

# Evaluation of immune status of young female during different phases of menstrual cycle

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## ABSTRACT


**Back ground:** Menstruation is characterized by monthly rhythmical changes in the rates of secretion of the female sex hormones and corresponding physical changes in the ovaries and other sexual organs. There are three phases of menstrual cycle namely, menstrual phase, proliferative phase, and secretory phase. There occur fluctuations in the levels of sex steroid hormones across different phases of menstrual cycle. **Aims and objectives:** This study was carried out to evaluate the immune response of healthy female students of first-year MBBS ( $n = 40$ ) in the age group of 18–25 years with regular menstrual cycle. **Materials and Methods:** The parameters analyzed were total leukocyte count (TLC); absolute eosinophil count (AEC); and differential leukocyte count (DLC) of neutrophils, lymphocytes, monocytes, eosinophil, and basophils. The data collected were statistically analyzed. **Result:** There was significant increase in TLC during menstrual and secretory phase of menstrual cycle. No significant difference was observed in AEC during different phases of menstrual cycle. In DLC, there was a significant increase in the neutrophil percentage during secretory phase. Lymphocyte count increased during proliferative and secretory phase but it is not statistically significant. No significant difference was observed in monocyte and eosinophil count. **Conclusion:** This study shows normal variation in the leucocyte count during different phases of menstrual cycle that may help in understanding various disorders.

**KEY WORDS:** Menstrual Cycle; Total Leukocyte Count; Differential Leukocyte Count; Absolute Eosinophil Count

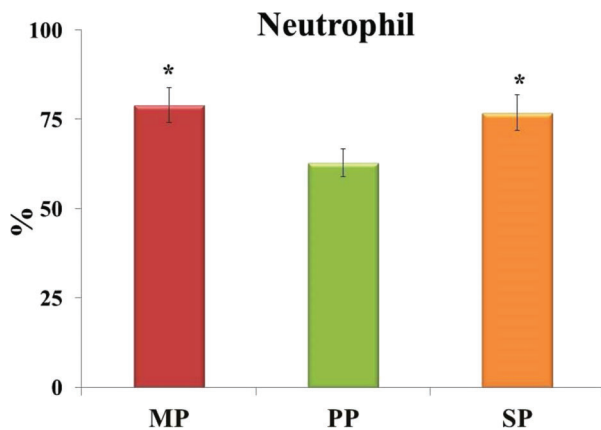
## INTRODUCTION

Menstruation is a cyclic physiological phenomenon that involves shedding of decidual layer of the endometrium along with the bleeding from uterine artery in women and in other female primates during their reproductive age. It is characterized by monthly rhythmical changes in the rates of secretion of the female sex hormones and corresponding physical changes

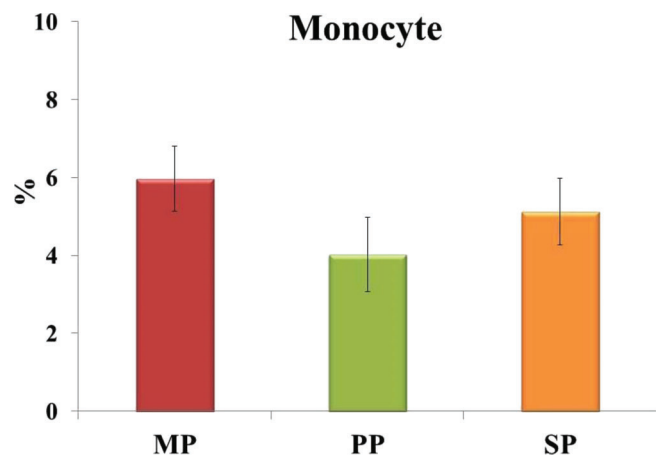
in the ovaries and other sexual organs.<sup>[1]</sup> The menstrual cycle alternates between two major phases, the follicular phase, typically persisting for 12–16 days, characterized by the presence of maturing follicles, and the luteal phase, most commonly persisting for 10–16 days, characterized by the presence of the corpus luteum in the ovary.<sup>[2]</sup> Basically there are three phases of menstrual cycle namely, menstrual phase (MP), proliferative phase (PP), and secretory phase (SP). The regular cyclic changes may be explained as a phenomenon for periodic preparations for fertilization and pregnancy.<sup>[3]</sup> Sex steroid hormones are known to induce immunological events such as altering immunological secretion and depression of suppressor T cell activity.<sup>[4]</sup> Available reports from animal and human studies suggest that the distribution of immune cells may change at different phases of the menstrual cycle.<sup>[5]</sup> The naturally occurring fluctuations in the levels of sex steroid hormones during the menstrual cycle provide a convenient

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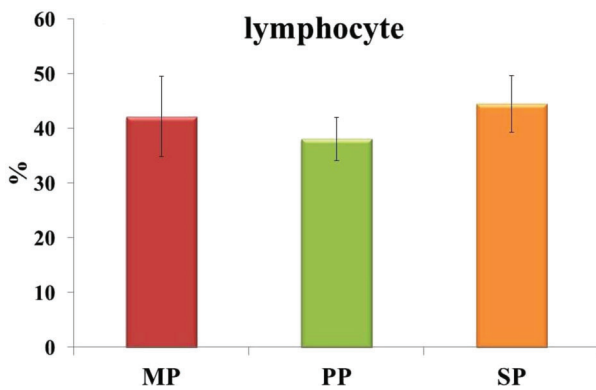
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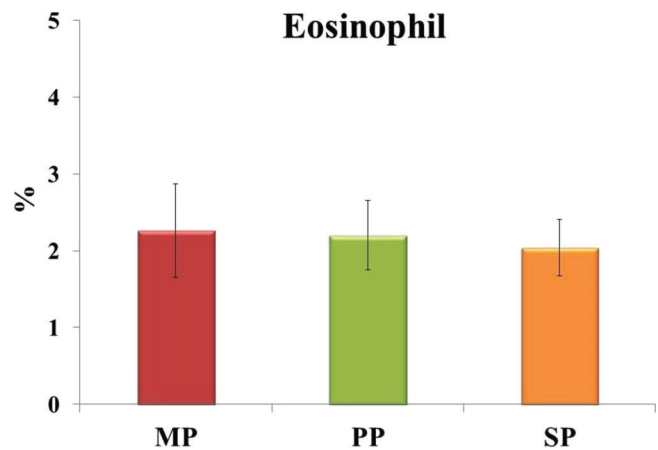
**Figure 1:** Effect of menstrual cycle on neutrophil count. \*Significance at  $p \leq 0.05$ . MP, Menstrual phase; PP, proliferative phase; SC, secretary phase.



**Figure 3:** Effect of menstrual cycle on monocyte count. MP, Menstrual phase; PP, proliferative phase; SC, secretary phase.



**Figure 2:** Effect of menstrual cycle on lymphocyte count. MP, Menstrual phase; PP, proliferative phase; SC, secretary phase.



**Figure 4:** Effect of menstrual cycle on eosinophil count. MP, Menstrual phase; PP, proliferative phase; SC, secretary phase.

basis for analyzing the interactions between sex steroid hormones and immune mechanisms.<sup>[6]</sup> Women have significantly higher neutrophil count than men, which suggests that physiological levels of estrogen or progesterone influence the leucocyte count. Thus, changes in the count might occur when the hormonal balance fluctuates during different phases of the menstrual cycle.<sup>[7]</sup>

Previous studies have not been carried out regarding variation of differential leucocyte count except neutrophils; we therefore, intend to investigate the variation in the leucocyte profile during different phases of the menstrual cycle.

## MATERIALS AND METHODS

This study was carried out on healthy female students of MBBS first-year ( $n = 40$ ) in the age group of 18–25 years with regular menstrual cycle. Subjects with irregular cycles, gynaecological disorders, anemia, history of drug intake

affecting menstrual cycle, or history of chronic diseases will be excluded from the study. Study protocol will be explained to the subjects and informed consent will be taken from each of them. Questionnaires will be distributed to the subjects to find out the duration of menstruation, age, and other relevant details. Blood samples will be taken during different phases of menstrual cycle. The first sample will be taken on the second day of the onset of menstruation (MP), second sample during 6–9th day (PP) and the third sample during 22–24th day of menstrual cycle (SP). Samples will be taken at the same time of the day to avoid diurnal variations. The parameters analyzed are total leucocyte count (TLC); absolute eosinophil count (AEC); and differential leucocyte count (DLC) of neutrophils, lymphocytes, monocytes, eosinophils, and basophils. TLC will be carried out using Improved Neubauer's chamber and Turk's fluid as a diluent and differential count will be carried out using Leishman's stain.

These counts will be done with the help of compound microscope in the hematology laboratory of Department of Physiology of People's College of Medical Science and Research Centre, Bhopal.

#### Statistical analysis:

Data are expressed as mean  $\pm$  standard deviation (SD). All data were analyzed with the SPSS for windows statistical package (version 20.0, SPSS Institute Inc., Cary, NC, USA). The statistical significance between the four different groups was analyzed using one way ANOVA test followed by Tukey's multiple comparison tests and the significance level was fixed at  $p < 0.05$ .

## RESULT

### Effect of Menstrual Cycle on Total Leucocyte Count

The results are summarized in (table) with mean  $\pm$  SD. The TLC did not get significantly altered in menstrual and PP when compared to normal TLC. However, SP showed significant increase in TLC, when compared to control normal TLC (4–11,000/cumm).

### Effect of Menstrual Cycle on Absolute Eosinophil Count

The results are summarized in (Table) with mean  $\pm$  SD. The absolute eosinophil count did not get significantly altered in all the three – menstrual, proliferative, and secretory – phases when compared to normal absolute eosinophil count (150–300/cumm).

### Effect of Menstrual Cycle on Neutrophil Count

The results are summarized in (Figure 1) with mean  $\pm$  SD. The neutrophil count did not get significantly altered in PP when compared to normal neutrophil count. However, in MP and SP, a significant increase was found in neutrophil count when compared to control normal neutrophil count (60–70%).

### Effect of Menstrual Cycle on Lymphocyte, Monocyte, and Eosinophil Count

The results are summarized in (Figures 2–4) with mean  $\pm$  SD. The lymphocyte, monocyte, and eosinophil count did not get significantly altered in all the three – menstrual, proliferative, and secretory – phases of menstrual cycle when compared to normal lymphocyte, monocyte, and eosinophil count (lymphocyte count 20–40%, monocyte count 2–8%, eosinophil count 1–4%).

## DISCUSSION

The menstrual cycle is the interval between the onsets of two successive menstruations; it is under the control of hypothalamic–pituitary–ovarian (HPO) axis. The endometrium is stimulated and regulated by ovarian steroid hormones, estrogen and progesterone, which in turn is controlled by an integrated of HPO axis through release of FSH and LH. The ovary plays a pivotal role in the menstrual cycle. It combines the gametogenic and steroidogenic functions in a unique manner (Longman).<sup>[8]</sup> Opinions regarding the cause of fluctuation in leukocyte levels during the menstrual cycle have been presented. The changes in the circulating leukocyte count during the menstrual cycle are associated with the presumptive changes in blood estrogen and the possibility that they are influenced by blood gonadotrophic hormone at the time of ovulation, or by blood progesterone or body temperature during the latter half of the cycle. Numerous studies have been undertaken to examine the effects of different phases of the menstrual cycle on differential leukocyte counts but results have often been inconclusive and contradictory. However, in this study, increase in the TLC during secretory phase is due to increase in all the subpopulations of leukocytes. This finding is similar to that observed in the previous studies by Mathur *et al.* and Tikare *et al.*<sup>[6, 9]</sup> Another study conducted by Rajnee *et al.* reveals that TLC rose from MP to PP and maximum level occurred around mid cycle.<sup>[8,10]</sup> Statistically significant increase in neutrophil percentage during secretory phase is probably due to the hormonal changes that are occurring in the ovaries. Estrogen and progesterone that is secreted by the ovaries regulate the neutrophil count. *In vitro* studies have suggested that estrogen enhances granulocyte proliferation.<sup>[7]</sup> An increase in 17 beta estradiol concentration during secretory phase probably causes the increase in the granulocyte number.<sup>[11]</sup> It has also been shown that progesterone enhanced chemotactic activity of neutrophils, while estrogens decreased the activity.<sup>[12]</sup> Increase in differential lymphocyte count during proliferative and secretory phase in comparison to MP is due to increase in number of helper T cells, cytotoxic T cells, and natural killer cells<sup>[13]</sup> that occurs under the influence of steroids which are present during the proliferative and the secretory phase. There is no significant change observed in the monocyte and eosinophil percentage as well as in the absolute eosinophil count. This result is contrary to the observations in other studies in which there was a significant drop in eosinophil count during midcycle followed by a rise in the secretory phase,<sup>[8]</sup> which occurred as a response to physiological stress whereas the levels of steroids increase causing eosinopenia.

**Table 1:** Total leucocyte count and absolute eosinophil count during different phases of menstrual cycle.

Parameter	Menstrual phase	Proliferative	Secretory phase
Total leucocyte count/mm <sup>3</sup>	6600 $\pm$ 1785	7020 $\pm$ 1580	15624 $\pm$ 1200*
Absolute eosinophil count/mm <sup>3</sup>	254 $\pm$ 67	266 $\pm$ 64	277 $\pm$ 53

## CONCLUSION

This study confirms the variations in total leukocyte count, absolute eosinophil count, and differential leukocyte count during the different phases of the menstrual cycle. This fluctuation shows the availability of immune cells in the peripheral blood. Optimal availability of these cells plays an important role in the disease process, body's response to disease process and may have therapeutic implications in diseases of autoimmunity in women.

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