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ORIGINAL ARTICLE

# Morphological Changes of Red Blood Cells in Peripheral Blood Smear of Patients with Pregnancy-related Hypertensive Disorders

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**Background and Aims.** Pregnancy-related hypertensive disorders are complications in which risk factors are identified such as nulliparity, age, malnutrition, obesity and social issues. Those statements are explained by theories of abnormal placentation, immunological inadequacy, genetics and oxidative stress, but all theories converge in endothelial damage, which is able to mechanically deform and hemolyze erythrocytes as they pass through the capillaries. Given the effects of endothelial damage, the aim of the study was to determine erythrocyte alterations in peripheral blood smear of patients with hypertensive disorders of pregnancy that could be used as prognostic condition.

**Methods.** We performed a prospective, descriptive and observational study where all patients with hypertensive disorders admitted to the obstetrics and gynecology service of a specialty hospital were recruited. Patients who provided signed informed consent underwent peripheral blood smear. Results were tabulated in percentage graphics and analyzed with Cramer's V based on  $\chi^2$ . The peripheral blood smear consisted of an extended drop of peripheral blood from the patient with subsequent hematological staining done with Romanowsky stain.

**Results.** A total of 119 samples were analyzed; 74% showed abnormal morphology of erythrocytes and the most frequent abnormality was the presence of schistocytes in up to 39% of samples. Descriptive analysis showed a degree of association to independent variables with Cramer's V = 0.41 value ( $p < 0.05$ ).

**Conclusions.** A high percentage of patients with hypertensive disorders of pregnancy show some morphologic alterations of erythrocytes in peripheral blood smear. © 2015 IMSS. Published by Elsevier Inc.

**Key Words:** Preeclampsia, Blood smear, Schistocytes.

## Introduction

Hypertensive disorders that complicate pregnancy are part of the triad along with hemorrhage and sepsis, which significantly contribute to maternal morbidity and mortality (1,2). The manner in which pregnancy incites or aggravates hypertension is still unclear and remains one of the most

important and unresolved issues in obstetrics. Hypertensive disease associated with pregnancy is characterized by reduction of systemic perfusion generated by vasospasm and activation of the coagulation system (3,4).

Within the spectrum of preeclampsia (PE)/eclampsia, various etiological possibilities are identified such as genetic, immunological, biochemical, hemodynamic, hemorheological, hematological, histopathological, oxidative stress and endothelial dysfunctions as well as changes in trophoblastic invasion in which vascular endothelial damage and vasospasm are the final pathophysiological events (5,6). However, it has been postulated that an initial

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event in PE reduces placental perfusion, which leads to a generalized dysfunction of the maternal vascular endothelium by as yet undefined mechanisms. The placenta has been considered as the origin of pathogenic focus because termination of pregnancy offers definitive treatment (7,8).

One of the systemic manifestations of this gestational pathology is intravascular hemolysis secondary to thrombotic microangiopathy, i.e., a process that basically results in endothelial damage of the small vessels followed by platelet consumption, microthrombosis and erythrocyte fragmentation with multiple cellular and tissue compromise, most notably in the liver, kidneys, brain and the fetoplacental structures themselves (9,10). Microangiopathies are uncommon disorders characterized by thrombocytopenia, microangiopathic hemolysis, and multiple organ dysfunctions. Histologically, these disorders are identified by endothelial damage and by hyaline microthrombus in the lumen of the arterioles and capillaries (11,12). It has been reported that pregnancy-associated thrombotic microangiopathy acts as a precipitating factor, implying that this may precipitate a cascade of events with disruption of the endothelium and vascular microthrombosis including recurrence in subsequent pregnancies (13,14).

Hemolysis is defined as the presence of microangiopathic hemolytic anemia, resulting in a mechanical lesion by the passage of red blood cells through the blood vessels with vasospasm (15,16). Similarly, fibrin deposits in endothelial cells also favor the lesion and destruction of the erythrocytes, which gives the appearance in the peripheral blood smear of triangular cells and burr cells, poikilocytosis, schistocytes and spherocytes with polychromasia in up to 54–86% of cases. Sensitivity of the smear is 64% and specificity 90% (4,11,17,18). There are other sensitive methods for the recognition of hemolysis in serum haptoglobin determination. This acute-phase protein with a very short half-life is reduced in 85–97% of cases with microangiopathic anemia (19). Nevertheless, we proposed to determine alterations of erythrocytes in peripheral blood smears of patients with hypertensive disorders of pregnancy, which have not been well elucidated and may be an early prognostic indicator.

## Patients and Methods

All patients admitted to the OB-GYN hospital with >20 weeks of gestational age and diagnosis of gestational hypertension, chronic hypertension, chronic hypertension with superimposed PE, mild PE, severe PE, and eclampsia according to the established international guidelines were included in the study (20). A prospective, descriptive and observational study was performed during the period from 2011 and 2012. Institutional ethics committee approval was obtained. Recruited patients provided signed informed consent and underwent peripheral blood smear. The result

was entered in an Excel spreadsheet. The peripheral blood smear consisted of an extended drop of peripheral blood from an earlobe puncture. Blood was placed on a slide with subsequent hematological staining done with Romanowsky stain (methylene blue, eosin and Wright stain). For the study of the smear, a Carl Zeiss microscope (Axio Star Plus, Carl Zeiss, Jena, Germany) was used. The smear was analyzed with 10, 40 and 100x objectives with oil immersion. Morphological changes of the erythrocytes present were identified in each of the hypertensive states in order of percentages. All blood analyses included an erythrocyte evaluation according to size, shape, color and inclusions. Morphology of the red cells was evaluated in the area of a smear where the red blood cells were united but not overlapping or at the end of the smear because in that area the cells flatten and show no central pallor. The results obtained were tabulated in percentage graphics. Cramer's V based on  $\chi^2$  (Excel 2010) was used for between-group comparison.

Morphological changes were classified as *echinocytes*, *burr cells*, *thorny cells*, which are cells with numerous short projections throughout their entire surface. Spiculated erythrocytes of the same size or smaller than normal erythrocytes maintain their central pallor and are not the result of membrane abnormalities and the change is reversible (Figure 1A). Schistocytes are broken or fragmented erythrocytes due to mechanical damage of the cell and may have different forms such as triangular, helmet, or comma shaped (Figure 1B). Spherocytes are spherical erythrocytes that have lost their biconcave character and are without a clear center. These have a dense content of hemoglobin and can be smaller than normal (microspherocytes). It is a consequence of a membrane alteration, which becomes rigid and may not deform (Figure 1C). Dacrocytes or "tear drop cells" are elongated red blood cells at one end forming a cell with the appearance of a tear drop and are of varying size. The pale center of the dacrocyte may be normal or increased and may need to stretch in order to enter into the circulation (Figure 1D).

## Results

A total of 1050 pregnant patients were registered during the study period. There were 119 patients with some degree of gestational hypertension, representing 13% of the total pregnancies. Table 1 shows the division by age group. There were 74% of patients with some form of morphological change: 39% schistocytes, 19% echinocytes, 12% spherocytes and 4% dacrocytes in the peripheral blood smear (Table 2).

Of the patients who presented echinocytes ( $n = 47$ ) in the peripheral blood smear, 54% had a diagnosis of severe PE, 24% with diagnosis of gestational hypertension, 10% had a diagnosis of mild PE, 6% had systemic arterial hypertension and 6% had arterial hypertension with

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