

Association Between Menstrual Cycle Phases and Physiological Hematological Variations

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Abstract:

Background: Physiological fluctuations in ovarian hormones across the menstrual cycle may influence hematological parameters in women of reproductive age. Understanding these variations is essential for accurate clinical interpretation of laboratory findings. The present study evaluated phase-wise changes in hematological indices across the menstrual cycle in healthy young women.

Material and Methods: A hospital-based analytical cross-sectional study was conducted at a tertiary care teaching hospital in India. A total of 110 apparently healthy women aged 18–35 years with regular menstrual cycles were included. Venous blood samples were collected during the menstrual (day 2–3), proliferative (day 8–10), and secretory (day 20–22) phases of a single cycle. Hemoglobin concentration, red blood cell (RBC) count, hematocrit, RBC indices, total leukocyte count, differential leukocyte count, and platelet count were analyzed using an automated hematology analyzer. Repeated measures ANOVA was applied for comparison across phases, with $p < 0.05$ considered statistically significant.

Results: Hemoglobin (11.7 ± 0.8 , 12.2 ± 0.9 , 12.4 ± 0.8 g/dL; $p < 0.01$), RBC count (4.12 ± 0.32 , 4.29 ± 0.34 , 4.35 ± 0.30 million/mm³; $p < 0.01$), and hematocrit (35.1 ± 2.4 , 36.8 ± 2.6 , $37.4 \pm 2.3\%$; $p < 0.01$) increased progressively from menstrual to secretory phase. Mean corpuscular hemoglobin concentration showed modest but significant variation ($p = 0.04$), while mean corpuscular volume and mean corpuscular hemoglobin remained unchanged. Total leukocyte count differed significantly across phases ($7,860 \pm 1,120$; $7,420 \pm 1,030$; $8,210 \pm 1,180$ cells/mm³; $p < 0.01$). Neutrophils increased and lymphocytes decreased during the secretory phase ($p < 0.01$), whereas monocytes and eosinophils showed no significant change. Platelet count was highest in the secretory phase (2.91 ± 0.44 lakhs/mm³; $p < 0.01$).

Conclusion: Significant menstrual phase-dependent variations occur in selected hematological parameters, emphasizing the need to consider cycle timing when interpreting laboratory results in reproductive-age women.

Keywords: Menstrual Cycle, Hematological Parameters, Hemoglobin, Leukocyte Count, Platelet Count, Physiological Variation.

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Introduction

The menstrual cycle is a recurring physiological process in reproductive-aged women characterized by orderly hormonal fluctuations that orchestrate endometrial remodeling and preparation for potential pregnancy. These hormonal changes,

particularly in estrogen and progesterone concentrations, influence multiple systemic processes beyond reproduction, including hematological mechanisms and immune function [1]. As cyclical bleeding and variations in blood

volume occur with each cycle, it is plausible that hematological indices such as hemoglobin concentration, red blood cell (RBC) count, leukocyte distribution, and platelet count may vary across different menstrual phases [2,3].

Several observational studies have reported phase-dependent variation in hematological parameters among healthy women. For instance, hemoglobin levels have been shown to increase from the menstrual to the secretory phase, likely reflecting recovery from acute blood loss during menstruation and hormonal modulation of erythropoiesis [4]. Similarly, differences in leukocyte counts have been documented across phases, suggesting an interaction between reproductive hormones and immune cell trafficking or activation [5]. Some investigations have also indicated that neutrophil and lymphocyte percentages may change in relation to cycle timing, although findings are not entirely consistent across populations [2,5].

Understanding these physiologic variations is important for accurate clinical interpretation of complete blood counts and for establishing appropriate reference frameworks for women of reproductive age. Moreover, recognizing normal cyclic hematological changes can inform the timing of diagnostic tests and avoid misclassification of normal physiologic variation as pathology. However, despite accumulating evidence, systematic characterization of these cyclic hematological shifts across all major complete blood count parameters remains limited in many settings, particularly in Indian populations.

Therefore, this study aimed to evaluate the association between menstrual cycle phases and routine hematological parameters in healthy young women with regular menstrual cycles.

Material and Methods

Study Design and Setting: A hospital-based analytical cross-sectional study was carried out at a tertiary care teaching hospital in India.

Study Population: Women of reproductive age attending the outpatient department, as well as undergraduate and postgraduate volunteers from the institution, were screened for eligibility. Participants aged 18–35 years with a self-reported history of regular menstrual cycles (cycle length 26–32 days for at least six consecutive months) were considered for inclusion.

Inclusion Criteria

- Apparently healthy females aged 18–35 years
- Regular menstrual cycles for ≥ 6 months
- Not using hormonal contraceptives in the preceding 6 months
- Willingness to provide written informed consent

Exclusion Criteria

- History of anemia, bleeding disorders, thyroid dysfunction, polycystic ovarian syndrome, or chronic systemic illness
- Current pregnancy or lactation
- Use of hormonal therapy, anticoagulants, or hematinic supplementation within the previous three months
- Acute infection within two weeks prior to sampling

Sample Size Determination: The sample size was calculated assuming a moderate effect size (Cohen's $d = 0.5$) for variation in hematological parameters across menstrual phases, with 80% power and a 5% level of significance. Using standard sample size estimation for comparison of means across repeated physiological states, the minimum required sample was 102 participants. To account for potential attrition and incomplete sampling across cycle phases, 120 participants were enrolled.

Study Procedure: After obtaining informed consent, demographic details, menstrual history, and anthropometric measurements were recorded using a structured proforma. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Participants were followed across a single menstrual cycle. Based on cycle chronology, blood samples were collected during three predefined phases:

- **Menstrual phase:** Day 2–3 of cycle
- **Proliferative (follicular) phase:** Day 8–10
- **Secretory (luteal) phase:** Day 20–22

Phase identification was based on self-reported cycle tracking, and participants with cycle irregularity during the study period were excluded from final analysis.

Blood Sample Collection and Laboratory Analysis: Under aseptic precautions, 3 mL of venous blood was drawn from the antecubital vein between 8:00 and 10:00 AM to minimize diurnal variation. Samples were collected in EDTA vacutainers and analyzed within two hours of collection. Hematological parameters were measured using an automated hematology analyzer calibrated according to manufacturer standards. The following variables were assessed:

- Hemoglobin concentration (g/dL)
- Total red blood cell (RBC) count (million/ mm^3)
- Hematocrit (packed cell volume, %)
- Total leukocyte count (cells/ mm^3)
- Differential leukocyte count (%)
- Platelet count (lakhs/ mm^3)
- Mean corpuscular volume (MCV, fL)
- Mean corpuscular hemoglobin (MCH, pg)
- Mean corpuscular hemoglobin concentration (MCHC, g/dL)

Outcome Measures: The primary outcome was variation in hemoglobin concentration across menstrual phases. Secondary outcomes included phase-wise changes in RBC indices, leukocyte parameters, and platelet count.

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using SPSS version 26.0. Continuous variables were expressed as mean \pm standard deviation. Normality was assessed using the Shapiro–Wilk test. Repeated measures analysis of variance (ANOVA) was applied to compare hematological parameters across menstrual, proliferative, and secretory phases. Post-hoc pairwise comparisons were performed with Bonferroni correction where applicable. A p-value <0.05 was considered statistically significant.

Results

Out of 120 women initially recruited, 110 participants completed phase-wise sampling and were included in the final analysis. The mean age of the study population was 23.8 ± 3.4 years. Participants had an average body weight of 57.6 ± 6.8 kg and a mean height of 159.4 ± 5.9 cm, corresponding to a mean body mass index of 22.6 ± 2.7 kg/m². The average menstrual cycle length was 28.4 ± 1.6 days, with a mean duration of menstrual bleeding of 4.7 ± 1.1 days (Table 1).

Hemoglobin levels exhibited significant variation across the three menstrual phases ($F = 18.42$, $p < 0.01$). The lowest mean value was recorded during the menstrual phase (11.7 ± 0.8 g/dL), followed by higher levels in the proliferative phase (12.2 ± 0.9 g/dL) and the secretory phase (12.4 ± 0.8 g/dL) (Table 2). A similar trend was observed for total red blood cell count, which increased progressively from 4.12 ± 0.32 million/mm³ in the menstrual phase to 4.35 ± 0.30 million/mm³ in the secretory phase,

demonstrating statistical significance ($F = 9.76$, $p < 0.01$). Hematocrit values also differed significantly across phases ($F = 14.35$, $p < 0.01$), with the lowest percentage during menstruation ($35.1 \pm 2.4\%$) and the highest during the secretory phase ($37.4 \pm 2.3\%$). Among red cell indices, mean corpuscular volume and mean corpuscular hemoglobin did not show statistically significant differences across the cycle ($p = 0.120$ and $p = 0.143$, respectively). In contrast, mean corpuscular hemoglobin concentration demonstrated a modest but statistically significant variation ($F = 3.21$, $p = 0.04$), with values increasing from the menstrual to the secretory phase (Table 2). Total leukocyte count varied significantly during the menstrual cycle ($F = 16.57$, $p < 0.01$). The highest count was observed in the secretory phase ($8,210 \pm 1,180$ cells/mm³), whereas the proliferative phase showed the lowest mean value ($7,420 \pm 1,030$ cells/mm³) (Table 3).

Differential leukocyte analysis revealed significant phase-related changes in neutrophil and lymphocyte percentages. Neutrophils were lowest in the proliferative phase ($57.8 \pm 4.9\%$) and peaked during the secretory phase ($62.3 \pm 5.6\%$) ($F = 12.44$, $p < 0.01$). Conversely, lymphocyte percentage was highest in the proliferative phase ($34.5 \pm 4.3\%$) and lowest in the secretory phase ($30.6 \pm 4.8\%$), with statistically significant variation ($F = 11.62$, $p < 0.01$). Monocyte and eosinophil percentages did not differ significantly across menstrual phases ($p = 0.342$ and $p = 0.434$, respectively) (Table 3).

Platelet count demonstrated significant cyclic variation ($F = 8.63$, $p < 0.01$). The highest mean platelet count was recorded during the secretory phase (2.91 ± 0.44 lakhs/mm³), while the proliferative phase showed the lowest value (2.64 ± 0.39 lakhs/mm³). The menstrual phase exhibited intermediate values (2.78 ± 0.42 lakhs/mm³) (Table 4, Figure 1).

Table 1: Baseline Characteristics of Study Participants (n = 110)

Variable	Mean \pm SD
Age (years)	23.8 ± 3.4
Body weight (kg)	57.6 ± 6.8
Height (cm)	159.4 ± 5.9
BMI (kg/m ²)	22.6 ± 2.7
Average cycle length (days)	28.4 ± 1.6
Duration of menstrual flow (days)	4.7 ± 1.1

Table 2: Comparison of Red Cell Parameters across Menstrual Cycle Phases (n = 110)

Parameter	Menstrual Phase	Proliferative Phase	Secretory Phase	F-value	p-value
Hemoglobin (g/dL)	11.7 ± 0.8	12.2 ± 0.9	12.4 ± 0.8	18.42	<0.01
RBC count (million/mm ³)	4.12 ± 0.32	4.29 ± 0.34	4.35 ± 0.30	9.76	<0.01
Hematocrit (%)	35.1 ± 2.4	36.8 ± 2.6	37.4 ± 2.3	14.35	<0.01
MCV (fL)	84.9 ± 4.1	85.6 ± 4.3	86.1 ± 3.9	2.14	0.120
MCH (pg)	27.8 ± 1.7	28.1 ± 1.6	28.4 ± 1.5	1.96	0.143
MCHC (g/dL)	32.6 ± 1.2	32.9 ± 1.1	33.1 ± 1.0	3.21	0.04

Table 3: Comparison of Leukocyte Parameters across Menstrual Cycle Phases (n = 110)

Parameter	Menstrual Phase	Proliferative Phase	Secretory Phase	F-value	p-value
Total leukocyte count (cells/mm ³)	7,860 ± 1,120	7,420 ± 1,030	8,210 ± 1,180	16.57	<0.01
Neutrophils (%)	60.4 ± 5.2	57.8 ± 4.9	62.3 ± 5.6	12.44	<0.01
Lymphocytes (%)	32.1 ± 4.6	34.5 ± 4.3	30.6 ± 4.8	11.62	<0.01
Monocytes (%)	4.9 ± 1.1	5.2 ± 1.0	5.1 ± 1.2	1.08	0.342
Eosinophils (%)	2.1 ± 0.8	2.3 ± 0.9	2.2 ± 0.7	0.84	0.434

Table 4: Comparison of Platelet Count across Menstrual Cycle Phases (n = 110)

Parameter	Menstrual Phase	Proliferative Phase	Secretory Phase	F-value	p-value
Platelet count (lakhs/mm ³)	2.78 ± 0.42	2.64 ± 0.39	2.91 ± 0.44	8.63	<0.01

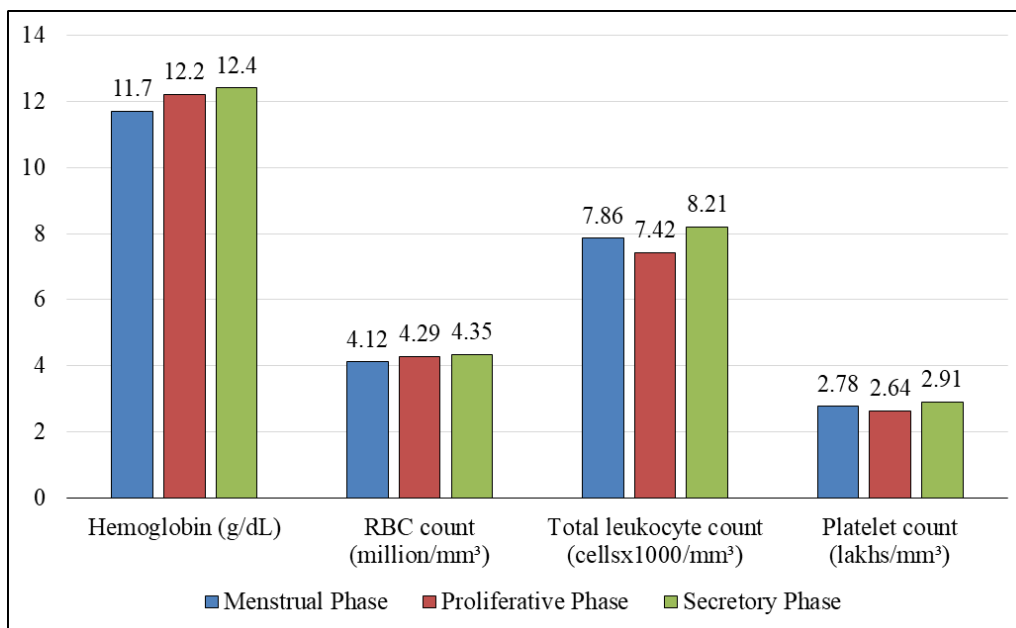


Figure 1: Key hematological variations in menstrual cycle phases

Discussion

The present investigation demonstrated significant cyclical variation in several hematological parameters across the menstrual cycle in healthy young women. Hemoglobin concentration, red blood cell count, and hematocrit values were lowest during the menstrual phase and progressively increased toward the secretory phase. Similar observations have been reported in previous studies, where erythrocyte-related indices showed lower values during menstruation, likely attributable to menstrual blood loss and subsequent recovery in the later phases of the cycle [6,7]. Hormonal influences, particularly rising estrogen levels during the proliferative phase and progesterone predominance in the luteal phase, may contribute to modulation of erythropoiesis and plasma volume dynamics, thereby influencing measured hematological values [6].

In addition to erythrocyte parameters, significant differences were noted in total leukocyte count and differential leukocyte percentages. The present study found higher total leukocyte counts and neutrophil percentages during the secretory phase, whereas lymphocyte percentage was relatively higher in the proliferative phase. These findings are consistent with evidence suggesting that ovarian hormones exert immunomodulatory effects, altering leukocyte distribution and activity during different menstrual phases [8,9]. Experimental and clinical studies indicate that estrogen and progesterone can influence cytokine production and immune cell trafficking, thereby contributing to phase-dependent variation in leukocyte subsets [8].

Platelet count was also observed to vary significantly across the cycle, with higher values in the secretory phase. Previous research has documented menstrual cycle-related changes in platelet function and aggregation, suggesting that sex steroids may influence hemostatic balance

[10,11]. The presence of estrogen receptors on platelets and hormone-mediated alterations in coagulation pathways provide plausible mechanisms for such variation [11,12]. These cyclical alterations may represent adaptive physiological responses aimed at maintaining hemostatic stability during and after menstrual bleeding.

Overall, the findings of the present study corroborate earlier reports that hematological parameters are not static across the menstrual cycle but are subject to physiological modulation. Recognition of these variations is essential to avoid misinterpretation of laboratory findings and to ensure appropriate clinical decision-making in women of reproductive age.

Conclusion

The present study demonstrates that several hematological parameters exhibit significant cyclical variation across the menstrual cycle in healthy reproductive-age women. Hemoglobin concentration, red blood cell count, hematocrit, mean corpuscular hemoglobin concentration, total leukocyte count, neutrophil and lymphocyte percentages, as well as platelet count, showed statistically significant phase-dependent differences, with relatively lower erythrocyte indices during menstruation and higher leukocyte and platelet values during the secretory phase. In contrast, mean corpuscular volume, mean corpuscular hemoglobin, monocyte percentage, and eosinophil percentage remained relatively stable throughout the cycle. These findings underscore the physiological influence of menstrual cycle phases on hematological parameters and highlight the importance of considering cycle timing when interpreting laboratory results in women of reproductive age.

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