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Comparison of Maternal Platelets in Preeclampsia and Normotensive Pregnancies: An Ultrastructural Study

Elif Erdem[1], Murat Akkus[2,*], Selcuk Tunik[2], Yusuf Nergiz[2], Mehmet Siddik Evsen[3], Mehmet Zeki Taner[3]

[1]Department of Nursing, School of Health Sciences, University of Artuklu, 47200, Mardin, Turkey
[2]Department of Histology and Embryology, University of Dicle, 21280, Diyarbakir, Turkey
[3]Department of Obstetrics and Gynecology, University of Gazi, 06500, Ankara, Turkey

Abstract

Background: *Preeclampsia is characterized by hypertension and proteinuria occuring after the 20th week of gestation. The purpose of this study was to compare the ultrastructural changes occuring in platelets of preeclamptic and normotensive pregnant women.*

Method: *Venous blood samples of whom 20 with preeclampsia and 20 of normotensive, for platelets extraction were firstly centrifuged. After prefixation and postfixation, was dehydrated at the increased alcohol degrees, left in infiltration and blocked. Then, semi-thin sections taken with ultramicrotome were stained with toludine blue. The assesment was made through light microscope following thin sections being taken and stained with contrast, platelets were examined ultrastructurally and their electron micrographs were taken.*

Results: *In the preeclampsia group the number of alpha and dense granules decreased, to a great extent, and the structures of channel systems in tubular structure, were observed to be irregular.*

Conclusion: *It is clearly seen that preeclampsia induces cellular damage in platelets and in case of aggravation in preeclampsia, it may cause significant changes in cell membranes of platelets and cytoplasmic organelles.*

Keywords: *Preeclampsia, platelets, ultrastructural structure*

[*] Corresponding author: E-mail:drmakkus@gmail.com, Tel.: +90 412 2488001 (ext. 4403), Fax: +90 412 2488440

Introduction

Platelets are anucleate cytoplasmic fragments derived from bone marrow megakaryocytes. The ultrastructure of the normal platelet reveals the surface-connected canalicular system, which is a continuous membrane system extending from the plasma membrane and the dense tubular system. Platelets contain several types of storage granules. These are alpha granules, lysosomes, and dense granules[1]. The platelets play a pivotal roles in certain metabolic diseases. They are firstly showed in haemostatic plugs in 1882 by Bizzozero and Hayem and the full appreciation of the intracellular architecture of these cells was showed with advent electron microscopic techniques[2,3]. A great deal of studies have now described the role that of the intracellular organelles to the functional of platelets, including thrombosis, inflammation, tumor progression as well as haemostasis[4,5].

Preeclampsia is a multisystem disease of unknown etiology and is characterized by hypertension and proteinuria occurring after the 20th week of pregnancy[6,7]. This metabolic and multisystem disorders, conjunction with abnormal vascularization, increased platelet aggregation, activation of the coagulation system and endothelial cell dysfunction occurs[6]. Endothelial damage in the uteroplacental and systemic circulation due to the insufficiency or total lack of trophoblastic invasion of maternal spiral arteries is considered as the main reason for the pathophysiology of preeclampsia[8]. Endothelial cell dysfunction occurs in the final stage of the pathogenesis of preeclampsia and is associated with the other characteristics of the disease, such as vasospasm, increased capillary permeability and platelet aggregation[9]. Serotonin, a vasoactive substance in the dense granule content of platelets, is released along with vasoconstrictor TXA2. An increase in the ratio of TXA2 to PGI2 may be the cause of platelet destruction and may have an explanatory significance for reduced uteroplacental blood flow along with spiral artery thrombosis and placental infarction occurring in preeclampsia. Platelets adhere to abnormal endothelial cells, and their subsequent activation occurs[10]. In this study, we aimed to compare ultrastructural changes that occur in the platelets of preeclamptic and normotensive pregnant women.

Materials and Methods

The present study was started immediately after obtaining approval of Ethics Committee of Dicle University Faculty of Medicine. Blood samples of both preeclampsia and control groups were drained from pregnant women who had been hospitalized at Dicle University, Department of Obstetrics and Gynecology. Every expectant mother was informed about and gave consent for the study. Preeclamptic group included pregnant women without a previous history of hypertension or renal disease and with a proteinuria of >300mg in 24-hour urine as well as persistent high blood pressure (> 140/90 mm Hg) in the current pregnancy. Control group excluded the pregnant women with hypertension, proteinuria or edema. The blood samples were obtained from blood samples were collected from 20 patients with the clinical presentation of preeclampsia for whom any treatment had not yet been commenced. Venous blood samples drained from the arms of pregnant women were taken into tubes containing EDTA and then centrifuged for 5 minutes at 250g to obtain platelet-rich plasma (PRP). The plasma portion was then taken and centrifuged at 4000 rpm for two minutes. Platelets obtained were fixed in 1.25% phosphate-buffered glutaraldehyde at the same rate for 45 minutes. Then, they were taken into 1% osmium tetroxide for 1.5 hours for post-fixation process, and kept in 50%, 70%, 80%, 90%, 100% alcohols each for 15 minutes. Tissues were kept in fresh resin in incubator at 60oC for two days and embedded. Then semi-thin sections were obtained by ultramicrotome (Ultracut R, Leica, Germany) and stained with toluidine blue. Subsequently, light microscopic evaluation was performed. Thin sections were collected into copper grids. Then, they

were contrast stained with lead citrate-uranyl acetate and ultrastructural examination of platelets was performed by transmission electron microscope (Evo LS 10+ED, Carl Zeiss).

Result

Ultrastructural examination of platelet sections of maternal normotensive group revealed that some of platelets maintained their discoidal shape, whereas others displayed morphologic changes and extending of the cell membrane extensions, therefore, further morphological changes including significant extending and tapering of the extensions. It was noted that granules of different densities in the platelet cytoplasm maintained the connection with tubular canalicular system towards the periphery and the organelles showed an irregular distribution especially between the granules. The shape changes due to the activation were observed to lead to the formation of spiral-shaped microtubular structures especially in different platelet sections. On the other hand, it was observed that endoplasmic reticulum had a regular tubular structure, mitochondria were located between the granules and glycogen particles presented solitary distribution in some platelets sections of the control group (Fig.1a-d).

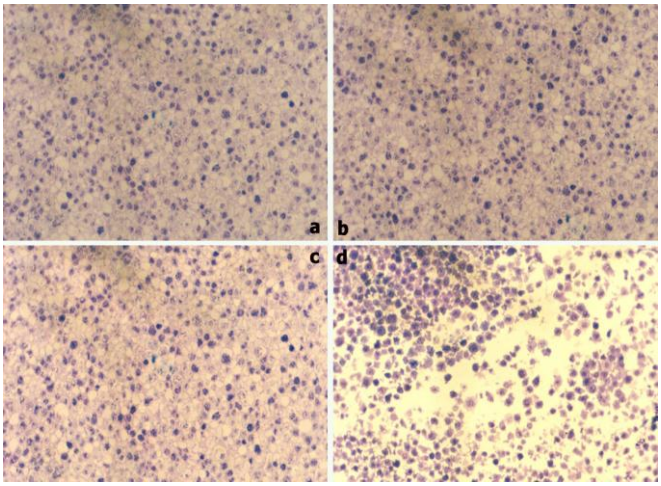

Figure.1: Panoramic view of platelets containing granules in their cytoplasm of nornotensive group **(a, b)**. Increase in cytoplasmic psedupods and decrease in granules **(c)**, loss of cytoplasm and dilation of some channel tubules are observed **(d)** in preeclamptic group sections (Semi-thin sections, original magnification X40)

Ultrastructural examination of cross-sections of the preeclampsia group showed that some of the granules in the cytoplasm were more concentrated, whereas some others were lighter. Between these granular structures, the presence of other granular structures in the form of fine particles was established. The scattered lipid droplets in the cytoplasm, as well as irregular and enlarged tubular canal system were noted. We observed a decrease in the amount of alpha granules and dense granules of some of the platelets in preeclampsia group, as well as an alteration in the diameter of the granules. Dense granules especially with lysosomal enzymes were found to be dark-stained in the peripheral parts in some platelet sections (Fig.2a-j).

Discussion

Under normal circumstances, platelets circulate freely in blood vessels without interacting with other platelets or the vascular endothelium. In the presence of endothelial damage, a chain of events is triggered, leading to platelet-rich clot formation. The responsible events represent a complex series of biochemical and cellular processes that can be loosely divided into four general categories: adhesion, activation, secretion and aggregation[11]. Platelets are complex circulating cellular elements that contribute both directly and indirectly to hemostasis and atherothrombosis. Their diverse biological effects are governed by increasingly well-characterized anatomic, biochemical, and molecular processes which lay the groundwork for novel diagnostic, prognostic and therapeutic investigations[12]. Platelet activation, a complex response to extracellular signals, prompts cytoskeleton rearrangements, membrane fusion, exteriorization and secretion of contents from within three different types of platelet storage granules: lysosomes, alpha granules, and dense bodies. Fusion of alpha granules with each other and with deep invaginations of the plasma membrane followed by an "emptying" of contents to the exterior has been demonstrated[13,14]. In our study, these granules that are closely associated with lysosomes displayed partial decrease in the platelets sections of preeclampsia group. In a study conducted on women with gray platelet syndrome characterized by thrombocytopenia, alpha granules were found to be decreased in number in platelet sections examined ultrastructurally[15]. In our study, maternal platelet sections of preeclampsia group also showed a decrease in alpha granule numbers of the platelet cytoplasm. The numerical decrease in alpha granules observed in the preeclampsia group was considered to suggest thrombocytopenia. Irregularity and dilatation of the canalicular system lead active molecules in the cytoplasm to easily be excreted and to structural change in the platelet plasma membrane. In the sections of the preeclampsia group, microtubule structure was found to be changed, which in turn led to the deterioration of discoidal structure of platelets.

Glycoprotein receptors of the platelets allow the transmission of impulses from extracellular to the intracellular environments, the collection of granules in the center of the cell, and the unification of granule membranes with open canalicular system. Granular content is carried outside platelets in this way, which contributes to the adhesion and aggregation. In the platelet sections of the control group, it has been clearly observed that granules were collected in the center of the cell and moved towards the peripheral part of the cells through the canal system. In the sections of preeclampsia group, the granules in the cytoplasm of several platelets were mostly moved from the central region to the peripheral part of the cells via dilated canalicular system[16]. In our study, this alteration in the preeclampsia group supports the platelet aggregation in the etiology of preeclampsia. Platelets are known to be one of the main sources of circulating serotonin. Increasing amounts of serotonin is secreted during platelet activation and aggregation. In preeclamptic pregnant women, platelet activation and aggregation are evidenced by the distribution of dense the granules in the cytoplasm, which are responsible for the secretion of serotonin. In this study, ultrastructural examination of the granular structure of the cytoplasm revealed the transport of secretions through tubular canals and the presence of significant activation.

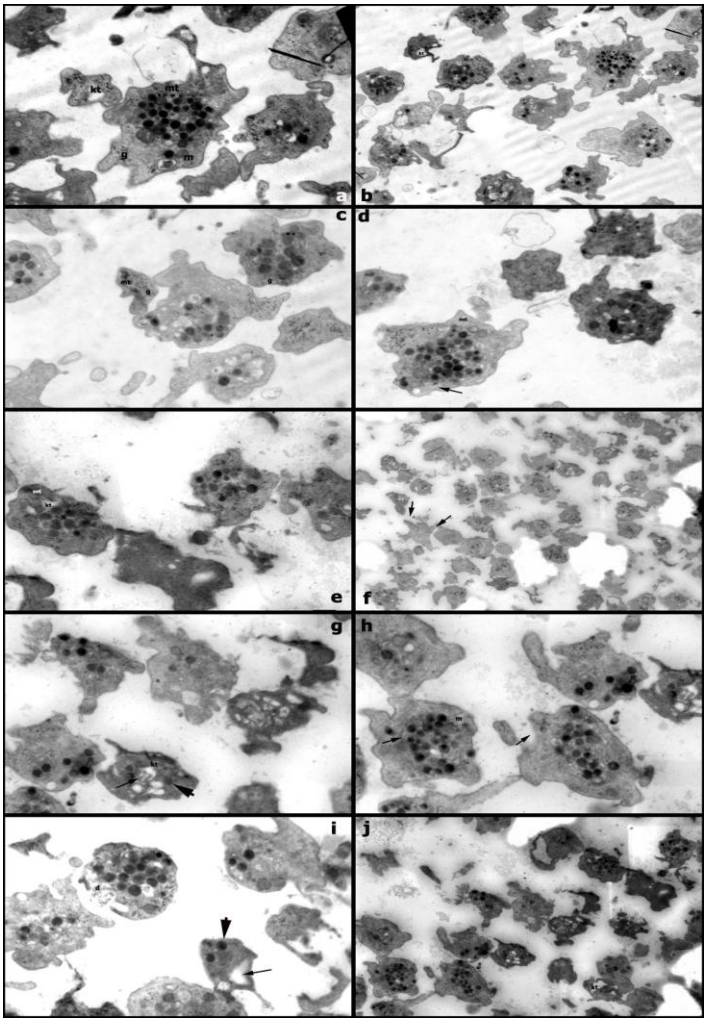

Figure.2: Electron dense and lesser dense granules, glycogen (g) and mitochondria are scattered in normal manner in the cytoplasm of normotensive group sections. Microtubules (mt) localized in peripheral side is remarkable in normotensive group **(a).** There are no any change in canaliculiar structures (kt) and tubular component of the rough endoplasmic reticulums **(b).** Finger-like invagination of plasma membrane into the cytoplasm, glycogen granules and microtubules were remarkable **(c).** Tubular structures of rough endoplasmic reticulum (arrow) and microtubules (mt) were preserved in normal appearance **(d)**. Dilatation of canaliculiar system (kt) which opened into plasma membranes of platelets, increased in pseudopods (arrow) and discoidal structures and organization of microtubules (mt) adversely affected in some platelets **(e,f).** Disarrangement of canaliculiar tubular system and presence of inclusion body (arrow) as well as α granules(arrowhead) decreased in some platelets **(g)**. Mitochondrial (m) cristolysis, dilatation of cisterna of endoplasmic reticulum (arrow) and irregularity of pseudopod (arrowhead) were observed **(h)**. Loss of cytoplasm and degeneration of plasma membrane of platelets (d), lysosomal hypertrophie (arrowhead) and dilatation of some tubular canaliculiar (arrow) were observed (i). Canaliculiar (kt) structures expanded in the cytoplasm of platelets **(j)**. Normotensive groups (a-d), preecplamsia group (e-j). Magnification for **a:**3700, **b:**1750, **c:**3600, **d:**3600, **e:**3650, **f:**1050, **g:**3700, **h:**3680**, i:**3750, **j:**1720, uranyl acetate and lead citrate.

In a study conducted on preeclamptic placentas, light and electron microscopic examinations showed a degeneration in mitochondria and smooth endoplasmic reticulum of the platelets in the capillary lumen[17]. In our study, a degeneration was observed especially in mitochondria due to loss of cristae, whereas smooth endoplasmic reticulums were found to be dilated in the cytoplasm of the platelet sections of preeclampsia group. In another study conducted on patients with thrombocytopenic purpura, ultrastructural examination of platelets revealed that discoidal and ellipsoidal structure was protected, the number of alpha granules was decreased in cytoplasm and vacuolar structures were observed in the cytoplasm[18]. In the literature, there is no study on ultrastructural examination of the

platelet alterations caused by preeclampsia. However, an ultrastructural assessment was conducted on the damage to the platelets caused by thrombocytopenia which is one of the clinical signs of preeclampsia. In contrast to this study[18], our study revealed that some of the platelet plasma membranes were altered and lost their discoidal and ellipsoidal structure, alpha granules showed an irregular distribution, while vacuolar structures were distributed very sparsely. Thrombocytopenia and platelet aggregation are a few of the characteristics of preeclampsia. Excessive platelet aggregation, maternal vasoconstriction and endothelial cell injury may be associated with placental infarction. Platelet aggregation causes the release of thromboxane (TXA2). Platelet-derived TXA2 increases the ratio of TXA2/PGI2 in preeclamptic women. In one study, larger platelet volumes were found to be the precursor to developing preeclampsia[19]. In our study, different sized platelet-sections in the preeclampsia group have attracted our attention in ultrastructural evaluation. However, an excessive volume increase is not expected.

Increased lipid peroxidation in preeclamptic pregnant women has been evidenced by the studies conducted. As a result of the increasing lipid peroxidation, prostacyclin (PGI2) synthesis is reduced and endothelial cell injury and platelet membrane damage occur. The balance between endothelium-derived prostacyclin (PGI2) and platelet-derived thromboxane is deteriorated against the favor of PGI2[20,21]. We have also found this damage of the plasma membrane in some of the platelet sections in the preeclampsia group, which is an important histopathological finding for our study in particular.

The damage of the plasma membrane was also found to cause a significant loss in the cytoplasm of platelets. A variety of platelet factors such as TXA2 and ADP, which are secreted from plasma membrane as well as granules, attracts more platelets to the damaged area, and following platelet activation, platelets adheres to the wall of blood vessels in the damaged area and ensures the aggregation. Platelet membrane system, surface-associated canalicular system, dense tubular system and microtubule circumferential band play an important role associated with platelet size and contraction and release of granules. In our study, surface-associated canalicular system showed a significant dilatation and an alteration of its morphological structure towards the cell membrane especially in the platelet sections of the preeclampsia group. It was observed that circumferential band appearance of microtubules was be deteriorated in the preeclampsia group and thus contractile structure was also altered. In some platelet sections of the preeclampsia group, it has been identified that granular secretion was developed with the tubular system and tubular system showed irregularities in some places. The platelet activation included in the etiology of preeclampsia is of great importance in terms of understanding how and to what extent the effects such as thrombocytopenia and platelet aggregation affects the histopathology of platelets.

In this study, granular changes in the platelet cytoplasm as well as irregularities in the canalicular structures, which were detected by ultrastructural evaluation of the platelet sections of the pregnant women with preeclampsia, are descriptive findings at the cellular level. The concurrent evaluation of the damage of the platelet cell membrane, which was caused by the increase in lipid peroxidation that is one of the most significant effects of preeclampsia, and cytoplasmic loss is the most important evidence of that we attribute the cell damage to the lipid peroxidation.

Conclusion

The evaluation of the damage to cytoplasmic organelles (the disruption of mitochondrial cristae, dilation of endoplasmic reticulum) clearly shows that preeclampsia triggers the cellular damage in the platelets and that aggravation of preeclampsia may lead to significant alterations and loss of organelles in the cell membrane and cytoplasm of the platelets. We believe that this study may help to reveal metabolic and functional effects caused by preeclampsia on the platelets ultrastructurally at the cellular level and to better explain the metabolism.

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