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## SPONTANEOUS PLATELET AGGREGATION IN THIRD TRIMESTER PREGNANCY AT ADAM MALIK HOSPITAL, MEDAN

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### ABSTRACT

Spontaneous Platelet Aggregation (SPA) is a condition when spontaneously platelet aggregate in plasma left in a tube at room temperature for 2-3 hours without adding any agonists. It is not entirely sure why this phenomenon happens. Pregnant females have a more tendency of hypercoagulability. Never the less, SPA demonstrates hyperaggregation of platelet and not the coagulation pathway. So far, there is no report regarding this phenomenon in pregnant females. This study aimed to find out whether pregnant females, especially in the third-trimester of the pregnancy, had the tendency of SPA. This cross-sectional study was established in the Department of Clinical Pathology and Department of Obstetrics-Gynecology, Adam Malik General Hospital in Medan between January–March 2016. Twenty-six females were included. Ultrasonography (USG) was used to know the age of pregnancy. From 26 females in this study, 10 were pregnant females in the third-trimester of pregnancy while 16 others were normal non-pregnant females as controls, with a mean age of 29.2±2.78 years old and 36.5±4.63 years old. There were significant differences between platelet aggregation using ADP agonist in pregnant females and controls ( $p < 0.01$ ). In the median Tmax 10%, there were no significant differences between platelet aggregation without ADP agonist in pregnant females and controls after 3 hours ( $p > 0.05$ ). Hyperaggregation and hypercoagulability in the third-trimester pregnancy tend to develop SPA. Eventhough there are only mild signs and symptoms, it can be an a rising risk of thrombus.

**Key words:** Spontaneous platelet aggregation, platelet aggregation, third-semester of pregnancy

### INTRODUCTION

In the 1980s, inadvertently in several European country laboratories a declining platelet count in EDTA blood by itself without any manipulation after more than two hours left at room temperature were reported. Later research on this situation showed spontaneous aggregation or also called Spontaneous Platelet Aggregation (SPA).

Over time, the condition is expressed in the mutual consensus that spontaneous platelet aggregation is defined as platelet aggregation obtained without agonist.<sup>1</sup> In SPA, from the image of the peripheral blood smear, "platelet clumping" can be found but there are also reports that the SPA without "platelet clumping" was found.

In pregnant females, strong evidence of hypercoagulation has been widely stated and the higher the period of pregnancy and, the hyper coagulation was also higher. But the evidence is almost entirely due to increased activation and concentration of blood clotting factors (procoagulation). According to Davis, pregnancy has a risk factor for thrombus with a frequency of

occurrence of about 0.67 per 1,000 pregnancies per year. In an extensive study of pregnant females in Sweden, there was a link between the occurrence of Venous thromboembolic (VTE) as a complication of the incidence of thrombus.<sup>2</sup> Hypercoagulability during pregnancy has been investigated using Thromboelastography (TEG) instruments, and an estimated increase in factor VII and fibrinogen was shown, although many other coagulation factors also increase during pregnancy.<sup>3</sup> The increase of gestational age will increase Plasminogen Activator Inhibitor-1 (PAI-1) and Plasminogen Activator Inhibitor-2 (PAI-2) coagulation factors which will then increase thrombogenesis.

So far, there has been no report on platelet assessment, especially regarding platelet aggregation, only partially reporting an increase in  $\beta$ -thromboglobulin which is a product of thrombus. Uchikova and Ledjev conducted a study of 3<sup>rd</sup>-trimester pregnant females with non-pregnant (healthy) controls to assess hemostasis variables and found an increase of Prothrombin Time (PT), Thrombin Time (TT), fibrinogen, factor VII, and alpha2-antiplasmin.<sup>4</sup>

During pregnancy, platelet aggregation, and secretion of ATP will increase compared with non-pregnant individuals (normal), with the peak occurring in the third-trimester and returning to normal at 6-12 weeks after delivery.<sup>5</sup>

In two studies of SPA in cardiovascular disease, it was found that SPA was a marker of the significant risk of arterial occlusion, and was clinically associated with a prothrombotic activity.<sup>6</sup> According to Ajayi, pregnant females who were given Unfractionated Heparin (UH) and Low-Molecular-Weight Heparins (LMWHs), dalteparin and enoxaparin therapy increased significantly by 37% compared to non-pregnant females by 16% ( $p < 0.01$ ).<sup>7</sup>

In Indonesia, especially in Medan, so far, data on the incidence of SPA occurring in the third-trimester of pregnant females have not been found. Thus, the authors were interested in researching research on platelet aggregation against the incidence of SPA in pregnant females, especially in the third-trimester at the Adam Malik Hospital (Medan District).

## METHODS

The design of this study was conducted by cross-sectional. Sampling by consecutive sampling, where the number of samples was limited to a minimum of the approximate number of samples or until the specified sample collection time limit. The variable measurement was done only once.

This research was conducted in the Laboratory of the Department of Clinical Pathology Medical Faculty of University of North Sumatra/Adam Malik Hospital Medan in cooperation with the Outpatient Clinic, Department of Obstetrics, and Gynecology Faculty of Medicine, University of North Sumatra/Adam Malik Hospital Medan, starting from January 2016 until March 2016. This study was discontinued when the minimum sample size was reached or when the sampling time had reached three months.

Case group comprised pregnant females in the third-trimester of pregnancy who came for treatment/control to Obstetric Outpatient Clinic of the Adam Malik Hospital Medan. Pregnant females in the third-trimester who were willing to participate in the research with the age of reproduction, without complications such as eclampsia, hypertension, diabetes mellitus were included. Third-trimester pregnant females with complications such as eclampsia, hypertension, diabetes mellitus, and using oral anticoagulant medication were excluded.

The material used in this study was blood collected in tubes with citrate and EDTA

anticoagulant. From all patients and controls who were willing to take part in the study, history, age, pregnancy, and birth history were recorded. Phlebotomy was performed on the median cubital vein. Asepsis with 70% alcohol was done beforehand and allowed to dry, then 12 mL of blood was collected using a venoject. The blood was divided into three tubes containing 3.2% sodium citrate (3 mL) and the rest to a 3 mL of K2EDTA tube. Complete blood count was checked using an automatic cell counter analyzer (Sysmex XN-1000) to assess the platelet count. The principle of this instrument was by flow cytometry. The examination was done immediately after the sample was collected and three hours after the sample was left at 37°C. Spontaneous platelet aggregation examination was performed using a Light Transmission Aggregometer AggRAM Analyzer Helena Laboratories instruments using turbidimetry principle and conducted by one examiner.

Data analysis was done by Oxstat V test, ver. 5.01.02 for Windows. The population was not normally distributed, so statistical analysis was done using Mann-Whitney U Test on both non-parametrics to show aggregate differences in plasma before and after 3h at room temperature. To compare the value of platelet aggregation between the two groups unpaired-T test was used. The test result was considered significant if the value of  $p < 0.05$ .

Ethical clearance was obtained from the Research Committee of Medical Field Faculty of Medicine, University of North Sumatra Medan with No. 96/KOMET/FK USU/2016. Informed consent was requested in writing from the research subjects or represented by his or her family who stated willingness to take part in the research after getting an explanation of the intent and purpose of this research.

## RESULT AND DISCUSSION

Twenty-six participants consisting of 10 pregnant females in the third-trimester (mean age  $29.2 \pm 2.78$ ) and 16 healthy controls (mean age  $36.5 \pm 4.63$ ) were recruited in this study. Laboratory tests of platelet levels and platelet aggregation were performed as soon as the samples were collected and 3h after being left at room temperature 37°C.

From Table 1, pregnant females who participated in this study were 10 females (38.5%) with a mean age of  $29.2 \pm 2.78$  and 16 controls (61.5%) with an average age of  $36.5 \pm 4.63$ . Of all study participants, the youngest was 25 years and the oldest 45 years.

Table 2, showed that the change of platelet value before and after 3h in patients was  $251.9 \pm 71.7239 \times 10^3/\mu\text{L}$ , and  $258 \pm 68.018 \times 10^3/\mu\text{L}$ , while platelets before and after 3h of controls were  $290.250 \pm 88.1374 \times 10^3/\mu\text{L}$  and  $286.937 \pm 66.6628 \times 10^3/\mu\text{L}$ . The difference before and after in Table 2 was considered as not significant,  $p > 0.1$ .

**Table 1.** Data on the characteristics of pregnant females and controls based on age

Characteristics	n (%)	Mean $\pm$ SD (years)
Pregnant females	10 (38.5)	29.2 $\pm$ 2.78
Controls	16 (61.5)	36.5 $\pm$ 4.63

**Table 2.** Mean values  $\pm$  SD of patients and controls platelet ( $10^3/\mu\text{L}$ ) level before and after 3h

Name	Mean ( $10^3/\mu\text{L}$ )	p
Patients 0 time	251.9 $\pm$ 71.7239	> 0.1
Patients 3h time	258 $\pm$ 68.018	
Controls 0 time	290.250 $\pm$ 88.1374	> 0.1
Controls 3h time	286.937 $\pm$ 66.6628	

Description: Data in mean  $\pm$  SD. Platelet \* Tests of significance with the unpaired-T test, significant if  $p < 0.05$

Table 3, there is no significant ( $p > 0.05$ ) between median value (range) of platelet counts ( $10^3/\mu\text{L}$ ) before and after 3h incubation in patients and controls, as well as the results in Figure 1 and Figure 2 which the median  $\pm$  range values of platelets in

**Table 3.** The median value (range) of platelet counts ( $10^3/\mu\text{L}$ ) before and after 3h incubation of patients and controls

	Lowest ( $10^3/\mu\text{L}$ )	Highest ( $10^3/\mu\text{L}$ )	Median ( $10^3/\mu\text{L}$ )	P
Patients 0 time	177	415	245	> 0.05
Controls 0 time	104	478	305.5	
Patients 3h time	183	409	246	> 0.05
Controls 3h time	171	451	289.5	

Data in the form of the median (range), lowest (lowest value), highest (highest score). \* Test significance with the unpaired-T test, meaning significant if  $p < 0.05$

**Table 4.** The median value  $\pm$  the patient's platelet aggregation range compared to control at  $T_{max} 10\mu\text{M}$  concentration immediately after it was taken

	Lowest	Highest	Median	P
Patients 0 time	6.4	555.4	59.7	< 0.01
Controls 0 time	1.9	81.8	33.05	> 0.05
Patients 3h time	9.4	81.3	51.4	
Controls 3h time	0.5	74	46.5	> 0.05
Increase of patients	-546	14.8	4.1	
Increase of controls	-45.6	67.7	11.25	> 0.05

patients and controls as soon as taken and after 3h incubation were describe in bar.

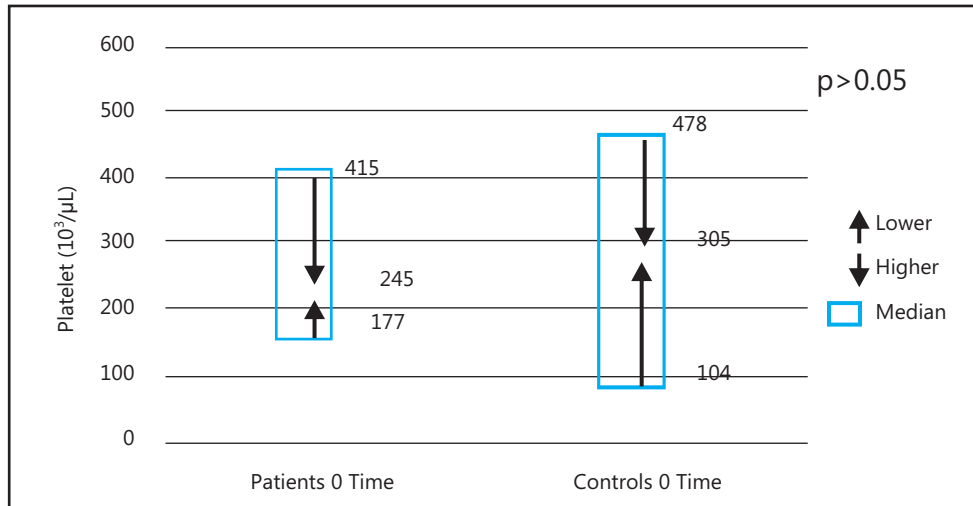
Table 4, the median (mean) aggregate of patient platelets and controls taken and immediately screened were 59.79% (6.4-555.4%) and 33.0% (1.9-81.8%), respectively,  $p < 0.01$ . Median (mean) of patient platelet aggregation after 3h at room temperature was 51.4% (9.4 - 81.3%); while the controls were 46.5% (0.5 - 74.0%),  $p > 0.05$ .

Pregnancy is a state of hypercoagulation where the condition is a mechanism prepared by the body to reduce the risk of bleeding during pregnancy and after delivery.<sup>3</sup> According Ajayi, which stated that of hypercoagulability in pregnancy indirectly would increase the occurrence of SPA events.<sup>7</sup>

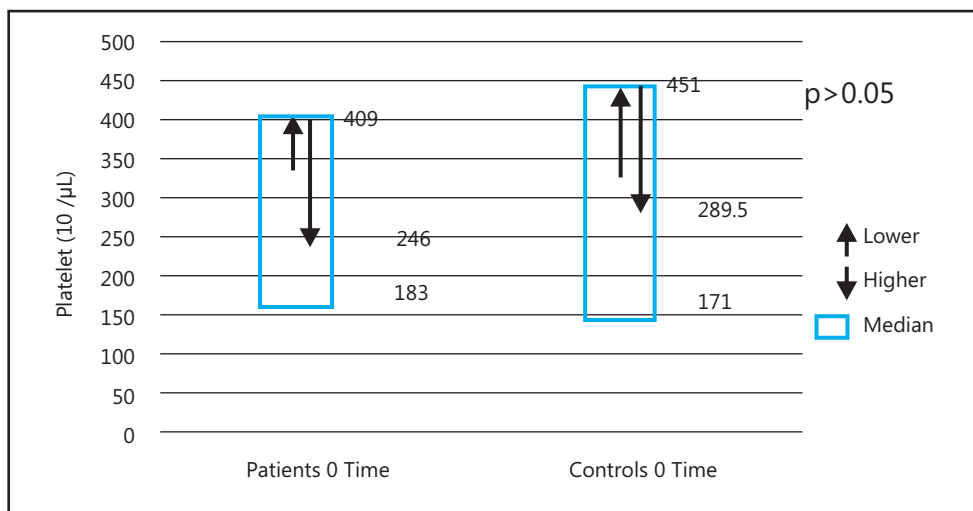
According to Davis et al. there are many studies that examine the hypercoagulation status in pregnant female. The results of the study stated that the higher the period of pregnancy then hypercoagulation was also increasing.<sup>2</sup> But it was almost entirely due to increased activation, and concentration of clotting factors (procoagulation).

In this study, an assessment of 10 (38.5%) pregnant females with gestational age in the 3<sup>rd</sup>-trimester (28-40 weeks); with the mean age of  $29.2 \pm 2.78$  and 16 (61.5%) of non-pregnant females as controls; with an average age of  $36.5 \pm 4.63$ .

From the results of this study, assessment of platelets was carried out and showed that thrombocytes of pregnant females in the 3<sup>rd</sup>-trimester were more hyperaggregative than



**Figure 1.** The median ± range values of platelets in patients and controls as soon as they were taken



**Figure 2.** The median ± range values of platelets in patients and controls after 3h incubation

normal non-pregnant females. This was consistent with a study conducted by Swanepoel *et al.*, who stated that during pregnancy, platelet aggregation, and secretion of ATP would increase compared with non-pregnant individuals, with the peak occurring in the third trimester and returning to normal at weeks 6-12 after delivery.<sup>5</sup> Juan *et al.* also stated that a normal pregnancy was characterized by an increase of platelet aggregation and a decrease in platelet values during pregnancy.<sup>8</sup>

In this study, the SPA was measured using a platelet aggregometer instrument which was treated without the addition of ADP agonists and expected to result in a flat wave. In this study, however, there was a slight change of the wave, with the median (mean) aggregation of platelets and controls taken and immediately screened were 59.79% (6.4 - 555.4%) and 33.0% (1.9 - 81.8%) respectively, p

<0.01. This result was at least in agreement with a study conducted by Vincelot *et al.*, which stated that assessment of platelet function was required in pregnant females because platelets in pregnant females may fall in the third-trimester of normal pregnancy, and 0.3% because of increased platelet aggregation.<sup>9</sup>

Platelets are very essential in the formation of hemostasis and play an important role in the occurrence of thrombus. This condition in pregnant females aims to protect the mother from the risk of bleeding during labor, but it is also likely to cause thromboembolism during pregnancy as well as at the time of the puerperium.<sup>10,11</sup>

In this study, the mean values of  $251.9 \pm 71.7239$  pregnant female thrombocyte values were found with the median value of  $245 \times 10^3/\text{mm}^3$  (177 -  $415 \times 10^3/\text{mm}^3$ ). This result suggested that platelet values

in pregnant females would be low but still within normal reference value limits. This statement was following research conducted by Vincelot *et al.*, which stated that platelets in pregnant females would experience a decrease or normal as "hemostatic challenge".<sup>9</sup>

The nonsignificant results were also obtained from the median (range) number of platelets baseline and 3h after incubation in patients of  $245 \times 103/\text{mm}^3$  ( $177-415 \times 103/\text{mm}^3$ ) and  $246 \times 103/\text{mm}^3$  ( $183-409 \times 103/\text{mm}^3$ ), while the initial platelet count and 3h after incubation in the controls were  $305.5 \times 103/\text{mm}^3$  ( $104-478 \times 103/\text{mm}^3$ ) and  $289 \times 103/\text{mm}^3$  ( $171 - 451 \times 103/\text{mm}^3$ ).

### CONCLUSION AND SUGGESTION

From this research, it can be concluded that platelets in the third-trimester pregnant females are found more hyperaggregative compared to non-pregnant females, but after the blood in the tube was being left for 3h at room temperature, the aggregation partially returned to the normal range. The presence of hypercoagulability in pregnant females, especially at the third-trimester of pregnancy showed that some have SPA at risk for thrombus. Platelet aggregation of the third-trimester pregnant females was not affected by platelet count. Hypercoagulability and hyperaggregation will trigger the occurrence of SPA in some pregnant females at the third-trimester of pregnancy.

The researchers suggest that further research is needed to find out whether pregnant females with high SPA levels have an increased risk of thrombus formation.

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