

Diurnal Variation of Leucocyte count in different Phases of Menstrual CycleSesha Pavani Gutti^{1*}, Madhav P², A Chandra Shekar³¹Tutor, Department of Physiology, Sri Balaji Medical College Renigunta, Andhra Pradesh, India²Assistant Professor, Department of Pharmacology, Apollo Medical College, Chittoor, Andhra Pradesh, India³Professor and HOD, Department of Physiology, Sri Balaji Medical College, Renigunta, Andhra Pradesh, India

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Abstract

Objectives: 1. To compare leucocyte count and types of leucocytes on day 2, 12 and 22nd day of the menstrual cycle 2. To study the diurnal changes in different types of leucocyte. **Materials and methods:** Permission from medical ethical committee of institute is obtained to select the subjects for the study. **Subjects:** The present study was conducted on twelve healthy women in their reproductive age group who volunteered for the study and having regular menstrual cycles. **Inclusion criteria:** All the 12 subjects that were chosen are having regular menstrual period of 21-30 days. **Exclusion criteria:** Subjects with anemia, endocrinal, gynecological and hemostatic disorders are excluded. The parameters analyzed were total leucocyte count, absolute neutrophil count, absolute eosinophil count, absolute lymphocyte count, absolute monocyte count. **Results:** The present study is carried out in women aged 20 to 22 years. All have reproductive cycles with duration of 28 days. As the range is narrow mean, standard deviation values are not done. According to table I total leucocyte count is done on day 2, 12 and 22nd of menstrual cycle. Four samples are collected at 6:00am, 12:00 pm, and 12:00am. The mean values of these samples on 2 and 12th day are compared by statistical analysis. 805625 ± 783.04 and 9293.75 ± 1099.59 and P value indicates significant and total leucocyte count is low on 2nd day. In the same way the mean, standard deviation and t values are calculated and compared between 12 and 22 day. **Conclusion:** It is clearly indicated that the total leucocyte count is increased during luteal phase of menstrual cycle. In the same way diurnal variations of total leucocytes count are observed, the count is high in evening time and less in morning time. This follows the cortisol rhythm and variation may be due to it. The changes in menstrual cycle indicates alteration in immune response and may be due to the estrogen-progesterone secretions in different phases of menstrual cycle.

Keywords: Leucocyte count, Menstruation, absolute neutrophil count, absolute eosinophil count, absolute lymphocyte count.

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Introduction

Leucocytes play an important role in immune responses of the body. Among different types of leucocytes, lymphocytes and monocytes play an important role in immunity. T lymphocytes provide cell mediated immune responses and also protects from hypersensitive reaction. B-lymphocytes provide humoral immunity by producing antibodies in the form of immunoglobulins. Activation of these cells require recognition of antigen by monocytes which release interleukins. Steroid hormones are known to alter the activity of these cells. Cortisol also decreases circulating levels of lymphocytes. The thymus contains germinal centers for lymphocytes [1-5]. The thymus contains germinal centers for lymphocytes and large numbers of lymphocytes are formed and mature within it. Sustained high concentrations of glucocorticoids produce a dramatic reduction in the mass of all lymphoid tissues including thymus, spleen and lymph nodes. Cortisol also decrease circulating levels of lymphocytes and particularly a class of white blood cells known as eosinophils. Curiously the total white blood cell count does not decrease because glucocorticoids also induce a substantial mobilization of neutrophils from bone marrow. Cortisol levels fall during night times that is 3 am and increases in the evening [6-9]. Thus it is responsible for circadian variations in leucocyte count. During menstrual cycle there are cyclical changes in ovarian pituitary hormones. So these hormones also affect the total leucocyte count by acting on bone marrow proliferation or on lymphoid tissue. Certain diseases are aggravated during different phases of menstrual cycle because of leucocyte

count changes. Women with rheumatoid arthritis which is cell mediated auto immune disorder experience improvement of the symptoms of ovarian/menstrual cycle. Skin disease-lupus erythematosus flare up during luteal phase of menstrual cycle. These are definitely due to changes in estrogen, progesterone levels [10-13]

Materials and methods

Permission from medical ethical committee of institute is obtained to select the subjects for the study.

Subjects: The present study was conducted on twelve healthy women in their reproductive age group who volunteered for the study and having regular menstrual cycles.

Inclusion criteria: All the 12 subjects that were chosen are having regular menstrual period of 21-30 days.

Exclusion criteria: Subjects with anemia, endocrinal, gynecological and hemostatic disorders are excluded. All the subjects are not using contraceptive pills or on hormone therapy.

Method of collection of blood sample

All the subjects were followed up during a single menstrual cycle. Three venous samples of 2ml each were drawn first sample on the 2nd day of menstrual cycle, second sample on the 12th day of menstrual cycle and third sample on the 22nd day of menstrual cycles. Each day four samples of venous blood were collected at different time intervals on 6:00 am, 12:00 pm, 6:00 pm and 12:00 am. All the samples were collected with anticoagulant solution citrate. The samples were analyzed immediately within 1-2 hour to avoid any variations due to storage. All the cell counts done by using MUNDRAV automated hematology analyzer and accurate measurements of cell concentrations were obtained. The parameters analyzed were total leucocyte count, absolute neutrophil count, absolute eosinophil count, absolute lymphocyte count, absolute monocyte count [14-19]

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Discussion

Twelve subjects with regular menstrual cycles are selected for this work. It is observed that there is increase in the total leucocyte count in the luteal phase (22nd day). Monocyte and granulocyte counts were significantly higher in the luteal phase than in the follicular phase (12th day). In the present study we observed that lymphocyte count is maximal in Day 12 when compared with Day 2 and maximum in the luteal phase i.e on 22nd day. But Subbi Mathur observed in their studies there is a minimal count at the mid cycle in the lymphocyte count. Generally women have higher leucocyte count than men, this is due to maximum amount of neutrophils. Mackinnon and Morley have suggested that there is cyclic variation of neutrophil count with menstrual cycle, these findings imply that important factors that regulate the white blood cells are female sex hormones i.e., estrogen and progesterone. In women during day 2 of menstrual cycle, we

observe one condition called Leucorrhoea where Leucocytes along with vaginal blood also sent out during Menstrual phase. So we see fall in the Leucocyte count during Bleeding phase. As far as Diurnal variation were considered there is an increase in cell count during 6 pm and 12 am. Then again fall at 6 am in the morning hours. These variations observed are due to cortisol. Cortisol secretion exhibits distinct diurnal variation, peak occurs just before the subject awakens in the morning and the nadir occurs from the late afternoon. The lowest level of cortisol secretion occurs just after the subject falls in to slow wave sleep. Cortisol decreases the circulating lymphocyte, monocyte and eosinophil. But substantial levels of cortisol increase mobilization of neutrophils from bone marrow. During the luteal phase increased levels of progesterone and androgen of the ovarian cycle play a role in deviation of the immune response toward a type-2 response. [20-26].

Table 1: Comparison of tlc between 2nd, 12th & 22nd days total leucocyte count

2 nd DAY (A)	8056.25±783.04	PROBABILITY AXB;T=6.35;P<0.001;S
12 th DAY(B)	9293.75±1099.5	BXC;T=3.30;P=0.001;S
22 nd DAY (C)	9997.92±984.23	CXA;T=10.69;P<0.001;S

Result :- p values are found significant

Table 2: Cyclical changes of absolute neutrophil count

2 nd DAY (A)	4145.83±534.73	PROBABILITY AXB;T=2.39;P<0.018;S
12 th DAY(B)	4393.75±478.65	BXC;T=2.30;P=0.0023;S
22 nd DAY (C)	4612.50±449.40	CXA;T=4.62;P,0.001;S

Result :- p values are found significant

Table 3: Cyclical changes of AEC

2 nd DAY (A)	151.81±23.61	PROBABILITY AXB;T=2.63;P<0.09;S
12 th DAY(B)	164.29±22.70	BXC;T=3.16;P=0.002;S
22 nd DAY (C)	178.83±22.30	CXA;T=5.76;P<0.001;S

Table 4: Cyclical changes of absolute lymphocyte count

2 nd DAY (A)	2145.52±336.91	PROBABILITY AXB;T=2.50;P=0.013;S
12 th DAY(B)	2527.08±372.88	BXC;T=2.10;P=0.0038;S
22 nd DAY (C)	2497.2±422.50	CXA;T=4.51;P<0.001;S

Result :- p values are found significant

Table 5: Cyclical Changes Of Absolute Monocyte Count

2 nd DAY (A)	291.67±38.55	PROBABILITY AXB;T=2.49;P<0.014;S
12 th DAY(B)	311.45±39.17	BXC;T=2.68;P=0.008;S
22 nd DAY (C)	333.96±42.77	CXA;T=5.08;P<0.001;S

Result :- p values are found significant

Table 2: Indicates the changes in absolute neutrophil count. The changes are similar to total leucocytes count. Absolute neutrophil count is high in luteal phase of menstrual cycle and the values indicate statistical significance. The results are observed by Tikare et al. They also indicate the changes are due to progesterone, estrogen seems to enhance granulocyte proliferation in vitro. This is indicated by Bain et al [2].

Table 3 : Indicates the change in absolute eosinophil count. The values are statistically compared. 2nd and 12th and 22nd day of menstrual cycle. The P values indicate the statistical significance. There is diurnal change in absolute eosinophil count.

Table 4: Indicate the changes in absolute lymphocyte count and variation is more on 12th day and P values are significant. Similar observations are noted by M. Raptopoulou et al [22]. The fall in leucocyte count during menstrual period may be due to release in hormone levels. There is significant release in lymphocyte count during luteal phase. In the study by Matijke Faas et al [14], the same is published. This indicates the changes in immune response during the different phases of menstrual cycle.

Table 5 : Indicates the changes in absolute monocyte count during menstrual cycle, the changes are similar to the changes in total leucocyte count and results are similar to the study by J.M. Cruickshank et al [10]. They observed a significant negative correlation between urinary estrogen. It has been suggested that increased estrogen, progesterone concentration may induce release of monocyte and play important regulatory role in immune responses [27-32].

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