Arneth Count In Different Phases of Menstrual Cycle in the Age Group 18-22 Yrs

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ABSTRACT

Background & Objectives: Blood leukocyte count and differential count varies from individual to individual and diurnal changes are noted in various studies. In present study we are investigating the effect of cyclic changes in gonadotropic hormones during normal menstrual cycle on the differential leukocyte count and in particular the nuclear lobe variation in the different phases of menstrual cycle in young women of 18-22 years old with normal past menstrual cycles.

Methodology: First year undergraduate students of Pushpagiri Medical College in the age group of 18-22 years were included in the study. 44 healthy female students with regular menstrual cycle of 28±2 days duration participated in the study, students with irregular cycles, gynaecological disorders; history of drug intake and acute illness were excluded from the study. The blood was collected under aseptic precautions and peripheral blood smears were formed. The smears were stained with Leishman’s stain following standard protocol and labelled them. The differential leukocyte count and arneth count were observed in the stained smear and recorded. To study variation the blood samples were collected on 5th day of the cycle (menstrual), 14th day of the cycle (proliferative) and 21st day of the cycle (luteal). The results were analysed using one way ANOVA between 3 groups and posthoc analysis if applicable.

Results & Conclusion: Different phases of menstrual cycle showed an statistically significant difference in Differential count of Neutrophils & Lymphocytes but other cells Eosinophil count, Monocyte & Basophil count had no significant difference which suggested that gonadotropic hormones had influence on leukocytes both granulocyte (Neutrophil) & agranulocyte (Lymphocyte) where we noticed increased Neutrophil count during luteal phase and least increase in number of Lymphocytes during luteal phase. In arneth count we had statistically significant difference in 2, 3, 4 and 5 lobed nuclei of Neutrophils and specifically 2 and 3 lobed Neutrophil count increased in luteal phase suggesting new (young) neutrophils released in the circulation

Keywords: Arneth count, menstrual cycle, white blood corpuscle.

INTRODUCTION

Menstruation is derived from a Latin word Menstrus, meaning monthly. It is a repetitive phenomenon occurring during the reproductive life of female that involves structural, functional, and hormonal changes in the reproductive life¹. There are three phases of menstrual cycle known as Menstrual phase (MP), Proliferative phase (PP) and the Luteal Phase (LP).
The menstrual cycle alternates between two major phases, the follicular phase, which typically persist for 12–16 days and is characterized by the presence of maturing follicles and the luteal phase, which most commonly persists for 10–16 days and is characterized by the presence of the corpus luteum in the ovary. The duration of menstrual cycle is between 21-35 days with a mean of 28 days. It is suggested that during reproductive age group immune responses are influenced by hormones, and since hormone levels change throughout the menstrual cycle, one would expect her immune responses to vary.

It is a well-known fact that sex hormones play a role in the immune response. Various studies have reported changes in leukocyte count during different phases of menstrual cycle but results have been inconclusive and contradictory.

**AIM**

This observation was undertaken to study the variation of differential leukocytes count during different phases of menstrual cycle along with nuclear lobe variation of Neutrophils in the different phases of the cycle.

**MATERIALS AND METHOD**

**RESULT**

| Parameters (%) | Menstrual Phase (M1) | Proliferative Phase (M2) | Luteal Phase (M3) | F-value | P-value | Post hoc test |
|----------------|----------------------|--------------------------|------------------|---------|---------|---------------|
| Neutrophil     | 64.55±1.18           | 61.93±1.20               | 68.16±1.66       | 225.48  | <0.0001 | M1VsM2* M2VsM3* M2VsM3* |
| Lymphocyte     | 30.46±1.54           | 32.83±1.63               | 26.97±2.15       | 115.53  | <0.0001 | M1VsM2* M1VsM3* M2VsM3 |
| Monocyte       | 2.63±0.68            | 2.79±0.67                | 2.83±0.78        | 0.78    | 0.46    | NS            |
| Eosinophil     | 2.20±0.70            | 2.41±0.58                | 2.27±0.76        | 1.02    | 0.36    | NS            |
| Basophil       | 0                    | 0                        | 0                | 0       | 0       | NS            |

* - p< 0.01- significant   NS - not significant

44 healthy female students in the age group of 18-22 years with regular menstrual cycle of 28±2 days duration were randomly selected and subjects with irregular cycles, history of drug intake and acute illness were excluded from the study. The study was approved by the institutional research & ethical committee of Pushpagiri Institute of Medical Sciences & RC, Thiruvalla. The study protocol was explained to the students and informed consent was obtained. Blood was collected under aseptic precautions and peripheral blood smears were prepared on immediate collection. The smears were stained with Leishman’s stain following standard protocol and labelled them. The differential leukocyte count and arneth count were observed in stained smear manually. To study variation in different phases, the blood samples were collected on 5th day of the cycle (menstrual), 14th day of the cycle (proliferative) and 21st day of the cycle (luteal).

**STATISTICAL ANALYSIS**

The results were analysed using one way ANOVA between 3 groups and post-hoc analysis done for statistical significance of p<0.05.
| Parameters (%) | Menstrual Phase (M1) | Proliferative Phase (M2) | Luteal Phase (M3) | F-value | P-value | Post hoc test |
|---------------|----------------------|-------------------------|-------------------|---------|---------|--------------|
| 2 lobed       | 25.30±6.20           | 16.69±5.38              | 32.86±5.48        | 86.48   | <0.0001 | M1 Vs M2*    |
|               |                      |                         |                   |         |         | M1 Vs M3 *   |
|               |                      |                         |                   |         |         | M2 Vs M3*    |
| 3 lobed       | 38.20±4.16           | 32.23±8.15              | 39.60±5.06        | 18.07   | <0.0001 | M1 Vs M2*    |
|               |                      |                         |                   |         |         | M1 Vs M3*    |
|               |                      |                         |                   |         |         | M2 Vs M3*    |
| 4 lobed       | 32.09±12.18          | 33.88±7.76              | 22.86±5.67        | 18.74   | <0.0001 | M1 Vs M2*    |
|               |                      |                         |                   |         |         | M1 Vs M3*    |
|               |                      |                         |                   |         |         | M2 Vs M3*    |
| 5 lobed       | 4.97±2.94            | 12.18±8.71              | 4.16±2.62         | 27.46   | <0.0001 | M1 Vs M2*    |
|               |                      |                         |                   |         |         | M1 Vs M3*    |
|               |                      |                         |                   |         |         | M2 Vs M3*    |
| 6 lobed       | 1±1.06               | 4.47±4.05               | 0                 | 36.36   | <0.0001 | M1 Vs M2*    |
|               |                      |                         |                   |         |         | M1 Vs M3#    |
|               |                      |                         |                   |         |         | M2 Vs M3*    |

*- p< 0.01- significant  
#- non-significant

As observed the subjects were matched to age, height and weight. The three phases of menstrual cycle showed a statistically significant difference in differential count of neutrophil & lymphocytes but other cells eosinophil, monocyte & basophil count difference was not significant.

We found a significant increase in neutrophil count in LP on comparing to the PP and MP (p<0.01), similarly lymphocyte count in PP was increased on comparison to LP and MP (p<0.01). There was an increasing trend in monocyte count from MP to LP but counts were statistically not significant (p>0.05). In arneth count we observed significant difference in the lobes of neutrophils during the different phases of cycle, increase in 2 lobed neutrophils was observed in the LP on comparing to MP and PP phase (p<0.01).

Statistical significant increase in 3 lobed neutrophil was found in LP phase (p<0.01) also statistical difference between PP and MP (p<0.01). Statistical significant increase in 4 lobed neutrophil was found in PP (p<0.01) and significant difference in MP and LP. 5 lobed and 6 lobed showed statistically significant increase in PP phase (p<0.01).

**DISCUSSION**

The present study showed variations in Leukocytes in different phases of menstrual cycle, neutrophil count was increased during the secretory phase and as observed in arneth count. Two lobed and three lobed neutrophils were increased during the secretory phase suggesting active leucopoiesis and young neutrophils released in the peripheral circulation.

In vitro studies have suggested that progesterone enhances granulocyte proliferation and release of neutrophils which explains the phenomenon. It has been shown that progesterone enhances chemotactic activity of neutrophils, whereas estrogen decrease the activity. In the present study lymphocyte count was increased during the proliferative phase in contrast to other studies showing decrease in lymphocyte count. Increase in lymphocytes during proliferative phase may due to increase in T- helper and T cytotoxic cells during this phase which will be shifted to Type 2 during secretory phase.

As a result disorders like SLE and other conditions mediated by excessive production of antibodies flares up during luteal phase and pregnancy. This suggests that immunity shifts from the cell-mediated immunity to innate immunity during the luteal phase and pregnancy.
Neuroendocrine regulation of immune response has a suggestive influence during ovarian cycle which may be critical for embryonic implantation and pregnancy. The physiologic meaning of this phenomenon may be preparation of the maternal immune system for potential implantation of the semi allogenic blastocyst.

CONCLUSION
This study showed a cyclical fluctuation of differential leukocyte count during the different phases of menstrual cycle. Increase in Neutrophils, specifically the two lobed and three lobed in the luteal phase suggested younger neutrophils are released into the circulation. The study also showed decreased lymphocyte count during luteal phase.

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