with short-term increases (following DDAVP infusion).[23] In this study we observed a continuous spectrum of results from non-pregnant women and women with uncomplicated pregnancies to those with pregnancies complicated by HELLP syndrome, with progressively higher VWF and lower ADAMTS-13 levels. We hypothesize that high VWF levels are the mechanism underlying the decrease of ADAMTS-13 in plasma, as a results of the *consumption* of the protease when high levels of the substrate must be disposed.

Can the measurement of ADAMTS-13 help to differentiate TTP from HELLP syndrome in pregnancy? In acute TTP, protease levels are typically very reduced or undetectable (less than 10% of normal) and are often accompanied by circulating ultralarge VWF multimers.[14] In patients with HELLP syndrome protease levels were always higher than those values (ranging from 12 to 43%), the proportion of high molecular weight multimers was similar to that found in normal plasma (Table 2) and in no case were ultralarge multimers detected in plasma (Figure 2). So, the behavior of VWF multimers and ADAMTS-13 in HELLP syndrome differs from the typical pattern in TTP.[7,8] On the other hand, cases of TTP with levels of ADAMTS-13 similar to those found in this study and the absence of ultralarge multimers have been reported,[24,25] so that ADAMTS-13 measurement helps in the differential diagnosis of pregnancy-associated microangiopathies only when plasma levels are very low or undetectable, a beacon of TTP.

Do low ADAMTS-13 levels play a role in the pathogenesis of the HELLP microangiopathy? It is claimed, but not unequivocally demonstrated, that ADAMTS-13 levels of 10% of normal or more, like those found in this study, are sufficient to prevent the presence of ultralarge multimers and intravascular platelet consumption.[26] On the other hand, the combined presence of low ADAMTS-13 levels with increased VWF concentrations[22] and the complex prothrombotic state typically associated with pregnancy, may engender a prothrombotic disequilibrium that in some cases could determine the onset of HELLP thrombotic microangiopathy. Even though the best and simplest treatment of HELLP syndrome is the induction of delivery, which successfully resolved also our cases, plasma exchange has been reported to accelerate recovery in patients with slow resolution of HELLP syndrome beyond 72 hours *post-partum*.[5,6] Perhaps the replacement of ADAMTS-13 is the reason for the success of this treatment.

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Pre-publication Report & Outcomes of Peer Review

Contributions

AL, CC and RC were responsible for the enrollment of the patients, AL for the laboratory methods and for the statistical analysis of the data. PMM wrote the article with AL and ER, and all the latter were responsible for the conception and planning of the study.

Disclosures

Conflict of interest: none.

Redundant publications: no overlapping with previous papers, except that lower ADAMTS-13 levels in normal pregnancy were previously reported by some of the authors.[22]

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Evan Sadler, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Sadler and the Editors. Manuscript received July 2, 2003; accepted July 21, 2003.

In the following paragraphs, Dr. Sadler summarizes the peer-review process and its outcomes.

What is already known on this topic

HELLP syndrome is a complication of pregnancy that can be very difficult to distinguish from thrombotic thrombocytopenic purpura, which may also occur during pregnancy. The distinction is important because plasma exchange is beneficial in TTP, whereas prompt delivery of the fetus is the treatment of choice in HELLP syndrome.

What this study adds

Idiopathic TTP in adults is usually caused by severe deficiency of ADAMTS-13, a metalloprotease that cleaves von Willebrand factor. The clinical differences between TTP and HELLP syndrome suggest that the conditions also have fundamentally different causes, so that ADAMTS-13 levels might not be low in HELLP syndrome. In fact, the results of the study indicate that women with HELLP syndrome generally do not have severe ADAMTS-13 deficiency. Therefore, ADAMTS-13 assays might be useful to discriminate between TTP and HELLP syndrome, and thus to select appropriate therapy for thrombotic microangiopathy during pregnancy.

Caveats

A relatively small number of patients were studied so the conclusions must be considered tentative. Studies of thrombotic microangiopathy in other groups of patients suggest that some cases of TTP may not be caused by severe deficiency of ADAMTS-13, but still may appear to respond to plasma exchange. Further study will be required to assess the clinical utility of ADAMTS-13 assays in patients with thrombotic microangiopathy.