A Study of Platelet Activation in Pre-Eclampsia

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The platelet behaviour during normal pregnancy seems to be quite modified like increased platelet aggregation, altered haemostatic mechanisms and enhanced fibrinolytic activity. The hyper-functioning endocrines and release of various platelet factors during pregnancy have been conjectured to cause the well-known features of pre-eclampsia. In order to evaluate the effects of pre-eclampsia on platelet behaviour, 125 women of child-bearing age were chosen from Services and Jinnah Hospitals, Lahore. The subjects/patients were grouped as control/non-pregnant (25), normal pregnant (50) and pre-eclamptic (50). Platelet aggregation study was performed on Aggregometer (Chronolog 540) and cell counts were performed on Coulter Counter Model T-660. It was observed that haemostatic mechanisms are stimulated during third trimester of normal pregnancy. The platelet reactivity remained unchanged despite a significant fall in platelet count. In pre-eclamptic group the platelet reactivity decreased significantly during third trimester. Platelet count also showed a highly significant decline in this group. The decreased responsiveness of platelets and fall in platelet count probably reflects platelet exhaustion. The routine performance of platelet function studies in pregnant women may help in predicting the onset of pre-eclampsia thereby providing a guideline for appropriate management of this condition.
Keyword: Platelet, Pregnancy, pre-eclampsia.

Normal pregnancy is associated with adaptation in maternal physiology, which supports fetal growth and development[1]. The changes in coagulation-endothelial interactions provide protection against haemorrhage at placental site[2]. The platelet reactivity is described by many workers to increase, while others have reported no significant change in platelet aggregation. The platelet activation in normal pregnancy is explained to be physiological rather than an abnormal state[3,4].

Nevertheless, platelets have been implicated in the pathogenesis of pre-eclampsia and may be involved in the formation of micro thrombi in utero-placental circulation[5]. The symptoms of pre-eclampsia elevated blood pressure, proteinurea, and oedema manifest late in pregnancy[6], but haematological changes ensue quite early in pre-eclampsia[7]. The study has been performed to focus the responses of platelets in pre-eclampsia and to correlate the findings with the cardinal features of pre-eclampsia.

Subjects and methods

For the study of platelet reactivity in normal pregnancy and evaluation of pre-eclampsia, 125 women of child-bearing age were selected from Services & Jinnah Hospitals, Lahore. They were grouped as control (25), normal pregnant (50), and pre-eclamptic (50). The pre-eclamptic women fulfilling the definition of pre-eclampsia by US National Institute of Health were included[8].

After complete history and thorough clinical examination, 15ml blood was drawn from the ante-cubital vein with minimum stasis using a disposable syringe. From the sample taken, 10ml was mixed with sodium citrate solution (3.8gm%) for studying platelet aggregation. Platelet Rich Plasma (PRP) was prepared by centrifuging blood at 200g for 15 minutes. In special cuvetes, labelled as "experimental", 450 microlitre (ul) PRP was taken. Platelet Poor Plasma (PPP) was used as control. The collagen and adenosine diphosphate solutions (50µl each) were added as an agonist to induce aggregation. Platelet aggregation as studied by Aggregometer Model-540 Chronolog is recorded on a paper. The results were calculated from the recorded graph as percentage change in aggregation and rate of aggregation (slope). 2ml of EDTA mixed blood was used for platelet counting. It was performed on Coulter Counter Model T-660. This instrument operates by counting the pulses of "definite sizes" as generated by the passage of suspended particles across the electrodes; which gives the platelet count in figures.

Results

During the third trimester of normal pregnancy, a non-significant change in ADP and collagen induced percentage aggregation was observed.(Fig 1&2). The slope (rate of aggregation) was also not affected when analyzed against non-pregnant control (P>0.05 Table 1, Fig.3).

Table 1. Collagen and ADP Induced %age aggregation and Rate of Aggregation in Non-pregnant & normal pregnant women

Aggregation		Non-Pregnant means ±SD	Normal Pregnant means±SD	P Value	Significance
Collagen Induced Aggregation	%age	24.04 ± 19.49	29.49 ± 19.11	>0.05	Non-*Sig.
	Rate	43.37 ± 29.55	38.87 ± 24.88	>0.05	Non-*Sig.
Collagen Induced Aggregation	%age	44.58 ± 15.98	46.96± 11.21	>0.05	Non-*Sig.
	Rate	61.87± 25.24	54.31 ± 20.11	>0.05	Non-*Sig.

*Sig: Significant

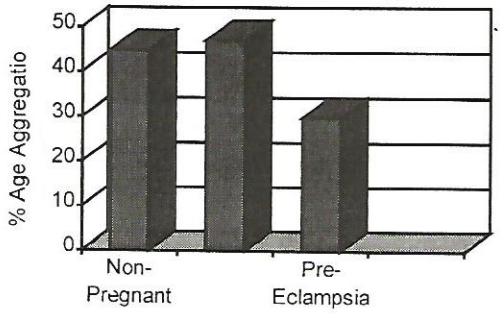

Fig.1 ADP Induced Mean Percentage of Aggregation In Control, Normal Pregnant & Pre-eclamptic Groups

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Collagen Induced Aggregation	%age	44.58 ± 15.98	46.96± 11.21	>0.05	Non-*Sig.
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*Sig: Significant

The ADP and collagen induced percentage aggregation showed a highly significant decline in pre-eclamptic pregnancies (P<0.001 Table 2, Fig. 1&2). The slope (rate of aggregation) also revealed a significant decrease in rate of aggregation in pre-eclampsia when compared with the normal pregnancy. (P<0.001, Table 2, Fig. 3).

Table 2. Comparison of Collagen and ADP Induced Aggregation in Normal Pregnant & Pre-eclamptic Group

Aggregation		Non-Pregnant means ±SD	Normal Pregnant means ±SD	P Value	Significance
Collagen Induced Aggregation	%age	29.49± 19.11	11.79 ± 6.73	<0.001	Highly *Sig.
	Rate	38.87 ± 24.88	17.80 ± 9.89	<0.001	Highly *Sig
Collagen Induced Aggregation	%age	46.96 ± 11.21	20.27± 9.43	<0.001	Highly *Sig
	Rate	52.98 ± 20.11	25.05± 8.88	<0.001	Highly *Sig

Sig: Significant

A highly significant decline in platelet count was observed during normal pregnancies (Fig.3). The platelet distribution curve during normal pregnancy showed maximum (64%) of the subjects in platelet range of 250–350 x 10^3 per cubic millimeter of blood while almost 100% of the pre-eclamptic women were having a count <250 x 10^3 /ml of blood.(Fig. 3).

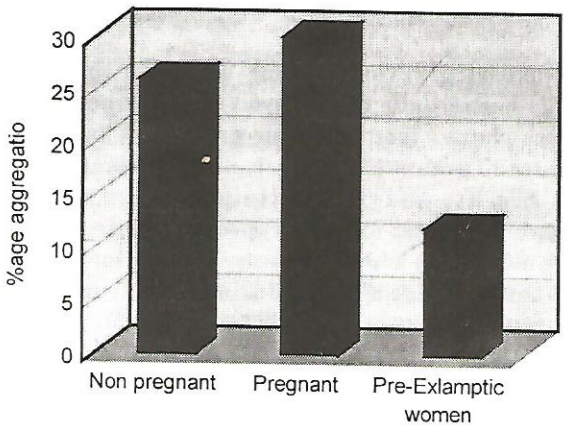

Fig. 2. Collagen Induced Percentage Aggregation in Control Normal Pregnant & Pre-Eclamptic Groups

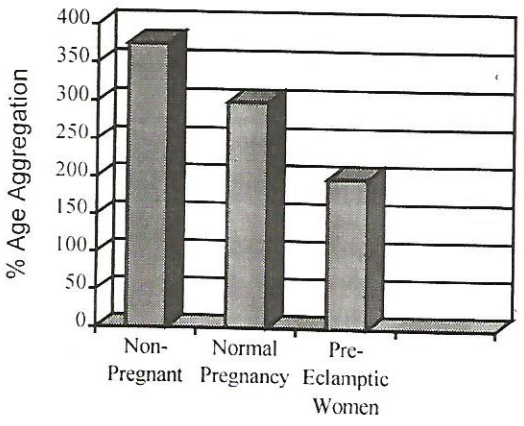

Fig. 3 Mean Platelet Count Pregnant and Pre-Eclamptic Groups

Discussion

Normal Pregnancy is associated with haematological changes and increased coagulopathy. Many previous studies have shown increased platelet reactivity in normal pregnancy[10,12]. It has been reported that platelet aggregation induced by a wide variety of aggregating agents was unchanged in normal pregnancy[9,10]. Many workers were unable to demonstrate any change in ADP induced platelet aggregation[9,10,11]. Results of the measurements of collagen induced aggregation during pregnancy have been have been inconsistent. Louden et al in 1990 failed to demonstrate any change in collagen induced platelet aggregation in normal pregnancy[5]. In the

present study the platelet response to collagen was unaffected.

Pre-eclamptic pregnancies have been associated with increased platelet reactivity[11]. Contrasting the evidence of increased platelet activation, we have demonstrated a reduced reactivity of the platelets to aggregating agents. Some workers observed decreased platelet aggregation in response to collagen and arachidonic acid[4,5]. Louden et al in 1991, described reduced platelet aggregation in response to collagen and ADP in pre-eclampsia[4].

A reduction in platelet count during or even before the onset of pre-eclampsia is a common reported finding[10,11,12]. However, Galten et al in 1997 found that severity of thrombocytopenia correlated with severity of pre-eclampsia[13]. This study supports the same findings by many other workers.

The reduced in-vitro response of platelets to aggregating agents in pre-eclampsia in this study may be the result of in-vivo platelet activation in micro-circulation. This abnormal activation leading to increased aggregation, dis-aggregation and release of granular contents is responsible for platelet exhaustion. The degranulated platelets are less responsive to aggregating agents, as demonstrated in the present study.

It may be argued that results obtained in pre-eclampsia may be influenced by low platelet count. Nevertheless, low platelet count would be more likely to produce 'false high values' than 'false low values' of platelet aggregation reaction. (Aggregation is measured as percentage change in transmission of light when platelets aggregate), which means that true levels of platelet aggregation are even lower than that observed in the study.

The decreased platelet count in pre-eclampsia appears to be related to the rate of activation and consumption of platelet and ability of bone marrow to compensate for the platelet loss. In pre-eclampsia the platelet production by bone-marrow is unable to keep pace with the shortened platelet life span, resulting in low platelet count.

Conclusion

In pre-eclampsia, platelets are less reactive to in vitro used agonsists. The reduced responsiveness of platelets is the result of in vivo activation of platelets during the course of the disease. Subsequently the hyperactive platelets release their granular contents, which result in vasospasm and other symptoms of pre-eclampsia. A serial study performed during first, second and third trimesters is suggested; which may further elaborate the functions of platelets in normal pregnancy and other sequential changes in pre-eclampsia and eclampsia.

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