Thrombosis Research 142 (2016) 40–43

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Are there any additional mechanisms for haemolysis in HELLP syndrome?

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ARTICLE INFO

Article history:
Received 11 October 2015
Received in revised form 6 March 2016
Accepted 12 March 2016
Available online 9 April 2016

Keywords:
HELLP syndrome
Haemolysis
Acute kidney injury
Complement system

ABSTRACT

HELLP syndrome is a microangiopathy that leads to severe maternal complications. The objective of this study was to identify any additional mechanisms that could have contributed to HELLP syndrome-induced haemolysis. This is a pilot, prospective and observational study that lasted 9 months. All patients with HELLP syndrome treated at academic tertiary care women hospital accepted to participate. Sixteen patients were included. In ten patients (63%), schizocytes were detected following a blood smear test. Six patients (38%) were diagnosed with a partial expression deficiency of proteins regulating the complement system (CD 55 or CD 59). In nine patients (56%), an activation of the complement classical pathway was detected. In two patients (13%), an ADAMTS 13 activity below 30% was detected. Three patients (19%) were diagnosed with a folate deficiency and one (6%) with an antiphospholipid syndrome. All patients developed maternal or fetal morbidity including nine (56%) an acute kidney injury. All patients but one had at least one additional mechanism that could contribute to haemolysis, besides a simple physical injury. Larger studies should be promoted to understand haemolysis in HELLP syndrome.

1. Introduction

Obstetric renal failure is a potentially life-threatening complication of pregnancy and preeclampsia one of its major risk factors [1]. The syndrome of haemolysis, elevated liver enzymes and low platelets (HELLP) is observed in 10% to 20% of cases of pre-eclampsia, and can induce severe maternal complications, including acute renal failure [2]. The HELLP syndrome-induced microangiopathic haemolysis, as evidenced by the presence of schizocytes on peripheral smear, has been attributed to mechanical crush of red blood cells due to micro arteriolar vasospasm and thrombosis [3]. The objective of this pilot study was to identify additional mechanisms that could contribute to HELLP syndrome-induced haemolysis.

2. Patients and methods

2.1. Study hypothesis

HELLP syndrome-induced haemolysis has been attributed to mechanical crush of red blood cells due to micro arteriolar vasospasm and thrombosis. We hypothesized that additional haemolytic mechanisms may be involved, including: 1) deficient expression of proteins regulating the complement system on blood cells (CD 55 and CD 59) as observed in paroxysmal nocturnal haemoglobinuria; 2) presence of complement activation, by the classical or alternative pathway, as documented in atypical Haemolytic and Uremic Syndrome; 3) ADAMTS 13 deficiency as observed in Thrombotic Thrombocytopenic Purpura (TTP); 4) auto immune disease (antiphospholipid syndrome or lupus); 5) allo immunization after blood transfusion or previous pregnancy; 6) vitamin B12 or folate deficiency; 7) hemoglobinopathy such as sickle cell disease and thalassemia; 8) classical Haemolytic Uremic Syndrome due to infection (*Escherichia coli* $O_{157}H_7$).

2.2. Settings

This was a pilot, observational, and prospective study conducted in a tertiary care academic hospital over a period of 9 months (November 2013 to July 2014). According to French law, the study was declared to the national legal commission (CNIL). Informed consent was obtained from each patient.

2.3. Study population

Patients were included in the study when they developed HELLP syndrome after 20 weeks of amenorrhea or in the early post-partum

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http://dx.doi.org/10.1016/j.thromres.2016.03.014
0049-3848/

period, according to the Tennessee Classification defined by Sibai [3]. Diagnostic criteria for HELLP syndrome were the association of haemolysis with increased LDH (>600 UI/L), elevated liver enzymes (AST > 70 UI/l), and thrombocytopenia (platelets < 100×10^9/L). Exclusion criteria were a chronic liver disease before pregnancy or patient refusal.

2.4. Data collection and biological methods

Biological data were obtained at the time of HELLP syndrome diagnosis. All patients underwent blood laboratory tests to assess haemolysis (hemoglobin, haptoglobin, LDH, free bilirubin, reticulocytes level, and schizocytes) and its causes. An expression deficiency of proteins regulating the complement system on blood cells (CD 55 and CD 59) was assessed by flow cytometry. Classical and alternative pathway complement activation (C3, C4, CH50, and C1 inhibitor) was assessed by turbidimetry. ADAMTS 13 activity was measured by the ELISA assay Technozym ADAMTS 13® activity (Technoclone) and VWF:Ag level by immunoturbidimetric assay, using Siemens reagent. The presence of an auto or allo immune disease was assessed by ELISA test, vitamin deficiency (vitamin B12 or folate) by electrochemiluminescence, hemoglobinopathy by capillary electrophoresis, and *Escherichia coli* $O_{157}H_7$ infection by PCR. Coagulation disorders were defined in our study by a plasma fibrinogen level < 0.9 μmol/L, another coagulation deficiency (factor II, factor V, or antithrombin deficiency) or an ISTH (International Society for Thrombosis and Haemostasis) score > 3. The ISTH score predicts the occurrence of disseminated intravascular coagulopathy (DIC) according to prothombin time, platelets, fibrin degradation products, D-dimers, and fibrinogen plasma levels [4]. All tests were analysed in the different laboratories of the "Centre de Biologie Pathologie" at Lille academic hospital, blindly to the study hypothesis. Normal ranges are reported in Table 1.

Clinical data were collected at inclusion by the study's investigators, including medical and obstetrical history, chronic and actual treatments (before and after delivery), recent infectious disease, clinical signs (high blood pressure, oedema, neurological signs, diuresis, epigastric pain, hyperthermia, diarrhoea), and intensive care unit admission.

Acute kidney injury, postpartum haemorrhage, or DIC were considered as maternal morbidity. Acute kidney injury was assessed by the RIFLE score, commonly used to evaluate glomerular filtration and correlated to kidney prognosis [5]. This score ranges from 0 to 5 according to creatinine plasma levels (Table 2). Postpartum haemorrhage was defined as blood loss of 500 mL or more from the genital tract within 24 h of the birth of a baby.

Fetal morbidity was considered in case of prematurity, intrauterine fetal death, intrauterine growth restriction or pathological uterine artery Doppler. Prematurity was defined as a birth before 37 weeks of amenorrhea. Intrauterine growth restriction refers to an estimated fetal weight below 10th percentile.

2.5. Objectives

Our primary objective was to explore the different causes of haemolysis in HELLP syndrome. Our secondary objective was to explore the link between these causes and feto-maternal morbidity.

2.6. Statistical analysis

Continuous variables were expressed as medians (interquartile range 25%–75%). Categorical variables were expressed as percentages.

3. Results

Sixteen consecutive patients with HELLP syndrome were included during the study period. All of them gave their informed consent. Median age was 30 years (23–34). Eight patients were primiparous. Among the eight other patients, only one had developed mild preeclampsia in her previous pregnancy. All pregnancies were spontaneous and single. Two patients presented with an untreated chronic arterial hypertension. Four patients had previously developed venous thromboembolism. One only, who suffered from a SAPL syndrome, was receiving antithrombotic prophylaxis when HELLP syndrome arose. HELLP syndrome occurred at 34 [28–36] weeks of gestation. HELLP syndrome biological data are reported in Table 3.

Table 1
Laboratory tests.

Blood test (plasma level)	Normal ranges	Results expected for study
Blood cells count	Hemoglobin 110–160 g/L Platelets > 150×10^9/L	Hemoglobin < 110 g/L Platelets < 100×10^9/L
Schizocytes	Negative	>1%
Reticulocytes	20 à 120×10^9/L	>120×10^9/L
Clone CD 55	Negative	Positive (%)
Clone CD 59	Negative	Positive (%)
Haptoglobin	4–20 μmol/L	<4 μmol/L
LDH	<430 UI/L	>600 UI/L
Free bilirubin	<1.7 μmol/L	>1.7 μmol/L
Uric acid	<300 μmol/L	>300 μmol/L
AST/ALT	<40 UI/L	>70 UI/L
Blood urea nitrogen	<8.3 μmol/L	>8.3 μmol/L
creatinine	<100 μmol/L	>100 μmol/L
D-dimer	<500 μg/L	>500 μg/L
Fibrin degradation products	<10 mg/L	>10 mg/L
PTT ratio/PT	<1.3 / >70%	>1.3 / <70%
Fibrinogen	0.9–1.8 μmol/L	<0.9 μmol/L
Factor II	>60%	<60%
Factor V	>60%	<60%
ADAMTS 13 activity	>70%	<70%
ADAMTS 13 antibody	Negative	Positive
Antithrombin activity	70–140%	<70%
Hemoglobin electrophoresis	Hemoglobin A	Hemoglobin S
Red blood cell agglutinins	Negative	Positive
Direct Coombs test	Negative	Positive
Indirect Coombs test	Negative	Positive
Anti nuclear antibody	Negative	Positive
C1 esterase inhibitor	17–345 mg/L	<170 mg/L
C3 complement	0.8–1.4 g/L	<0.8 g/L
C4 complement	0.2–0.4 g/L	<0.2 g/L
CH50	70–120%	<70%
Serum folate	>6.8 nmol/L	<6.8 nmol/L
RBC folate	>385 nmol/L	<385 nmol/L
Vitamin B12	>148 pmol/L	<148 pmol/L
Stool test		
Escherichia coli $O_{157}H_7$ (PCR assay)	Negative	Positive

In all patients but one, there were one or more additional mechanisms involved in the haemolytic process (see Table 4). Six patients (38%) presented a partial expression deficiency of complement regulating proteins: these CD 55 deficiencies were observed in 0.4 to 17% of granulocytes cells. Median level of ADAMTS 13 activity was 59% [7–100] and two patients presented an ADAMTS 13 activity below 30% (14% and 8%). The median ADAMTS 13 to VWF:Ag ratio was 0.20 [0.16–0.3], in the 12 tested patients. Three patients (19%) presented a vitamin deficiency affecting serum folates for one and vitamin B12 for two. One patient (6%) suffered from antiphospholipid syndrome. In nine patients (56%), complement activation using the classical pathway was identified by a decrease in C4 levels. Median levels of CH50, C3, and C4 were 174% [108–215], 1.25 g/L [0.95–1.56], and 0.18 g/L [0.14–0.26], respectively. Neither hemoglobinopathy nor infection was found.

All patients developed maternal or fetal morbidity, as described in Table 4. Nine patients (56%) developed acute kidney injury. The RIFLE score reached 1 point for seven patients, 2 and 3 points for the two remaining patients. Three patients (19%) developed a mild postpartum haemorrhage. Thirteen patients (81%) developed haemostasis disorders: twelve patients (75%) had an ISTH score > 3, six (38%) had a fibrinogen plasma level < 0.9 μmol/L, and four (25%) another coagulation deficiency. Fetal morbidity was found in twelve patients (75%): eleven