MÆDICA - a Journal of Clinical Medicine

EDITORIALS

The endothelial-platelet dysfunction in preeclampsia

Ana-Maria VLADAREANU, MD, PhD[a]; Cristian ANDREI, MD[b]; Minodora ONISAI, MD[a]; Veronica VASILACHE, MD[a]; Horia BUMBEA, MD[a]; Radu VLADAREANU, MD, PhD[b]

[a]Hematology Department, Emergency Universitary Hospital, Bucharest, Romania
[b]Obstetrics-Gynecology Department, Elias Emergency Universitary Hospital, Bucharest, Romania

ABSTRACT

Preeclampsia is a common, pregnancy-specific syndrome defined by clinical findings of elevated blood pressure combined with proteinuria and edema. It is characterized by a generalized dysfunction of the maternal endothelium, as demonstrated by the increased levels of factor VIII, total and cellular fibronectin, thrombomodulin, endothelin and a disturbance of prostacyclin/thromboxane balance. The new concept of endothelial-platelet dysfunction shows the central role of endothelial cell and platelet: endothelial dysfunction, increased vascular reactivity and platelet activation. The aim of this review is to reveal the recent data regarding the role of endothelial-platelet dysfunction in the pathogenesis of preeclampsia.

Key words: preeclampsia, endothelial dysfunction, platelet, inflammation, cytokine.

PREGNANCY INDUCED HYPERTENSION

High blood pressure (HBP) represents the most common complication during pregnancy, being the first cause of morbidity and mortality in pregnant women. The incidence of HBP in pregnancy is around 5 to 10% of all pregnant women and it represents an issue of public health.

According to the American College of Obstetricians and Gynecologists, high blood pressure in pregnancy is classified as following:

A. Pregnancy induced hypertension (pre-eclampsia, eclampsia)
B. Chronic hypertension before pregnancy
C. Chronic hypertension with superimposed preeclampsia
D. Transient hypertension

The various pathogenic aspects of pre-eclampsia and eclampsia are not completely known. Impaired placentation seems to be an important predisposing factor for preeclampsia. Poor placental blood supply triggers local release of vasoactive factors. These factors are responsible for endothelial systemic dysfunction,

Address for correspondence:
Ana-Maria Vladareanu, MD, PhD, Chairman, Assistant Professor Hematology Department, Emergency Universitary Hospital, 169 Splaiul Independentei, Zip Code 050098, Bucharest, Romania
email address: anamariavladareanu@yahoo.com

increased vascular reactivity, altered coagulation pattern with procoagulant changes and platelet dysfunctions, diminished utero-placental blood supply (the first in order of appearance) and generalized vasoconstriction.

Maternal complications of preeclampsia are: eclampsia, acute renal failure, stroke, placental abruption, HELLP (**h**emolytic **a**nemia, **e**leveted **l**iner **e**nzymes and **l**ow **p**latelet **c**ount) syndrome. The main fetal complications are: abortion, intrauterine death, fetal hypotrophy, premature birth. ❑

THE ENDOTHELIAL-PLATELET DYSFUNCTION – KEY ROLE IN PREECLAMPSIA

The haemostasis alterations are well defined in the new concept of endothelial-platelet dysfunction. This concept shows the central role of the endothelial cell and of the platelet, which interact in a vicious circle: systemic endothelial dysfunction induces vasoconstriction, adhesion and aggregation of platelets and activates coagulation; this generates further hypoxic damage to the endothelium. Platelet activation begins in the first months of pregnancy in women with risk factors for preeclampsia.

The endothelium plays a very important role in controlling the vascular tonus, in hemostasis, in inflammation and in immune responses (1), roles that are realized through the interaction with the circulatory cells. The studies proved that leukocytes' adhesion to the endothelial monolayer and their migration through the vascular wall is an essential step in multiple physiologic processes – such as inflammation – and especially in pathologic processes. It is actually very important in the response to any kind of injury (2, 3, 4). Circulatory platelets play an important role in haemostasis, but also in inflammation. The haemostatic function initiates when the platelets come into contact with damaged endothelial wall or with circulatory coagulation factors, especially thrombin, resulting in platelet activation, aggregation and clot formation. Thus, the endothelial dysfunction and the increase in vascular reactivity are proven to be involved in preeclampsia pathogenesis: the generalized endothelial alteration from preeclampsia causes vasoconstriction and promotes the adhesion and aggregation of the platelets, as well as the activation of the coagulation factors, inducing supplementary hypoxic injury of the endothelium. The data from the literature show that preeclampsia is characterized by an extensive activation of the thrombocytes, demonstrated through an increase of the expression specific platelet surface markers (P-selectin, CD63 and PECAM 1 – platelet endothelial cell adhesion molecule 1); those were studied from week 14 of gestation through flowcytometry and aggregometry (5).

At the international level, although there are some clear protocols for the investigation of pregnant women with high blood pressure, there is not yet a unanimous concept concerning haemostasis modifications. The actual studies are in different stages of evolution and there are some concerns about developing some reference centers in this field.

Gathering new information concerning molecular and cellular changes in the function of platelets and endothelium seems to deliver a clearer image concerning the mechanism of appearance of the pathological state. These data have to be correlated with clinical observable manifestations. The endpoint is to establish a practical method for the early determination of the risk of disease appearance (through the selection of some relevant tests).

The specific methods include: i) flow-cytometric study of the expression of platelet membrane receptors, with the aim of determining the platelet's activation status, ii) the analysis of platelet-derived microparticles, iii) the importance of the leukocyte-thrombocyte aggregates, iv) the impaired platelet aggregation, v) the thrombomodulin level, vi) the level of the reactive oxygen species generated in the platelets, vii) the intracellular calcium level in the platelets, viii) the study of flow mediated vasodilatation, ix) the plasmatic level of von Willebrand factor, x) the plasmatic level of homocysteine.

The present studies did not show the presence of a genetic predisposition for preeclampsia. There were described many risk factors for the appearance of preeclampsia, but neither one is highly predictive, in order to use its monitoring for successfully predicting preeclampsia: a) nulliparity; b) preeclampsia at an anterior pregnancy; c) age below 18 years or more than 35 years; d) family history of pregnancy – inducing high blood pressure (6); e) chronic renal disease; f) chronic arterial hypertension; g) antiphospholipid syndrome; h) vascular or connective tissue diseases; i) multiple

pregnancies; j) diabetes mellitus; k) high body mass index; l) thrombophilia; m) fetal hydrops; n) intrauterine growth restriction (IUGR).

Several tests were suggested for the detection of the subgroups of pregnant women with high risk for developing preeclampsia: 1) the measurement of arterial pressure, the roll-over test, the urine analysis; 2) peripheral complete blood count (platelets' number, haemoglobin, haematocrit), uric acid, creatinine, liver enzymes, LDH (7); 3) Doppler ultrasound examination of the uterine arteries (high predictive value – protodiastolic notch from the second trimester), biometry for IUGR and amniotic fluid index (AFI); 4) the abnormal Doppler velocity of the umbilical arteries – one of the best clinical tests for the selection of pregnant women with high risks for developing preeclampsia (8, 9); 5) Medium Platelet Volume seems to be a very important parameter, which is significantly increased in women with altered Doppler velocty of uterine arteries (10); 6) unexplained abnormal triple test indicating a high risk for Down syndrome; 7) seric and urinary determination of three urinary proteases synthesized by placenta: VEGF, P1GF, and the soluble receptor VEGF (sFlt1). The sFlt1/VEGF ratio has a higher sensitivity (88%) and specificity (100%) compared to proteinuria for the identification of severe preeclampsia (11); 8) seric determination of hCG and α-fetoprotein; 9) positive test for D-Dimers in almost 50% of patients with preeclampsia comparative to normal pregnancies. A woman with preeclampsia and positive D-Dimers test has a high risk for HELLP Syndrome (12,10) coagulation tests that confirm thrombophilia (protein C, S, Antithrombin III (AT III) deficiency, activated C protein, anticardiolipin antibodies, Factor V Leiden) – associated with an increased risk for severe preeclampsia; 11) the seric level of C reactive protein in patients with preeclampsia can be correlated with the severity of the process (13). ❑

THE STUDY OF ENDOTHELIUM

The endothelium expresses two large classes of receptors for leukocytes: selectins (P and E) and immunoglobulins; their synthesis, expression and connection affinity is regulated especially by proinflammatory cytokines and chemokines (14). The endothelium-leukocyte interaction modifies the endothelium-mediated vasodilatation, with increased permeability and activation of coagulation. Many leukocyte products (including reactive oxygen species, proinflammatory cytokines), may alter endothelial function and may generate a positive feedback loop between inflammation and coagulation. Prothrombotic states are proven to be associated with an alteration of endothelial factors and with the presence of some activation markers: von Willebrand Factor, soluble P-selectin that promotes migration of leukocytes through the blood vessel wall (15). The release of these factors is an early and essential process in thrombosis and inflammation. It is important to take into consideration that von Willebrand factor and P-selectin are also stored in platelets granules and they can be released in response to endothelial injury (15).

Laboratory evaluation that sustain the hypothesis of systemic endothelial dysfunction in women with preeclampsia include: 1) increased circulatory cellular fibronectin, factor VIII and thrombomodulin; 2) alteration of flow-mediated vasodilatation and acetylcholine-mediated vasorelaxation; 3) decreased endothelial vasodilators (such as nitric oxide, prostacyclin) and increased endothelial vasoconstrictors (endothelin and thromboxane); 4) increased vascular reactivity to angiotensin II; 5) serum from preeclamptic women induces cell activation in endothelial cultures from umbilical vein in studies in vitro; systemic arteriolar spasm appears consecutive to endothelial alteration. (16).

Determination of flow-mediated vasodilatation (FMV) through ultrasound measurement of the brachial artery diameter is simple, noninvasive and reproducible. FMV is well correlated with the endothelial function, especially with the presence of serological markers of endothelial alteration (17, 18). Ultrasound determined FMV in brachial artery in the second trimester is a simple and noninvasive method of predicting preeclampsia.

Thrombomodulin (endothelial membrane glycoprotein) functions as a thrombin receptor. The thrombin-thrombomodulin complex is responsible for the inhibition of coagulation through C-protein: it rapidly activates C-protein which, together with S-protein, inactivates factors Va and VIIIa. The thrombomodulin levels are significantly higher in patients with preeclampsia, as a result of both endothelial injury, and inflammatory reaction (19).

It is certain that procoagulant and pro-inflammatory markers also influence platelet function. It was proven that serum soluble CD40 ligand (sCD40L) has an important role in preeclampsia pathogenesis – it is responsible for the increase of proinflammatory and procoagulant properties of the endothelium, for increased endothelium cytokine production and for the dysfunction of the endothelial cells in pregnancies complicated by preeclampsia (20).

ILβ and TNFα cytokines influence platelet aggregation, and TNFα may stimulate IL6 synthesis, while IL6 inhibits TNFα release. The oxidative stress and endothelial activation can also stimulate the IL-6 release, which increases the endothelial permeability and may reduce prostacyclin synthesis by inhibiting the cyclo-oxygenase. The result is an increased thromboxane A2/prostacyclin ratio, which is found in preeclampsia. IL6 also stimulates PDGF (platelet derived growth factor), which is also increased in preeclampsia. Free radicals can induce IL6 synthesis by the endothelium. They are involved in preeclampsia because they can produce endothelial injuries that decrease NO synthesis and imbalance prostaglandins production. Some studies demonstrated that the presence of an increased level of IL6 can contribute to the pathogenesis of the pre-eclampsia (21).

Another cause of altered vascular reactivity – with implications in preeclampsia and eclampsia – is hyperhomocysteinemia, which produces vascular lesions and promotes thrombosis (the aggression and death of the endothelial cell, the oxidation of the LDL and thrombin generation at the vascular level) (22). Homocysteine is lower in normal pregnancy than in the non-pregnant state (23); the increased level of homocysteine in pregnancy is associated with a 3.2 fold increased risk of pre-eclampsia (24).

Von Willebrand factor (synthesized by the endothelial cell and by the megakaryocytes), is also an indirect marker of endothelial dysfunction. The von Willebrand multimers released in the blood circulation rapidly combine with the GP Ib-IX-V and GP IIb-IIIa receptors and induce platelet aggregation.

HELLP syndrome appears in 0.2-0.8% of all pregnancies and 2-20% of pregnancies complicated with severe preeclampsia. It was first observed by Pritchard in 1954 and described by Weinstein in 1982. It is a complication of preeclampsia, characterized by the presence of intravascular thrombosis. The onset of the syndrome is in the third trimester of pregnancy, sometimes in the second trimester; it rarely appears in the first 48-72 hours postpartum. Endothelial dysfunction plays an important role in preeclampsia and HELLP syndrome. It leads to the release of von Willebrand factor multimers, with an increased ability of adhesion to the platelets. Normally, these multimers will undergo cleavage by von Willebrand factor-cleaving protease (ADAMTS13), resulting fractions with decreased biological activity. In HELLP syndrome, endothelial dysfunction and decreased activity of ADAMTS13 is followed by high levels of von Willebrand active factor (25). This can explain thrombocytopenia and thrombotic microangiopathy. The discovery of a structure than can block von Willebrand factor activity could be a potential therapy for HELLP syndrome. The platelet has a very important role in onset of HELLP syndrome, especially fibrinogen receptors which are involved in the final phase of platelet aggregation. In patients with preeclampsia, a large number of platelets express fibrinogen receptors.

In order to differentiate between impaired primary haemostasis (vascular-platelet) from other causes that could complicate a pregnancy with hypertension, the following steps are necessary: 1) to exclude the Leiden mutation, one of the most frequent causes of hereditary thrombophilia – which is a risk factor for pre-eclampsia; the Leiden mutation is by performing the APCR-V test (activated protein C resistance V test), which can identify all types of resistance to activated protein C through mutations of factor V; this test is not influenced by the pregnancy physiological changes; 2) to evaluate the hypercoagulability state through the D-Dimer test using the immunologic method (turbidimetry) on the automated coagulation analyzer.

The endothelial cell dysfunction can be explored through the quantitative determination (immunologic method) of the plasmatic level of von Willebrand factor antigen and through the electrophoretic analysis of the multimeric structure.

It is also necessary to evaluate the plasmatic level of homocysteine in patients suspected of eclampsia, in order to establish a relationship between the incidence and the severity of the disease and the plasmatic level of homocysteine.

The homocysteine may be tested with an immunological method on an automated coagulation analyzer. ❑

THE STUDY OF THE PLATELET

The platelet is a multifunctional cell, implicated in hemostasis and inflammation (Figure 1). The platelets are normally present in the blood stream in an inactive state, but they can activate instantly when they come in contact with the subendothelium (exposed by a damaged vessel) or with coagulation factors (mostly thrombin – Figure 2), inducing activation and aggregation thus forming the platelet thrombus.

Flow cytometry is a very useful technique for the study of activated circulating platelets, allowing platelet phenotyping, platelet activation study – compared to aggregation, it can detect platelet hyperactivation – an increase on platelet function, which determines the syndrome of platelet hyperactivity (CD 63+, P-selectin +); flow cytometric studies can also determine the presence of platelet microparticles and of leukocyte-thrombocyte aggregates (26). This method can demonstrate *in vitro* the presence of platelet activation as a marker of pro-thrombotic activity present *in vivo*, that appears in many pathologic conditions such as pregnancy-induced arterial hypertension, with a marked increase of activation in preeclampsia. The investigation of platelet procoagulant activity is closely connected to increased plasmatic levels of the markers of thrombin production and fibrinolytic activity.

The platelet activation markers are:

- glycoprotein IIb/IIIa (GP IIb/IIIa) (CD 41), demonstrated through the binding to specific monoclonal antibodies anti LIBS1, anti RIBS and PAC1;
- P-selectin (CD 62P);
- glycoprotein 53 (CD 63);
- procoagulant platelet activity, demonstrated through
  - increased levels of annexin V which binds to the prothrombinase complex from the surface of activated platelet
  - monoclonal antibodies against activated coagulation factors Xa and Va, that also bind to the surface of activated platelets.

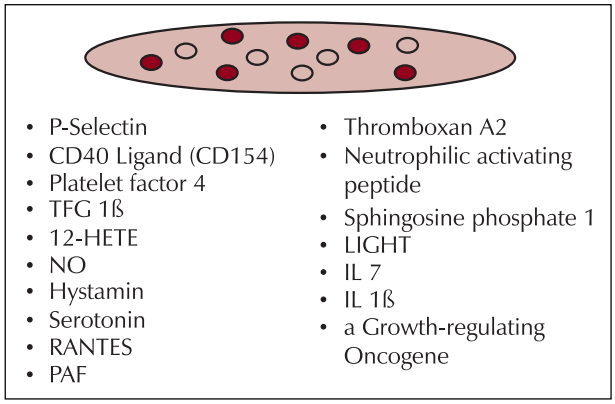

FIGURE 1. The platelet role in inflammation – mediators produced and released by the platelet *(Adapted after Ref 37)*

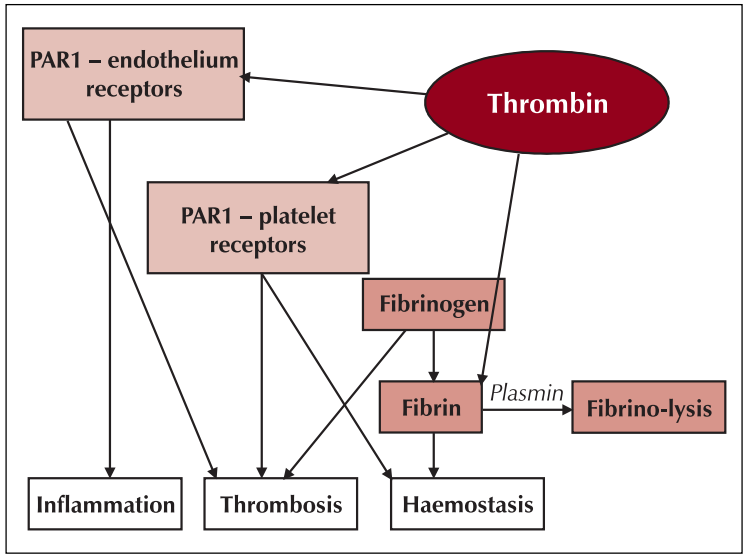

FIGURE 2. The multiple roles of thrombin *(Adapted after Ref 37)*

In preeclampsia, the platelets seem to circulate in a highly activated state. By using flowcytometry to determine the expression of surface platelet antigens, it was proven that in preeclamptic women during the third trimester, the platelets showed a more extensive activation, indicated by the increased expression of P-selectin (CD62P) (27; 28), CD 63, plasmatic β-thromboglobulin (29), platelet endothelial cell adhesion molecule 1 (PECAM-1). CD63 may be useful in the identification of a subgroup of patients with a high risk of preeclampsia, especially if it is correlated with diastolic hypertension during the first trimester of pregnancy (27).

Glycoprotein	HLDA classification	Function	Observations
Fundamental receptors – large number, clear function			
GP Ib-IX-V	CD42b/CD42a	Receptor for Von Willebrand Factor	Deficit –Bernard-Soulier Syndrome
GP IIb-IIIa	CD41/CD61	Receptor for Fibrinogen and von Willebrand Factor	Deficit – Glanzmann thrombasthenia
GP 53	CD63	Platelet secretion marker	Also on the endothelial cells, neutrophils, monocytes
GMP-140 (P-selectin)	CD62P	Platelet activation marker	Also on the endothelial cells
Collagen receptors			
GP Ia-IIa	CD49b/CD29	Collagen receptor	Deficit – variable hemorrhage
GP IV	CD36	Collagen/ thrombospondin receptor	Deficit – no hemorrhage
GP VI		Collagen receptor	Deficit – medium hemorrhage
Extra-cellular matrix receptors – low number, no known clinical manifestations in deficits			
GP Ic'-IIa	CD49e/CD29	Fibronectin receptor	
GP Ic'-IIa	CD49f/CD29	Laminin receptor	
Surface receptors with an uncertain role in haemostasis			
GP IIa' (PECAM-1)	CD31	Heparin receptor	Unclear function – Adhesion?

TABLE 1. Platelet receptors with roles in adhesion/aggregation

Leukocyte-thrombocyte aggregates are important and specific markers of platelet activity. The circulatory level of leukocyte-thrombocyte aggregates CD 11b/CD42b and of CD 62b/CD11b complexes demonstrated through flowcytometry is higher in acute coronary syndromes and – recently proven – in patients with preeclampsia (30).

Platelet microparticles are fragments resulted from platelet activation that were initially discovered through flow cytometry. They express GP IIb/IIIa and GP Ib/IX and are highly thrombogenic, interacting with other procoagulant factors: V, VII, annexin. Platelet microparticles are found in high levels in many thromboembolic disorders, including acute coronary syndromes. So, their study in the multisystemic affection from preeclampsia is perfectly justified.

The previous studies on biophysical properties of the thrombocyte demonstrated the existence of correlations between thrombocytes and different pathological states – as diabetes mellitus. Among the methods that were used, are: dosing of reactive oxygen species (ROS) that seem to play a key role in most thrombotic syndromes (31), as well as membrane fluidity. Although it is known that exogenous reactive oxygen species affect the pathways of platelet activation, recent data also suggest the existence of the platelet production of ROS. But the actual data are insufficient, so it is not very clear if ROS are indispensable or not to the platelet activation (32, 33).

The reactive oxygen species – O_2^- and H_2O_2 – at cellular level can be determined through chemiluminescent methods, based on lucigenin, luminol or tetrazolic compounds (34, 35).

Another category of biophysical studies in the analysis of pathological states of the platelet refers to the determination of concentration of free intracellular calcium. Usually, the intracellular free calcium concentration is very low; its increase during the activation represents a very efficient method of monitoring this process (36). This phenomenon was already used to determine the subjects' susceptibility to arterial hypertension. The level of free intracellular calcium is determined through fluorescence methods using specific fluorofors (Fura2, FuraRed, Fluo3).

Conclusion

Preeclampsia represents an important complication of pregnancy which may have tremendous consequences for both mother and fetus/newborn. The study of the ethiopathogenical factors is very complex: the endothelium is the key in understanding a multi-step process in which the platelet also plays an important part. It is vital to understand the molecular and cellular alterations, because those precede with several months the clinical features, hence offering a significant advantage and the possibility of prevention.

REFERENCES

1. **Moncada S, Vane JR** – The discovery of prostacyclin: A fresh insight into arachidonic acid metabolism. In: Kharasch N, Fried J, Eds. *Biochemical Aspects of Prostaglandins*. New York: Academic Press, 1997; 155-177
2. **Seligman SP, Buyon JP, Clancy RM, et al.** – The Role of Nitric Oxide in the Pathogenesis of Preeclampsia. *Am J Obstet Gynecol*, 1994; 171(4):944-948
3. **Albelda SM, Smith CW, Ward PA** – Adhesion molecules and inflammatory injury. *FASEB J,* 1994; 8:504-512
4. **Westlin WF, Gimbrone MA** – Role of cytokine activation. *Am J Pathol* 1993; 142:117-128
5. **Konijnenberg A, van der Post J, Mol B, et al.** – Can flow cytometric detection of platelet activation early in pregnancy predict the occurrence of preeclampsia? A prospective study. *Am J Obstet Gynecol*, 1997; 177(2):434-442
6. **Heiskanen N**, **Heinonen S, Kirkinen P** – Obstetric prognosis in sisters of preeclamptic women – implications for genetic linkage studies. *BMC Women's Health,* 2003; 3:1
7. **Villar J, Say L, Shennan A, et al.** – Methodological and technical issues related to the diagnosis, screening, prevention, and treatment of pre-eclampsia and eclampsia. *Int J Gynaecol Obstet*, 2004; 85:S28-S41
8. **Chien PF, Arnott N, Gordon A, et al.** – How useful is uterine artery Doppler flow velocimetry in the prediction of pre-eclampsia, intrauterine growth retardation and perinatal death? An overview. *Br J Obstet Gynecol* 2000; 107:196-208
9. **Papageorghiou A, Yu C, Nicolaides K** – The role of uterine artery Doppler in predicting adverse pregnancy outcome. *Best Pract Res Clin Obstet Gynaecol*, 2004; 18(3):383-396
10. **Gioia S, Piazze J, Anceschi M, et al.** – Mean platelet volume: Association with adverse neonatal outcome. *Platelets*, 2007; 18(4):284-288
11. **Levine R, Thadhani R, Qian C, et al** – Urinary Placental Growth Factor and Risk of Preeclampsia. *JAMA,* 2005; 293:77-85
12. **Neiger R, Trofatter MO, Trofatter KF Jr** – D-dimer test for early detection of HELLP syndrome. *South Med J,* 1995; 88(11):1182-1183
13. **Paternoster D, Fantinato S, Stella A, et al.** – C-Reactive Protein in Hypertensive Disorders in Pregnancy. *Clin Appl Thromb Hemost*, 2006; 12(3):330-337
14. **Imhof A, Dunan D** – Basic Mechanism of Leukocyte Migration. *Horm Metab Res,* 1997; 29:614-621
15. **Migliacci R, Becattini C** – Endothelial dysfunction in patients with spontaneous venous thromboembolism. *Thromb Haemost,* 2007; 97:444-504
16. **Shu F, Sugimura M, Kanayama N, et al.** – Immunohistochemical Study of Annexin V Expression in Placentae of Preeclampsia. *Gynecol Obstet Invest,* 2000; 49:17-23
17. **Anderson TJ, Uehata A, Gerhard MD, et al. –** Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*, 1995; 26:1235-1241
18. **Leeson P, Thorne S, Donald A, et al.** – Non-invasive measurement of endothelial function: effect on brachial artery dilatation on graded endothelial dependent and independent stimuli. *Heart,* 1997; 78:22-27
19. **Dusse LM, Carvalho MG, Getliffe K, et al. –** Increased circulating thrombomodulin levels in pre-eclampsia. *Clin Chim Acta,* 2007; 5 (10):1016-1023
20. **Laskowska M, Laskowska K, Leszczynska-Gorzelak B, et al. –** Comparative analysis of the maternal and umbilical interleukin-8 levels in normal pregnancies and in pregnancies complicated by preeclampsia with intrauterine normal growth and intrauterine growth retardation. *J Matern Fetal Neonatal Med*, 2007; 20(7):527-532
21. **Tavakkol Afshari J, Ghomian N, Shameli A, et al.** – Determination of Interleukin-6 and Tumor Necrosis Factor-alpha concentrations in Iranian-Khorasanian patients with preeclampsia. *BMC Pregnancy Childbirth*, 2005; 5:14
22. **Rees MM, Rodgers GM** – Homocysteinemia: association of a metabolic disorder with vascular disease and thrombosis. *Thromb Res,* 1993, 71:337-359
23. **Walker M, Smith G, Perkins S, et al.** – Changes in homocysteine levels during normal pregnancy. *Am J Obstet Gynecol,* 1999; 180(3):660-664
24. **Sorensen TK, Malinow MR, Williams MA, et al.** – Elevated second-trimester serum homocysteine levels and subsequent risk of preeclampsia. *Gynecol Obstet Invest,* 1999; 48:98-103
25. **Hulstein J, van Runnard Heimel PJ, Franx A, et al.** – Acute activation of the endothelium results in increased levels of active von Willebrand factor in hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. *J Thromb Haemost*, 2006; 4 (12):2569-2575
26. **Matzdorff AC, Berchner D, Kühnel G, et al.** – Relative and Absolute Changes of Activated Platelets, Microparticles and Platelet Aggregates after Activation *in vitro. Haemostasis,* 1998; 28:277-288
27. **Konijnenberg A, Stokkers E, van der Post J, et al.** – Extensive platelet activation in preeclampsia compared with normal pregnancy: Enhanced expression of cell adhesion molecules. *Am J Obstet Gynecol,* 1997; 176(2):461-469
28. **Toth B, Lok C, Böing A, et al.** – Microparticles and Exosomes: Impact on Normal and Complicated Pregnancy. *Am J Reprod Immunol,* 2007; 58 (5):389-402
29. **Janes SL, Goodall AH** – Flow cytometric detection of circulating activated platelets and platelet hyper-responsiveness in pre-eclampsia and pregnancy. *Clin Sci,* 1994; 86:731-739
30. **Holthe MR, Staff AC, Berge LN, et al** – Calprotectin plasma level is elevated in preeclampsia. *Acta Obstet Gynecol Scand,* 2005; 84:151-154
31. **Wachowicz B** – Binding of adenosine diphosphate to turkey thrombocytes. *Haemostasis*, 1982; 11(3):139-148
32. **Iuliano L, Colavita AR, Leo R, et al.** – Oxygen free radicals and platelet activation. *Free Radic Biol Med*, 1997; 22(6):999-1006
33. **Krotz F, Sohn HY, Pohl U** – Reactive oxygen species: players in the platelet game. *Arterioscler Thromb Vasc Biol,* 2004; 24:1988-1996
34. **Fridovich** – Superoxide anion radical (O2&Mac183;-), superoxide dismutases and related matters, *J Biol Chem,* 1997; 272: 18515-18517.

35. **Tiano L, Kantar A, Falcioni G, et al.** – Oxidative response and membrane modification of diabetic platelets challenged with PAF. *Prostaglandins Other Lipid Mediat*, 2000; 62:351-366

36. **Parekh A, Riley A, Potter B** – Adenophostin A and ribophostin, but not inositol 1,4,5-trisphosphate or *manno*-adenophostin, activate the Ca^{2+} release-activated Ca^{2+} current, I_{CRAC}, in weak intracellular Ca^{2+} buffer. *Biochem J*, 2002; 361:133-141

37. **Levy JH** – Inflammation, Hemostasis and Blood Conservation Strategies *Hematol Oncol Clin North Am*, 2007; 21(1)