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*SHORT COMMUNICATION*

# BLOOD LEUKOCYTE PROFILE IN DIFFERENT PHASES OF MENSTRUAL CYCLE

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**Abstract :** The optimal availability of immune cells in the peripheral blood stream of women plays a critical role in their response to disease and therapeutic interventions. Interaction between the reproductive and immune system plays an important immunoregulatory role. This study was designed to examine the impact of different phases of menstrual cycle on the blood leukocytes. Twenty-four healthy women in their reproductive age group and having regular menstrual cycle were studied during menstrual, proliferative and secretary phases of menstrual cycle. Total leukocyte count, absolute and differential counts of neutrophils, lymphocytes and mixed cells (includes eosinophils, basophils and monocytes) were analyzed. Results showed that the variations in the different types of leukocytes during different phases of menstrual cycle were not statistically significant. No significant inter group difference, except for the significant decrease in differential lymphocyte percentage in proliferative phase as compared to menses were observed.

**Key words :** menstrual cycle immune cells leukocytes

## INTRODUCTION

Steroid hormones are known to induce immunological events such as altering immunological secretion and depression of suppressor T cell activity (1). Available reports from animal and human studies suggest that the distribution of immune cells may change at different phases of the menstrual cycle (2). The naturally occurring fluctuations in the levels of sex steroid hormones during the menstrual cycle provide a convenient basis for analyzing the interactions between sex steroid hormones and immune mechanisms (3). Numerous studies have been undertaken to examine the changes in various type of blood cell counts and hormonal profile in different phases of menstrual cycle, but results have often been variable and contradictory (4).

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The present study aims to assess the impact of different phases of menstrual cycle on blood leukocyte profile.

## MATERIAL AND METHODS

The present study was conducted on twenty-four healthy women in their reproductive age group who volunteered for the study and having regular menstrual cycles. Exclusion criteria included presence of anemia, endocrinal, gynecological and haemostatic disorders, and evidence of infection at the time of sampling. The protocol was explained to all the subjects who volunteered for the study. Informed consent was obtained from each of the participant. An institutional ethical committee approved the entire experimental protocol. Necessary care was taken during the experimental procedure according to the Declaration of Helsinki 1964 (5). All the subjects were followed up during a single menstrual cycle. Three venous samples of 2 ml each were drawn; first sample within 48 h of onset of menses, second sample during days 8–10 of menstrual cycle, i.e. during the proliferative phase (6–9), and the third sample was taken during days 22–24 of menstrual cycle, i.e. during the secretory phase (6). All the samples were taken between 11.00 h–12.00 noon to avoid diurnal variations. The samples were analyzed immediately within 1–2 h to avoid any variations due to storage. The sample analysis was done by Sysmex KX-21, Automated Hematology Analyzer, since electronic cell counters generally provide reliable, reproducible, and accurate measurements of cell concentrations (10). The parameters analysed were total leukocyte count, absolute and differential counts of neutrophils, lymphocytes and mixed cells (includes eosinophils, basophils and monocytes). The significance of difference among the groups was assessed by one way analysis of variance (ANOVA) followed by post hoc t test using Stat cal, version 4.0.100.1190 of Stat Pac, Inc, USA. Significance was set at $P<0.05$. The percentage changes in the various leukocyte counts during different phases of menstrual cycle were also calculated.

## RESULTS AND DISCUSSION

The anthropometric profile of the subjects is depicted in Table I. All the subjects belong to the age group of 18 to 20 years. The mean ± SD of total leukocytes count, absolute and differential leukocyte counts in various phases of menstrual cycle are shown in Table II. One-way ANOVA showed that the variations in the various leukocytes were not statistically significant. Also post hoc t test showed no statistically significant inter group difference, except for the significant decrease in differential lymphocyte percentage in proliferative phase in comparison to menses. However certain noticeable changes were observed in various leukocyte counts during different phases of menstrual cycle. These changes have been depicted as percentage change in Fig. 1. During proliferative phase there was percent change decrease in all the counts except absolute and differential lymphocyte counts compared to menstrual

TABLE I: Anthropometric measurements of the subjects (n=24).

| *Parameters* | *Mean±S.D.* |
|---|---|
| Age (y) | 18.12±0.53 |
| Height (cm) | 156.62±4.72 |
| Weight (kg) | 52.89±9.36 |

TABLE II : One-way ANOVA with post hoc test of results for various leukocyte counts during different phases of menstrual cycle.

| *Parameters* | *A* | *B* | *C* | *F-ratio* | *P* | *Post hoc t test* |
|---|---|---|---|---|---|---|
| TLC (cells/cumm) | 8191.6 ±2152.6 | 7595.8 ±1787.2 | 8300.0 ±1977.0 | 0.882 | 0.418 | A vs B df=46, t=1.043, P=0.3022<br>B vs C df=46, t=–1.233, P=0.2237<br>A vs C df=46, t=–0.190, P=0.8503 |
| ANC (cells/cumm) | 5202.1 ±1783.8 | 4362.5 ±1187.6 | 4975.0 ±1378.4 | 2.092 | 0.131 | A vs B df=46, t=1.977, P=0.0540<br>B vs C df=46, t=–1.442, P=0.1560<br>A vs C df=46, t=0.535, P=0.5954 |
| ALC (cells/cumm) | 2300.0 ±519.1 | 2495.8 ±712.9 | 2562.5 ±723.3 | 1.020 | 0.365 | A vs B df=46, t=–1.025, P=0.3108<br>B vs C df=46, t=–0.349, P=0.7286<br>A vs C df=46, t=–1.374, P=0.1761 |
| AMC (cells/cumm) | 666.6 ±259.8 | 620.8 ±275.0 | 770.8 ±348.2 | 1.609 | 0.207 | A vs B df=46, t=0.534, P=0.5956<br>B vs C df=46, t=–1.750, P=0.0867<br>A vs C df=46, t=–1.216, P=0.2302 |
| DNC (%) | 62.79 ±7.77 | 58.92 ±6.52 | 59.84 ±6.87 | 1.961 | 0.148 | A vs B df=46, t=1.895, P=0.0643<br>B vs C df=46, t=–0.451, P=0.6544<br>A vs C df=46, t=1.445, P=0.1553 |
| DLC (%) | 28.8 ±5.97 | 33.05 ±6.04 | 31.07 ±5.74 | 3.124 | 0.050 | A vs B df=46, t=–2.498, P=0.0161<br>B vs C df=46, t=1.164, P=0.2506<br>A vs C df=46, t=–1.334, P=0.1888 |
| DMC (%) | 8.35 ±3.26 | 8.02 ±2.93 | 9.16 ±3.23 | 0.836 | 0.437 | A vs B df=46, t=0.364, P=0.7178<br>B vs C df=46, t=–1.256, P=0.2154<br>A vs C df=46, t=–0.893, P=0.3767 |

n=24; TLC: total leukocyte count; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; AMC: absolute mixed cell count; DNC: differential neutrophil count; DLC: differential lymphocyte count; DMC: differential mixed cell count; A: menses; B: proliferative phase; C: secretory phase.

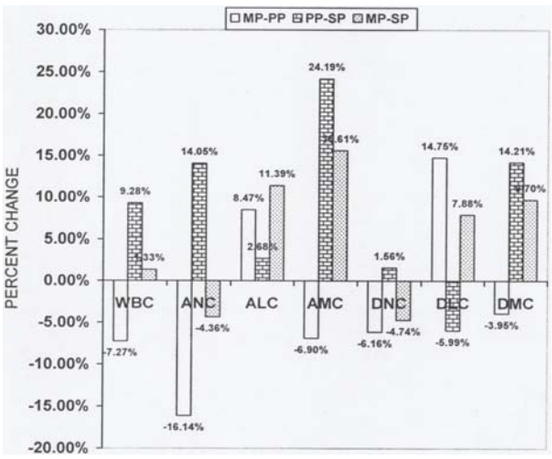


Fig. 1 : The percentage change in various leukocytes during different phases of menstrual cycle. MP, Menstrual phase; PP, Proliferative phase; SP, Secretory phase; WBC, Total leukocyte count; ANC, Absolute neutrophil count; ALC, Absolute lymphocyte count; AMC, Absolute mixed count; DNC, Differential neutrophil count; DLC, Differential lymphocyte count; DMC, Differential mixed count.

phase. A maximum fall of 16.14% was noticed in case of absolute neutrophil count. In comparison to proliferative phase, the counts increased during the secretory phase ranging from 1.56% to 24.19%. The percent change increase in total WBC count in the secretory phase found in our study corroborated with earlier studies (1, 3, 8, 9) and is due to increase in all subpopulations (lymphocytes, monocytes and granulocytes). An increase of 14.05% in absolute neutrophil count was found in the secretory phase when compared to proliferative phase. This may also be due to hormonal changes during luteal phase of ovarian cycle. The levels of estrogen or progesterone are important factors in regulating the neutrophil count. A rise of neutrophil count is also associated with increasing hormone levels in pregnancy. Estrogen seems to enhance granulocyte proliferation in vitro (11). The absolute and

differential lymphocyte counts increased during proliferative and secretory phase in comparison to menstrual phase. It has been reported that numbers of helper and cytotoxic T cells as well as natural killer cells increased during secretory phase (9). It has also been reported that during luteal phase of the normal ovarian cycle the immune response shifts towards a type 2 response. During the luteal phase of ovarian cycle (as in pregnancy), women with rheumatoid arthritis, a cell mediated autoimmune disorder, often experience improvement of their symptoms. Diseases mediated by excessive antibody production such as systemic lupus erythematosus, tend to flare up during pregnancy (12) and in the luteal phase of menstrual cycle (13). The reason may be that both the luteal phase and pregnancy are associated with a type 2 immune response shifting immunity away from the cell-mediated immune response. The pregnancy-associated changes in the immune response are already observed in the luteal phase. The physiologic meaning of this phenomenon may be preparation of the maternal immune system for potential implantation of the semi allogenic blastocyst (9). Both absolute and differential mixed cell count were increased (24.19% and 14.21%) during secretary phase in comparison to their respective proliferative phase. Increase in granulocyte and monocyte numbers in the secretary phase have already been reported earlier and also suggested to be due to increased 17ß-estradiol concentration (14).

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