New Parameters of Hemostasis, some of their Research Results during Pregnancy

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Abstract

The article presents up-to-date information about the hemostasis system is a biological system that ensures the preservation of the liquid state of the blood, maintaining the integrity of the walls of blood vessels, preventing and stopping bleeding from the latter by thrombosis. Hemostasis is implemented mainly by three interacting functional and structural components: the walls of blood vessels (primarily intima), blood cells (platelets, erythrocytes, etc.) and plasma enzyme systems (coagulation, fibrinolytic, kallikrein-kinin, etc.). The article presents data from original studies of 20 women with their first pregnancy between the ages of 25 and 28 (the gestation period was 18 to 20 weeks). And 10 non-pregnant women (control group). We studied the aggregation of enterocytes and to study the aggregation of platelet aggregation (Rheoscan apparatus). Further, by transforming the data with the technical analysis program (Tas-Plus apparatus), we calculated the platelet and erythrocyte aggregation indexes (PAI and EAI). The research was conducted in accordance with the Declaration of Helsinki. Pregnant and healthy control women were notified of the study. We have received informational consent from them. Our statistical data showed that the aggregation of erythrocytes (EAI) is increased in comparison with the norm by 15% (*), the aggregation of platelets (PAI) is increased in comparison with the norm by 25% (*).

KEY WORDS: platelet; erythrocyte; D-dimer; pregnancy

Introduction

The hemostasis system is a biological system that ensures the preservation of the liquid state of the blood, maintaining the integrity of the walls of blood vessels, preventing and stopping bleeding from the latter by thrombosis. Hemostasis is implemented mainly by three interacting functional and structural components: the walls of blood vessels (primarily intima), blood cells (platelets, erythrocytes, etc.) and plasma enzyme systems (coagulation, fibrinolytic, kallikrein-kinin, etc.). Until recently, the plasma blood coagulation system was of decisive importance in the implementation of hemostasis. However, the vessels themselves (spasm, opening of shunts above the injury site) and blood cells (platelets and erythrocytes) are the first to react to vascular damage. It is platelets, and not blood clotting, that play a leading role in the primary arrest of bleeding from microvessels, the most vulnerable and most often the source of hemorrhages. Thus, it is possible to distinguish primary hemostasis – platelet-vascular reaction and secondary hemostasis – blood coagulation. More often than not, these mechanisms function simultaneously and in conjunction. Platelet-vascular (primary) hemostasis Intact vascular endothelium has thromboresistance.

This quality of the endothelium is due to the following properties:

- Prevention of platelet aggregation due to negative surface charge and synthesis and secretion of prostacyclin antiplatelet (PGI);
- Suppression of coagulation hemostasis due to the binding of thrombin by thrombomodullin and inactivation of other procoagulants (V, VIII, IX and X plasma factors);
- By activation of anticoagulants:
  - ◊ by the thrombin-thrombomodullin complex of the protein C system, inactivating factors V and VIII;
  - ◊ the synthesis of protein S – a cofactor of protein C;
  - ◊ the synthesis of heparin-like proteoglycans (heparin sulfate, etc.), which activate antithrombin III, inactivating, in turn, all enzymatic plasma coagulation factors;
  - ◊ synthesis of an inhibitor of the tissue factor pathway (IPTP), which inactivates the tissue factor (TF) – FYIIa complex;
- Activation of the fibrinolytic system due to the synthesis of tissue plasminogen activator (TPA);
- The ability to metabolize biologically active substances that affect hemostasis

(biogenic amines, atherogenic lipoproteins, PAF, etc.);
- Production of endothelium-relaxing factor (nitric oxide).

The participation of platelets in hemostasis is determined by the performance of the following functions:
- Angiotrophic (about 15% of circulating platelets are consumed daily for the role of physiological “breadwinners” of the endothelium);
- The ability to maintain the spasm of damaged vessels by secreting vasoactive substances (adrenaline, serotonin);
- Participation in the activation of secondary coagulation hemostasis due to plate factor 3 – a component of the platelet membrane, which plays the role of a phospholipid matrix on which coagulation hemostasis reactions take place, and the release of other procoagulants during degranulation;
- The ability to clog a damaged vessel with a primary platelet thrombus resulting from the implementation of the adhesive-aggregation function of platelets;
- The implementation of the reparative function due to the release of a growth factor that causes migration to the site of damage of fibroblasts, macrophages, smooth muscle cells;
- Retraction of a blood clot with the participation of contractile proteins.

The damaged endothelium is transformed into a powerful procoagulant surface:
- As a result of the release of adrenaline and the secretion of endothelin-1, a transient spasm of the vessel develops at the site of injury, which slows down blood flow and improves the interaction between platelets, coagulation factors and the site of damage;
- The production of the physiological antiaggregant prostacyclin decreases and the release of platelet activators, stimulators of their adhesion and aggregation increases: adrenaline, ADP, von Willebrand factor, thromboxane A2, platelet aggregation factor (PAF), etc.
- The anticoagulant activity of the endothelium weakens: the activity of thrombomodulin decreases, the synthesis of protein S, the activation of antithrombin III, the synthesis of a tissue factor pathway inhibitor (IPTP);
- The procoagulant potential increases: tissue factor (FIII), factor V, factor XIII are released, factor XII or Hageman’s factor is activated (contact activation);
- The synthesis of tissue plasminogen activator (TPA) decreases and the release of its inhibitors increase;
- The subendothelium and basement membrane are exposed, the components of which cause the activation of hemostasis mechanisms.

The process of hemostasis in case of damage to the vascular wall can be conditionally divided into several interrelated stages:

- Local vasoconstriction (neurohumoral, metabolic, axon reflex);
- Adhesion of activated platelets to the damaged site; aggregation of platelets with the formation of a primary platelet thrombus;
- Activation of coagulation hemostasis localized by the anticoagulant system at the site of vessel damage; stabilization of a platelet thrombus by the formed fibrin filaments;
- Recanalization of the vessel due to activation of the fibrinolytic system (plasminogen system) [1, 2].

About Platelet Adhesion-Aggregation Function were a lot of scientific literature [3, 4, 5]. Summarizing, we can conclude that.

For full adhesion of platelets to the damaged site, the following conditions are necessary:

- Contact with the main stimulator of adhesion collagen subendothelium in the presence of a plasma cofactor of calcium ions;
- Change in the shape of platelets from discoid to spherical with pseudopodia (see Fig. 1, 2);
- Synthesis of adhesive proteins by endothelial cells (von Willebrand factor, etc.);
- Expression on the platelet membrane of von Willebrand factor receptors – glycoproteins Ib (GP Ib). The absence of these receptors (Bernard-Soulier thrombocytodystrophy) and von Willebrand factor deficiency (von Willebrand disease) lead to the development of hemorrhagic syndromes.

In parallel with adhesion, the process of platelet aggregation proceeds. Stimulants of aggregation are collagen, which gives the primary stimulus, to the greatest extent ADP, as well as catecholamines (adrenaline, norepinephrine), serotonin, thromboxane A2, PAF, which are released from the vascular wall, hemolyzed in the area of damage to erythrocytes and initially adhered platelets and activated platelets and activated platelets and leukocytes. Subsequently, aggregation stimulants (adrenaline, serotonin, ADP, antiheparin factor 4, etc.) release the platelets themselves during degranulation. Stimulants of aggregation, in particular adrenaline, act by exposing and expressing glycoproteins IIb / IIIa of fibrinogen receptors on the platelet membrane. Fibrinogen, interacting with these receptors, binds neighboring platelets with bridges to form reversible aggregates. The formation of irreversible aggregates provides thrombin, which is formed in the hemostasis zone within a few seconds, due to the activation of coagulation hemostasis by an external mechanism in small amounts, as well as other adhesive proteins (fibronectin, thrombospondin) [6, 7, 8].

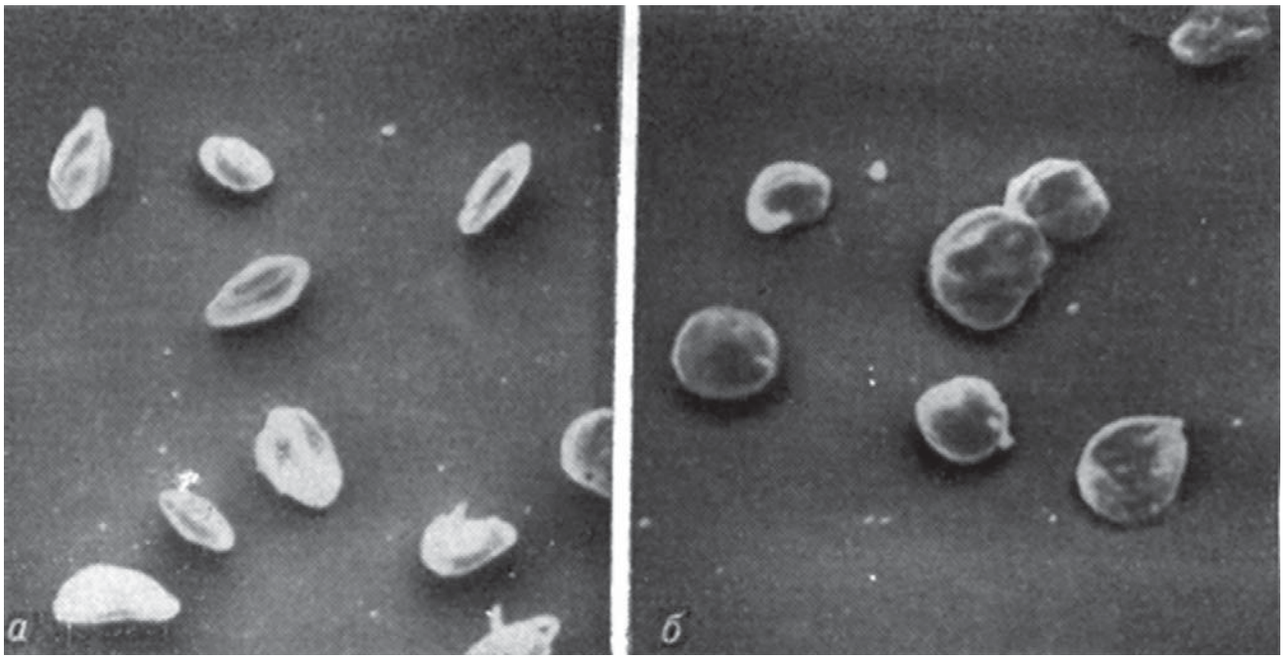

Fig. 1. *Scanning electron diffraction patterns of non-activated (a) and weakly activated (b) platelets*

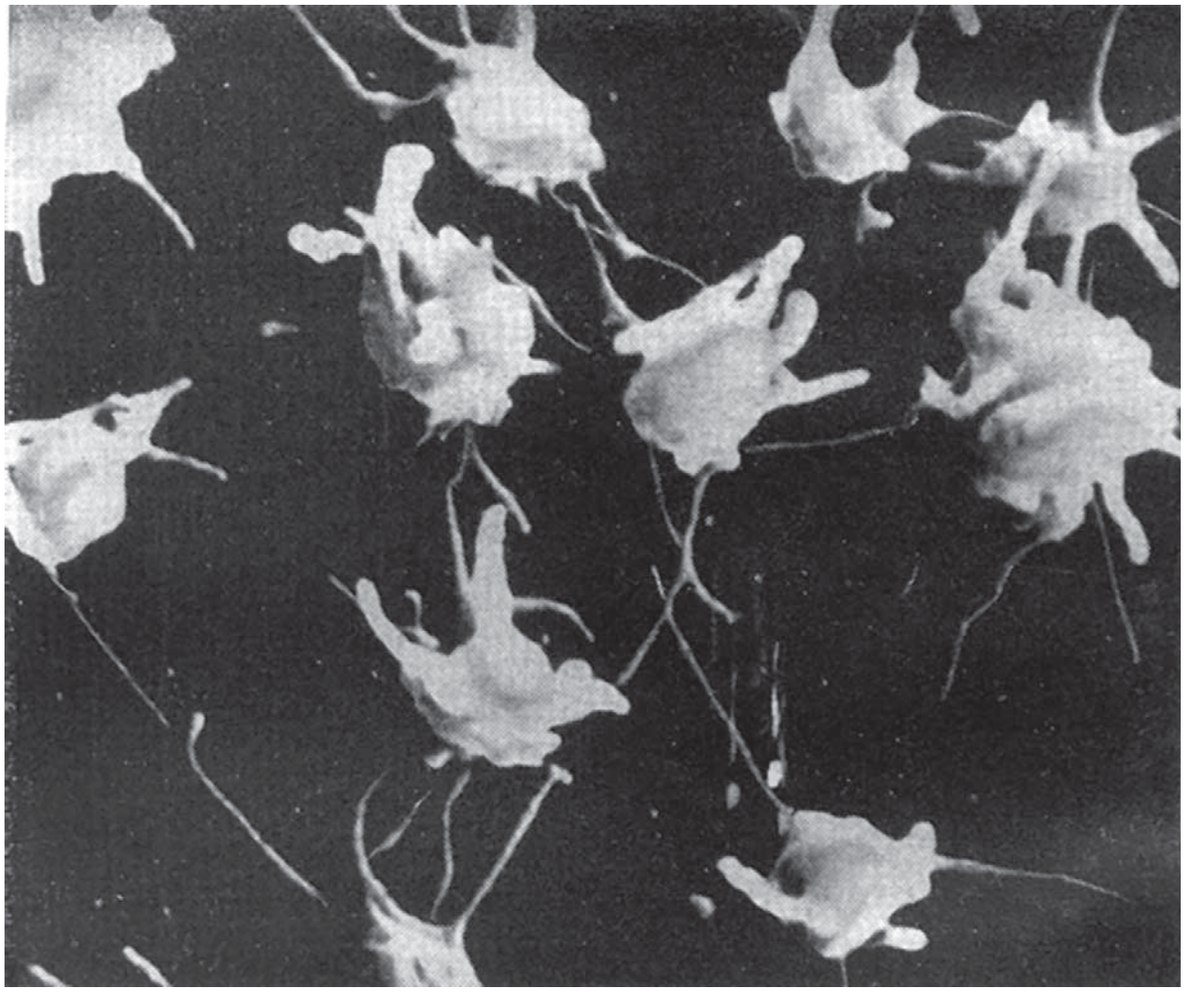

Fig. 2*. Scanning electron diffraction pattern of activated platelets*

The ability to activate aggregation is also possessed by such agents produced by other cells (leukocytes, etc.) as tumor necrosis factor (TNF), interleukins, leukotrienes, etc. Magnesium ions, protein cofactors, denoted as aggrexones, phospholipid cofactor, etc. are involved in the implementation of AAFT. Ultimately, a hemostatic platelet plug is formed and bleeding from microcirculatory vessels stops in 2-4 minutes (Duke's test or bleeding time – VC). Platelets also contain activators of polymerization of fibrin monomers, factor V, and many plasma coagulation factors are concentrated on the surface and in the channels, as a result of which their high concentration is created in the hemostatic plug. Due to this, platelets significantly affect the intensity and rate of local blood coagulation in the thrombus formation zone [8,10].

The study of hemostasis in clinical practice is necessary and compulsory. The study of hemostasis in pregnant women is especially relevant.

Pregnancy is not a pathological phenomenon; it is a physiological process accompanied by changes in all body systems.

A normal pregnancy is accompanied by many changes to ensure the growth of the fetus. Changes also occur in the hemostatic system, while any deviations from the norm can be fraught with serious complications for both the mother and the child.

Changes in the hemostasis system in pregnant women are primarily associated with the emergence of a new circle of blood circulation. This is the uteroplacental circulation. The uteroplacental circulation is necessary for the full supply of the fetus with oxygen and nutrients. The number of platelets changes [8].

In most cases, the content of platelets in the blood remains unchanged, but in about 10% of women, the concentration of these cells decreases. Thrombocytopenia develops. It is usually associated with three conditions:

- Hypertensive disorders, such as preeclampsia;
- Gestational thrombocytopenia caused by an increase in total blood volume;
- Idiopathic thrombocytopenic purpura.

It is known that an increase in the coagulation potential is associated with a significant increase in the level of all blood coagulation factors, except for factors XI and XIII. The concentration of fibrinogen in plasma increases.

Changes in the indicators of hemostasis in pregnant women, the general picture:

- Plasma fibrinogen levels at the end of pregnancy may be higher than normal;
- The content of factor VII can increase several times;
- The level of von Willibrand factor and factor VIII increases in late periods, when the activity of the coagulation system is more than doubled compared to the non-pregnant state;
- Factor IX levels increase slightly;
- Factor XI level decreases slightly;

- Factor XIII levels gradually decline after an initial increase, reaching half the normal value for non-pregnant women;
- The level of factors II and V does not change significantly;
- Antithrombin often remains at the same level;
- Protein C activity presumably unchanged;
- Protein C antigens tend to increase in the second trimester, however they remain within the normal range;
- Total and free protein S decrease with increasing gestational age;
- Fibrinolytic activity decreases during pregnancy, remaining low during labor and in the postpartum period.

However, it is not known specifically how erythrocyte aggregation and platelet aggregation behave.

The aim of our work was to find out how erythrocyte aggregation and platelet aggregation change during normal pregnancy. For this, we have worked out a special research protocol. It should be noted that a parallel study of the aggregation ability of red blood cells is very relevant and will bring innovation to the study of hemostasis in pregnant women.

Materials and Methods

To achieve our goal, we studied 20 women with their first pregnancy between the ages of 25 and 28. In addition to the standard set of tests, each woman was examined for the erythrocyte aggregation index and platelet aggregation index. The gestation period was 18 to 20 weeks. The weight gain of pregnant women corresponded to the norm. The complete blood count was normal. The pregnant women did not have any particular complaints. The control group included 10 non-pregnant women. The group of pregnant women and the control group were statistically shuttered between themselves.

To study the aggregation of enterocytes and to study the aggregation of platelet aggregation (Rheoscan apparatus). Further, by transforming the data with the technical analysis program (Tas-Plus apparatus), we calculated the platelet and erythrocyte aggregation indexes (PAI and EAI).

The research was conducted in accordance with the Declaration of Helsinki. Pregnant and healthy control women were notified of the study. We have received informational consent from them.

Results

Our statistical data showed that the aggregation of erythrocytes (EAI) is increased in comparison with the norm by 15% (*), the aggregation of platelets (PAI) is increased in comparison with the norm by 25% (*). (See Fig. 3, 4).

It is necessary to pay attention to such reliable facts, despite the fact that the groups studied by us are small.

Discussion

As we can see, the intensity of aggregation increases in comparison with the control. What caused such a reliable increase in the process of aggregation of red blood corpuscles. During pregnancy, significant changes occur in the hemostasis system, aimed at increasing the total activity of blood coagulation factors. This is due to the fact that in the walls of the vessels that provide placental blood flow and, therefore, the vital activity of the fetus, there is no layer that prevents blood clotting inside the vessels. Fibrin threads regularly accumulate on the tissues of the placenta. So that they do not disturb the blood flow, it is necessary to constantly dissolve them, and for this the fibrinolytic system of the blood must be much more active than before conception. That is why the indicators reflecting the level of aggregation in healthy women who are expecting a baby are increased.

The change in aggregation by its clinical value is similar to an increase in the concentration of D-dimer with increasing gestational age. Thus, during pregnancy, physiological changes in the hemostatic system are observed.

As part of our study, we want to add the parameters we are investigating to the required list of studies of the hemostasis system in pregnant women. These are erythrocyte aggregation and platelet aggregation.

Most experts agree that the assessment of hemostasis must be carried out at different stages of pregnancy, starting from the moment of the initial examination.

To assess hemostasis, the level of several indicators is examined, each of which plays an important role in the functioning of the blood coagulation system.

The minimum examination of hemostasis includes the determination of the following parameters: Activated partial thromboplastin time, prothrombin time, fibrinogen, D-dimer.

In some laboratories, activated partial thromboplastin is called the activated partial thromboplastin time. This is the time required for the clotting of blood plasma after adding calcium, phospholipids and kaolin to it. A shortening of the activated partial thromboplastin time indicates an acceleration of clotting and an increase in the likelihood of developing disseminated intravascular coagulation, as well as the possible presence of antiphospholipid syndrome or deficiency of coagulation factors. Prolongation of the activated partial thromboplastin time is characteristic of insufficient blood coagulation capacity and the risk of bleeding during childbirth or in the postpartum period. Prothrombin time is an indicator of hemostasis, showing how long it takes for blood plasma to coagulate when calcium and tissue factor are added to it. Reflects the outer folding path. Shortening of prothrombin time is characteristic of disseminated intravascular coagulation. Elongation may indicate an increased likelihood of postpartum hemorrhage due to a deficiency of a number of clotting factors, liver disease, vitamin K deficiency, and some other conditions and diseases.

In different laboratories, prothrombin time can be represented in three ways:

- Prothrombin index, which is the ratio of a given prothrombin time result to that of normal blood plasma;
- Prothrombin according to Quick, which reflects the level of various coagulation factors in percent;
- INR, or INR – international normalized ratio, an indicator reflecting the comparison of blood coagulation of the test sample with coagulation of standardized blood in the norm.

Fibrinogen – a protein from which fibrin is formed, which is involved in the formation of a red blood clot. A decrease in the content of this protein is observed with disseminated intravascular coagulation syndrome, liver pathology. Increased fibrinogen levels during pregnancy are normal. You should also determine the number of platelets in the blood to exclude thrombocytopathies.

D-dimer is a breakdown product of fibrin, a small protein fragment present in the blood after the destruction of a blood clot. That is, its increase indicates an active process of thrombus formation. At the same time, this indicator physiologically increases during pregnancy. However, measuring the D-dimer level is not enough to confirm that a patient has developed thrombosis. To confirm the diagnosis, additional instrumental research methods (ultrasound duplex angioscanning, CT angiography) should be performed and the presence of clinical signs of the disease should be assessed. If antiphospholipid syndrome (APS) is suspected, doctors may determine the presence of lupus anticoagulant, anticardiolipin antibodies, and antibodies to β2-glycoprotein 1. Also, in some cases, doctors may suggest the presence of hereditary thrombophilia (the genetically determined ability of the body to form blood clots).

Along with these parameters, we insist to include the parameters studied by us in the recommended list of tests for pregnant women, since they are especially informative from the point of view of placental blood flow [11, 12].

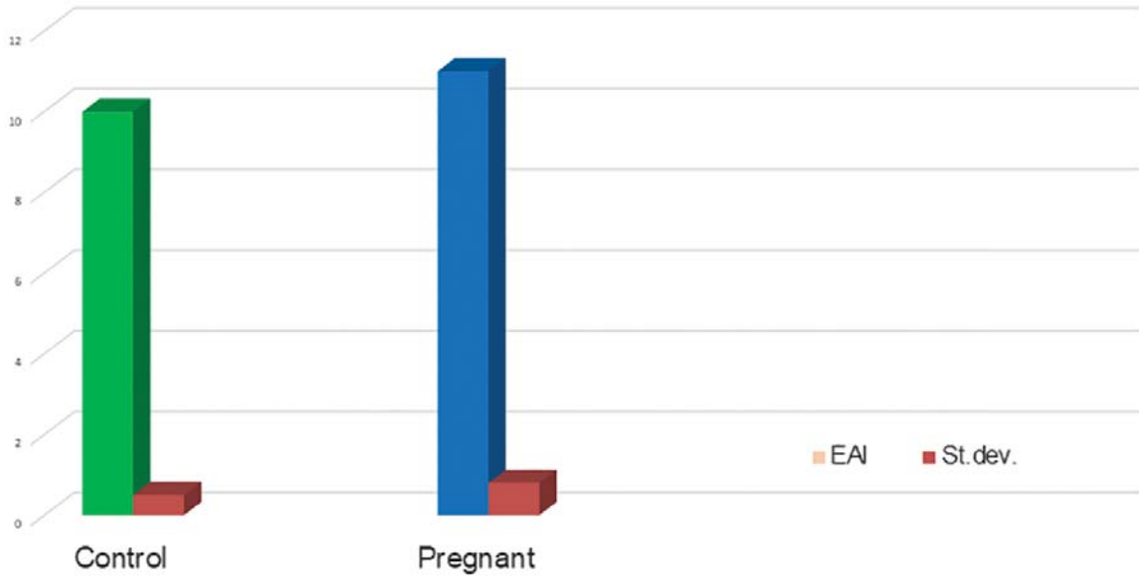

Fig. 3. *Aggregation of erythrocytes (PAI) in control group and in group with*

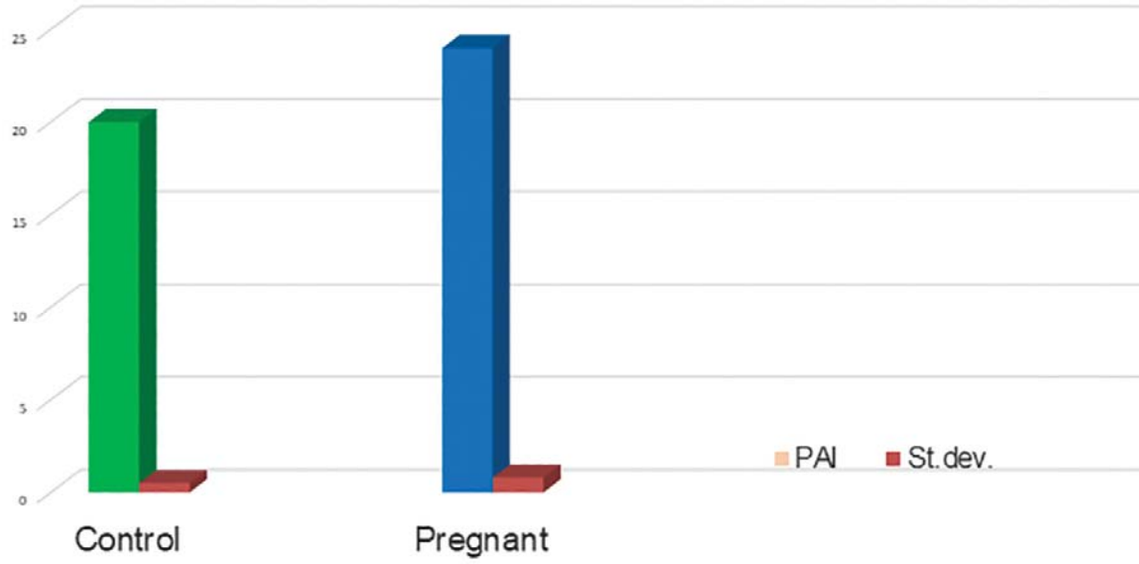

Fig. 4. *Aggregation of platens (EAI) in control group and in group with pregnant women. (*). [*] – high mathematical validity*

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