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Changes of Platelets' Function in Preeclampsia

Research Article

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Abstract: Increased aggregation of platelets during preeclampsia was shown in several studies, yet several others reported no change. The aim of our study was to investigate platelet aggregation in a group of patients suffering from preeclampsia. In a cross-sectional study blood samples were taken from 89 hospitalized patients in the third trimester of pregnancy: 38 were suffering from mild to moderate preeclampsia and 51 patients were without preeclampsia. From the blood samples platelet aggregation, secretion of adenine nucleotides from platelets, concentration of energy-rich adenine compounds and levels of cyclic adenosine-mono-phosphate and cyclic guanosine mono-phosphate in platelets were measured. In the patients with preeclampsia, the adenosine diphosphate threshold for biphasic aggregation [odds ratio (OR): .75; 95% Confidence Interval (CI): 0.55-1.02; $p<0.05$], total adenine nucleotides concentration in the metabolic pool of platelets (OR:0.99; CI: 0.62-1.57; $p<0.01$) and cyclic adenosine-mono-phosphate (OR:0.81; CI: 0.57, 1.14; $p<0.05$) and cyclic guanosine mono-phosphate (OR: .78; CI: 0.55-1.09; $p<0.05$) levels in platelets were decreased in comparison with the control group, while adenylate energy charge in the metabolic pool of platelets (OR: >100.00; CI: 0.00->100.00; $p<0.05$) and secretion of adenosine triphosphate (OR: .13; CI: 0.00-14.26; $p<0.05$) and adenosine diphosphate (OR: .77; CI: 0.08-36.79; $p<0.05$) were increased. The results of our study show increased activation and aggregation of platelets in pregnant females with preeclampsia.

Keywords: *Preeclampsia • Pregnancy • Platelets • Aggregation*

1. Introduction

Preeclampsia is a syndrome occurring usually after the 20th week of pregnancy and characterized by new-onset hypertension and proteinuria [1]. It is a disorder with a high incidence (2-7%) and potentially severe consequences to both mother and child [2,3].

The pathogenesis of preeclampsia is still incompletely understood, but a majority of researchers consider placental ischemia to be an initial phenomenon which triggers the syndrome [4,5]. However, disfunctioning of endothelium and its interaction with platelets seems essential for the development of preeclampsia [6]. Disfunctioned and damaged endothelium in preeclampsia (concomitant chronic disorders like diabetes mellitus, hypertension and hyperlipidemia contribute significantly to this process) decreases its production of prostacycline, leading to an imbalance between prostacycline

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and thromboxane in the blood, vasoconstriction and increased aggregation of the platelets in majority of the patients [7,8]. Aggregation of platelets further compromises blood flow through placenta and else, and is often accompanied with decrease of platelet count in the blood. A subset of preeclampsia, HELLP syndrome (Hemolysis, Elevated Liver Enzymes, Low Platelets) is always accompanied with a low platelet count, and further 10% of patients with more prevalent form of preeclampsia have low platelet count [9,10]. However, even when platelet count is normal, during preeclampsia aggregation of platelets could be increased, as confirmed by in vitro tests in several studies [11-13]. On the other hand, there are still reports on normal aggregation of platelets in patients with preeclampsia [14].

The aim of our study was to investigate platelet aggregation in a group of patients suffering from preeclampsia.

2. Material and Methods

Study population. The study population consisted of pregnant patients hospitalized at Gynecology and Obstetrics Clinic, Clinical Center "Kragujevac", Kragujevac, Serbia, during a one-year period. The inclusion criteria were: (1) the third trimester of pregnancy; (2) pregnancy with natural conceiving. The exclusion criteria were: (1) history of chronic hypertension; (2) history of a hematological disorder; (3) therapy with aspirin or other non-steroid anti-inflammatory drugs during the last 10 days; (4) gestational diabetes and (5) patients not willing to sign the informed consent form. The investigators did not interfere with the patients' treatment, which was chosen and prescribed by gynecologists not involved with the study, at their own preference. Out of 122 screened patients, 38 patients suffering from preeclampsia (the cases: patients with [1] arterial blood pressure equal or more than 140/90 mm Hg after 20 weeks gestation; [2] proteinuria>0,3 g per day) and 51 patients without preeclampsia (hospitalized for the treatment of premature uterine contractions) were enrolled in the study. The study was approved by the Ethics Committee of Clinical Center "Kragujevac".

Study protocol. After signing the informed consent form, a history was taken from the patients and they were examined physically. Arterial blood pressure was measured in lying position, after 15 minutes of rest. Then a fetal ultrasonography was performed by Aloka 680 SD machine, with 3.5 MHz probe, including measurement of blood flow through umbilical artery. A 20 ml blood sample was taken from the cubital vein during morning hours (from 8 to 9 o'clock, a.m.), and the following measurements were made with the blood: (1) biochemistry; (2) full blood count; (3) platelets aggregation index according to Bowry's modification of Wu and Hoak's method [15]; (4) platelets aggregation caused by collagen and adenosine according to turbidimetric method [16]; (5) secretion of adenine nucleotides from platelets by fluorimetry; (6) concentration of energy-rich adenine compounds (adenosine-tri-phosphate, adenosine di-phosphate, adenosine mono-phosphate) by fluorimetry, and (7) levels of cyclic adenosine-mono-phosphate (cAMP) and cyclic guanosin mono-phosphate (cGMP) by immunoassays. After the blood sample was taken, participation of a patient in the study was completed.

Study design. The study was set up as cross-sectional investigation, with the same diagnostic tests undertaken in all enrolled patients, regardless of their diagnoses and treatments. Based on the expected difference of 10% in aggregation of platelets among the patients with and without preeclampsia, standard deviation of platelets aggregation in each of the groups of 10%, probability of type I error 0.05 (alpha) when using two-tails Student's T-test, and power of the study of 90%, minimal sample size was calculated by G*Power3 software [17,18] to be 23 patients per group.

2.1. Statistical analysis

For each group mean and standard deviation of measured parameters were calculated. The differences between mean values of measured parameters in the study groups were tested for significance by two-tails Student's T-test [19]. The differences in frequencies of qualitative variables among the treatment groups were tested for significance by Chi-square test [17]. The probability of null hypothesis was set to 0.05 values. All calculations were made by statistical software SPSS version 18.

3. Results

The patients with preeclampsia (n=38) were 26.3±8.5 years old, and the average age of control patients (n=51) was 27.1±7.8 years (T=0.4606, df=87). In the control group, the ratio of females with one, two or three children was 32/12/7, while in the preeclampsia group this ratio was 29/9/0 (c2=0.5499, df =5). There was one case of twin pregnancy (2%) in the control group, and 3 cases (8%) in the preeclampsia group (c2=0.1814, df=2). Symptoms, signs and values of laboratory tests in both groups are shown in Table 1. Values of platelet function tests and adenine nucleotides concentrations are shown in the Table 2. Concentrations of total adenine nucleotides in platelets and adenine nucleotides from the metabolic pool of platelets are shown separately in the Table 2.

Table 1 Simtoms, signs and values of laboratory tests in preeclampsia and control groups (variabilities shown in the table represent standard deviation).

	Control group (n=51)	Preeclampsia group (n=38)	OR (95% CI)
MAP (mmHg)	88.8±5.5	110.9±15.1*	1.22 (1.15, 1.29)
Level of proteinuria (g/day)	.	1.35±0.22	
Hemoglobin (g/l)	107.8±10.6	107.5±13.2	
Erythrocyte count (10^{12}/l)	3.3±0.2	3.2±0.3	
Serum protein (g/l)	63.7±5.8	62.1±8.5	
Fibrinogen (g/l)	4.4±0.8	4.1±0.7	
AST (SGOT)	19.4±4.9	17.9±5.7	
ALT (SGPT)	13.9±3.6	13.8±3.1	
Serum glucose (mmol/l)	4.9±1.1	4.3±0.7	
Urea (mmol/l)	3.8±0.9	3.9±1.0	
Creatinine (mmol/l)	66.3±7.1	67.1±6.8	
Uric acid (mmol/l)	192.2±49.4	292.2±90.5*	1.01 (1.00, 1.02)
LDH (U/l)	322.4±48.3	407.2±92.5*	1.01 (1.00, 1.02)

**Significant difference among the groups, p<0.01; MAP, Mean arterial pressure; CI, confidence interval; OR, odds ratio.*

Table 2 Values of platelet function tests in control and preclampsia groups (variabilities shown in the table represent standard deviation).

	Control group (n=51)	Preeclampsia group (n=38)	OR (95% CI)
Platelet count (x10^9/l)	289.40±09.20	287.70±12.98	
Aggregation index	0.89±0.01	0.92±0.05	
Maximum aggregation (%)	80.8±03.0	88.9±03.0	
ADP threshold for biphasic aggregation (μmol/l)	5.79±0.27	4.68±0.47*	0.75 (0.55, 1.02)
ATP (μmol/10^{11} platelets)	4.93±0.41	5.34±0.40	
ADP (μmol/10^{11} platelets)	2.13±0.18	1.70±0.24	
AMP (μmol/10^{11} platelets)	0.05±0.01	0.04±0.01	
TAN (μmol/10^{11} platelets)	7.22±0.48	6.75±0.39	
ATP/ADP (μmol/10^{11} platelets)	2.53±0.28	3.49±0.60	
AEC (μmol/10^{11} platelets)	0.84±0.01	0.86±0.01	
mATP (μmol/10^{11} platelets)	4.68±0.44	4.50±0.37	
mADP (μmol/10^{11} platelets)	2.19±0.20	1.02±0.21**	1.11 (0.99, 1.20)
mAMP (μmol/10^{11} platelets)	0.04±0.01	0.04±0.01	
mTAN (μmol/10^{11} platelets)	7.37±0.48	4.88±0.03**	0.99 (0.62, 1.57)
mATP/mADP (μmol/10^{11} platelets)	2.70±0.40	5.61±0.70**	1.02 (0.89, 1.17)
mAEC (μmol/10^{11} platelets)	0.78±0.04	0.89±0.02*	>100.00 (0.00, >100.00)
sATP (μmol/10^{11} platelets)	0.57±0.09	0.72±0.12*	0.13 (0.00, 14.26)
sADP (μmol/10^{11} platelets)	0.38±0.07	0.87±0.24*	1.77 (0.08, 36.79)
sATP/sADP	2.17±0.74	0.81±0.26*	0.51 (0.12, 2.25)
cAMP (nmol/10^{11} platelets)	22.93±4.51	9.97±0.62*	0.81 (0.57, 1.14)
cGMP (pmol/10^9 platelets)	21.36±4.25	6.49±1.58*	0.78 (0.55, 1.09)
cAMP (nmol/ml of plasma)	9.21±0.27	10.81±1.13	
cGMP (pmol/ml of plasma)	9.40±1.27	8.91±1.74	

**Significant difference among the groups, p<0.05;*

***Significant difference among the groups, p<0.01; AMP, Adenosine monoposphate; ADP, Adenosine diphosphate; ATP, Adenosine triphosphate; TAN, Total adenine nucleotides=ATP + ADP + AMP; AEC, Adenylate energy charge=(ATP+ 1/2 ADP) / TAN; mATP, Adenosine triphosphate in the metabolic pool; mADP, Adenosine diphosphate in the metabolic pool; mAMP, Adenosine monophosphate in the metabolic pool; mTAN, Total adenine nucleotides in the metabolic pool=mATP + mADP + mAMP; mAEC, Adenylate energy charge in the metabolic pool=(mATP+ 1/2 mADP) / mTAN; sATP, Secreted adenosine triphosphate; sADP, Secreted adenosine diphosphate; cAMP, Cyclic adenosine monophosphate; cGMP, Cyclic guanosine monophosphate; CI, confidence interval; OR, odds ratio.*

4. Discussion

Platelets count could be decreased in up to 8% of normal pregnancies [20,21], but it rarely falls below 150 x 109/l, and is not accompanied by immunological disturbances [21,22]. In our study, the platelets counts in patients with and without preeclampsia were within the normal limits, and without significant differences between the groups. Normal platelets count in patients with preeclampsia could be explained by mild to moderate severity of the disorder in our study sample, since it was shown that decreased platelets count accompanied only 12-15% of pregnancies with moderate preeclampsia, and even 30-50% of pregnancies with severe preeclampsia and eclampsia [10]. Possible pathogenetic mechanism of preeclampsia-associated decreased platelets count could be accelerated destruction of platelets caused by non-immunological factors [23].

In most studies, in vitro platelet aggregation is decreased compared with the normal increase characteristic of pregnancy [35]. However, in our study the adenosine diphosphate threshold for biphasic aggregation was significantly lower, suggesting increased platelet aggregation in patients with preeclampsia, probably caused by hypertension-induced endothelial damage [24]. An indirect sign of platelet activation and aggregation is increased secretion of substances from dense granules of the platelets: adenosine triphosphate (ATP) and adenosine diphosphate (ADP) [24]. In our study, levels of secreted adenosine triphosphate and adenosine diphosphate were much higher in the preeclampsia group (see Table 2).

All phases of platelet activation are very energy-demanding [25-27]. Energy utilization in platelets which are not activated ranges from 3 to 5 μmol ATP/min/1011 platelets, and it is increased 3 to 6 times after maximal activation by thrombin [28]. In our study, adenylate energy charge and ATP/ADP ratio in the metabolic pool of platelets were significantly increased in patients with preeclampsia, while total adenine nucleotides level was decreased. Such results suggest increased activation of platelets in our patients with preeclampsia [29,30]. Further confirmation of increased activation of platelets in patients with preeclampsia comes from observed decrease of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) levels in their platelets [31,32].

Decreased levels of cGMP in platelets of the patients with preeclampsia also could be partially explained by decreased plasma levels of nitric oxide (NO) in these patients, which was previously described [33]. There are numerous case reports of beneficial effects of nitrates (which release NO) in patients with HELLP syndrome, demonstrating decrease in platelets activation and increase in cGMP [34].

The results of our study show increased activation and aggregation of platelets in pregnant females with preeclampsia with potentially deleterious consequences. Monitoring and early detection of increased activation and aggregation of platelets in preeclampsia patients could significantly contribute to timely administration of appropriate therapy and prevention of complications in preeclampsia.

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