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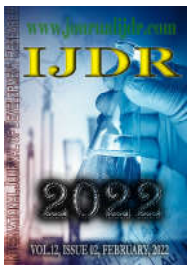

RESEARCH ARTICLE OPEN ACCESS

BIOCHEMICAL ANALYSIS OF OXIDATIVE STRESS AND PURINERGIC SYSTEM IN HYPERTENSIVE PREGNANT WOMEN

Bianca Devens Oliveira[1], Bruna Laís Hardt[1], Maiara Vanusa Guedes Ribeiro[2], Matheus Ribeiro Bizuti[3], Aline Mânica[4], Érica de Brito Pitilin[5], Julyane Felipette Lima[5], Margarete Dulce Bagatini[6] and Débora Tavares de Resende e Silva[6]

[1]Graduated in Nursing, Federal University of Fronteira Sul (UFFS), Chapecó, SC, Brazil; [2]Master's student of the Postgraduate Program in Biosciences and Pathophysiology (PBF), from the State University of Maringá (UEM), Maringá, Brazil; [3]Department of Medicine, Federal University of Fronteira Sul (UFFS), Chapecó, SC, Brazil; [4]Health Science Department, Community University of the Region of Chapecó (UNOCHAPECÓ), Chapecó, SC, Brazil; [5]Department of Nursig, Federal University of Fronteira Sul (UFFS), Chapecó, SC, Brazil; [6]Health Science Department, Community University of the Region of Chapecó (UNOCHAPECÓ), Chapecó, SC, Brazil

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Corresponding author:
Bianca Devens Oliveira

ABSTRACT

Introduction: Pregnancy is a physiological event, as well as a period of many expectations and questions, constituting a unique moment in the lives of many women. However, some women may present complications during pregnancy, among them, we can highlight Gestational Hypertension (GH) as causing several harmful effects to the maternal, fetal and neonatal organism. In this sense, recent studies indicate that there is a close relationship between hypertension and the purinergic system. **Objective:** To analyze the purinergic system and oxidative stress of hypertensive and non-hypertensive pregnant women in a Brazilian city. **Methods:** A quantitative survey was performed in which a total of 30 pregnant women, 15 of whom were at low risk (LR) and 15, with GH. **Results:** It was found that the hydrolysis of ATP and ADP indicated a greater activity of NTPDase in lymphocyte cells in pregnant women with GH. **Conclusion:** The analyzed components help in the vascular and endothelial regulation proper to the gestational period. Additionally, with regard to the parameters of the oxidative profile of the studied population, they showed a higher production of reactive oxygen species during the gestational period, which contributed to cell damage.

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INTRODUCTION

Pregnancy is a physiological event, understood as a period of many expectations and questions, constituting a unique moment in the life of many women. However, some women may present complications during the gestational period, among which, Gestational Hypertension (GH) stands out, responsible for several harmful effects to the maternal, fetal and neonatal organism [1]. During the gestational period, several changes occur in the maternal organism, namely: physiological, hormonal, vascular, platelet changes and changes in clotting factors. These changes are attenuated and are intrinsically linked to the predominance of GH. Because of this, the body acts by quickly controlling bleeding, activating and releasing biochemical receptors, thus preventing hemorrhagic complications [2].

GH is the main cause of hypertension during pregnancy. It is present around 17% in healthy nulliparous women and around 4% in multiparous women [3]. In this sense, recent studies indicate that there is an intimate relationship between hypertension and the purinergic system. This system is composed of nucleotides, enzymes and receptors that, together, help to maintain vascular integrity, as well as the regulation of the immune system. In hypertension, changes in the activity of enzymes in this system were observed in lymphocytes and platelets, cells that are closely related to thromboregulation [4]. In addition, women in the gestational period show an increase in reactive oxygen activities, as well as in the failure of antioxidant protection mechanisms, which results in oxidative damage. Therefore, oxidative stress is related in situations of hypoxia during high-risk pregnancies, in cases of pre-eclampsia and hypertension during pregnancy. Thus, there are several explanations

about the role of oxidative stress in pregnancy and the association in fetal and neonatal changes [5].

MATERIALS AND METHODS

Kind of study: The research was quantitative and experimental in nature. The experimental research is similar to the clinical trial, which seeks to compare similar elements from different groups, in this case, hypertensive and non-hypertensive pregnant women. The research was carried out in the Family Health Centers in the municipality of Chapecó, State of Santa Catarina, Brazil and in the reference unit Women's Clinic.

Ethical aspects: This study is approved by the Research Ethics Committee of the Federal University of Fronteira Sul (UFFS) Chapecó, Santa Catarina, under the Certificate of Presentation for Ethical Appraisal number 67328417.3.0000.5564, respecting resolution No. 466 of December 12, 2012 from the National Health Council, which considers respect for human dignity and protection of life for participants in scientific research involving human beings [6].

Study participants: The study included 30 pregnant women residing in Chapecó, Brazil, who were divided into two groups. In the first group, 15 low-risk pregnant women participated, without comorbidities associated with pregnancy (diabetes mellitus, gestational diabetes mellitus and hypertensive syndrome). In the second group, 15 hypertensive pregnant women participated, without other associated comorbidities (diabetes mellitus or gestational diabetes mellitus).

Data collection procedure: Through the verification of the control notebook of pregnant women who were attended at the Reference Center for High-Risk Pregnant Women in the municipality of Chapecó, as well as in the Family Health Centers, participants who identified the inclusion criteria of this study were identified. Based on this information, contact was made with the participants via telephone and the face-to-face meeting was scheduled to explain how the research would be conducted, its objectives and its likely outcome.
Data collection was carried out from October 2017 to December 2018. After signing the Free and Informed Consent Form, the following information was collected: gestational age, weight, height, maternal age, parity, date of last menstruation, probable date of delivery, if calcium or multivitamin was used, presence of renal impairment, if she was diagnosed with gestational hypertensive syndrome and/or gestational diabetes mellitus and continued medication. 10 mL of blood was also collected from each participant. Platelets, lymphocytes and serum were separated in the biochemistry laboratory at UFFS for further analysis. In addition, information was collected from their newborns about sex, birth weight, height, apgar score and prematurity.

Blood sample separation: The blood sample (lymphocytes, platelets and serum) was separated in order to analyze the activity of the purinergic system and oxidative stress. The separation of lymphocytes was performed from blood collected with EDTA and separated through a density gradient [7]. The serum was obtained by centrifugation at 3,500 rpm for 15 minutes. Platelet-rich plasma was prepared by the method of Pilla et al. modified by Lunkes et al. [8, 9]. Whole blood was collected with sodium citrate as an anticoagulant and centrifuged at 1500 rpm for 10 minutes. Then, the platelet-rich plasma was centrifuged at 5000 rpm for 30 minutes and washed with 3.5 mM HEPES buffer, pH 7.0 at least twice. The platelet pellets were suspended in the HEPES buffer and the protein was adjusted to 0.4-0.6 mg / mL.

Analysis of oxidative stress: The determination of substances reactive to thiobarbituric acid (TBARS) was according to Ohkawa, Ohishi, and Yagi [10]. The formation of malondialdehyde, due to the breakdown of polyunsaturated fatty acids, was the method adopted to determine the degree of lipid peroxidation. The activity of glutathione peroxidase (GPx) was according to Paglia and Valentine [11]. The nitric oxide test, which detects the presence of organic nitrite in the sample, was carried out according to the protocol of Choi et al. [12]. Protein carbonylation analyzes were according to protocols described by Levine et al. [13]. The activity of the myeloperoxidase enzyme (MPO), was according to Suzuki et al. [14]. Plasma levels of vitamin C were determined according to Galley et al. [15]. This method aims to generate an orange chromogen produced by reacting vitamin C with dinitrophenylhydrazine at 37ºC, which can be measured spectrophotometrically at 520 nm. Finally, the quantification of thiol groups, classic biomarkers of oxidative stress, was according to Ellman [16].

Analysis of the purinergic system: The analysis of the enzymatic activity of the purinergic system occurred in a similar way to the analysis of oxidative stress, however, with some modifications: E-NTPDase, determined according to Pilla et al. modified by Lunkes et al. and the activity of the ADA enzyme according to the method described by Giusti and Galanti [8, 9, 17]. The data obtained were analyzed using the GraphPad Prism 6.0 software using the Student's T test.

Statistical analysis: The data obtained were analyzed using the GraphPad Prism 6.0 software using the Student's T test. In addition, a correlation analysis was performed for the researched variables, using Pearson's correlation. The results were presented with mean and standard deviation. Differences in which the probability of rejection of the null hypothesis is less than 5% ($p < 0.05$) will be considered statistically significant.

RESULTS

General features: The characteristics of the participants indicated that the predominant age group of hypertensive pregnant women included in this study was 20 to 35 years old (60.0%), multiparous (86.7%), with a prevalence of gestational age between 27 and 36 weeks (53. 3%), women who had obesity (80.0%), history of abortion (13.3%) and previous history of hypertension (20.0%). Regarding the use of antihypertensive medication, 86.5% of the pregnant women used it, and the predominant medication was methyldopa in 40.0%. As for calcium supplementation, 26.6% underwent supplementation and 40% of them used the multivitamin during pregnancy. In low-risk pregnant women, the age group was 20 to 28 years old (60%), mostly primiparous (66.7%), women who were obese (26.7%), history of abortion (6.7%) and none of them had a previous history of hypertensive disease. Regarding calcium supplementation, 46.7% underwent supplementation and 93.3 %% of them used the multivitamin during pregnancy. The characteristics of hypertensive and low-risk pregnant women according to risk factors, reproductive history and treatment of hypertension during pregnancy are described in Table 1. Regarding the neonatal outcomes of pregnant women with HG, we can highlight that 40.0% of the neonates were male and 40% female. Regarding gestational age, 60.0% at full term (38 weeks to 40 weeks and 6 days), 80.0% without asphyxia at birth (with apgar score in the first and fifth minutes between 7 and 10), 66, 6% of newborns with adequate weight (weighing more than 2,500 g and less than 4,000g) and 60.0% with length between 45 and 50 cm (Table 2). Regarding the neonatal outcomes of LR pregnant women, it was shown that 60.0% of the neonates were female and 40%, male. Regarding gestational age, 60.0% at full term, 93.3% without asphyxiation at birth, 80.0% of newborns with adequate weight and 7.3% with length between 45 and 50 cm (Table 2).

Purinergic system: Laboratory analyzes of the purinergic system indicated a significant decrease in ATP hydrolysis in the platelet sample in the group of hypertensive pregnant women, $p=0.0221$ (Figure 1A), as well as a significant increase in ATP lymphocyte hydrolysis for the same group, $p=0.0482$ (Figure 1B). Regarding the hydrolysis of the ADP nucleotide, it appears that there was a significant reduction in the hydrolysis of ADP platelets in pregnant women with gestational hypertension, $p=0.0033$ (Figure 2A), and a significant increase in the hydrolysis of ADP lymphocytes also in the group with gestational hypertension, $p=0.0238$ (Figure 2B).

Table 1. Characteristics of hypertensive and low-risk pregnant women in the city of Chapecó/SC

	VARIABLES	GH	%	LR	%
PARITY	PRIMIPARA	2	13,3	10	66,7
	MULTIPAROUS	13	86,7	5	33,3
	20-35 YEARS	9	60,0	12	80,0
AGE RANGE	>35YEARS	6	40,0	3	20,0
	<19WEEKS	2	13,3	0	0,0
GESTATIONAL AGE	19-27WEEKS	4	26,7	10	66,6
	27-36WEEKS	8	53,3	4	26,7
	>36WEEKS	1	6,7	1	6,7
	METILDOPA	6	40,0	*	*
USE OF MEDICATION DURING	AAS	1	6,7	*	*
PREGNANCY	METILDOPA ANDAAS	4	26,7	*	*
	OTHERS MEDICATIONS	2	13,3	*	*
	WITHOUT MEDICATION	2	13,3	*	*
USE OF MEDICATION DURING					
PREGNANCY	YES	6	40,0	14	93,3
	NO	9	60,0	1	6,7
CALCIUM SUPPLEMENTATION	YES	4	26,7	7	46,7
	NO	11	73,3	8	53,3
ABORTION HISTORY	YES	2	13,3	1	6,7
	NO	13	86,7	14	93,3
PREVIOUS GH HISTORY	YES	3	20,0	0	0,0
	NO	12	80,0	15	100,0
	LOW WEIGHT	0	0,0	1	6,6
	ADEQUATE	0	0,0	6	40,0
BMI	ABOUT WEIGHT	2	13,3	4	26,7
	OBESITY	12	80,0	4	26,7
	UNINFORMED	1	6,7	0	0,0
TOTAL		15	100,0	15	100,0

Legend: Data expressed as a percentage. The asterisk indicates that antihypertensive drugs are not applied / not used based on the information about the diagnosis provided in the research form.

Table 2. Neonatal outcomes of hypertensive and low-risk pregnant women according to their clinical conditions in the city of Chapecó/SC

	VARIABLES	GH	%	LR	%
	MASCULINE	6	40,0	6	40,0
CHILD'S GENDER	FEMININE	6	40,0	9	60,0
	UNINFORMED	3	20,0	0	0,0
	<37WEEKS	1	6,7	4	26,6
GESTATIONAL AGE AT BIRTH	37-41WEEKS	9	60,0	9	60,0
	>41WEEKS	0	0,0	1	6,7
	UNINFORMED	5	33,3	1	6,7
	0-3	0	0,0	0	0,0
	4-6	0	0,0	0	0,0
APGAR INDEX	7-10	12	80,0	14	93,3
	UNINFORMED	3	20,0	1	6,7
	<2.500Kg	1	6,7	0	0,0
	2,500-4,000Kg	10	66,6	12	80,0
WEIGHT	>4,000Kg	1	6,7	3	20,0
	UNINFORMED	3	20,0	0	0,0
	<45	1	6,7	1	6,7
	45-50cm	9	60,0	11	73,3
LENGTH	>50cm	1	6,7	3	20,0
	UNINFORMED	4	26,6	0	0,0

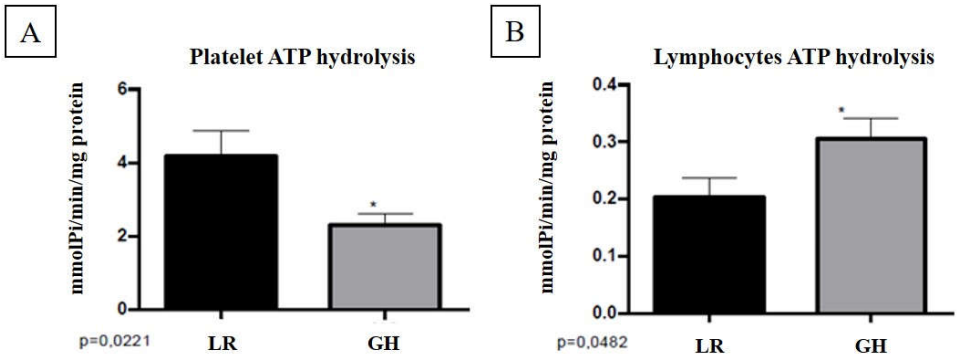

Figure 1. Hydrolysis of ATP in low-risk pregnant women and in pregnant women with gestational hypertension. Hydrolysis of platelet ATP (A) and hydrolysis of ATP lymphocytes (B). *p<0.05, n=15

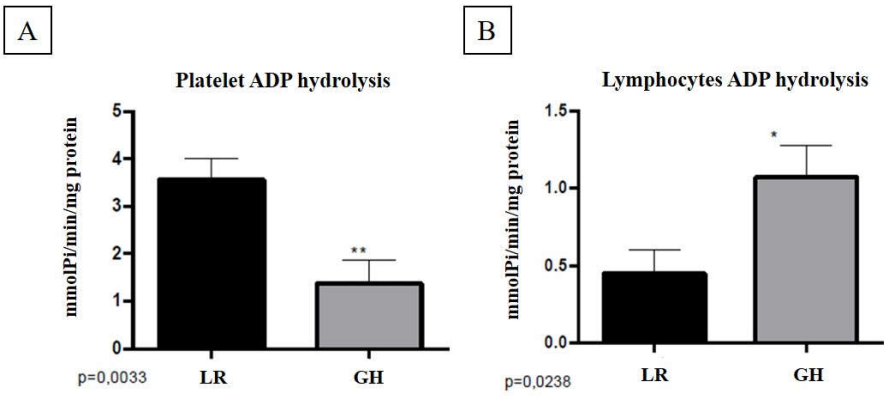

Figure 2 . Hydrolysis of ADP in low-risk pregnant women and in pregnant women with gestational hypertension. Hydrolysis of ADP platelets (A) and hydrolysis of ADP lymphocytes (B). *p<0.05, n=15

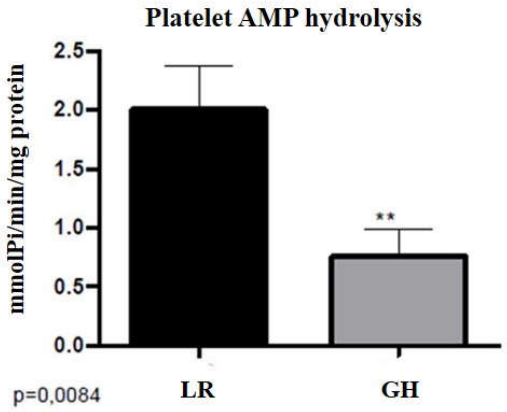

Figure 3. Hydrolysis of platelet AMP in low-risk pregnant women and in pregnant women with gestational hypertension. *p<0.05, n=15

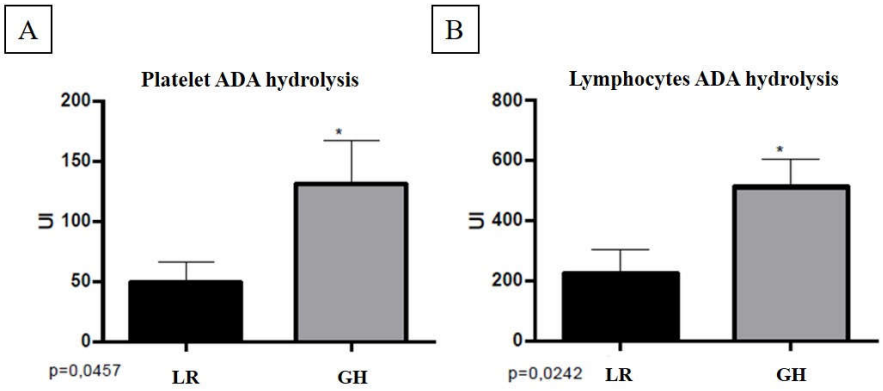

Figure 4. Hydrolysis of ADA in low-risk pregnant women and in pregnant women with gestational hypertension. Hydrolysis of ADA platelets (A) and hydrolysis of ADA lymphocytes (B). *p<0.05, n=15

Regarding AMP hydrolysis, there is an increased trend in hydrolysis in the platelet sample in the low risk group and a significant decrease in the group with gestational hypertension, p=0.0084 (Figure 3).

Finally, regarding adenosine hydrolysis (ADA), there was a significant increase in the hydrolysis of this nucleotide in the platelet sample, p=0.0457 (Figure 4A), as well as in the lymphocyte sample, p=0,0242 (Figure 4B), both in the group of pregnant women with gestational hypertension.

Oxidative stress

The oxidative profile of the study participants was assessed by analyzing the concentrations of oxidative damage indicators, such as TBARS lipid peroxidation and the activity of the myeloperoxidase enzyme (MPO). Components of the antioxidant defense system were also evaluated, such as protein (PSH) and non-protein (NPSH), glutathione (GSH), carbonyl protein and vitamin C.

Regarding vitamin C levels, there was no significant difference between groups (Figure 5A). Regarding carbonyl protein, at the 5% significance level, with p=0.0001, there was a significant reduction in the group of hypertensive pregnant women (Figure 5B). Regarding GSH levels, there was no significant difference between groups (Figure 5C). On the other hand, in relation to TBARS lipid peroxidation, at the significance level of 5%, with p=0.0311, it was found that the mean of TBARS is significantly lower in pregnant women with gestational hypertension (Figure 5D). Finally, regarding the MPO levels, there was no significant difference between the groups studied (Figure 5E). With regard to antioxidant defense components, represented by protein thiols (PSH) and non-protein thiols (NPSH), there was no significant difference in relation to the levels of PSH between the groups analyzed (Figure 6A). However, at the significance level of 5%, with p=0.0485, there was an increase in the concentration of NPSH in the group of pregnant women with gestational hypertension (Figure 6B).

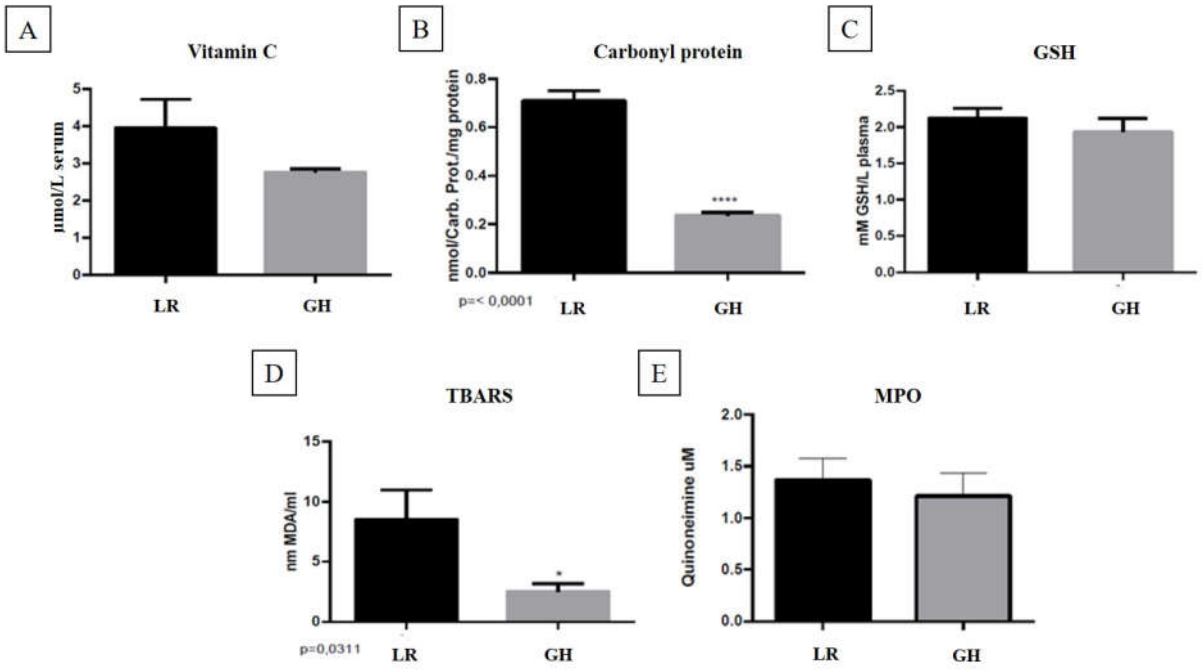

Figure 5. Analysis of oxidative stress indicators in low-risk pregnant women and in pregnant women with gestational hypertension. Vitamin C (A), protein carbonyl (B), GSH (C), TBARS (D) and MPO (E). *p<0.05, n=15

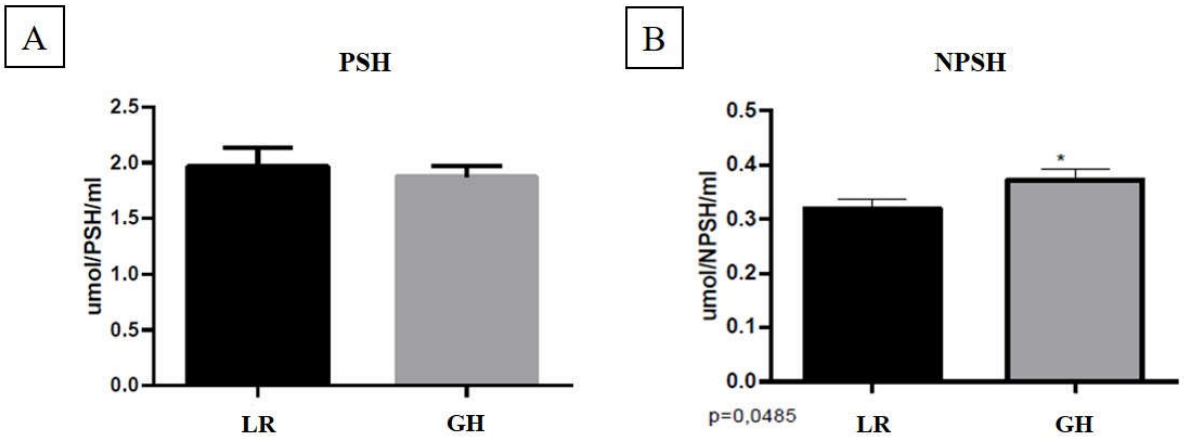

Figure 6. Analysis of oxidative stress indicators in low-risk pregnant women and in pregnant women with gestational hypertension. PSH (A) and NPSH (B). *p<0.05, n=15

DISCUSSION

Currently, it is a problem to raise the prevalence of HG, which has been affecting women of childbearing age and women in pregnancy, facing a higher risk of complications and death as a result of pregnancy. According to the Pan American Health Organization, 75% of maternal deaths are due to complications such as hypertension, severe bleeding and infections. Problems associated with poor eating habits, impaired quality of life, obesity, lack of physical activity and increased maternal age are also associated with gestational complications [18]. As in the result of this study, there are other studies that demonstrate relevant statistical data regarding the nutritional profile, as the obesity index is growing in many countries, in order to influence the manifestation of hypertensive diseases during pregnancy [19]. In this study, pregnant women with GH in the age group of 20 to 35 years prevailed and, in the literature, other studies show that GH generally affects women under the age of 20 and over 35 years of age [20, 21]. In another study, hypertensive pregnant women over the age of 30 years were found [22]. And in relation to parity, multiparous pregnant women are more likely to develop hypertensive diseases in the pregnancy period compared to nulliparous women [20].

This situation, which was found among the pregnant women participating in this research, with the majority of pregnant women diagnosed with GH predominating as being multiparous. Although the data regarding the previous history of some type of comorbidity related to pregnancy were not significant, women who have a personal or family history of hypertension are at a higher risk of developing a given condition during pregnancy or in a future pregnancy [21]. Regarding the neonatal losses resulting from gestational hypertension, no damage was identified in this study, which may be in line with the early diagnosis, in order to favor the therapeutic intervention and, thus, immediately initiate the control of the physiological metabolism of these pregnant women [23]. In this sense, studies consider that the most used drugs are: labetalol, nifedipine and hydralazine. In addition to the pharmacological treatment, it is also necessary to monitor the fetus in order to have proper monitoring [24]. The drug treatment indicated by the studies as being first-rate in these cases is labetalol, followed by methyldopa and nifedipine as alternative drugs, stressing that the choice of the drug is related to the safety of the pharmacological compounds that can and are safely used during pregnancy [25]. Regarding the purinergic system, the hydrolysis of ATP and ADP indicated a greater activity of NTPDase in lymphocyte cells in pregnant women with GH. This condition was already expected since E-NTPDase is an enzyme known to play an important role in inflammation and

thromboregulation. This increase in its activity results in an increase in the hydrolysis of nucleotides (ATP and ADP) released in cases of hypoxia or cellular damage with the intention of stabilizing physiological levels, since high levels of ATP in the extracellular environment are responsible for triggering a greater tissue and inflammatory damage [26, 27]. Furthermore, a decrease in the hydrolysis of ATP, ADP and AMP was evidenced in the platelet samples of the HG group, with a significant decrease ($p<0.05$), which indicates a lower activity of NTPDase, an enzyme that is the major promoter of platelet inhibition. Thus, the decrease in their activity contributes to the increase in platelet recruitment and activation [28].
In cases of hypoxia and inflammation, there is an increase in the formation of adenosine in order to modify the balance of pro-inflammatory signaling elicited by ATP towards the anti-inflammatory response of adenosine. Considering that the purinergic signaling enzymes cascade and that the activity of ecto-5'-nucleotidase has not been evaluated in the lymphocytes of pregnant women with GH, we can suggest that their activity is also increased, causing an increase in the level of adenosine to the extracellular medium in order to compensate for the pro-inflammatory and aggregating reactions of the signaling of the ATP and ADP molecules [28].

Ecto-5'-nucleotidase is an important enzyme capable of hydrolyzing the AMP molecule leading to the formation of adenosine, a platelet antiaggregant. In platelets, the activity of ecto-5'-nucleotidase was evaluated, but the hydrolysis of the AMP nucleotide was not altered, thus, no significant difference was evidenced in the activity of this enzyme in platelets of pregnant women with GH when compared with pregnant women of LR. A study was carried out for biochemical assessment of pregnant women, where it showed an increase in adenosine in practically all studied groups. These results show that these enzymes are involved in the thromboregulatory and immune system during pregnancy. In addition, the nucleotides certainly play an important role in homeostatic regulation in pregnancy, and ADP is the major promoter of platelet aggregation. However, adenosine acts in the opposite direction, inhibiting aggregation and modulating vascular tone [27]. In this sense, this study showed high adenosine activity in the GH group. It is considered that the nucleoside adenosine rises rapidly in conditions in which the organism needs to prevent some tissue damage, which can be induced by numerous factors, adenosine stimulates a wide variety of signals in order to preserve the injured tissue [27, 28]. The increase in ADA in the intracellular environment is responsible for triggering a higher consumption of oxygen. This is due to increased cellular effort. Another factor that could explain this condition, in relation to oxygen consumption, is that during the third trimester the pregnant woman demands a greater metabolic contribution, so there is an important index of the amount of platelets and leukocytes in these pregnant women, which may be corroborating for the high activity and release of adenosine, favoring the increase in uteroplacental blood supply, explaining the increase in adenosine activity [26, 27].

Regarding the evaluation of the components of oxidative stress, this study demonstrated significant differences in the pro and antioxidant compounds between pregnant women with LR and with GH. Thus, the increase in antioxidant components may be associated with the dietary composition of pregnant women and, possibly, supplementation with multivitamins, indicating an improvement in antioxidant capacity after the supplementation period [27, 29]. Superoxide radicals can reach phospholipids (the main component of the plasma membrane) by reacting with fatty acids causing oxidative degradation of lipids and consequent cell damage. These reactive oxygen species have been investigated as agents that promote lipid peroxidation and cause changes and endothelial dysfunction, also associated with disorders during pregnancy [29]. Some research shows that the enzymes belonging to the antioxidant system and that are present in the tissue of the placenta can, in a way, preserve and protect maternal blood from the excessive content of hydrogen peroxide. And if this protection becomes compromised during pregnancy, being able to trigger a pro-thrombotic state, it can generate coagulation problems that become increased after delivery [27, 29]. In short, the regulation of nucleotides in a compensatory response to supposed cellular damage (hypoxia and inflammation), represents an important control in the regulation of this system, as well as in the concentration of these substrates. In addition, the increase in adenosine hydrolysis contributed to the reduction of cell damage. With that, we can say that the purinergic system plays a very important role in inflammatory and vascular processes resulting from gestational hypertension. This change contributes to the improvement and reversal of the inflammatory and thrombotic process [28]. Therefore, it was possible to verify that the components of the purinergic system participate in hemostatic control during pregnancy. Despite the existence of other parameters in platelet regulation and in the hemostatic process, it is evident that the components of the purinergic system assist in vascular regulation. Furthermore, the participation of the ADA enzyme in conjunction with the platelet aggregation process is in line with the other evidenced results, in order to attest that they play a fundamental role in thromboregulatory functions, since they help in the prevention of future vascular damage resulting from the gestational period. Additionally, in relation to the parameters of the oxidative profile, it appears that the production of reactive oxygen species during the gestational period contributes to the control of cell damage, with no significant differences between LR pregnant women and GH pregnant women.

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