INTRODUCTION

The ability to arrest bleeding, whether from a small puncture or a blood vessel, is largely a function of primary haemostasis. This process is dependent on sufficient numbers of functional platelets. The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury.

Normal pregnancy may be associated with a lower mean platelet count than nonpregnant women ***(Boehlen et al., 2000)***. Platelet count is furtherly decreased at the end of the third term of normal pregnancy in about 0.3% of women despite an increased platelet agreggabiliy. On the other hand approximately 30% – 50% of cases of severe preeclampsia are associated with thrombocytopenia (platelet count <150,000/mm^3) ***(Sharma et al., 1999)***.

There is a contradiction whether haemostatic disorders occurring during preeclamsia is due to excessive platelet activation or platelet dysfunction. Excessive platelet activation occurs by dysfunctional endothelium, as a result of abnormal nitric oxide, prostaglandin and endothelin release and metabolism. Work with flow cytometry, using a variety of activation markers, confirms that this abnormal activation is

associated specifically with preeclampsia, as opposed to normal pregnancy or essential hypertension during pregnancy.This accounts for the increased platelet turnover, and ultimately, reduced numbers ***(Harlow et al., 2002)***. This activation may be involved in the pathogenesis of pre-eclampsia since its inhibition using low dose aspirin has been shown to modify the disease in high risk pregnancies (***Norris et al., 1993***).

The existence of intrinsic platelet dysfunction in preeclampsia, independent of number, is supported by aggregometry studies demonstrating reduced aggregation in severe preeclampsia compared with normal pregnancy ***(Norris et al., 1993).***

In pre-eclamsia patients,the platelet count above which anaesthesia may be performed safely is not known (***Yuen et al., 1999)***. Also two tests could be performed to evaluate platelet function in a routine clinical testing. Bleeding time, which lacks specificity and sensitivity and platelet aggregation tests which like the bleeding time, cannot predict the risk of haemorrhage ***(OKelly et al., 1992)***. The PFA-100 platelet function analyzer may be of value in such circumstances. It evaluates in vitro primary haemostasis by measuring the time required for whole blood to occlude an aperture in the membrane of a test

cartridge,which is coated with platelet agonists and is recorded as closure time (CT) ***(Kundu et al., 1995).***

The PFA-100 has been proposed as a replacement for both template bleeding time and platelet aggregation as a screening test for platelet dysfunction and it has been demonstrated to identify individuals with impaired haemostasis preoperatively ***(Koscielny et al., 2004)***. In women with thrombocytopenia and preeclamsia, PFA-100 evaluates the platelet function and correlates it with the severity of preeclampsia (***Vincelot et al., 2001).***

AIM OF THE WORK

In this study we aim to investigate platelet function among pregnant women suffering from pre-eclampsia with or without thrombocytopenia in comparison to healthy pregnant women by the use of PFA 100 in correlation with the severity of preeclampsia.

PREECLAMPSIA

Pre-eclampsia is an important disorder of pregnancy, with potentially severe consequences for mother and child. It is associated with increased maternal and fetal mortality and morbidity ***(Sibai et al., 2005, Khan et al., 2006).*** It is a multi-system disorder of pregnancy, which is characterized by new onset hypertension (systolic and diastolic blood pressure (BP) of ≥ 140 and 90 mm Hg, respectively, on two occasions, at least 6 hours apart) and proteinuria (protein excretion of ≥ 300 mg in a 24 h urine collection, or a dipstick of ≥ 2+), that develop after 20 weeks of gestation in previously normotensive women ***(Redman and Sargent 2005, Sibai et al., 2005).***

INCIDENCE:

The frequency of pre-eclampsia varies between 2% and 7% in healthy nulliparous women ***(Cnossen et al., 2008)*** and 2 to 3% of all pregnancies. It is responsible for about 60,000 maternal deaths every year, mainly in poor countries ***(World Health Organization, 2005).*** The disorder has a higher incidence among nulliparous women, in women who conceive with assisted reproduction techniques, and in women affected by autoimmune disorders, reflecting the probable influence of a dysregulated maternal immune system in its emergence ***(Sargent et al., 2006, Saito et al., 2007).*** On the other hand, women with pre-existing metabolic, vascular or renal disease are especially at increased risk for superimposed preeclampsia,

possibly due to their elevated sensitivity to the normal physiological changes imposed by pregnancy itself ***(Grill et al., 2009)*** Increased risk of preeclampsia has been reported with increased obesity ***(O`Brien et al., 2003).***

AETIOLOGY:

Although the precise mechanism of disorder remained elusive but according to new emerging consensus it is a complex polygenetic trait in which maternal and fetal genes as well as environmental factors are involved ***(Laivuori et al., 2007).*** In preeclampsia the interaction between the placenta and maternal constitution is influenced by genetic and environmental factors, causing a hypertensive inflammatory response ***(Redman and Sargent, 2005).***

DIAGNOSIS:

The minimal criteria for diagnosis of preeclampsia are proteinuria defined as 300 mg or more of urinary protein per 24h, and hypertension of 140/90 mmHg or higher and first diagnosed after 20 wk of gestation ***(National High Blood Pressure Education Program 2000).***

Blood Pressure:

It has been shown that women destined to develop pre-eclampsia have higher mean arterial pressures in the first and second trimester and even before pregnancy than women with normal pregnancies ***(Rang et al., 2004).*** Blood pressure

measurement is a screening test routinely used in antenatal care to detect or predict hypertensive disease ***(Sibai et al., 2005).*** Accurate prediction of women at risk for pre-eclampsia is crucial to judicious allocation of monitoring resources and use of preventive treatment with the prospect of improving maternal and neonatal outcome ***(Duley et al., 2004).*** Studies investigating the predictive accuracy of blood pressure measurement report conflicting results. In view of these conflicting reports it is uncertain whether blood pressure measurement should be used routinely as a predictive test or used only to diagnose hypertensive disorders in pregnancy once they are suspected (***Cnossen et al., 2008).***

Proteinuria:

Proteinuria is one of the essential criteria for the clinical definition of pre-eclampsia. It is part of the fundamental investigations performed by healthcare professionals in primary and secondary care to monitor disease severity and predict complications in women with pre-eclampsia. Urine analysis by visual reagent strip tests is widely performed in antenatal clinics and in the community by various health professionals. Total protein estimation in a 24-hour urine sample is also frequently used to assess the severity of pre-eclampsia in patients admitted to the hospital. Also spot urine protein:creatinine ratio has been used to provide an accurate quantification of 24-hour proteinuria ***(Chan et al., 2005).*** Estimation of the accuracy of the predictive value of proteinuria by any of the above methods in predicting maternal and fetal complications will aid in

clinical management by identifying the highest risk women who may need aggressive management, and the lower risk women in whom unnecessary interventions may be avoided ***(Thangaratinum et al., 2009).***

Preeclampsia may be mild or severe depending on the degree of blood pressure elevation, degree of proteinuria, extent of edema and the presence of signs and symptoms, including epigastric pain, severe headache and blurred vision. However, severe preeclampsia can result in bleeding disorders and death ***(Sibai, 2005).***

Edema and coagulopathy is also present and has been linked to maternal systemic endothelial cell dysfunction. Delivery of the placenta results in clinical resolution, and placenta is viewed as the essential organ in the development of preeclampsia ***(O`Brien et al., 2003)***

Development of insulin resistance in the third trimester of pregnancy together with adipose tissue accumulation is a possible adaptation of the maternal metabolism to optimize fetal nutrition. Insulin resistance has a potential role in pregnancy-induced hypertension, and extensive insulin resistance is often observed during preeclampsia. Furthermore, placenta secretes a variety of hormones that may play a role in both gestational insulin resistance and preeclampsia ***(Ehrenberg et al., 2003).***

Dependent on the systemic involvement, several other symptoms, such as edema, disturbance of hemostasis, renal or

liver failure, and the HELLP syndrome (hemolysis, elevated liver enzymes and low platelet counts) also complicate the clinical picture ***(Grill et al., 2009).***

Preeclampsia can have an early onset (preeclampsia starting before 34 weeks of gestation) or late onset (preeclampsia starting after 34 weeks of gestation), can show mild or severe symptoms (systolic BP ≥ 160 mmHg or diastolic BP ≥ 110 mmHg, proteinuria >5 g/24 hours, oliguria, neurological symptoms, other clinical symptoms such as deranged liver function, thrombocytopenia < 100 000 mm^3, HELLP syndrome), and can evolve in eclampsia in the most severe cases. In addition, it can manifest as a maternal disorder only, with an appropriate fetal growing, or it can present itself with a growth restricted fetus (in utero growth restriction (IUGR)) or sudden fetal distress ***(Grill et al., 2009).***

SEVERITY OF PREECLAMPSIA:

The differentiation between mild and severe preeclampsia can be misleading because apparently mild disease may progress rapidly to severe disease. Blood pressure alone is not always a dependable indicator of its severity. For example, a thin adolescent woman may have 3+ protienuria and convulsions while her BP is 140/85 mm Hg, whereas most women with BP as high as 180/120 mm Hg do not have seizures. Convulsions are usually preceded by an unrelenting sever headache or visual disturbances; thus, these symptoms are considered ominous ***(Cunningham et al., 2001).***

The severity of preeclampsia is assessed by the frequency and intensity of several abnormaities (Table 1):-

Table (1): The severity of preeclampsia

Abnormality	Mild	Severe
Diastolic blood pressure	<100mgHg	110 mmHg or higher
Proteinuria	Trace to 1+	Persistent 2+ or more
Headache	Absent	Present
Visual disturbances	Absent	Present
Upper abdominal pain	Absent	Present
Oliguria	Absent	Present
Convulsions	Absent	Present (eclampsia)
Thrombocytopenia	Absent	Present
Fetal growth restriction	Absent	Obvious
Pulmonary edema	Absent	Present

(Cunningham et al., 2001)

DIFFRENTIAL DIAGNOSIS:

Preeclampsia should be differentiated from other causes of hypertention during pregnancy (Table 2).

Table (2): Hypertension in Pregnancy: Classification and Definitions ***(Craici et al., 2008).***

Preeclampsia-eclampsia	Preeclampsia is a pregnancy-specific disorder characterized by hypertension and proteinuria of 300 mg or greater in a 24-hour urine. Eclampsia is a convulsive form of preeclampsia that affects 0.1 % of all pregnancies.
Chronic hypertension	BP greater than or equal to140/90 mm Hg prior to pregnancy, or before the 20^{th} week of gestation.
Preeclampsia superimposed on chronic hypertension	Up to 30% of women with chronic hypertension develop preeclampsia and proteinuria occurs for the first time in the third trimester.
Gestational hypertension	Hypertension occurring for the first time during the second half of pregnancy in the absence of proteinuria. It includes i) women with preeclampsia who have not yet developed proteinuria ii) those with hypertension only, and iii) a subset of patients in whom blood pressure remains elevated after delivery, leading to the diagnosis of chronic hypertension.

(Craici et al., 2008)

MANAGEMENT:

The only known cure for pre-eclampsia is delivery of the placenta. This creates a conflict of interest between the individuals (mother and baby) on either side of the placenta: the mother stands to benefit from early delivery, while the baby

may suffer complications of prematurity if born too early. Conservative management of pre-eclampsia to gain time for the baby to mature inevitably places the mother at risk ***(Bombrys et al., 2008).*** Pre-eclampsia is usually a progressive disease, but the rate of progression and the occurrence of catastrophic complications such as eclampsia, cerebrovascular accident, severe HELLP syndrome, pulmonary edema or renal failure are difficult to predict. Any marker which could reliably predict the likelihood of serious complications would be very valuable for helping choose the optimal time for delivery ***(Hofmeyr and Belfort , 2009).***

Despite extensive clinical trials, up to date, no therapeutic approaches are available for either treatment or prevention of preeclampsia. Anti-hypertensive drugs, corticosteroids for lung maturation or magnesium sulfate to prevent from eclampsia are given to handle (or prevent the worsening of) the symptoms and can thus temporize over the short term to allow for safe delivery with a more mature fetus. However, the maternal risks must be carefully weighted against the possible fetal benefits in temporizing management, as the risk of fatal deterioration of the maternal and/or fetal health condition is high ***(Grill et al., 2009).***

Several prophylactic therapies (anti-oxidant, vitamins, calcium or folic acid supplementation, Aspirin) have so far failed to prove efficacious in the prevention of preeclampsia in healthy, nulliparous subjects, although some benefit has been shown in high risk groups for a review on the different trials. As a consequence, the sole, though radical, resolution of preeclampsia is the removal of the placenta, and in case of prematurity, with the adverse consequence of delivering a pre-term baby. Therefore, preeclampsia, with or without IUGR, remains a major cause of maternal and neonatal mortality and morbidity worldwide ***(Grill et al., 2009).***

Normal Haemostatic Mechanism

HAEMOSTASIS:

Haemostasis is achieved by several mechanisms: (1) vascular constriction, (2) formation of a platelet plug, (3) formation of a blood clot as a result of blood coagulation, and (4) eventual growth of fibrous tissue into the blood clot to close the hole in the vessel permanently (Figure 1) ***(McGeown,2007*)**. Haemostasis is either primary or Secondary. Primary haemostasis refers to the formation of platelet plug, a process that occurs within 1-3 minutes of injury. This involves platelet activation, platelet, vessel wall and platelet interaction. Secondary haemostasis refers to the action of the coagulation cascade to form a clot **(*Banerjee, 2005*).**

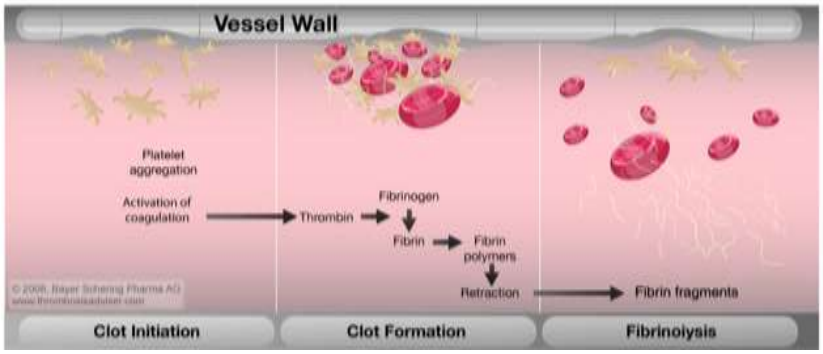

Figure (1): Reactions involved in haemostasis ***(Thrombosis Adviser).***

Vascular Constriction

Immediately after a blood vessel have been cut or ruptured, the trauma to the vessel wall itself causes the smooth muscle wall to contract, this instantaneously reduces the flow of blood from the ruptured vessel. Vascular spasm, however, can only

reduce or arrest blood loss in the short term as the vessels eventually relax again. Other haemostatic mechanisms are required if bleeding is to be arrested permanently (***McGeown, 2007*****).**

Platelet Plugging:

If the cut in the blood vessel is very small it can be sealed by a platelet plug rather than a blood clot. Platelet repair of vascular openings is based on several important functions of the platelet itself. When platelets come in contact with a damaged vascular surface, especially with the collagen fibers in the vascular wall, the platelets themselves immediately changes their own characteristics drastically. They become sticky so that they adhere to collagen and to a protein called von Willebrand factor (VWF) that leaks into the traumatized tissue from the plasma, they secrete large quantities of adenosine diphosphate (ADP) and their enzymes form thromboxane A2 ***(Guyton and Hall, 2006***). The ADP and thromboxane in turn act on nearby platelets to activate them as well and the stickiness of these additional platelets causes them to adhere to the original activated platelets. Therefore, at the site of any opening in a blood vessel wall, the damaged vascular wall activates successively increasing number of platelets that themselves attract more and more additional platelets, thus forming a platelet plug, but it is usually successful in blocking blood loss