

Original Research Article

Evaluation of Haematological Factors in Various Phases of Menstrual Cycle

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ABSTRACT

Background: Menstrual cycle is a physiological process that occurs in women. Changes in the levels of female sex hormones during menstrual cycle are known to affect the coagulation cascade by producing parallel changes in the prothrombotic tendency and the fibrinolytic activity of healthy women. Objective-Evaluation of haematological factors in various phases of menstrual cycle.

Materials and Methods: The study was conducted on 20 female subjects in the age group of 18-22 years over a period of 1 year. Three venous samples were taken during single menstrual cycle in menstrual, proliferative, and secretory phases in each subject and were followed up. The various parameters noted were absolute platelet count, prothrombin time and whole blood clotting time.

Results: Comparison of absolute Platelet count and Prothrombin time during menstrual versus proliferative phases showed statistically significant difference ($p < 0.0001$). Again both values were less in comparison with the secretory phase and the difference was significant statistically ($p < 0.001$) and ($p < 0.0001$) respectively. Comparison of clotting time during menstrual versus proliferative phase shows statistically significant difference ($p = 0.001$).

Key words: phases of menstrual cycle, absolute platelet count, prothrombin time and whole blood clotting time.

INTRODUCTION

The menstrual cycle is unique to female human beings and a few primates. Periodic and cyclic shedding of uterine endometrium accompanied by loss of blood occurs during their reproductive age and this is outcome of a complex interaction between hypothalamus, anterior pituitary gland, ovaries and uterus. [1] Haemostasis is achieved through a delicate equilibrium between the coagulation and the fibrinolytic cascades. The formation of a stable fibrin clot is preceded and regulated by sequential activation of coagulation factors in events called the coagulation cascade. Activation of blood coagulation is associated with accelerated clot formation, whereas

activation of blood fibrinolysis enhances the breakdown of the blood clot. Intact haemostatic potential is essential for the control of menstrual bleeding whereas, platelet dysfunction has been reported as the most prevalent bleeding disorder among women with menorrhagia. [2]

Changes in the levels of female sex hormones during menstrual cycle are known to affect the coagulation cascade by producing parallel changes in the prothrombotic tendency and the fibrinolytic activity of healthy women. It appears that platelet function is increased during the luteal phase. [3] There is also variation in the number of platelets and platelet retention during various phases of menstrual cycle.

All these cyclical changes result in prothrombotic tendency and low fibrinolytic activity during menstrual and luteal phases which coincide with lower levels of estrogen. [4]

There are great inter- and intra-individual variations in coagulation markers in women due to different physiological conditions such as age, ethnicity, blood group and phases of the menstrual cycle. [5] Various studies reported that hemostatic factors reach the lowest levels during menstrual and early follicular phase, especially for von Willibrand factor, antihemophilic factor A (Factor VIII) and platelet function tests. [6] Hemostatic parameters show variation in different phases of menstrual cycle. Fibrinogen and fibrinogen degradation products were significantly increased in the luteal phase as compared with the follicular phase. Platelet functions were altered during the ovarian cycle under the influence of progesterone and estrogen on von Willebrand Factor. [7]

Alterations in haematological parameters in different phases of menstrual cycle have been an interest of numerous research workers in past. Therefore present study was taken up to evaluate the haematological parameters in different phases of menstrual cycle.

MATERIALS AND METHODS

This study was undertaken over a period of one year, to evaluate some haematological factors in various phases of menstrual cycle. A total of 20 female in age group of 18 to 20 years were recruited from Jammu city for the study. Each subject was requested to individually visit the laboratory of the Department of Physiology before the beginning of the study and detailed instructions were given regarding the experimental protocol. All the subjects were provided with the knowledge about different phases of menstrual cycle and were requested to maintain a diary of the cycle. A written informed consent was taken from each of them and their relaxed mental condition was prerequisite for appropriate

results. The subjects were asked to visit the laboratory during each phase of the menstrual cycle. All the eligible subjects were interviewed by the investigator herself regarding their age, menstrual history, personal habits, relevant recent or past medical disease, smoking habits, and dietary habits. Age of all the subjects was recorded as on their previous or next birthday, whichever near. General physical examination and clinical examination of the respiratory and cardiovascular systems were performed. Anthropometric measurements were made. Weight of the subjects was measured to the nearest kilogram, with their light clothing on without shoes. A digital, portable weighing machine (Avery) was used for this purpose. Height was noted to the nearest full cm with the help of nonstretchable measuring tape in standing and erect posture. Body mass index was calculated in kg/m² as per the standard formula. Various haematological parameters performed as part of present study were absolute platelet count, whole blood clotting time and prothrombin time (PT). These tests were conducted three times in each individual: (i) during menstrual phase, (ii) during proliferative phase, and (iii) during secretory phases of the same menstrual cycle. Accordingly, the first blood sample from each subjects was taken on the second day of onset of menstruation (menstrual phase), second sample between 6 and 9 days (proliferative phase), and third sample between 22 and 24.

Inclusion criteria: for subjects were as follows:

- I. Age 18-22 years.
- II. Regular menstrual cycle.
- III. No physical activity. (No leisure time physical activity as per standard definition of the term.

Exclusion criteria: Subjects were excluded, if they had any of the following factors:

- I. Married.
- II. Obese (according to body mass index).
- III. Irregular periods (menorrhagia, polymenorrhoea, dysmenorrhoea).

- IV. Diabetes mellitus.
- V. Cardiovascular abnormality like hypertension.
- VI. Hepatic or renal disorders.
- VII. Psychiatric illness
- VIII. Intake of drugs affecting coagulation profile
- IX. Chronic drug intake.

Collection of Sample and Method

The subjects were asked to report within 48 h after onset of menses. From each subject, 7.5 ml of blood was drawn from the median cubital vein under aseptic precaution with the help of a disposable syringe. The needle was detached from the syringe. Of this freshly drawn blood, 1.5 ml was transferred gently into each of the three glass test tubes reserved for whole blood clotting time test and 2.7 ml blood was transferred into a vial containing 3.2% sodium citrate with citrate and blood in the ratio of 1:9 (to be used for PT). For the purpose of platelet count, the tip of left hand finger was thoroughly cleaned with methylated spirit, allowed to dry, and a bold prick was given with the help of a sterile, disposable lancet. A well-formed blood drop was obtained. For each subject, blood was immediately sucked into a separate red blood cell pipette up to 0.5 mark followed by platelet dilution fluid up to 101 mark. Whole blood clotting time was measured by Wright and Lee multiple test tube method. The platelet count was performed by the ammonium oxalate method. Prothrombin time was calculated as per the method described by Dacie and Lewis. [8]

Statistical Analysis

Data obtained were compiled using computer software MS Excel for Windows. Statistically significant differences among quantitative variables were evaluated by independent *t*-test and with the help of SPSS software (version 2.0). A *p*-value less than less than 0.05 were considered as statistically significant.

RESULTS

During menstrual phase, proliferative phase and secretory phase

absolute platelet count ranged from 1.56 to 2.56 lakhs/mm³, 2 to 3.17 lakhs/mm³ and 1.53 to 3lakhs/mm³ respectively. During menstrual phase, prothrombin time ranged from 10 to 13 seconds while during proliferative phase, it ranged from 14 to 19 seconds and during secretory phase, it ranged from 13 to 19 seconds. During menstrual phase, proliferative phase & secretory phase clotting time ranged from 301 to 546 seconds, 305 to 660 seconds and 301 to 600 with seconds.

Comparison of mean Absolute Platelet Count between menstrual, proliferative and secretory phase

| Comparison | Independent 't' test | Statistical inference |
|--|----------------------|-----------------------|
| Menstrual phase versus Proliferative phase | p<0.0001 | Significant |
| Menstrual phase versus Secretory phase | p < 0.001 | Significant |
| Proliferative phase versus Secretory phase | p = 0.148 | Not significant |

Comparison of absolute platelet count during different menstrual phases, showed that in menstrual phase it was significantly less (p<0.0001) when compared with both proliferative phase and secretory phase (p < 0.001).

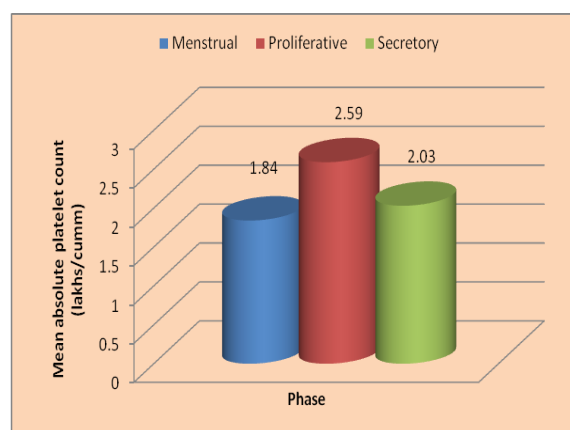


Fig:1 Graph depicting mean absolute platelet count during menstrual, proliferative and secretory phases

Comparison of prothrombin time between menstrual, proliferative and secretory phase

| Comparison | Independent 't' test | Statistical inference |
|--|----------------------|-----------------------|
| Menstrual phase versus Proliferative phase | p<0.0001 | Significant |
| Menstrual phase versus Secretory phase | p<0.0001 | Significant |
| Proliferative phase versus Secretory phase | p<0.001 | Significant |

On comparing the Prothrombin time during different phases of their menstrual

cycle it was found that prothrombin time during menstrual phase was significantly ($p < 0.0001$) less when compared with both proliferative phase and secretory phase. Between the proliferative and secretory phases the prothrombin time was significantly ($p < 0.001$) higher in the former

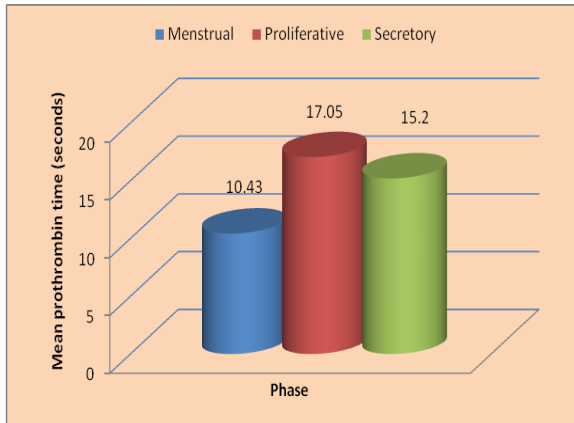


Fig.2 : Graph depicting mean prothrombin time during menstrual, proliferative and secretory phases

Comparison of mean Clotting Time between menstrual, proliferative and secretory phases

| Comparison | Independent 't' test | Statistical inference |
|--|----------------------|-----------------------|
| Menstrual phase versus Proliferative phase | $p = 0.001$ | Significant |
| Menstrual phase versus Secretory phase | $p = 0.134$ | Not significant |
| Proliferative phase versus Secretory phase | $p = 0.065$ | Not significant |

On comparing the clotting time values, it was found that menstrual phase showed a reduced clotting time when compared with proliferative phase and difference was statistically significant ($p = 0.001$).

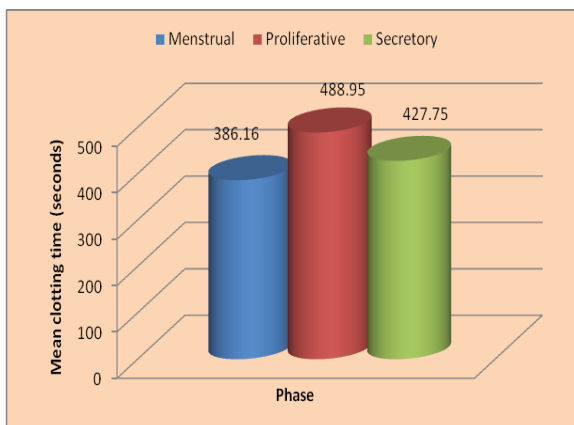


Fig 3; Graph depicting mean clotting time during menstrual, proliferative and secretory phases

DISCUSSION

The human menstrual cycle involves a complex and regular change in female anatomy and physiology over a month period. The menstrual cycle that is interval between the onsets of two successive menstruations, is under the control of hypothalamic-pituitary-ovarian axis. The endometrium is stimulated and regulated by ovarian steroid hormones oestrogen and progesterone which in turn is controlled by an integrated HPO axis through release of LH and FSH. It is also now recognized that important systemic as well as haematological changes are accompanying the various phases of menstrual cycle. In our study we estimated absolute platelet count, whole blood clotting time and prothrombin time during various phases of cycle. The absolute platelet count during menstrual phase was statistically significantly lower ($p = 0.004$) when compared with the value obtained during proliferative phase and secretory phase. The decrease in the platelet levels at the onset of menstruation may be due to an endocrine reaction via the spleen or haemopoietic system. [9]

A mid-cycle peak elevation in platelet count, followed by a gradual decline may be due to luteal hormone inhibiting the spleen from releasing platelets. [10] It is believed that cause of this peak lies in the physiological stress mechanism occurring at the time of ovulation with an alarm reaction. [11]

These results are similar to some of the earlier studies. Study conducted by Pepper H and Lindsay [12] is comparable with the present study. They reported low platelet count during the menstrual phase. Work done by Saxena SC and Mishra S [13] is consistent with results of present study. They observed low platelet count during menstrual phase and high during proliferative phase. Dusse LMSA [14] also reported low platelet count during menstrual phase. Similarly Tikare SN et al, [15] observed that platelet count was low during menstrual phase and high during the proliferative phase. The present study is in

agreement with the study conducted by, who reported an increased platelet count during proliferative phase in their study group. Similar studies conducted by Abbott R et al [16] also observed low total absolute platelet count during menstrual phase. Th. Pricila et al [17] observed low platelet count during menstrual phase and high during proliferative phase

Prothrombin time during menstrual phase was statistically significantly lower ($p < 0.0001$) than proliferative phase as well as with secretory phase ($p < 0.0001$). Our findings are supported by Gaur S et al, [18] who in a study group comprising of 18 medical students reported that blood samples taken during the menstrual phase, pre-ovulatory phase, post-ovulatory phase and pre-menstrual phase, fibrinolytic activity showed peak during 1st phase, while fibrinogen content, prothrombin time and clotting time were increased during 2nd phase and 3rd phase as compared with 1st and 4th phase.

Clotting time in our study increased in proliferative and secretory phase as compared with menstrual phase. Clotting time during menstrual phase was statistically significantly lower ($p = 0.001$) when compared with proliferative phase and secretory phase Gaur S et al [18] too observed low clotting time during menstrual phase and highest during the proliferative phase.

CONCLUSION

We concluded that platelet count was lowest in menstrual phase and highest in proliferative phase. Clotting time was lowest in menstrual phase and highest in proliferative phase whereas Prothrombin time was lowest in menstrual phase and highest in proliferative phase. This observation may be induced by phasic changes in female sex steroids.

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