Research Article

Cyclic fluctuation of bleeding time and clotting time in various phases of menstrual cycle

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Received January 30, 2015. Accepted March 25, 2015

Abstract

Background: The physiologic mechanisms whereby the human endometrium maintains hemostasis during endovascular trophoblast invasion, yet permits menstrual hemorrhage, are unknown. **Aims and Objective:** To find out whether hemostatic responses alter during various phases of normal menstruation. **Materials and Methods:** In this study height, weight, body mass index, pulse, and blood pressure were measured in 60 female volunteers having normal, regular menstrual cycle. Bleeding and clotting time were measured during three phases of single menstrual cycle: menstrual, follicular, and luteal. One-way analysis of variance test with Bonferroni correction was used to find out statistical significance. **Results:** This study shows mean bleeding time at follicular phase was significantly ($P < 0.05$) less as compared to that at menstrual and luteal phases. Mean clotting time at follicular phase was comparatively less than that at menstrual and luteal phases, but the difference was not significant ($P > 0.05$). **Conclusion:** Our observations indicate that primary and secondary hemostatic mechanism activities are at their high in the follicular phase than in the luteal and menstrual phases.

KEY WORDS: Menstrual Cycle; Bleeding Time; Clotting Time; Hemostasis

Introduction

Gynecological problems of adolescents occupy a special place in the spectrum of gynecological disorder of all age groups. The prevalence of menorrhagia in adolescent populations with bleeding disorders varies from 14% to 48%. Among the inherited bleeding disorders, platelet function defects are also an important cause of menorrhagia. Studies from West have also found an increased incidence of platelet dysfunction in black women compared to Caucasians.[1]

The physiologic mechanisms whereby the human endometrium maintains hemostasis during endovascular trophoblast invasion, yet permits menstrual hemorrhage, are unknown. One study found elevated endometrial expression of tissue factor (initiator of hemostasis) and plasminogen activator inhibitor-1 (primary inhibitor of fibrinolysin) by progestin, whereas estradiol augmented the action of progestin during luteal phase and withdrawal of this may be the basis for menstrual flow.[2] It is well known that onset of menstruation is preceded by sudden decrease in blood level of estrogen and progestin about 2 days before and cessation of bleeding occurs with regaining of estrogen levels.[3] Fibrinolysin (plasmin) present in this blood does not allow clotting and stasis of blood in uterus. Flow stops as a result of combined effect of vasoconstriction, myometrial contraction, and local aggregation of platelets.[3,4]

Studies conducted during menstruation show fibrinogen level; coagulation factors II, VII, and X; and platelet retention are lower in menstrual phase than in luteal phase and fibrinolytic activity is higher in menstrual phase.[5] Platelet function is periodically altered during the ovarian cycle due to the influence of progestin and estrogen on von Willebrand factor (vWF) concentrations.[6] Estrogen is found to increase gene expression of factor XII.[7] Estrogen receptor-β (ER-β) protein is present in glycoprotein IIb + ve megakaryocytes and platelets.[8] Estradiol

Access this article online	
Website: http://www.njppp.com	Quick Response Code:
DOI: 10.5455/njppp.2015.5.3001201536	

synthesized in megakaryocytes triggers proplatelet formation.[9] It is evidenced that deletion of ER-β is associated with modulation of specific platelet functions such as aggregation, dense granule ATP secretion, basal expression of fibrinogen receptors, P-selectin, and annexin V binding.[10] This suggests that estrogen not only increases coagulation factors but also promotes formation and activation of platelets.

To treat essential menorrhagia, mefenamic acid and tranexamic acid are being used. These drugs also act by improving platelet aggregation, degranulation, vasoconstriction, and antithrombolytic activities.[5,11] This shows that cyclic change in hemostatic mechanism might be associated with each phase of menstruation as well as with changes in levels of estrogen and progestin. The purpose of this study was to find out whether this relation exists during normal menstruation.

Hemostatic evaluation in women with unexplained menorrhagia is uncommon in gynecological practice. It is hypothesized that the identification of hemostatic disorders through these evaluations might improve care for these women.[12]

Materials and Methods

This was an observational study conducted among 60 female volunteers aged 18–22 years, having regular menstrual cycle (28–30 days) with normal blood flow (1–2 pads/day, i.e., 20–80 mL/day) lasting for 4–6 days.

Exclusion Criteria

1. History of menorrhagia or irregular menstrual cycles
2. History of bleeding or clotting disorders
3. Recent history of viral infection such as dengue and hepatitis (thrombocytopenia is common with viral infections)
4. History of any major illness, hospitalization, or surgery

Study Procedure

Proper informed written consent of the volunteers was taken. Thorough menstrual and family history was collected. Height, weight, pulse, and blood pressure were measured. Bleeding time (by Duke's filter paper method) and clotting time (by Wright's capillary tube method) were measured in three phases during a single menstrual cycle as follows:

1. Menstrual phase: Day 1–3
2. Follicular phase: Day 9–14 (nearer to peak level of estrogen hormone)
3. Luteal phase: Day 19–24 (nearer to peak level of progestin hormone)

Table 1: Profile of menstrual cycle of participants

Parameters (n = 60)	Mean	SD	Range
Age of menarche (years)	12.98	0.965	11–16
Length of cycle (days)	29.18	2.175	21–40
Duration of bleeding (days)	04.90	0.752	4–6

Statistical Analysis

Statistical analysis was performed using SPSS software, version 10. The mean and standard deviation (SD) were calculated for all the parameters. One-way analysis of variance test and post hoc test with Bonferroni correction were used. The P-value of <0.05 was considered to be statistically significant.

Result

The data collected from all 60 subjects were analyzed. In the menstrual history, none of the participants had a history of menorrhagia or irregular menstrual cycle. Table 1 shows that ages of menarche of the participants were ranging from 11 to 16 years with average being 12.98 years. Mean length and duration of cycle were 29.18 and 4.90 days, respectively, that is, within normal limits of a normal menstrual cycle.

Table 2 shows mean height (159.03 cm), weight (53.73 kg), and body mass index (21.23 kg/m^2) of the participants to be within normal limits for respective age of females. As per the observation, physical examination of vital parameters such as pulse rate and blood pressure was within normal limits. Table 3 shows mean pulse rate to be 77.17/min, mean systolic blood pressure to be 114.53 mmHg, and mean diastolic blood pressure to be 78.13 mmHg.

As shown in Table 4, mean bleeding time at follicular phase was 128.17 s, which was significantly ($P < 0.05$) less as compared to 158.00 and 149.25 s among menstrual and luteal phases, respectively.

In our study, we encountered (Table 5) that mean clotting time at follicular phase was 248.75 s, which was comparatively less than that seen at menstrual and luteal phases (260.25 and 252.75 s, respectively), but the difference was not significant ($P > 0.05$).

Discussion

Abnormal uterine bleeding is accounting for approximately 50% of the visits of adolescent girls to gynecologists. The prevalence of menorrhagia in adolescent populations with bleeding disorders varies between 14% and 48%. In fact, menorrhagia may be the initial symptom of rare bleeding disorders as well as von Willebrand disease and platelet disorders.[13] As per the studies conducted among women with

Table 2: Demographical parameters of study group

Parameters (n = 60)	Mean	SD	Range
Age (years)	18.35	01.13	18.00–22.00
Height (cm)	159.03	05.63	145.00–170.00
Weight (kg)	53.73	09.17	37.00–78.00
Body mass index (kg/m^2)	21.23	03.31	15.40–29.36

Table 3: Profile of vital parameters of participants

Physical examination (n = 60)	Mean ± SD
Pulse rate (/min)	77.17 ± 7.73
Systolic blood pressure (mmHg)	114.53 ± 4.64
Diastolic blood pressure (mmHg)	78.13 ± 6.42

Table 4: Comparison of mean bleeding time between three phases of menstrual cycle, that is, menstrual, follicular, and luteal phases using ANOVA with Bonferroni correction

Phase	n	Mean bleeding time (s) (mean ± SD)
Menstrual	60	*158.00 ± 44.29
Follicular	60	128.17 ± 40.99
Luteal	60	*149.25 ± 42.40

Table 5: Comparison of mean clotting time between three phases of menstrual cycle, that is, menstrual, follicular, and luteal phases using ANOVA

Phase	n	Mean clotting time (s) (mean ± SD)
Menstrual	60	260.25 ± 55.08
Follicular	60	248.75 ± 55.87
Luteal	60	252.75 ± 51.80

menorrhagia, inherited platelet dysfunctions most commonly seen were isolated platelet factor 3 availability defect (48.4%), Glanzmann thrombasthenia (8.9%), storage pool disease (2.4%), arachidonic acid pathway defect (1.5%), and Bernard–Soulier syndrome (1.8%).[14,15]

But for better understanding of pathology, we must know physiology underlying it. We must know whether quantitative and functional changes occur in platelets and coagulation factors during various phases of menstrual cycle or not?

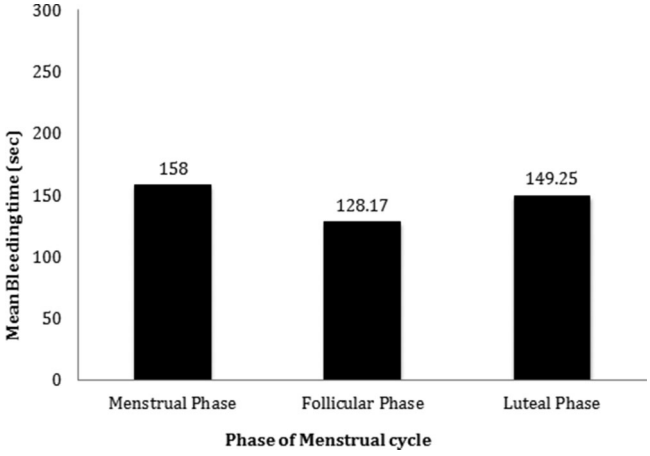

Figure 1: Comparison of mean bleeding time between three phases of menstrual cycle, that is, menstrual, follicular, and luteal phases using ANOVA with Bonferroni correction

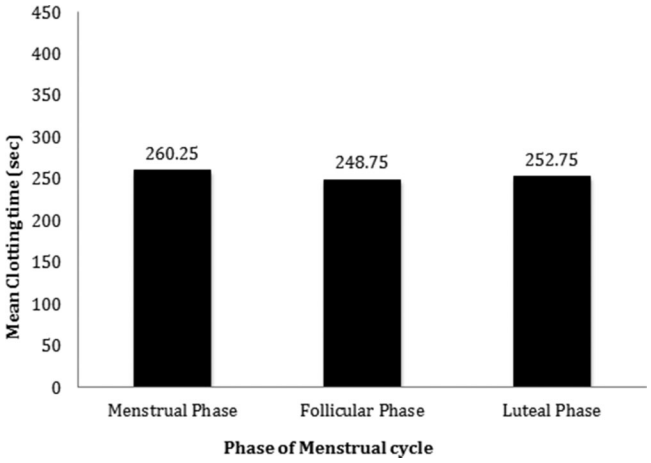

Figure 2: Comparison of mean clotting time between three phases of menstrual cycle, that is, menstrual, follicular, and luteal phases using ANOVA

Whether these changes are localized to endometrium of uterus or encompass whole-blood hemostasis?

In our study, we found that mean bleeding time at follicular phase was significantly ($P < 0.05$) less as compared to menstrual and luteal phases. It might be the combined effect of increased platelet count as well as aggregation around the pre-ovulatory peak of estrogen, that is, during follicular phase.

During menstruation, platelet count decreases by 50–70% which rises to normal again by fourth day. Ovulation count may rise by 1.4 lakhs/mm^3 of blood.[16] Some studies have indicated that platelet function is periodically altered during the ovarian cycle due to the influence of progesterone and estrogen on vWF concentrations.[17] In uteri of the women with menorrhagia treated with mefenamic acid, hemostatic plugs were further transformed and fewer vessels without a plug were observed than in uteri of those receiving placebo. These data suggest that mefenamic acid may act through an improvement of platelet aggregation and degranulation and through increased vasoconstriction, absence of which leads to menorrhagia.[11]

In our study, we observed that mean clotting time at follicular phase was 248.75 s, which was comparatively less than that observed at menstrual and luteal phases (i.e., 260.25 and 252.75 s, respectively), but the difference was not significant ($P > 0.05$).

Some studies[17–19] reported the lowest vWF levels during menstruation or early follicular phase (cycle day 1–7). But few studies[6,20] reported no cyclic variation of vWF. A cyclic variation of factor VIII is reported during menstrual cycle. The lowest levels were found during menstruation and early follicular phase.[19] Higher rate of coagulation and fibrinolysis was found among the endometrium of women with menorrhagia compared to women with normal blood losses. The hypothesis is supported by results of studies in which tranexamic acid, an inhibitor of fibrinolysis, was administered to reduce the menstrual blood loss.[5]

Some studies reported the lowest fibrinogen levels during the follicular or mid-cycle phase. Few studies reported the

lowest levels during the luteal phase and all other studies reported no cyclic variation. The strong association between fibrinogen and the acute-phase reaction could be an explanation for these conflicting results.[21]

All studies mentioned earlier have directly measured coagulation factor levels, fibrin degradation products, and so on in blood, whereas in our study we have estimated clotting time. Clotting time is an external output of total effect of coagulation and fibrinolytic system. Hence, it is a sum total of each and every component of hemostatic system. Change in only one component of hemostatic system may or may not show change in clotting time depending on change or constancy of other components.

Limitation

A larger sample including both normal and subjects with menorrhagia could have been evaluated simultaneously. Also, subjects could have been followed more than three times in single cycle but invasiveness of tests was the limiting factor for participant's compliance.

Duke's filter paper method for bleeding time and Wright's capillary tube method for clotting time, though easy, rapid, inexpensive, and bedside screening tests, still are not very sensitive tests and are subjected to show inconsistent changes due to many reasons.

Exact ovulation time was not calculated using body temperature method, expecting that a cycle with normal duration and blood flow must be ovulatory one. Anovulatory cycles hardly can be regular.

Future Perspectives

Our observations indicate that primary and secondary hemostatic mechanism activities are at their high in the follicular phase than in the luteal and menstrual phases. Hence, optimal timing of hemostatic testing seems to be the menstrual and early follicular phase.

In future, similar study must be conducted on larger sample size in Indian population and with more precise tests such as platelet function analyzer closure time, platelet aggregation studies, prothrombin time, activated partial thromboplastin time, and coagulation factor assays.

We need these kinds of studies for evaluating hemostatic variables during normal menstrual cycle and in women with menorrhagia because perhaps women with menorrhagia may show more or less variation. This could give us more insight in the pathogenesis of menorrhagia. This will lead to the development of more targeted interventions for the management of abnormal uterine bleeding in the future.

Conclusion

In this study, mean bleeding time at follicular phase was found to be significantly ($P < 0.05$) less as compared to that at menstrual and luteal phases. Also, mean clotting time at follicular phase was comparatively less than that at menstrual and luteal phases, but the difference was not significant ($P > 0.05$).

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How to cite this article: Sulaxane YD, Patel SG. Cyclic fluctuation of bleeding time and clotting time in various phases of menstrual cycle. Natl J Physiol Pharm Pharmacol 2015;5:328-332.

Source of Support: Nil, **Conflict of Interest:** None declared.