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ASSESS IMPACT OF MENSTRUATION PHASES ON TOTAL LEUCOCYTE COUNT AND DIFFERENTIAL LEUKOCYTE COUNT IN HEALTHY FEMALES AND CORRELATE IT WITH STRESS SCORE.

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ABSTRACT **Background:** Menstruation is defined as "periodic and cyclic shedding of endometrium accompanied by loss of blood. A series of utero-ovarian and hormonal events take place over a period of 21-35 days, with a mean of 28 days. To assess impact of menstruation phases on total leucocyte count and differential leukocyte count in healthy females and correlate it with stress score. **Method:** Observational study, a total one hundred thirty five healthy female's age group 18 to 35 years old were included in the study. During each visit blood sample was collected and analyzed using hemocytometer for TLC and counting method for DLC and PSS questionnaire were given to subjects for stress scoring. **Results:** Total leukocyte count, neutrophil count, lymphocyte count, monocyte count increases during secretory phase of menstrual cycle and a significant positive correlation found between WBCs count and stress. **Conclusion:** Stress and hormonal changes during reproductive aged may influence the availability of immune cells in the peripheral blood.

KEYWORDS : menstrual cycle; total leukocyte count; phases of menstrual cycle; perceived stress scale.

INTRODUCTION

Menstruation is defined as "periodic and cyclic shedding of progestational endometrium accompanied by loss of blood".[1] During menstrual cycle, a series of utero-ovarian and hormonal events take place over a period of 21-35 days, with a mean of 28 days. Hormones act as a keyregulatory factor in the menstrual cycle, where their secretion is highly influenced by the negative and positive feedback during the follicular and luteal phases[2]. Hypothalamus plays an important role in regulating ovarian functions by controlling the secretion of gonadotropins from anterior pituitary. The ovarian functions are controlled by hormones secreted from hypothalamus, anterior pituitary and ovary. It was reported that fluctuations in ovarian hormones during menstrual cycle alters immune cells.[3] Estimation of leucocyte count (4,500-11,000) helps in women to identify reproductive morbidities. They are known to alter the immune system like depression of the suppressor T cell activity.[4] Human and animal studies suggest that there is a change in the distribution of immune cells during different phases of menstrual cycle.[5] Prevalence of dysmenorrhea 5–20% of women reporting severe dysmenorrhea (painful menstruation) which may be associated with reproductive morbidities like infection, thus estimation of leucocyte count is an important tool.[6] In developing countries, abnormal uterine bleeding appears to affect about 5–15% of women of reproductive age. It is a major cause of gynecological morbidity, affecting up to one in five women some point during their reproductive life span.[7]

Stress can influence people in every age and situation can result in both physical and physiological health.[8] Stress leads the way in causing changes to the menstrual cycle. When the body is under stress, it releases the hormone cortisol. Cortisol affects multiple areas of the body, including estrogen, and a change in estrogen levels alters menstrual cycle lengths.[9] Sanders et al. menstrual cycle characteristics have not only been shown to be associated with self-reported levels of stress, but also with physiologic measures of stress. Sanders and Bruce found that cortisol levels were highest among women with long menstrual cycles.[10] It is suggested that stressful situations during ovulatory periods and menstruation may cause increased 17-hydroxy corticosterone levels with resulting eosinopenia. Elevated levels of the end product i. e. cortisol, has a range of side effects including disruption of normal luteinizing hormone (LH) rhythm, hence affecting the menstrual cycle.[11] During menstruation, eosinopenia, thrombocytopenia and higher 17- hydroxycorticosteroid (17-OHCS) levels occurred in the majority of studies. It is suggested that stressful situations during ovulatory periods and menstruation may cause increased 17- hydroxycorticosteroid levels with resulting eosinopenia.[12] Nakanishi et al., 2003 reported that slightly elevated white blood cells (WBCs) count to be significantly associated with stressful lifestyles.[13] Aim of the present study to assess impact of menstruation phases on totalleucocyte count and differential leukocyte count in healthy females and correlate it with stress score.

METHODS

It is observational study. The present study was carried out in 135 healthy female between the aged groups of 18-35 years with normal regular menstrual cycle were selected. The duration of the cycle was 28±2 days. Subject with irregular cycles, pregnant female, gynecological disorders like endometriosis, fibroids, anemia, cancer, history of drug intake affecting menstrual cycle or history of chronic disease were excluded from the study. Study protocol was explained to the subjects and written informed consent was obtained from each of the subjects before took blood sample. Institutional ethical committee clearance was obtained. First sample was collected within 48 hours of onset of menstruation, second sample during days 8th-10th of menstrual cycle. Third sample was taken during days 22nd-24th of menstrual cycle. All the subjects were followed up during a single cycle. Samples were taken at morning time at 9am-10am to avoid diurnal variation. Total leucocyte count was made under Improved Neubauer's chamber using Turk's fluid which was examined under compound microscope and their undiluted blood is calculated. The Granulocyte consist of Neutrophils, eosinophils and agranulocytes i.e. monocytes, lymphocytes were examined by using Leishman's stain under compound microscope. A minimum of 100 WBCs are identified in a systematic manner and counting were made by using Tally bar method in the Haematology Laboratory of Department of Physiology, Rajasthan University of Health & Sciences College of Medical Sciences, Jaipur, Rajasthan. Cohen's perceived stress score questionnaire was distributed to all included subjects to assess stress score.

Data Analysis

The parameters were statistically analysed by using descriptive statistical i.e., mean and standard deviation. Association between the variables and significant were calculated by using t- test. The p value <0.05 was considered statistically significant and Pearson's correlation test were used to correlate differential leukocytes count, total leukocytes count and stress score. After completion of data collection, the data were entered in Microsoft Excel. Data were analyzed by using SPSS 16.0 version (Chicago.,USA).

RESULTS

Table I: Differential Leucocyte Count (DLC) And Total Leucocyte Count In Different Phases Of Menstrual Cycle.

Hematological Parameters	Menstrual phase Mean ± SD (n= 135)	Proliferative phase Mean ± SD (n= 135)	Secretory phase Mean ± SD (n = 135)
Neutrophil count (%)	59.9 ± 8.49	55.8 ± 7.84	60.6 ± 8.74

Lymphocyte count (%)	27.1 ± 3.83	27 .1 ± 3.83	32 ± 3.72
Monocyte count (%)	2.73 ± 0.98	2.73 ± 0.98	2.93 ± 1.42
Eosinophil count (%)	2.17 ± 0.59	2.21 ± 0.69	2.17 ± 0.60
Total leukocyte count (cell/cumm)	7035 ± 1524	6975 ± 1444	7130 ± 1531

The p<0.05 was considered as statistically significant.

Table 1: depicts statistical comparison of total leucocyte counts, neutrophil counts, monocyte counts and lymphocyte counts in proliferative phase with that of secretory phase, a significant increase were observed during secretory phase and eosinophil count (2.21±0.69%) were high in proliferative phase.

Table II: Pearson Correlation Analysis Of Differential Leucocyte Count And Total Leucocytes Count With Stress In Different Menstruation Phases.

Hematological Parameters	Menstrual Phase PSS			Proliferative Phase PSS			Secretory Phase PSS		
	Mild	Moderate	Sever	Mild	Moderate	Sever	Mild.	Moderate	Sever
Differential leukocyte count									
Neutrophil count	.275	.077	1.000**	.373	.064	1.000**	.230	.111	.680
Lymphocyte count	.248	.044	1.000**	.808**	.055	1.000**	.279	.016	.943
Monocyte count	.300	.114	1.000**	.167	.067	1.000**	.197	.105	.801
Eosinophil count	.555	-.120	1.000**	.125	-.111	1.000**	.555	-.057	.967*
Total leukocyte count	.164	.031	1.000**	.151	.024	1.000**	.247	.789	.692

The p<0.05 was considered as statistically significant.

Table 1I: Depicts Pearson correlation analysis of differential leucocyte count (neutrophils, lymphocytes and monocytes count) and total leucocytes count shows significant positive correlation (r = 1.000**) with stress in different menstruation phases and eosinophil count show significant negative correlation (r = -1.000**) with stress in menstrual cycle.

DISCUSSION

The present observational study was conducted in the hematology laboratory of department of Physiology, RUHS College of medical sciences, Jaipur. The population evaluated under the study was 135 healthy females of age group between 18 to 35 years. In this present study WBCs count and differential count were assessed in different phases of menstruation. Total leucocytecounts in proliferative phase (6975±1444) with that of secretory phase (7130 ± 1531), a significant increase were observed during secretory phase. An increase in total leukocyte count in SP compared to MP and PP was observed in the present study, which was in agreement with Tikare et al [14]. Similar study conducted by Faas et al results were reported the increased levels of oestradiol and progesterone in the SP might be playing a role in the deviation of immune response toward a type 2 response.[15] Another similar study found Makinoda et al results were reported that estradiol also helps in promoting the release of monocytes and granulocytes from bone marrow. IL-4 was significantly increased in T helper cells in SP as compared with the PP of the cycle. Increase in natural killer cells and cytotoxic T cells might also be a reason for increased leukocyte counts. A statistically significant increase in neutrophil count was found in SP compared to MP and PP. [16] In the present study monocyte count was increased in SP (2.93 ± 1.42) and decreased in PP (2.73 ± 0.98) were statistically significant. Similar study conducted by Thongngarm et al [75]results were reported monocytes play a key role in immune responses and their count is highly dependent on the changes in oestrogen and progesterone levels. These hormones induce mitotic arrest, apoptosis in monocytes, and promotion of monocyte release from bone marrow.[17] In the present study statistically significant increase in neutrophil count was found in SP (60.6 ± 8.74) compared to MP (59.9 ± 8.49) and PP (55.8 ± 7.84). This may be due to oestrogen influence on granulocyte proliferation and may be due to promotion of neutrophil release from bone marrow. Similar study conducted by Molloy et al, This study were examines the specific effects of estradiol and progesterone on neutrophil apoptosis and function in healthy adult men and women. They also examined the contribution of these hormones to the persistence and resolution of an inflammatory response.[18] In this present study we found increase lymphocyte count in SP (32 ± 3.72) with statistical significance. Heuvel V.D et al reported similar results Increase in differential lymphocyte count might be a result of large influx of natural killer cells, cytotoxic T cells, and T helper cells.[19] In the present study we found significant positive correlation (r value is 1.000**) of total leucocyte count with perceived stress. Similar results reported by Shafiee M, et al mean WBC count increased with increasing severity of symptoms of depression and anxiety. Results suggest that higher depression and anxiety scores are associated with an enhanced inflammatory state, as assessed by higher hematological inflammatory markers including WBC.[20]

CONCLUSION

Total leukocyte count and differential leukocyte count during the menstrual cycle are highly dependent on the phasic changes in the immune response mechanism and hormones. Menstrual, proliferative and secretory phases of menstrual cycle gives variations of blood count may be due to physiological stress and hormonal changes occurring during reproductive aged. A significant positive correlation found with stress and total leucocytes count, neutrophils count, lymphocytes count and monocytes count with stress. This study gives knowledge about availability of immune cells in the peripheral blood which may help in understanding burden of various disorders like reproductive tract infections.

Study Limitations:

Study in larger sample size and by increased reproductive age group (18-45 years old) could have generated more accurate results**.**

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Conflict Of Interest: None to declare.

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