ARE HYPERTENSIVE DISORDERS OF PREGNANCY CAPABLE OF INFLUENCING THE STABILITY OF THE ERYTHROCYTE MEMBRANE?

DESORDENS HIPERTENSIVAS DA GESTAÇÃO SÃO CAPAZES DE INFLUENCIAR A ESTABILIDADE DA MEMBRANA DE ERITRÓCITOS?

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RESUMO: Os distúrbios hipertensivos da gestação são processos complexos associados a alterações placentárias e incluem elevações de enzimas hepáticas, alterações no perfil lipídico e na membrana eritrocitária. **Objetivo:** Avaliar a existência de alterações na estabilidade da membrana eritrocitária (*EMS*) nas doenças hipertensivas da gravidez. **Métodos:** Uma população de 32 gestantes foi estratificada em grupo controle, hipertensão gestacional, pré-eclâmpsia sobreposta à hipertensão crônica e pré-eclâmpsia grave. A estabilidade osmótica da membrana eritrocitária foi representada pelos valores mínimo (Amin) e máximo (Amax) da absorbância da hemoglobina livre, o inverso da concentração de NaCl que pode promover 50% de hemólise (1/H50) e a variação na concentração de NaCl necessário para causar 100% de lise (dX). **Resultados:** Observou-se aumento significativo ($p < 0,05$) de 1/H50 no grupo com hipertensão gestacional, embora na pré-eclâmpsia sobreposta à hipertensão crônica tenha ocorrido redução limítrofe ($0,05 < p < 0,10$) nesta variável. Além disso, foi observada diminuição significativa ($p < 0,01$) nos valores de Amin entre gestantes com pré-eclâmpsia grave. **Conclusão:** Todas as doenças hipertensivas da gestação consideradas neste estudo estiveram associadas a algum tipo de alteração na estabilidade da membrana eritrocitária.

Palavras-chave: Hipertensão; Pré-eclâmpsia; Glóbulos vermelhos; Membrana celular.

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ABSTRACT: Hypertensive disorders of pregnancy are complex processes associated with placental changes and include elevations of liver enzymes, changes in lipid profile and erythrocyte membrane. **Objective:** To evaluate the existence of changes in erythrocyte membrane stability (EMS) in hypertensive diseases of pregnancy. **Methods**: A population of 32 pregnant women was stratified into control group, gestational hypertension, preeclampsia superimposed on chronic hypertension and severe preeclampsia. The osmotic stability of the erythrocyte membrane was represented by minimum (A_{min}) and maximum (A_{max}) values of the free hemoglobin absorbance, the inverse of the NaCl concentration that can promote 50% hemolysis ($1/H_{50}$) and the variation in the concentration of NaCl required to cause 100% lysis (dX). **Results**: A significant increase ($p < 0.05$) in $1/H_{50}$ was observed in the group with gestational hypertension, although in preeclampsia superimposed on chronic hypertension occurred a borderline reduction ($0.05 < p < 0.10$) in this variable. Furthermore, a significant decrease ($p < 0.01$) was observed in the A_{min} values among pregnant women with severe preeclampsia. **Conclusion**: All of gestation hypertensive diseases considered in this study was associated with some kind of change in the erythrocyte membrane stability.

Keywords: Hypertension; Pre-eclampsia; Red blood cells; Cell membrane.

1 INTRODUCTION

Hypertensive disorders of pregnancy are a common problem of high maternal and fetal mortality. In pregnancy hypertension is associated with placental changes and, in more severe cases, there is change in the profile of liver enzymes, hemolysis and reduction in the number of platelets (CARVALHO; FAUNDES; SANTOS, 1997; LEEMAN; FONTAINE, 2008). Hypertensive disorders of pregnancy are classified into four groups: pre-eclampsia, chronic hypertension (of any etiology), preeclampsia superimposed on chronic hypertension and gestational hypertension (Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy, 2013).

In mild preeclampsia there is increase in pressure accompanied by proteinuria (excretion of 0.3 g protein in the 24 hours urine or intensity ≥ 1+ on urine dipsticks). Severe preeclampsia is diagnosed if the systolic blood pressure is ≥ 160 mmHg and the diastolic blood pressure is ≥ 110 mmHg, when measured in patients at rest at four-hour intervals, or when hypertension is associated with thrombocytopenia (platelet count < $10^3/mm^3$), worsening in the hepatic function (elevation of blood levels of hepatic enzymes more than twice the upper reference range limit), worsening in the renal function (creatinine > 1.1 mg/dL), pulmonary edema and signs of hypertensive encephalopathy (Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy, 2013).

Chronic hypertension is characterized by elevated blood pressure before pregnancy or before completing 20 weeks of gestation. In preeclampsia superimposed on chronic hypertension occurs worsening in the arterial blood pressure. In preeclampsia the change in pressure is accompanied by proteinuria. Gestational hypertension is characterized by an isolated change in the blood pressure (Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy, 2013; MAGEE; PELS; HELEWA; REY *et al.*, 2014).

Hypertension was associated with changes in the erythrocyte membrane, such as changes in the behavior of its sodium channels and lithium (CANESSA; ADRAGNA; SOLOMON; CONNOLLY *et al.*, 1980; TSUDA; MINATOGAWA; TSUDA; SHIMA *et al.*, 1987) and also in its lipid composition, due to changes in the blood lipid levels, such as decrease in HDL-C and increase in VLDL- and LDL-C (ZICHA; KUNES; DEVYNCK, 1999).

The erythrocyte membrane's ability to change its shape and resist the force determined by blood flow and by friction with the vessel wall (MOHANDAS, N.; EVANS, E., 1994) is mainly due to the cytoskeleton proteins and to its proper lipid composition (MOHANDAS, NARLA; EVANS, EVAN, 1994). The erythrocyte membrane's ability to preserve its structure before any kind of situation is called stability (CUNHA; ARVELOS; COSTA; PENHA-SILVA, 2007; PENHA-SILVA; ARVELOS; CUNHA; AVERSI-FERREIRA *et al.*, 2008; PENHA-SILVA; FIRMINO; DE FREITAS REIS; DA COSTA HUSS *et al.*, 2007).

The erythrocyte membrane stability can be influenced by intrinsic factors of the membrane and the cell, but also by extrinsic factors, which include the composition and properties of the medium and the wide range of factors that can affect metabolism. The proper dynamics of erythropoiesis, which will affect qualitatively and quantitatively erythrocytes, is influenced by several key nutritional factors, such as iron, amino acids, folate, cobalamin, pyridoxine and lipid membrane.

Furthermore, the lipid composition of the membrane of mature erythrocytes is an intrinsic condition that can be influenced by the nature and levels of circulating lipids (PACETTI; GAGLIARDI; BALZANO; FREGA *et al.*, 2016). This influence is related to the dynamics of the exchanges that take place between the membrane of mature circulating erythrocyte and plasma lipoproteins. The circulating erythrocytes is capable of receiving cholesterol from LDL (COOPER; LESLIE; FISCHKOFF; SHINITZKY *et al.*, 1978), which is an important physiological mechanism for regulating the fluidity and

stability that cell membrane. That is why increase in blood cholesterol levels is associated with increase in counts not only of erythrocytes, but also of other blood cells such as platelets, at least in certain segments of the population (FESSLER; ROSE; ZHANG; JARAMILLO *et al.*, 2013).

However, an excessive increase in LDL-cholesterol (LDL-C) leads to excessive incorporation of cholesterol in the erythrocyte membrane, with a decrease in the fluidity and stability of the RBC membrane. That is why a greater increase in blood cholesterol is associated with the decrease of erythrocytes and platelets counts in other segments of the population (FESSLER; ROSE; ZHANG; JARAMILLO *et al.*, 2013). If the blood cholesterol levels reach the pathological levels present in individuals with familial hypercholesterolemia, excessive incorporation of cholesterol in the RBC membrane will cause the so-called spur-cell anemia (COOPER, 1969).

Thus, it is not without sense that the variables usually determined in erythrogram are affected by the cholesterol levels in the blood (BERNARDINO NETO; DE AVELAR; ARANTES; JORDAO *et al.*, 2013; DE ARVELOS; ROCHA; FELIX; DA CUNHA *et al.*, 2013; DE FREITAS; MARQUEZ-BERNARDES; DE ARVELOS; PARAISO; GONCALVES; MASCARENHAS NETTO RDE *et al.*, 2014). Indeed, an increase in the RDW was associated with high cholesterol content in the erythrocyte membrane (TZIAKAS; CHALIKIAS; GRAPSA; GIOKA *et al.*, 2012). Certainly this is the reason why the erythrocyte membrane stability was associated with the blood cholesterol levels and the RDW (DE ARVELOS; ROCHA; FELIX; DA CUNHA *et al.*, 2013; DE FREITAS; MARQUEZ-BERNARDES; DE ARVELOS; PARAISO; GONCALVES; F. *et al.*, 2014; NETTO; FABBRI; DE FREITAS; NETO *et al.*, 2014). This is very important and can have significant clinical implications, since the RDW has been associated with the prediction of a wide range of degenerative diseases (MALANDRINO; WU; TAVEIRA; WHITLATCH *et al.*, 2012; PATEL; FERRUCCI; ERSHLER; LONGO *et al.*, 2009; PATEL; MOHANTY; KANAPURU; HESDORFFER *et al.*, 2013; PATEL; SEMBA; FERRUCCI; NEWMAN *et al.*, 2010; TZIAKAS; CHALIKIAS; GRAPSA; GIOKA *et al.*, 2012; TZIAKAS; CHALIKIAS; STAKOS; BOUDOULAS, 2010; ZALAWADIYA; VEERANNA; PANAICH; AFONSO, 2012; ZALAWADIYA; ZMILY; FARAH; DAIFALLAH *et al.*, 2011), because it is able to reflect the harmful implications of dyslipidemia in the etiology of endothelial and gas exchange dysfunctions, what puts the erythrocyte as a protagonist in the etiology of the vascular degenerative disorders and their pathophysiological complications.

The membrane stability variables have shown to be promising to reflect changes in the lipid profile and erythrocyte indices associated with aging (DE FREITAS; MARQUEZ-BERNARDES; DE ARVELOS; PARAISO; GONCALVES; MASCARENHAS NETTO RDE *et al.*, 2014; PENHA-SILVA; FIRMINO; DE FREITAS REIS; DA COSTA HUSS *et al.*, 2007), energy restriction imposed by bariatric surgery (DE ARVELOS; ROCHA; FELIX; DA CUNHA *et al.*, 2013), physical activity (PARAISO; DE FREITAS; GONCALVES; DE ALMEIDA NETO *et al.*, 2014) and even infectious diseases as malaria (NETTO; FABBRI; DE FREITAS; NETO *et al.*, 2014).

As hypertensive pregnancy diseases are generally associated with changes in serum lipids and in the turnover rate of erythrocytes, it is possible that those diseases are also associated with changes in the erythrocyte membrane stability. In this context, this study aimed to evaluate the existence of changes in erythrocyte membrane stability in hypertensive diseases of pregnancy.

2 MATERIAL AND METHODS

2.1 POPULATION

The study project was approved by the Local Ethics Committee (3008/2014). The study included 32 pregnant patients who sought the Clinical Hospital of the Federal University of Uberlândia, Minas Gerais, Brazil, from July 2015 to April 2016. Pregnant patients who showed no changes in blood pressure, blood count and their biochemical tests were included in the control (C) group (n = 9). Patients who had an elevation in arterial blood pressure after completing 20 weeks of gestation were included in the gestational hypertension (GH) group (n = 7).

Patients who already had a history of hypertension before pregnancy and developed a more severe pressure frame associated with proteinuria were included in preeclampsia superimposed on chronic hypertension (PSCH) group (*n=7*). The patients with blood pressure ≥ 140 x 90 mmHg after 20 weeks of pregnancy associated with proteinuria or hypertension associated with thrombocytopenia, hepatic, renal and cerebrovascular disorders or blood pressure ≥ 160 x 110 mmHg were included in the severe preeclampsia (SP) group (*n=9*). The study excluded pregnant women who have other diseases and/or were users of tobacco or abuse drugs.

2.2 COLLECTION OF BLOOD SAMPLES

Blood samples were collected by venipuncture into tubes containing K_3EDTA (for hematologic analysis and determining the stability of erythrocytes) and in tubes without anticoagulant (for biochemical analysis) (Vacutainer; Becton Dickinson, Juiz de Fora, Brazil).

2.3 DETERMINATION OF THE OSMOTIC STABILITY OF HUMAN ERYTHROCYTES

The determination of the osmotic stability of erythrocytes was performed as described by Penha-Silva et al (PENHA-SILVA; FIRMINO; DE FREITAS REIS; DA COSTA HUSS *et al.*, 2007). Initially, duplicate sets of test tubes containing 1 ml of 0.1-1.5 g/dL NaCl solutions (Labsynth, Diadema, SP, Brazil) were preincubated at 37 °C for 10 min. After addition of 10 μL of total blood and gentle agitation, the tubes were incubated at 37 °C for 30 min. After centrifugation at 1500 x *g* for 10 min, the supernatants were used to evaluate the optical density at 540 nm (A_{540}) in a UV-VIS spectrophotometer (Shimadzu™, model UV1650TC, Japan). The graphs of A_{540} as a function of the NaCl concentration (X) were fitted by non-linear regression in accordance with the Boltzmann equation:

$$A_{540} = \frac{A_{max} - A_{min}}{1 + e^{(X - H_{50})/dX}} + A_{min} \quad (1),$$

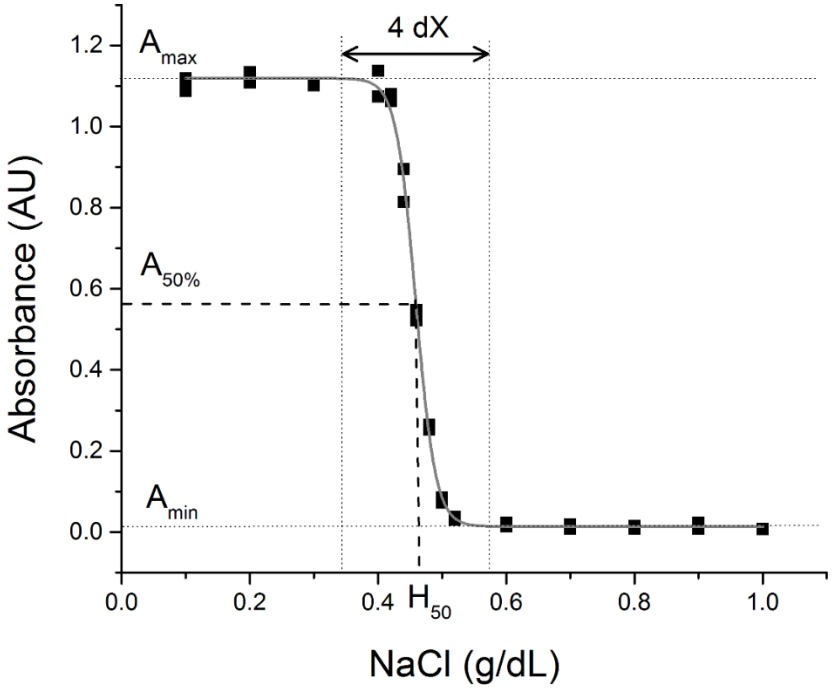
where A_{max} and A_{min} represent respectively the minimum and maximum plateaus of A_{540}, H_{50} is the NaCl concentration capable of promoting 50% hemolysis, and dX is the variation in concentration of NaCl responsible for 100% hemolysis.

A typical curve used in determining the erythrocyte membrane stability variables is shown in Figure 1. A condition of isotonicity with the blood occurs in the right region of the curve, where there is a plateau that defines the variable A_{min}, which represents the amount of basal hemolysis present in the sample of blood taken from each volunteer. A decrease in the medium tonicity is associated with an increase in absorbance at 540 nm, due to increase in the amount of hemoglobin that is released into solution by the hemolysis process, which defines a sigmoidal curve whose upper plateau sets the variable A_{max}.

Additionally, this curve passes through an intermediate point that defines the variable H_{50}, which represents the concentration of NaCl required to promote 50% hemolysis. The curve also defines the variable dX, which represents the change in salt concentration needed to promote 100% hemolysis. The variable dX has a same direction

relationship with the osmotic stability of erythrocytes, but the H_{50} variable has an opposite direction relationship and, therefore, it was used as a $1/H_{50}$, so that an increase in the values of both dX and $1/H_{50}$ could indicate increased osmotic stability of erythrocytes.

Figure 1: Typical curve of osmotic fragility haemolysis.



A_{min} and A_{max} represent respectively the minimum and maximum mean value of absorbance. H_{50} is the NaCl concentration capable of promoting 50% haemolysis. dX is the NaCl concentration range responsible for 100% haemolysis. **Source:** Prepared by the authors.

2.4 DETERMINATION OF HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

The hematological parameters were obtained using an automated system (Sysmex K4500; Sysmex Corporation™, Mundelein, IL, USA). These parameters include erythrocytes (RBC), platelets (Plt) reticulocytes counts and the values of hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV) and prothrombin time (PT).

The biochemical parameters were measured using an automated analyzer (Architect c8000, IL, USA). These parameters include triglycerides (TGC), total cholesterol (t-C), very-low density lipoprotein cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase

(ALT), alkaline phosphatase (AP), creatinine (C), uric acid (UA), sodium (Na^+), potassium (K^+), indirect bilirubin (iB), total bilirubin (tB), serum iron (Fe) and human serum albumin (HSA).

The reference values (women over 16 years of age) were: RBC, 4.3-5.0 x $10^6/mm^3$; Hb, 12.0–16.0 g/dL; Ht, 35.6–48.6%; MCV, 82–98 fL; MCH, 27–31 pg; MCHC, 32.9–36%; RDW, 12-15%; RI, 0.5-2.5%; Plt, 150-450 x $10^3/mm^3$; MPV, 7-10 fL; t-C, <200 (optimum) and ≥240 mg/dL (high); TGC, <150 (optimum) and >201 mg/dL (high); VLDL-C, up to 40 mg/dL; LDL-C, <100 (good) and >160 mg/dL (high); HDL-C, <45 (low) and >65 mg/dL (ideal); AST, 5-34 IU/L; ALT, 0-55 IU/L; LDH, 100-190 IU/L; AP, 35-104 IU/L; urea, 10-45 mg/dL; creatinine, 0.7-1.2 mg/dL; uric acid, 2.4-6.0 mg/dL; Na^+, 135-145 mEq/L; K^+, 3.7-5.6 mEq/L; iB, 0.2-0.8 mg/dL; tB, < 1.2 mg/dL; Fe, 65-175 μg/dL; ferritin, 6-159 ng/mL; and HAS, 3.5-5.2 g/dL.

2.5 STATISTICAL ANALYZES

The normality of the data was assessed by the Shapiro-Wilk test. Most variables were not normally distributed. Median and interquartile range were used to represent the measurements of biochemical, hematological and membrane stability variables. The comparison between groups was performed using the Mann-Whitney test. Differences associated with p values < 0.05 were considered statistically significant. Differences with *p* values between 0.05 and 0.10 were considered borderline. All analyzes were performed using the software Origin 8.5 Professional (Microcal, Northampton, MA, USA) and/or GraphPad Prism 6.01 (La Jolla, CA, USA).

3 RESULTS

Table 1 shows the baseline characteristics of the four groups that constitute the study population. The variables values were presented as median ± interquartile range. The groups were compared with respect to all variables using the Mann-Whitney test.

The group with gestational hypertension (GH) showed a significant increase in membrane stability parameter $1/H_{50}$ compared with the group with preeclampsia superimposed on chronic hypertension (PSCH) (Figure 2).

The PSCH group showed a decrease in the parameter $1/H_{50}$ compared to the group of women with severe preeclampsia (SP) (Figure 2). When compared with the control group, the SP group showed significant elevations in ALT and creatinine and borderline

elevations in ferritin. A borderline decrease in LDH was also observed in the SP group compared to the control group (Table 1).

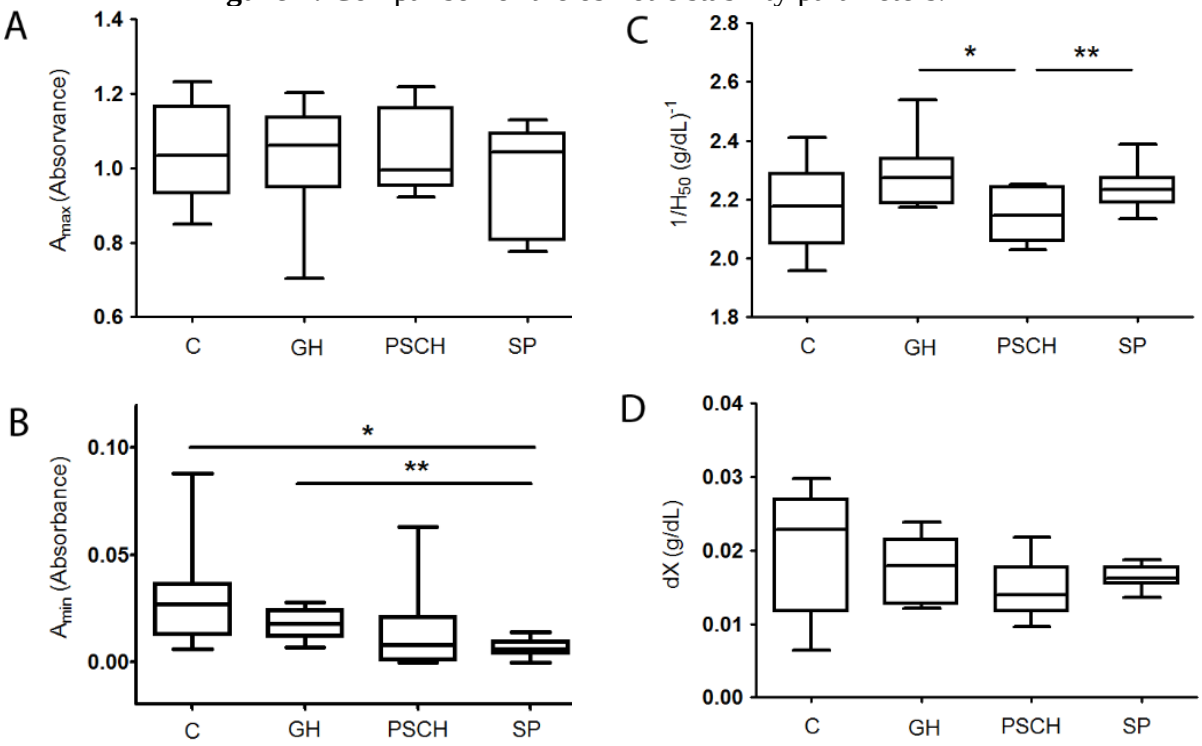
Table 1 Baseline characteristics of the study population

Variables	Control (N=9)	Gestacional Hypertension (N=7)	Superimposed Preeclampsia (N=7)	Severe Preeclampsia (N=9)
Maternal age (years)	20 (6)	31 (7)	32 (18)	33 (7)
Gestacional age (weeks)	40.7 (0.75)	37 (4.5)	38 (15)	33.7 (9.5)
SBP (mmHg)	115 (10)	150 (00)	145 (40)	160 (10)
DBP (mmHg)	73 (10)	90 (10)	100 (10)	110 (20)
Ht (%)	34.5 (3.8)	38.4 (8.5)	35.1 (6.7)	36.9 (5.4)
Hb (mg/dL)	11.3 (1.7)	12.6 (3.0)	11.8 (2.7)	12.3 (2.0)
RBC (10^6/mm^3)	4.33 (0.45)	4.21 (0.7)	4.16 (1.2)	4.15 (0.9)
MCV (fL)	80.8 (9.2)	89.1 (11.3)	87.0 (7.5)	87.3 (5.0)
RDW (%)	14.0 (1.0)	13.0 (1.0)	13.0 (1.0)	13.0 (3.0)
MCH (pg)	27.0 (4.4)	29.9 (3.2)	30.6 (3.1)	28.8 (1.0)
MCHC (%)	33.4 (1.3)	33.6 (1.6)	33.8 (1.7)	33.5 (0.8)
RI (%)	1.8 (2.1)	0.85 (0.3)	1.0 (1.8)	0.65 (0.8)
Plt (10^3/mm^3)	2.65 (8.6)	1.79 (6.6)	1.66 (7.8)	2.09 (7.3)
MPV (fL)	7 (2)	9 (3)	9 (1)	8 (2)
PT (%)	89.85 (20.3)	1 (49.56)	0.99 (0.03)	1 (54)
t-C (mg/dL)	237 (69)	271.5 (73)	214 (93)	211 (35)
TGC (mg/dL)	191.5 (72)	221 (122)	204 (128)	166 (146)
VLDL-C (mg/dL)	39 (4)[a,d]	67.4 (33.82)[b,d]	48 (36.6)	30 (11.8)[a,b]
LDL-C (mg/dL)	137 (58)	102.6 (62.3)	112.9 (47)	131.15 (38.25)
HDL-C (mg/dL)	67 (14)	69.45 (24.9)	66.5 (20.8)	61.5 (8.5)
AST (IU/L)	14.4 (2.6)[d]	12 (5)[a]	15 (4)	18 (6)[a,d]
ALT (IU/L)	7 (2) [a,b]	8 (6)[d]	11 (4)[a]	13 (6)[b,d]
LDH (IU/L)	215 (50)[d,e]	176.5 (35)[a,d]	183 (35)[b,e]	255 (73)[a,b]
AF (IU/L)	-	125 (62)	108 (68)	76.5 (50)
Urea (mg/dL)	15.5 (3.6)	22 (14)	17 (25)	27 (26.5)
Creatinine (mg/dL)	0.49 (0.09)[a,b,c]	0.7 (0.2)[a]	0.6 (0.1)[b,d]	0.7 (0.1)[c,d]
Uric Acid (mg/dL)	4.6 (0.4)[a]	5.09 (1.52)[d]	4.55 (2.52)[b]	6.65 (1.49)[a,b,d]
Na^+ (mEq/L)	138 (2)	137 (4)	137 (5)	139 (2)
K^+ (mEq/L)	4.25 (0.57)	4.1 (1)	4 (1.2)	4.1 (0.7)
iB (mg/dL)	0.20 (0.08)	0.15 (0.035)	0.17 (0.08)	0.28 (0.27)
tB (mg/dL)	0.3 (0.01)	0.28 (0.08)	0.31 (0.3)	0.39 (0.34)
Fe (μg/dL)	65 (45)	119.1 (93.2)	73.7 (5.8)	82.1 (57.05)
Ferritin (ng/mL)	17.55 (16.79)[a,d]	67.43 (0)	60.44 (41.53)[d]	84.25 (185.48)[a]
HSA (g/dL)	3.495 (0.26)[a]	3 (0)	-	3.07 (0.52)[a]

[a, b and c] and [d and e] indicate statistically significant ($p<0.05$) and borderline differences ($0.05<p<0.10$), respectively, when present as pairs of common letters. **Abbreviations:** SPB, systolic blood pressure; DBP, diastolic blood pressure; A_{max}, absorbance at 540 nm associated with lysis of the whole population of erythrocytes; A_{min}, absorbance at 540 nm associated with residual lysis of the erythrocytes population; $1/H_{50}$, inverse the NaCl concentration capable of promoting 50% haemolysis; dX, variation in the concentration of NaCl responsible for total haemolysis; N, number of participants; Ht, hematocrit; Hb, hemoglobin; RBC, erythrocytes; MCV, mean corpuscular volume;

Abbreviations continuation, table 1:RDW, red cell distribution width; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RI, reticulocytes index; Plt, platelets; MPV, mean platelet volume; PT, prothrombin time; t-C, total cholesterol; TGC, triglycerides; VLDL-C, very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; AF, alkaline fosfatase; Na^+, sodium; K^+, potassium; iB, indirect bilirubin; tB, total bilirubin; Fe, serum iron; HSA, human serum albumin. **Source:** Prepared by the authors.

Figure 2: Comparison of the osmotic stability parameters.



A_{min} (**A**), A_{max} (**B**), $1/H_{50}$ (**C**) and dX (**D**) between groups (C, control; GH, gestational hypertension; PSCH, preeclampsia superimposed on chronic hypertension; SP, severe preeclampsia). *p<0.05 indicates statistically significant difference and **0.05<p<0.10 indicates borderline difference. **Source:** Prepared by the authors.

The SP group showed a decrease in membrane stability parameter A_{min} when compared to the groups GH and C (Figure 2). When compared with the control group, the SP group showed significant elevations of creatinine, uric acid, ferritin and ALT and a borderline elevation of AST, as well as a significant decrease in the levels of VLDL-C. The blood levels of AST and LDH of the SP group were significantly higher and the levels of VLDL-C were significantly lower in relation to the GH group. Blood levels of ALT and uric acid in the SP group also showed borderline elevations when compared to group with chronic hypertension. Furthermore, the blood levels of creatinine, uric acid

and LDH of the SP group were significantly higher when compared to the PSCH group (Table 1).

4 DISCUSSIONS

In the hypertensive diseases erythrocytes were subjected to aggressions of high blood pressure and friction with the vascular endothelium, which alter the shape of these cells and raise the rate of hemolysis (HERNANDEZ HERNANDEZ; VILLASENOR; DEL RIO ALVARADO; LUCACH *et al.*, 2015; HOFMEYR; BELFORT, 2009).

Indeed, in the group of pregnant women with PSCH the parameter $1/H_{50}$ was lower when compared to the SP group, but not in relation to the control group. However, the preservation of the value of $1/H_{50}$ in patients with gestational hypertension compared to the control group means that these erythrocytes remained osmotically resistant (Figure 2).

This preservation of the osmotic stability of erythrocytes could be due to an intrinsic factor, as the increase in the turnover of these cells. In fact, the turnover of red blood cells rises during pregnancy (LURIE; MAMET, 2000) and particularly in preeclampsia (TROEGER; HOLZGREVE; LADEWIG; ZHONG *et al.*, 2006).

On the other hand, it is possible that the preservation of the erythrocytes stability may be due to extrinsic factors such as an increase in the cholesterol exchange rate. A larger amount of circulating nHDL-C would enhance the availability of cholesterol for the erythrocyte membrane, with increase in its stability (BERNARDINO NETO; DE AVELAR; ARANTES; JORDAO *et al.*, 2013; CHABANEL; FLAMM; SUNG; LEE *et al.*, 1983; DE ARVELOS; ROCHA; FELIX; DA CUNHA *et al.*, 2013; DE FREITAS; MARQUEZ-BERNARDES; DE ARVELOS; PARAISO; GONCALVES; MASCARENHAS NETTO RDE *et al.*, 2014). Thus, the erythrocyte would become more resistant, which would imply higher values of $1/H_{50}$, as occurred in patients with gestational hypertension. Indeed, higher VLDL-C values were observed in patients with gestational hypertension compared to the control group of this study (Table 1). In addition, higher levels of triglycerides, VLDL- and LDL-C are common in hypertensive patients (BAGDADE; BUCHANAN; POLLARE; LITHELL, 1995), which could affect the membrane of erythrocytes (ZICHA; KUNES; DEVYNCK, 1999). The existence of more stable erythrocyte would mean that the *in vivo* hemolysis would not be contributing to elevation of the LDH levels, since red blood cells are an important source of the LDH found in plasma (CATANZARITE; STEINBERG; MOSLEY; LANDERS *et al.*, 1995;

VAZQUEZ-RODRIGUEZ; RIOS-GUTIERREZ; PAREDES-LOZANO; GARCIA-FLORES, 2016). Indeed, there was a borderline decrease in LDH levels in the group of pregnant women with gestational hypertension.

The significant decrease of Amin values in pregnant women with severe preeclampsia in the control group and borderline in the group with gestational hypertension (Figure 2) indicates that the red blood cells of pregnant women with SP had increased membrane stability. This variable is proportional to the rate of hemolysis already present even in a condition which is isotonic with blood. This means that the blood collected from pregnant women with preeclampsia had more stable erythrocytes even in the *in vivo* conditions of tonicity.

In light of the fact that, compared to GH and PSCH groups, there was an increase in LDH levels in the SP group, the improved stability of erythrocytes in the SP group may appear contradictory if *in vivo* hemolysis was the main source of this biomarker. However, since the AST and ALT levels were also increased in this group, this indicates that the major source of these biomarkers should not be erythrocytes but more probably other cells such as the hepatocytes. In fact, the liver is a known target of hypertensive aggression present in preeclampsia. Further evidence that the liver is the source of these enzymes is the decrease in the levels of VLDL-C observed in this group, since the hepatic impairment harm lipogenesis, esterification and assembly of VLDL. The decrease in HSA levels in this group is further evidence of hepatic dysfunction.

Thus, the increased stability of erythrocytes of pregnant women with pre-eclampsia should not be due to environmental factors, but rather from a factor that is intrinsically associated with erythropoiesis. This makes sense, since elevation of erythropoietin levels and increased renewal rate of erythrocytes occur during pregnancy (LURIE; MAMET, 2000) and particularly in severe preeclampsia (TROEGER; HOLZGREVE; LADEWIG; ZHONG *et al.*, 2006).

The results of this study seem quite consistent, especially because they also included increases in the blood levels of creatinine, uric acid and ferritin in the group of pregnant women with severe preeclampsia, as commonly occurs in this pathologic condition. (BARTON; SIBAI, 2004; BERHAN, 2016).

5 CONCLUSIONS

In summary, although the populations of these study groups are small, we can say that both in the gestational hypertension and in severe pre-eclampsia there is preservation

of the erythrocyte membrane stability, although by different mechanisms. These study findings suggest that the maintenance of the erythrocytes stability in gestational hypertension is associated with lipid exchange with lipoproteins, while the stability increase observed in severe preeclampsia is associated with increase in the erythrocytes turnover.

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REFERENCES

BAGDADE, J. D.; BUCHANAN, W. F.; POLLARE, T.; LITHELL, H. Abnormal lipoprotein phospholipid composition in patients with essential hypertension. **Atherosclerosis**, 117, n. 2, p. 209-215, Oct 1995.

BARTON, J. R.; SIBAI, B. M. Diagnosis and management of hemolysis, elevated liver enzymes, and low platelets syndrome. **Clin Perinatol**, 31, n. 4, p. 807-833, vii, Dec 2004.

BERHAN, Y. No Hypertensive Disorder of Pregnancy; No Preeclampsia-eclampsia; No Gestational Hypertension; No Hellp Syndrome. Vascular Disorder of Pregnancy Speaks for All. **Ethiop J Health Sci**, 26, n. 2, p. 177-186, Mar 2016.

BERNARDINO NETO, M.; DE AVELAR, E. B., Jr.; ARANTES, T. S.; JORDAO, I. A. *et al.* Bivariate and multivariate analyses of the correlations between stability of the erythrocyte membrane, serum lipids and hematological variables. **Biorheology**, 50, n. 5-6, p. 305-320, 2013.

CANESSA, M.; ADRAGNA, N.; SOLOMON, H. S.; CONNOLLY, T. M. *et al.* Increased sodium-lithium countertransport in red cells of patients with essential hypertension. **N Engl J Med**, 302, n. 14, p. 772-776, Apr 3 1980.

CARVALHO, M. A.; FAUNDES, A.; SANTOS, L. C. Pregnancy-induced hypertension and hyaline membrane disease. **Int J Gynaecol Obstet**, 58, n. 2, p. 197-202, Aug 1997.

CATANZARITE, V. A.; STEINBERG, S. M.; MOSLEY, C. A.; LANDERS, C. F. *et al.* Severe preeclampsia with fulminant and extreme elevation of aspartate aminotransferase and lactate dehydrogenase levels: high risk for maternal death. **Am J Perinatol**, 12, n. 5, p. 310-313, Sep 1995. Case Reports.

CHABANEL, A.; FLAMM, M.; SUNG, K. L.; LEE, M. M. *et al.* Influence of cholesterol content on red cell membrane viscoelasticity and fluidity. **Biophys J**, 44, n. 2, p. 171-176, Nov 1983.

COOPER, R. A. Anemia with Spur Cells - a Red Cell Defect Acquired in Serum and Modified in Circulation. **Journal of Clinical Investigation**, 48, n. 10, p. 1820-&, 1969.

COOPER, R. A.; LESLIE, M. H.; FISCHKOFF, S.; SHINITZKY, M. *et al.* Factors influencing the lipid composition and fluidity of red cell membranes in vitro: production of red cells possessing more than two cholesterols per phospholipid. **Biochemistry**, 17, n. 2, p. 327-331, Jan 24 1978.

CUNHA, C. C.; ARVELOS, L. R.; COSTA, J. O.; PENHA-SILVA, N. Effects of glycerol on the thermal dependence of the stability of human erythrocytes. **J Bioenerg Biomembr**, 39, n. 4, p. 341-347, Aug 2007.

DE ARVELOS, L. R.; ROCHA, V. C.; FELIX, G. P.; DA CUNHA, C. C. *et al.* Bivariate and multivariate analyses of the influence of blood variables of patients submitted to Roux-en-Y gastric bypass on the stability of erythrocyte membrane against the chaotropic action of ethanol. **J Membr Biol**, 246, n. 3, p. 231-242, Mar 2013.

DE FREITAS, M. V.; MARQUEZ-BERNARDES, L. F.; DE ARVELOS, L. R.; PARAISO, L. F. *et al.* Influence of age on the correlations of hematological and biochemical variables with the stability of erythrocyte membrane in relation to sodium dodecyl sulfate. **Hematology**, 19, n. 7, p. 424-430, Oct 2014.

DE FREITAS, M. V.; MARQUEZ-BERNARDES, L. F.; DE ARVELOS, L. R.; PARAISO, L. F. *et al.* Influence of age on the correlations of hematological and biochemical variables with the stability of erythrocyte membrane in relation to sodium dodecyl sulfate. **Hematology**, 19, n. 7, p. 424-430, Oct 2014.

FESSLER, M. B.; ROSE, K.; ZHANG, Y.; JARAMILLO, R. *et al.* Relationship between serum cholesterol and indices of erythrocytes and platelets in the US population. **J Lipid Res**, 54, n. 11, p. 3177-3188, Nov 2013.

HERNANDEZ HERNANDEZ, J. D.; VILLASENOR, O. R.; DEL RIO ALVARADO, J.; LUCACH, R. O. *et al.* Morphological changes of red blood cells in peripheral blood smear of patients with pregnancy-related hypertensive disorders. **Arch Med Res**, 46, n. 6, p. 479-483, Aug 2015.

HOFMEYR, G. J.; BELFORT, M. Proteinuria as a predictor of complications of pre-eclampsia. **BMC Med**, 7, p. 11, 2009.

Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. **Obstet Gynecol**, 122, n. 5, p. 1122-1131, Nov 2013. Consensus Development Conference.

LEEMAN, L.; FONTAINE, P. Hypertensive Disorders of Pregnancy. **American Family Physician**, 78, p. 93-100, 2008.

LURIE, S.; MAMET, Y. Red blood cell survival and kinetics during pregnancy. **Eur J Obstet Gynecol Reprod Biol**, 93, n. 2, p. 185-192, Dec 2000.

MAGEE, L. A.; PELS, A.; HELEWA, M.; REY, E. *et al.* Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: executive summary. **J Obstet Gynaecol Can**, 36, n. 7, p. 575-576, Jul 2014.

MALANDRINO, N.; WU, W. C.; TAVEIRA, T. H.; WHITLATCH, H. B. *et al.* Association between red blood cell distribution width and macrovascular and microvascular complications in diabetes. **Diabetologia**, 55, n. 1, p. 226-235, Jan 2012.

MOHANDAS, N.; EVANS, E. Mechanical properties of the red cell membrane in relation to molecular structure and genetic defects. **Annu Rev Biophys Biomol Struct**, 23, p. 787-818, 1994.

MOHANDAS, N.; EVANS, E. Mechanical Properties of the Red Cell Membrane in Relation to Molecular Structure and Genetic Defects. **Annual Review of Biophysics and Biomolecular Structure**, 23, p. 787-818, 1994.

NETTO, R. D. M.; FABBRI, C.; DE FREITAS, M. V.; NETO, M. B. *et al.* Influence of Plasmodium vivax malaria on the relations between the osmotic stability of human erythrocyte membrane and hematological and biochemical variables. **Parasitology Research**, 113, n. 3, p. 863-874, Mar 2014.

PACETTI, D.; GAGLIARDI, R.; BALZANO, M.; FREGA, N. G. *et al.* Changes in the Fatty Acid Profile and Phospholipid Molecular Species Composition of Human Erythrocyte Membranes after Hybrid Palm and Extra Virgin Olive Oil Supplementation. **J Agric Food Chem**, Jun 29 2016.

PARAISO, L. F.; DE FREITAS, M. V.; GONCALVES, E. O. A. F.; DE ALMEIDA NETO, O. P. *et al.* Influence of acute exercise on the osmotic stability of the human erythrocyte membrane. **Int J Sports Med**, 35, n. 13, p. 1072-1077, Dec 2014.

PATEL, K. V.; FERRUCCI, L.; ERSHLER, W. B.; LONGO, D. L. *et al.* Red blood cell distribution width and the risk of death in middle-aged and older adults. **Arch Intern Med**, 169, n. 5, p. 515-523, Mar 9 2009.

PATEL, K. V.; MOHANTY, J. G.; KANAPURU, B.; HESDORFFER, C. *et al.* Association of the red cell distribution width with red blood cell deformability. **Adv Exp Med Biol**, 765, p. 211-216, 2013.

PATEL, K. V.; SEMBA, R. D.; FERRUCCI, L.; NEWMAN, A. B. *et al.* Red cell distribution width and mortality in older adults: a meta-analysis. **J Gerontol A Biol Sci Med Sci**, 65, n. 3, p. 258-265, Mar 2010.

PENHA-SILVA, N.; ARVELOS, L. R.; CUNHA, C. C.; AVERSI-FERREIRA, T. A. *et al.* Effects of glycerol and sorbitol on the thermal dependence of the lysis of human erythrocytes by ethanol. **Bioelectrochemistry**, 73, n. 1, p. 23-29, Jun 2008.

PENHA-SILVA, N.; FIRMINO, C. B.; DE FREITAS REIS, F. G.; DA COSTA HUSS, J. C. *et al.* Influence of age on the stability of human erythrocyte membranes. **Mech Ageing Dev**, 128, n. 7-8, p. 444-449, Jul-Aug 2007.

TROEGER, C.; HOLZGREVE, W.; LADEWIG, A.; ZHONG, X. Y. *et al.* Examination of maternal plasma erythropoietin and activin A concentrations with regard to circulatory erythroblast levels in normal and preeclamptic pregnancies. **Fetal Diagn Ther**, 21, n. 1, p. 156-160, 2006. Research Support, Non-U.S. Gov't.

TSUDA, K.; MINATOGAWA, Y.; TSUDA, S.; SHIMA, H. *et al.* Alterations in calcium-handling of erythrocytes in spontaneously hypertensive rats. Calcium-induced changes in the osmotic fragility of erythrocytes in hypertension. **Jpn Heart J**, 28, n. 6, p. 923-931, Nov 1987.

TZIAKAS, D.; CHALIKIAS, G.; GRAPSA, A.; GIOKA, T. *et al.* Red blood cell distribution width: a strong prognostic marker in cardiovascular disease: is associated with cholesterol content of erythrocyte membrane. **Clin Hemorheol Microcirc**, 51, n. 4, p. 243-254, 2012.

TZIAKAS, D. N.; CHALIKIAS, G. K.; STAKOS, D.; BOUDOULAS, H. The role of red blood cells in the progression and instability of atherosclerotic plaque. **Int J Cardiol**, 142, n. 1, p. 2-7, Jun 25 2010.

VAZQUEZ-RODRIGUEZ, J. G.; RIOS-GUTIERREZ, C. D.; PAREDES-LOZANO, E. P.; GARCIA-FLORES, A. [Frequency and maternal complications of the criteria of hemolysis in preeclamptic patients with HELLP syndrome treated in an intensive care unit]. **Ginecol Obstet Mex**, 84, n. 1, p. 19-26, Jan 2016.

ZALAWADIYA, S. K.; VEERANNA, V.; PANAICH, S. S.; AFONSO, L. Red cell distribution width and risk of peripheral artery disease: analysis of National Health and Nutrition Examination Survey 1999-2004. **Vasc Med**, 17, n. 3, p. 155-163, Jun 2012.

ZALAWADIYA, S. K.; ZMILY, H.; FARAH, J.; DAIFALLAH, S. *et al.* Red cell distribution width and mortality in predominantly African-American population with decompensated heart failure. **J Card Fail**, 17, n. 4, p. 292-298, Apr 2011.

ZICHA, J.; KUNES, J.; DEVYNCK, M. A. Abnormalities of membrane function and lipid metabolism in hypertension: a review. **Am J Hypertens**, 12, n. 3, p. 315-331, Mar 1999.