

# Study of Cyclical Variations of Leukocyte Profile during different Phases of Menstrual Cycle in 1st MBBS Students

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## ABSTRACT

**Introduction:** Menstrual cycle is the periodic shedding of endometrium accompanied by loss of blood during reproductive age. The period extending from the beginning of menses to the beginning of next one. The regulation of the normal cycle is under the control of neuro hormonal mechanisms with its feedback system present in the hypothalamic-pituitary-ovarian axis. This study was taken up to see the alterations in the immune cells (leukocytes cells) during menstrual cycle in the Medical students of Jawaharlal Institute of Medical sciences.

**Material and Methods:** This longitudinal study was carried out in the Department of Physiology, Jawaharlal Nehru Institute of Medical Science, Imphal. A total of 50 healthy female medical students were selected in the age group of 18-25 yrs with regular cycles of 28±2 days duration. Irregular cycles, gynaecological disorders, history of drug intake and history of recent infections or fever were excluded from the study. The immune cells were examined during menstrual, proliferative and secretory phases during a single cycle. The data collected was statistically analyzed by one way Anova.

**Results:** There was statistically significant increase in total leukocyte count during secretory phase. Granulocyte count was increased significantly in secretory phase and small rise in agranulocyte count in secretory phase but was not statistically significant. Absolute neutrophil shows significant rise in secretory as well as menstrual phase. Significant increase in the differential neutrophil count in secretory phase and lymphocytes was increased in proliferative phase but was found not statistically significant. Eosinophil and monocyte count, there was not much variation in menstrual, proliferative and secretory phases.

**Conclusion:** In the present study, there was statistically significant increase in total leukocyte count, Granulocyte count and differential neutrophil count in secretory phase. Our study also shows rise in agranulocyte count in secretory phase. Differential lymphocytes count was increased in proliferative phase but was not statistically significant but differential eosinophil and monocyte count shows not much change during menstrual cycle. These alterations in immune cells play important roles in knowing the disorders commonly affecting females.

**Keywords:** Menstrual Cycle, Immune Cells, Medical Students

## INTRODUCTION

Menstrual cycle is the periodic shedding of endometrium accompanied by loss of blood during reproductive age.<sup>1</sup> The period extending from the beginning of menses to the beginning of next one. The endometrium consists of surface epithelium, glands, stroma and blood vessels. It consists of two distinct zones i.e basal zone or stratum basale and stratum

functionale. Basal zone is uninfluenced by hormones and no cyclic changes occur. Basal zone is all that remains at the end of menstruation and regeneration occurs from this zone. The superficial two-thirds of the endometrium that is shed during menstruation is the stratum functionale. The cycle consists of menstruation, proliferative, and secretory phase. During menstruation phase, with the corpus luteum regressing, the hormonal support for the endometrium is withdrawn producing menstrual blood flow for 3-5 days. From the endometrial function, the proliferative phase represents restoration of the epithelium from the preceding menstruation, and the secretory phase represents preparation of the uterus for implantation of fertilized ovum. The length of secretory phase is remarkably constant at about 14 days and the variations seen in menstrual cycle are due to variations in the length of proliferative phase. The regulation of the menstrual cycle is under the control of neuro hormonal mechanisms with its feedback system present in the hypothalamic-pituitary-ovarian axis.<sup>11</sup> Many studies have reported some variations of immune cells during menstrual cycle but still the findings are inconclusive. The objective of this study was to assess the variations of immune cells during different phases of menstrual cycle and also to identify the disorders commonly affecting females.

## MATERIAL AND METHODS

This is a longitudinal study carried out in the Department of Physiology, Jawaharlal Nehru Institute of Medical Science, Imphal. A total of 50 healthy female medical students were selected in the age group of 18-25 yrs with regular cycles. Irregular cycles, gynaecological, endocrinal and haemostatic disorders, history of drug intake and history of recent infections or fever were excluded from the study. The immune cells were examined during menstrual, proliferative and secretory phases during a single cycle. Study protocol was explained to the students and informed consent was obtained from each of the students. Institutional ethical

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committee clearance was obtained. The collection of sample i.e. 2ml of venous blood were collected 3 times from each participant's antecubital vein. All the samples were collected in a plastic vial containing EDTA. The subjects were asked to report with two days of onsets of menstruation. The second sample during 6-9<sup>th</sup> days of proliferative phase. Third sample was taken during 22<sup>nd</sup>-24<sup>th</sup> days during secretory phase. The count was done within an hour to avoid diurnal variations due to storage. The parameters analyzed were total and differential leukocyte count, absolute leukocyte count. All the samples were analyzed by manual cell counting method i.e. total leukocyte counting was made under Improved Neubauer's chamber using Turk's fluid and differential counting by using Leishman's stain under Compound microscope under oil immersion and different types were examined until 100 cells were counted. Knowing the total and differential leukocyte count, the absolute count of each type were calculated (DLC/100 TLC) in the Hematology laboratory of Department of Physiology, JNIMS, Imphal, Manipur. The data were analyzed by using SPSS software. Mean and standard deviation were calculated and one way ANOVA was adopted for significance test. P value <0.05 was used for statistical significance.

## RESULTS

Total leukocyte count was increased significantly in secretory phase as shown in Table 1.

Table-2 shows significant increased in granulocyte count in secretory phase whereas decreased count in proliferative phase which was not statistically significant. A small rise in agranulocyte count in secretory phase was observed.

A significant rise in absolute neutrophil count during secretory and menstrual phase, decreased in absolute monocyte count in proliferative phase to secretory phase. No significant change was observed in absolute eosinophil count, absolute basophil count is zero throughout the phases (Table-3).

Table-4 shows a significant rise in neutrophil percentage during secretory and proliferative phase. Lymphocyte count was increased in proliferative phase but was found to be not significant. No significant changes are observed in eosinophil, monocyte and also basophil count is zero throughout the menstrual phases

## DISCUSSION

Menstruation is a cyclical shedding of endometrium during reproductive age. The regulation of the normal cycle is under the control of neuro hormonal mechanisms with its feedback system present in the hypothalamic-pituitary-ovarian axis through release of FSH and LH. Changes in various blood parameters due to the naturally occurring fluctuations in the levels of sex steroid hormones concentration occur during the menstrual cycle.<sup>1,3,4</sup>

There was significant increased in the total leukocyte count

Total leukocyte count (TLC)	No of subjects (n)	Menstrual phase	Proliferative Phase	Secretory phase	p-value
	50	8934.00±241.4	8964.00±203.134	9890.00±187.7	0.01

All datas are expressed as mean ±SEM, P<0.05 is considered significant

**Table-1:** Total leucocyte counts/mm<sup>3</sup> of blood in three phases of the menstrual cycle

parameters	No of subjects (n)	Menstrual phase	Proliferative phase	Secretory phase	p-value
Granulocyte count	50	4145.98±290.7	3884.74±220.4	6166.80±164.6	0.000
Agranulocyte count	50	2989.9±83.9	3108.7±85.0	3238.7±86.7	.122

P<0.05 is considered significant

**Table-2:** Granulocyte and Agranulocyte /mm<sup>3</sup> of blood in three phases of the menstrual cycle

No. of subjects (n)	Absolute counts	Menstrual phase	Proliferative phase	Secretory phase	p-value
50	ANC	4016.7±290.0	3754.2±219.2	6023.1±163.9	0.000
	AMC	110.2±6.1	101.04±5.1	110.04±6.2	0.4
	AEC	129.3±7.4	130.6±145.2	143.68±7.6	.33
	ALC	2879.8±81.8	3007.7±83.3	3128.7±85.3	0.11
	ABC	00	00	00	-

All datas are expressed as mean ±SEM, P<0.05 is considered significant. ANC: Absolute neutrophil, AMC: Abs monocyte, AEC: Abs eosinophil, ALC: Abs lymphocyte, ABC: Abs basophil count.

**Table3:** Absolute leukocyte count/mm<sup>3</sup> of blood during different phases of menstrual cycle

No. of Subjects (n)	Differential counts (%)	Menstrual phase	Proliferative phase	Secretory Phase	p-value
50	Neutrophil	58.58±4.8	64.34±6.36	78.66± 5.2	.03
	Lymphocyte	30.46±4.1	36.74±4.69	33.24±7.6	.05
	Monocyte	1.26±.4	1.08±.27	1.18±.4	.17
	Eosinophil	1.58±.60	1.38±.490	1.44±.5	.18
	Basophil	00	00	00	-

( p <0.05) is considered as significant

**Table-4:** Differential leukocyte counts during different phases of menstrual cycle

during the secretory phase which may be due to hormonal effects that increase the subpopulations of leukocytes. These observations were in contradictory to some studies, according to them menstrual blood loss does not affect the complete blood count.<sup>17</sup> However the finding is in agreement with the findings of Tikare et al and Faas M et al.<sup>16,15</sup> Granulocyte count was found increased in secretory phase which was statistically significant, this may be due to increased in 17 beta estradiol concentration in circulatory pool of blood during secretory phase that promotes granulopoiesis, thereby increase in the granulocyte numbers.<sup>9,20</sup> However, rise in granulocyte counts in menstrual phase is as a result of the release of tremendous number of leukocytes during menstruation.<sup>5</sup> The finding of the present study is in consistent with the finding of Tikare et al, Dixon et al.<sup>16,6</sup>

These results identify significant fluctuation in number and patterns of circulating immune cells in women's immune response.<sup>19</sup> However, the agranulocyte count shows only slight increased during secretory and proliferative phase but was not statistically significant. The increased count may be due to increased 17βestradiol concentration.<sup>19</sup>

Decreased in absolute neutrophil count in proliferative phase was not statistically significant. Increased in absolute neutrophil count in secretory phase was statistically significant. The highest neutrophil count during secretory was not in agreement with the study by Apseloff G et al.<sup>13</sup> A small rise in neutrophil count in menstrual phase when compared to proliferative was in agreement with the results of Faas M et al and Subbi M et al.<sup>15,10</sup> This significant increased in secretory phase is due to phasic changes in estrogen or progesterone during menstrual cycle which play an important role in regulating the neutrophil count. Estrogen seems to enhance granulocyte proliferation in vitro.<sup>8,14</sup> The rise in absolute neutrophil count during menses may play role in phagocytic defense at the site of breached endometrium. Absolute lymphocyte count was found gradually increased in different phases of the menstrual cycle. The present finding was in agreement with the study by Faas M et al and Tikare et al.<sup>14,16</sup> This may be due to increased numbers of helper and cytotoxic T cells as well as NK cells. And also probably due to combined effects of increased concentrations of progesterone and 17 β –Estradiol acting in concert to establish a shift towards T- helper type-2( Th2) response. This finding may have clinical implications. During the luteal phase of ovarian cycle (as in pregnancy), women with rheumatoid arthritis, a cell mediated auto immune disorder, often experience improvement of their symptoms. Diseases mediated by excessive autoantibody production such as systemic lupus erythematosus, tend to flare up during pregnancy<sup>21</sup> and in the luteal phase of menstrual cycle. The reason may be that both the luteal phase and pregnancy are associated with a type -2immune 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.<sup>22</sup> In proliferative phase the count was decreased. The count was increased in menstrual and secretory phases but was not significant. This variation in cell count may be

due to increased 17 β – E<sub>2</sub> concentrations that promotes the release of granulocyte and monocyte from the bone marrow and also possible that monocyte may have receptors for progesterone.<sup>9</sup> As the above studies differ in explaining the cause for the increase in monocyte count, the exact cause remains to be determined which requires more sophisticated techniques of investigation. The count was increased in secretory phase but not statistically significant. The finding is in agreement with the finding reported Begum S et al and Malipatil et al.<sup>18,19</sup>

Neutrophil (%) was significantly increased in secretory phase. This might probably due to the hormonal changes occurring in the ovaries. Estrogen enhances granulocyte proliferation resulting to enhancement of the neutrophils release from bone marrow rather than from marginated pool.<sup>19,20</sup> The neutrophil (%) count was decreased in menstrual phase were reported by some authors.<sup>23</sup> The increased in neutrophil count during secretory phase was not in agreement with the study of Apseloff G et al.<sup>13</sup> The study showed significant increased of lymphocytes in proliferative as well as slight increase in secretory phase. This probably might be resulted due to increase in number of Helper T cells, cytotoxic T cells and Natural killer cells that occurs under the influence of steroids hormone.<sup>12,14,24</sup> In several studies, monocyte was significantly increased in the luteal phase than in the follicular phase which was found to follow the pattern closely that of progesterone or due to increased in 17β-estradiol concentration.<sup>5,9</sup> No basophil cell can be detected in all the phases of the menstrual cycle. This finding is similar to Malipatil et al<sup>19</sup> No significant changes were observed in eosinophil count. This observation was contrary to Subbi M et al, which showed a marked drop in eosinophil count during mid cycle followed by increase in the secretory phase.<sup>10,26</sup> Some authors also reported that cyclical variation of eosinophil count which was inversely related to those of the neutrophil and monocyte count, that enhance during menstruation and reduced throughout the rest of the cycle.<sup>7,20</sup>

## CONCLUSION

From this study we observed some hematological changes occurring during different phases of menstrual cycle. Total leukocyte count, absolute neutrophil and differential neutrophil counts were significantly increased in secretory phase when compared to menstrual and proliferative phase. There was significant increased in differential lymphocyte count during proliferative phase. No significant changes in eosinophil, basophil and monocyte cells in both absolute as well as differential count were detected. The reason could be due to the hormonal influence in female during menstrual cycle. It is also seen in few studies due to increased in 17 beta estradiol concentrations in secretory phase which helped to promotes granulopoiesis which in turn lead to increased the leukocyte count. These observations were in contradictory to some studies, according to them menstrual blood loss does not affect the complete blood count in relation to the menstrual cycle. This study also gives us the knowledge about the distribution of immune cells in peripheral blood

during different phases of menstrual cycle which might be importance in understanding various disorders, natural history of disorders occurring in female. It may also explain and answer the question of why few disorders are commonly suffered by women population and also help in therapeutic interventions. Since sample size is small in this study further studies is required to get more information.

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