Platelet Aggregability And Fibrinolytic Activity In Different Phases Of Menstrual Cycle

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Abstracts Background:- Sexual dimorphism in coronary artery disease (CAD) mortality is attributed to the cardioprotective effects of estrogen.This is reinforced by the observation that incidence of myocardial infarction is higher in menstrual phase, corresponding with low estrogen levels, in people who are predisposed to CAD due to the presence of modifiable risk factors. Cyclical variability of estrogen and progesterone in normal menstruating women may be associated with variability of platelet aggregation and fibrinolytic activity.There exists a delicate balance between fibrinolytic activity and platelet aggregation governing the haemostatic status. Objectives:- Platelet aggregability and fibrinolytic activity were measured and compared during menstrual (1-5 days), follicular (9-12 days) and luteal (20-25days) phases of menstrual cycle. Method:- In this cross sectional study of 50 normal menstruating females in age group of 18-35yrs, Platelet aggregability was measured by ADP induced platelet aggregation on a spectrophotometer. Fibrinolytic activity was estimated by euglobulin clot lysis time. Results :- Results were analyzed by students unpaired 't' test. Change in platelet aggregability was found 0.12 ± 0.15, 0.04 ± 0.04 and 0.08 ± 0.07 in menstrual, follicular and luteal phase respectively. Platelet aggregability was found significantly ($p < 0.001$) higher in menstrual and luteal phases than follicular phase. The mean euglobulin clot lysis time was found 277.6 ± 43.96, 147.6 ± 52.78 and 244.6 ± 59.12 in menstrual, follicular and luteal phase respectively. Fibrinolytic activity was found significantly ($p < 0.0001$) lower in menstrual and luteal phases than follicular phase. Conclusion :- According to the present study, in both luteal and menstrual phases, not only platelet aggregability was found higher, but fibrinolytic activity was also found lower as compared to follicular phase, thereby pointing towards thrombotic tendency in these phases. Hence, these phases require careful monitoring in women who are susceptible to thrombotic disorders. However, follicular phase with lower platelet aggregability and higher fibrinolytic activity is relatively free from thrombotic risk. [Gaule A NJIRM 2012; 3(4) :80-85]

Key Words: Platelet aggregability, fibrinolytic activity, Phases of menstrual cycle

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Introduction: The incidence of coronary artery disease (CAD) in women at all ages remains lower than men until menopause.[1] This fact is attributed to the cardio protective effects of estrogen.[2] This cardio protective effect of oestrogen is reinforced by the observation that incidence of myocardial infarction is higher in menstrual phase, when oestrogen level falls. [2,3] Platelet adhesiveness and their aggregation play an important role in thrombo-embolic disorders. Physiologically, whenever procoagulatory mechanism is activated there is corresponding activation of thrombolytic mechanism such as fibrinolytic activity.

A literature review on platelet aggregability and fibrinolytic activity in different phases of menstrual cycle yields conflicting results. [4,5, 6, 7] There is also a definite paucity of Indian data in this regard.
On assumption that cyclical variability of oestrogen and progesterone in normal menstruating women may be associated with variability of platelet aggregation and fibrinolytic activity, [8] this study was planned to know the possible fluctuations in these parameters during normal ovarian cycle in healthy young women.

This will also provide insight in diagnosis and management of thromboembolic episodes in young women who are susceptible to thrombotic tendency due to smoking, hypertension and hypercholesterolemia.

Material and Methods: This was an observational cross–sectional study conducted on female medical students and female staff members of a medical college. A written informed consent was obtained from subjects and ethical clearance was obtained from Institutional ethics committee (IEC). This study was conducted over18 months, fromFebruary 2010 to June 2011. 50 healthy

normal menstruating women were selected for the study.

The inclusion criteria consisted of females of age group 18–35 years with normal, regular menstrual cycle of 28 ± 2 days, for over the last 6 months, with normal body mass index (BMI).[8,1]

Females with history of irregular menstrual cycle and females with BMI >25 were excluded from the study. Pregnant & lactating females were excluded.[8,1] Those with history of intake of oral contraceptive pills (OCP) or any other steroidal hormones and NSAIDS were excluded from the study. In addition, females with history of regular smoking, regular alcohol intake or any known history of polycystic ovarian disease (PCOD), coronary heart disease, hypertension, diabetes mellitus, coagulation disorder, bleeding disorder and liver disease were excluded. [9] Females doing regular exercise were also excluded. [10]

The phases of menstrual cycle were ascertained based on basal body temperature (BBT) using a thermometer. Fasting venous samples were collected in menstrual phase (1^{st}-5^{th} day), follicular phase (9^{th}–12^{th} day) and luteal phase (21^{st}–25^{th} day), over one cycle.[11]

Subjects were investigated for:-

1) Platelet aggregability, measured by adenosine-di-phosphate (ADP) induced platelet aggregation as described by O'Brien.[12] Principle-Absorbance (optical density) of platelet rich plasma (PRP) is directly proportional to the concentration of platelets in it. When ADP is added to the constantly stirred PRP, it induces platelet aggregation and forms platelet clumps. The clumps settle down to the bottom of the test tube and a decrease in the absorbance by plasma is observed. More the platelet aggregability less is the absorbance. The change in the absorbance reading is expressed as change in platelet aggregability.

2) Fibrinolytic activity, measured by estimating euglobulin clot lysis time (ECLT) as described by Buckell.[13]Principle-When plasma is diluted and acidified, the precipitate formed contains plasminogen activator, plasminogen and fibrinogen. Natural inhibitors of fibrinolysis are not precipitated. The precipitate is then re-dissolved by adding borate solution and fibrinogen is clotted with calcium chloride. Then the time taken for clot lysis is estimated. Thus the test predominantly measures the plasminogen activator activity. Euglobulin clot lysis time bears inverse relation with fibrinolytic activity. Normal Euglobulin clot lysis time:- 90-240mins.

Method of Data analysis- Observations obtained in different phases were compared and results were analysed statistically by applying students unpaired't' test. $p< 0.05$ was considered statistically significant. Test of correlation was also used to analyse the parameters.

Result: Table 1(a) shows that platelet aggregability was least in follicular phase, intermediate in luteal phase and maximum in menstrual phase.

Table 1(a): Phase wise change in platelet aggregability

Phase (n=50)	Platelet aggregability Mean ± SD
Menstrual	0.12 ± 0.15
Follicular	0.04 ± 0.04
Luteal	0.08 ± 0.07

Table 1(b) shows the difference in platelet aggregability was statistically significant between menstrual versus follicular and luteal versus follicular phases. However, the difference in platelet aggregability between menstrual and luteal phase was statistically not significant. Thus the change in platelet aggregability was least in follicular phase and higher in luteal and menstrual phases.

Table 2(a) shows that ECLT was within normal limits in follicular phase, whereas it exceeded the normal limits in menstrual and luteal phases. ECLT was least in follicular phase, intermediate in luteal phase and maximum in menstrual phase. Thus fibrinolytic activity was maximum in follicular,

minimum in menstrual and intermediate in luteal phases.

Table 1(b): Phase wise comparison of difference in platelet aggregability

Phases (n=50)	Difference in change in platelet aggregability Mean ± SD	Standard error	't'	P value
Menstrual vs Follicular	0.08±0.14	0.53	3.84	<0.001*
Menstrual vs Luteal	0.04±0.16	0.08	1.59	>0.05
Luteal vs Follicular	0.04±0.08	0.02	3.65	<0.001*

* Statistically significant

Table 2(a): Phase wise euglobulin clot lysis time (ECLT in mins)

Phase (n=50)	Euglobulin clot lysis time (mins) Mean ± SD
Menstrual	277.6 ± 43.96
Follicular	147.6 ± 52.78
Luteal	244.6 ± 59.12

Table 2(b) shows that the difference in ECLT i.e., fibrinolytic activity between menstrual vs follicular phase, menstrual vs luteal phase and luteal vs follicular phase. It was found statistically significant in all the cases.

Table 2(b): Phase wise comparison of euglobulin clot lysis time (ECLT) in minutes

Comparison of Phases	Difference in ECLT Mean ± SD	Standard error	't'	P value
Menstrual vs Follicular	130±73.65	0.12	13.39	<0.0001*
Menstrual vs Luteal	33±58.91	0.13	3.96	<0.0001*
Luteal vs Follicular	97±78.15	10.42	13.82	<0.0001*

*Statistically significant

Table 3 shows, that platelet aggregability and fibrinolytic activity were positively correlated in follicular phase and negatively correlated in menstrual and luteal phases. However the correlation was not statistically significant in all the three phases.

Table 3. Phase wise correlation between platelet aggregability and fibrinolytic activity

Phase	Correlation between Platelet aggregability and fibrinolytic activity (r)	P Value
Menstrual	-0.16	0.25*
Follicular	0.16	0.28*
Luteal	-0.03	0.86*

*Statistically not significant

Discussion:

The main function of the hemostatic mechanism in body is to ensure that circulating blood remains in a fluid state while in the vascular bed and to arrest bleeding at the site of injury.Normal hemostasis is maintained by a delicate balance and a complex interaction between procoagulants and fibrinolytic system.Platelets play an important role in all the steps of haemostasis. Platelets are normally quiescent in the circulation, but when exposed to collagen of injured blood vessel wall or to a glass surface they become active & exhibit properties of adhesiveness & aggregation.

Platelet aggregability is a significant index to detect prothrombotic tendency and propensity for coronary artery disease, making it one of the most important markers of cardiovascular health.

Fibrin deposition occurs frequently, intravascularly and extravascularly, in both health and disease. The resolution of such deposits is achieved through a basic repair mechanism involving the enzymatic dissolution of insoluble fibrin polymers, a phenomenon termed as 'fibrinolysis'. This phenomenon is controlled & regulated by the activity of a normally circulating plasma proteolytic system termed as plasminogen-plasmin system. It is also called as fibrinolytic system, which comprises of plasminogen, plasmin, plasminogen activators & inhibitors. Poor fibrinolytic activity may be implicated in the onset and course of thromboembolic disease.[14]

The present study showed reduction in platelet aggregation in mid follicular phase, where as Tehran et al reported similar results in pre ovulatory phase of oestrogen.[15,16]Oestrogen, which is the chief hormone of follicular phase is said to increase release of anti-platelet aggregating agents such as nitric oxide (NO) & PGI_2, while decreasing platelet aggregating agents such as fibrinogen, TxA2 and P-selectin.[17,18] Oestrogen associates with co-repressor gene to inhibit synthesis of fibrinogen, an essential component in the process of platelet aggregation.[2] PGI2 inhibits release of ADP and Ca^{2+} ions from platelets during aggregation. Simultaneously, oestrogen also lowers TxA2 that promotes platelet aggregation. P-selectin a cell adhesion molecule lowered by oestrogen has an important role in platelet recruitment and aggregation.

In addition, both vWF and fibrinogen, essential for platelet aggregation, exhibit cyclical variation during menstrual cycle with peak levels in luteal phase. This also explains lower platelet aggregability in follicular phase and higher platelet aggregability in luteal phase in the present study.[5]In luteal phase, progesterone blocks the anti aggregating action of oestrogen, thus providing an additional cause for the raised platelet aggregabilty in luteal phase.[19]Plasma beta thromboglobulin and PF-4 which are secreted from platelets in platelet release reaction are found elevated in menstrual phase.[20] This may reflect enhanced platelet aggregability obtained in menstrual phase, in addition to the absence of anti platelet aggregating action of oestrogen in the same phase.

In the present study, fibrinolytic activity was found maximum in follicular phase, minimum in menstrual and intermediate in luteal phase. Higher fibrinolytic activity was seen with higher oestrogen levels. Oestrogen and progesterone affect fibrinolytic activity by influencing the release of fibrinolytic factors such as PAI-1 and tPA. There are studies which suggest elevated fibrinolytic activity due to the rise of tPA activity and fall of PAI-1 levels, in postmenopausal women receiving oestrogen replacement.[8,21,22,23,24,25] These observations were corroborated with rise in fibrinolytic activity in follicular phase as obtained by us in the present study.

Higher alpha-2 anti plasmin levels could contribute to low fibrinolytic activity.[26,27]In addition,PGE_2 that decreases fibrinolytic activity was found to be higher in luteal and menstrual phases and lower in follicular phase.[28]

In the present study, though statistically not significant, as platelet aggregability was found to decrease, fibrinolytic activity was found to increase in follicular phase. On the other hand with increase in platelet aggregability, fibrinolytic activity was also found to decrease in both luteal and menstrual phases.

Conclusion: According to the present study, in both luteal and menstrual phases, not only platelet aggregability was higher, but fibrinolytic activity was also lower as compared to follicular phase, thereby pointing towards thrombotic tendency in these phases. Hence, these phases require careful monitoring in women who are susceptible to thrombotic disorders. However, follicular phase with lower platelet aggregability and higher fibrinolytic activity is relatively free from thrombotic risk.

Acknowledgment: Staff members of Department of Physiology and Biochemistry of Bharati Vidyapeeth University Medical College, Dhanakawadi, Pune 43. (INDIA). Biostatistician Mr P S Borle and participants of the study. Special thanks to Dr R R Melinkeri, Prof and Head of Biochemistry for her support and encouragement.

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