

Association between the Quantitative Assessment of Schistocytes in Peripheral Blood Smear and Prognosis of Patient Initially Diagnosed as HELLP Syndrome

Thesis Protocol

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Introduction

HELLP syndrome probably represents a severe form of preeclampsia, but the relationships between the two disorders remain controversial. As many as 15 to 20 percent of patients with HELLP syndrome do not have antecedent hypertension or proteinuria, leading some authorities to believe that HELLP syndrome is a separate disorder from preeclampsia (*Reubinoff – Schenker ., 1991*).

The most common symptom is abdominal pain and tenderness in the midepigastrium, right upper quadrant, or below the sternum (*Sibai – Ramadan ., 1993*).

Many patients also have nausea, vomiting, and malaise, which may be mistaken for a nonspecific viral illness or viral hepatitis, particularly if the serum aspartate aminotransferase and lactate dehydrogenase are markedly elevated (*Catanzarite ., et al 1995*).

Less common signs and symptoms include headache, visual changes, jaundice, and ascites. Mistaking abdominal pain, nausea, vomiting, and malaise for viral illness is a common pitfall that has resulted in maternal death or severe morbidity (*Isler ., et al 1999*).

Hypertension (blood pressure $\geq 140/90$ mmHg) and proteinuria are present in approximately 85 percent of cases,

but it is important to remember that either or both may be absent in women with otherwise severe HELLP syndrome ([Sibai, 2004](#)).

Signs and symptoms typically develop between 28 and 36 weeks of gestation, but second trimester or postpartum onset is also common. In an illustrative series of 437 women who had 442 pregnancies complicated by the HELLP syndrome, 70 percent occurred prior to delivery. Of these patients, approximately 80 percent were diagnosed prior to 37 weeks of gestation and fewer than 3 percent developed the disease between 17 and 20 weeks of gestation ([Sibai, et al 1993](#)).

Serious maternal morbidity may be present at initial presentation or develop shortly thereafter. This includes disseminated intravascular coagulation, abruptio placentae, acute renal failure, pulmonary edema, subcapsular or intraparenchymal liver hematoma, and retinal detachment, bleeding related to thrombocytopenia is an unusual presentation ([Sibai, et al 1993](#)).

Outcome and prognosis of HELLP syndrome is associated with a variety of maternal morbidities, which can rarely result in a fatal outcome. The risk of serious morbidity

correlates with increasing severity of maternal symptoms and laboratory abnormalities ([Martin ., et al 2006](#)).

The majority of patients will show evidence of resolution of the disease process within 48 hours after delivery([ACOG 2004](#))

Differential diagnosis HELLP syndrome may occasionally be confused with other diseases complicating pregnancy: acute fatty liver of pregnancy, gastroenteritis, hepatitis, appendicitis, gallbladder disease, immune thrombocytopenia, lupus flare, antiphospholipid syndrome, hemolytic-uremic syndrome, thrombotic thrombocytopenic purpura, and nonalcoholic fatty liver disease ([Page- Girling ., 2011](#)).

Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome are systemic disorders characterized by endothelial injury and the formation of small vessel platelet-rich thrombi. The resulting thrombotic microangiopathy produces microangiopathic hemolytic anemia and thrombocytopenia; presenting features may also include neurologic and/or renal abnormalities ([Scully ., et al 2012](#)).

If untreated, thrombotic thrombocytopenic purpura-hemolytic uremic syndrome in adults typically follows a progressive course in which irreversible renal failure, progressive neurologic deterioration, cardiac ischemia, and death are common outcomes ([Remuzzi ., 1987](#)).

The mortality rate prior to the use of plasma exchange was approximately 90 percent ([von ., 2002](#)).

The mainstay of treatment for most patients with thrombotic thrombocytopenic purpura-hemolytic uremic syndrome is plasma exchange, which in the context of this syndrome refers to the removal of the patient's plasma by pheresis and the replacement with donor plasma rather than another replacement fluid such as albumin ([Scully., et al 2012](#)).

Compared with the mortality rate of 90 percent prior to the use of plasma exchange, the mortality rate for patients treated with plasma exchange is 25 percent or less ([Kremer., et al 2010](#)).

Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome should be considered in all pregnant women with severe thrombocytopenia, severe anemia, and elevated lactate dehydrogenase enzyme ([Stella .,et al 2009](#)).

The distinction between thrombotic thrombocytopenic purpura-hemolytic uremic syndrome and severe preeclampsia is important for therapeutic and prognostic reasons. However, the clinical and histological features are so similar that establishing the correct diagnosis is often difficult; furthermore, these disorders may occur concurrently ([Burns ., etal 2004](#)).

The diagnosis of thrombotic thrombocytopenic purpura rests on evidence of microangiopathic hemolytic anemia and thrombocytopenia in the absence of disseminated intravascular coagulation and other known causes of thrombotic microangiopathy. Highly specific diagnostic tools such as serum levels of ADAMTS13 are not routinely available for immediate clinical diagnosis. The presence of schistocytes on a blood smear is the morphologic hallmark of the disease. During the early 1970s plasma exchange was introduced as an effective treatment for TTP, making early and rapid diagnosis urgent. (*Burns ., etal 2004*)

So we are searching for the association between the quantitative assessment of schistocytes in peripheral blood smear and prognosis of patient initially diagnosed as HELLPsyndrome.

Aim of the work

Research question:

In patients who are initially diagnosed as HELLP syndrome does the quantitative assessment of schistocytes will predict deterioration of HELLP syndrome after delivery ?

Research hypothesis:

We supposed that there might be a difference in the quantitative assessment of schistocytes in peripheral blood smear between women initially diagnosed as HELLP syndrome who showed no spontaneous resolution within 48 hrs after delivery and those who showed spontaneous resolution within 48 hrs after delivery.

Aim of study:

The aim of the current study is to search for a diagnostic test which might predict microangiopathic hemolytic anemia and persistent HELLP syndrome from cases who initially diagnosed as HELLP syndrome in order to start PEX/ FFP which might decrease the maternal mortality rate at Ain Shams maternity hospital

Patients and Methods

Study Design:

- o diagnostic test accuracy. (pilot study)

Study Setting:

- o Ain Shams University Maternity Hospital from January 2015 to December 2015.
- o Intensive Care Unit , Wards, Labour ward at Ain Shams University Maternity Hospital and Hematology Unit and Clinical Pathology Unit at Ain Shams University.

Study Population:

A total of 100 women with an initial diagnosis of HELLP syndrome will be recruited in the study.

Blood sample taken within 12hrs peripartum and patients will be followed up 48hrs after delivery and subdivided in to 2 groups according to improvement or deterioration after 48hrs after delivery .

Inclusion criteria :

- o Gestational age more than 20 weeks.
- o Age from 18 to 40 years old.
- o Fulfilled criteria for the diagnosis of severe preeclampsia.
- o Fulfilled criteria for the diagnosis of HELLP syndrome.

Diagnostic criteria for severe preeclampsia(one of the following):

- Blood pressure of 160 mm Hg systolic or higher or 110 mm Hg diastolic or higher on two occasions at least 6 hours apart while the patient is on bed rest (unless antihypertensive therapy is initiated before this time).
- New onset Cerebral or visual disturbances.
- Pulmonary edema or cyanosis.
- Severe persistent epigastric or right upper-quadrant pain unresponsive to medication and not accounted for by alternative diagnosis, or both.
- Impaired liver function as indicated by abnormally elevated blood concentration of liver enzymes (to twice normal concentration).
- Thrombocytopenia (platelet count less than 100, 000 per microliter. *(ACOG., 2013)*

Diagnostic criteria for HELLP syndrome:

- Hemolysis documented by an increased LDH level and progressive anemia .
- Hepatic dysfunction documented by an LDH level >600 IU/L, elevated liver enzymes documented by AST >40 IU/L, ALT >40 IU/L, or both .
- Thrombocytopenia documented by a platelet nadir less than 150, 000 cells/mm³. Thrombocytopenia is subclassified as class one HELLP syndrome: platelet nadir \leq 50, 000 cells/mm³, class two HELLP syndrome: platelet nadir \leq 100, 000 cells/mm³, or class three HELLP syndrome: platelet nadir \leq 150, 000 cells/mm³.*(Martin ., et al 1999)* .

Anticipation of Microangiopathic Hemolytic Anemia:

Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome should be considered in all pregnant women with severe thrombocytopenia, severe anemia, and elevated lactate dehydrogenase enzyme (*Stella ., et al 2009*).

Methodology:

All included women will be subjected to the following:

- o History taking and consent
- o Consent form:
 - o Patient name:
 - o Hospital number:
 - o Information to the participant:
 - o The procedure
 - 1. The results of your blood results and hospital data will be used in this clinical trial.
 - 2. An extra blood sample will be withdrawn at the time of diagnosis which is a simple procedure with no known complications.
 - 3. All your data and blood results, will saved confidentially and it will appear as a number in the results.
 - 4. The results of these investigation will not affect your management plan.

- o General, abdominal and local examination.

Laboratory test:

complete blood count, using EDTA-anticoagulated peripheral blood sample, for concentration and platelet counts.

Peripheral blood smears will be taken within 12hrs peripartum and prepared within two hours of blood collection using the EDTA-anticoagulated peripheral blood sample. The blood smear then spread, air-dried, and fixed and stained according to standard procedures with panoptical stains Romanowsky stain as reported by (*International Council for Standardization in Hematology; 1984*).

Schistocytes are fragments of red blood cells (RBCs) produced by extrinsic mechanical damage within the circulation. The detection of schistocytes is an important morphological clue to the diagnosis of thrombotic microangiopathic anemia (TMA). Reporting criteria between different laboratories, however, are not uniform, owing to variability of shape and nature of fragments, as well as subjectivity and heterogeneity in their morphological assessment. Lack of standardization may lead to inconsistency or misdiagnosis, thereby affecting treatment and clinical outcome. The Schistocyte Working Group of the

International Council for Standardization in Hematology 2012 (ICSH) has prepared specific recommendations to standardize schistocyte identification, enumeration, and reporting. They deal with the type of smear, method of counting, morphological description based on positive criteria (helmet cells, small, irregular triangular, or crescent-shaped cells, pointed projections, and lack of central pallor). A schistocyte count has a definite clinical value for the diagnosis of TMA in the absence of additional severe red cell shape abnormalities, with a confident threshold value of 1%. Automated counting of RBC fragments is also recommended by the ICSH Working Group as a useful complement to the microscope, according to the high predictive value of negative results, but worthy of further research and with limits in quantitation.

International Council for Standardization in Haematology recommendations for schistocyte counting 2012:

1. Schistocytes should be evaluated on peripheral blood smears using an optical microscope at medium magnification and estimated as a percentage after counting at least 1000 red blood cells.
2. A schistocyte count should be requested and carried out when a diagnosis of thrombotic microangiopathies caused by red cell mechanical damage is suspected, usually in patients with thrombocytopenia.
3. Schistocytes should be identified by specific positive morphological criteria. Schistocytes are always smaller than intact red cells and can have the shape of fragments with sharp angles and straight borders, small crescents, helmet cells, keratocytes, or microspherocytes*.
4. A schistocyte count should be considered clinically meaningful if schistocytes represent the main morphological red blood cells abnormality in the smear (other than signs of erythropoietic regeneration).
5. A robust morphological indication for the diagnosis of thrombotic microangiopathic anemia in adults should be recognized when the percentage of schistocytes is above 1%.
6. Fragmented red cell enumeration by automated blood cell counters should be considered a useful complement to microscopic evaluation, as it provides rapid results with a high predictive value of negative samples. A microscope check is needed for positive and macrocytic samples†.

*Microspherocytes only in the presence of other mentioned RBC shapes.

†Macrocytic samples are at risk of underestimation or absence of flag ('false negative' test) (***International Council for Standardization in Hematology 2012***).

Schistocytes counting: Schistocytes will be identified and counted on the peripheral blood smear using optical microscopy. The results of microscopic examination then will be expressed as a percentage, after counting at least 1000 RBCs in optimal areas of the film. Interpretation of the examination results had been done following the (***International Council for Standardization in Hematology, 2012***) recommendations for identification, diagnostic value, and quantitation of schistocytes.