Assessment of Immature Platelet Fraction in Pregnancy-Associated Thrombotic Microangiopathy

Thesis

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By

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Introduction

Thrombotic microangiopathy (TMA) syndromes are extraordinarily diverse. They may be hereditary or acquired. They occur in children and adults. The onset can be sudden or gradual. Despite their diversity, TMA syndromes are united by common, defining clinical and pathological features, which are microangiopathic hemolytic anemia (MAHA), thrombocytopenia, and organ injury **(Moake, 2002).**

Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are the two most well known, and are considered to be the most serious, TMA syndromes **(Ab Rahman et al., 2013).** TTP/HUS occurs more commonly in women and among women is commonly associated with pregnancy **(George and Nester, 2014).** Nevertheless, there are other pregnancy conditions that may manifest with TMA, including preeclampsia, eclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), in addition to acute fatty liver of pregnancy, antiphospholipid syndrome, and systemic lupus erythematosis **(Scully et al., 2012).**

TTP may be caused by an acquired (inhibitory antibodies) or more rarely, constitutional deficiency in

ADAMTS13, a metalloprotease that cleaves the ultralarge multimers of von Willebrand factor (vWF). vWFs are potent inducers of platelets aggregation **(Furlan et al., 1998).** In the setting of ADAMTS13 deficiency, ultralarge vWF multimers initiate thrombi formation with ensuing microvascular platelet clumping, resulting in MAHA, fragmented erythrocytes (schistocytes), consumptive thrombocytopenia, renal dysfunction, and neurological symptoms. Since TTP can present without the full pentad, the revised diagnostic criteria state that TTP must be considered in the presence of thrombocytopenia and MAHA alone **(Galbusera et al., 2006).**

HUS describes TMA that affects mainly the kidney. It is characterized by MAHA, thrombocytopenia and acute renal failure. HUS includes various subtypes: verotoxin-associated typical (diarrheal) HUS, secondary HUS (of which, pregnancy-associated HUS is one type), and idiopathic atypical HUS (aHUS). Owing to similarity in clinical status & complications, diagnostic overlap with TTP can occur **(Fakhouri et al., 2012).**

Even in patients with congenital ADAMTS13 deficiency and recurrent acute episodes of TTP/HUS since infancy, pregnancy is one of the events that act as precipitating factors; occurring in approximately 5– 25% of

TTP cases **(Scully et al., 2008).** This is because pregnancy may provide multiple risk factors that can provoke an acute episode of TTP/HUS in a susceptible woman. These risk factors include increasing concentrations of procoagulant factors, decreasing fibrinolytic activity, loss of endothelial cell thrombomodulin, and decreasing activity of ADAMTS13 **(George, 2003).** These abnormalities become progressively more severe through the course of pregnancy, with the maximum abnormalities occurring at delivery and immediately postpartum. This is also the time of greatest risk for the occurrence of the more common pregnancy-related syndromes (pre-eclampsia, eclampsia, HELLP syndrome, and HUS). Differentiating these other syndromes from TTP may difficult or impossible because of similar clinical presentation in some aspects **(Stirling et al., 1984).** In many women, the distinction can only be determined by the course of illness following delivery **(McMinn and George, 2001).**

Reliable laboratory criteria specific for TTP are largely lacking. The diagnostic value of ADAMTS13 deficiency remains uncertain since not all TTP patients have severe deficiency **(Kremer Hovinga et al., 2010),** and not all documented severe ADAMTS13 deficiency patients meet TTP diagnostic criteria **(Scully et al., 2012).** Moreover, it is not feasible to constantly monitor ADAMTS13 activity

during therapeutic plasma exchange (TPE) in order to adjust therapy and monitor disease progress. Conjointly, relying on the therapeutic response to TPE harbors its own hazards and, besides, it has not been demonstrated that TPE is beneficial in treating non-idiopathic TTP. Thus, additional tests that could aid in the diagnosis and management of TTP are needed **(Sadler et al., 2008).**

Assessment of immature platelets was introduced as a non-invasive test of real time thrombopoeisis. They are newly released in the circulation with a larger size & greater RNA content than mature platelets, and can be measured by automated hematology analyzer equipped with reticulocyte detection channel and described as immature platelet fraction (IPF-%) and absolute immature platelet count (A-IPC). A high IPF-% has been suggested as an indicator of thrombocytopenia due to rapid platelet consumption, while a low IPF-% is characteristic of bone marrow suppression states. IPF-%/A-IPC has the competency to be performed routinely and, therefore, can provide therapeutic and diagnostic feedback in the compatible life threatening conditions **(Hong et al., 2013).**

Aim of work

The aim of this work is to show the utility of estimating IPF-% and A-IPC using a reticulocyte detection channel CBC auto analyzer as a simple reproducible blood analysis to be employed in the differential diagnosis of pregnancy-associated thrombotic microangiopathic conditions.

Patients and Methods

This study aiming to show the utility of estimating IPF-% and A-IPC using a reticulocyte detection channel CBC auto analyzer as a simple reproducible blood analysis to be employed in the differential diagnosis of pregnancy-associated thrombotic microangiopathic conditions. Patients were recruited from Ain Shams University Maternity Hospital.

Study sample was 57 women and were classified into three groups:

Group 1: *SPE/HELLP group*. This group will include 24 pregnant women (gestational age of >20 weeks) who are diagnosed as having TMA with provisional diagnosis of pre-eclampsia, HELLP syndrome.

Group 2: *TTP/HUS group*. This group will include 13 pregnant women (gestational age of >20 weeks) who are diagnosed as having TMA with provisional diagnosis of TTP/HUS.

Group 3: *Control group*. This group will include 20 pregnant women (gestational age of >20 weeks) having normal pregnancy with normal blood pressure and platelet count.

Inclusion criteria

Women will be included if they are:

- Older than 20 years of age
- Pregnant with singleton intrauterine pregnancy
- More than 20 weeks of gestation

Exclusion criteria

Patients will be excluded from the study if there was any of the following:

- Congenital malformation and fetuses with chromosomal or genetic syndrome.

- Recent blood transfusion.
- Refusal to participate in the study.
- BMI <18.
- Placental abnormalities like velamentous insertion.
- Multiple pregnancies.
- Known kidney disease.
- History of auto immune disease.

Ethical Considerations

An informed consent will be taken from each patient. The aim of the research was explained to patients. No harm was caused to patients as blood samples were routinely collected from patients.

Consent form:

Patient name:

Hospital number:

Information to the participant:

The procedure

- The results of your blood results and hospital data will be used in this clinical trial.
- An extra blood sample will be withdrawn at the time of diagnosis which is a simple procedure with no known complications.
- All your data and blood results, will saved confidentially and it will appear as a number in the results.
- The results of these investigation will not affect your management plan.

Methodology

An informed consent will be taken from each participant.

For all patients included in the study the following were done

1- *Thorough history taking* with special emphasis on:

- The gestational age will be estimated according to the last menstrual period and confirmed by a first trimesteric ultrasound.
- Past history to ensure the absence of any medical disorder included in exclusion criteria.

2- *General, abdominal and local examination* with particular emphasis on vital sign monitoring records.

Laboratory Investigations:

- **Haematological tests**
  - *Sampling:*

Five (5.0) ml of venous blood will be collected from each patient, it will be divided as follows:

- **Two (2.0) ml** added to an EDTA containing sterile tube for assessment of:

a) *Complete blood count (CBC)*, for estimation of hemoglobin concentration and platelet count using XE-2100® (Sysmex Corporation, Kobe, Japan) automated hematology system.

b) *Morphological assessment of different blood cells* with special emphasis on estimation of schistocyte counts (if present), achieved by microscopic examination of Romanwsky-stained PB smears prepared from an EDTA-anticoagulated PB sample**.** Schistocytes counting will be performed in at least 1000 RBCs in optimal areas of the film, with interpretation of results following the ICSH recommendations for identification, diagnostic value, and quantitation of schistocytes **(Zini et al., 2011).**

c) *Reticulocyte count:* by microscopic examination of supravitally stained PB smear.

d) Estimation of IPF-% and A-IPC: All samples will be analyzed within 4 h of collection, using the XE-2100® (Sysmex Corporation, Kobe, Japan) automated hematology system. Using a carefully designed gating system in the XE-2100 optical (fluorescence) reticulocyte/platelet channel it is now possible to reliably quantify the IPF, employing upgraded software (XE-Pro Series; Sysmex). The flow cytometric IPF determination uses a proprietary fluorescent dye containing polymethine and oxazine. These two dyes penetrate the cell membrane, staining the RNA in the red cell and platelet reticulocytes. The stained cells are passed through a semiconductor diode laser beam and the resulting forward scatter light (cell volume) and fluorescence intensity (RNA content) measured. A computer algorithm discriminates the mature and IPF by the intensity of forward scattered light and fluorescence. The XE-2100 instrument used in the study was equipped with upgraded software for the data analysis of the IPF, which applies a preset gate to separate the two platelet populations. Mature platelets appear as blue dots and the immature platelets are displayed as green dots, the latter constituting the IPF parameter. Immature platelet fraction is usually expressed as a proportional value of the total optical platelet count to indicate the rate of platelet production, although an absolute count can also be obtained **(Briggs et al., 2004).**

- **Three (3.0) ml** are to be added to a sterile plain tube for serum assessment of: lactate dehydrogenase (LDH), serum bilirubin, liver enzymes (ALT & AST), serum uric acid, kidney function tests, using HITACHI 912 automatic analyzer (Roche Diagnostics GmbH. Sandhofer Str. Mannheim, D-68298 Germany).

Statistical methods:

Data will be analyzed using using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA) and MedCalc© version 14 (MedCalc© Software bvba, Ostend, Belgium).

The Shapiro-Wilk test to examine the normality of numerical data distribution. Normally distributed numerical variables will be presented as mean ± SD and inter-group differences were compared using one-way analysis of variance (ANOVA) for multiple group comparison with application of the Schéffé test for post hoc pair-wise comparison, whenever the ANOVA test revealed a statistically significant difference among the groups.

Skewed numerical will be presented presented as median (interquartile range) and inter-group differences were compared non-parametrically using the Kruskal-Wallis test.

Categorical variables were presented as number (%) and differences will be compared using Fisher's exact test.

Correlations among numerical variables will be tested non-parametrically using the Spearman rank correlation.