Research Article

Cyclical variation of leucocyte profile in healthy females: a comparison with males

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Abstract

Aim: Aim of our study is to observe the cyclical variation of total WBC count in women and between men and women as sex hormones are having effect on these counts.

Methods: A total of 56 girls and 74 boys have participated in the study, fingertip blood sample was taken to count total and differential WBC count, for boys only one sample was taken, for girls three sample at different phases like first sample on 2^{nd} or 3^{nd} day of menstruation, second during proliferative phase (around 8^{th} to 10^{th} day) and third during secretory phase (around 22^{nd} to 24^{th} day).

Statistical analysis: Mann Whitney U test was used for comparing two independent groups. Comparison of study variables between different phases in girls was done by using Kruskal Wallis test. A p value of less than 0.05 was considered as statistically significant results.

Results- Among women there is a significant high Total WBC count during proliferative phase compared to menstrual phase(p=0.002) and secretory phase(p=0.000). There is a significant high total WBC count and Lymphocytes in all the phases of women compared to men.

Conclusion-there is a physiological variation of WBC count among women and also our study showed that significant variation of total WBC count among men and women can be attributed to the female sex hormones.

Keywords: hormone, WBC count, different phases, gender

1. Introduction

Menstruation is a process that occurs during the reproductive age of women from menarche to menopause involving structural, functional and hormonal changes in reproductive organs. The Hormonal change includes specially estrogen and progesterone, which shows cyclical variation during menstruation that is well known fact. But this cyclical variation of hormones is having effect on one or the other factors like immune cells, as these hormones are known to alter the immune system like depression of the suppressor T cell activity ¹, effect on erythrocyte sedimentation rate (ESR) ², Electrocardiogram (ECG), platelets ³ and WBC etc. There are many studies that have focused on this aspect of correlation between the female hormones and WBCs count. Many researches are done to find out the physiological variation in white blood cells (WBCs) during different phases of menstrual cycle but results are conflicting. So first aim of our study is to find out cyclical variation of Total WBC count and differential count in women.

Even gender wise these hormones have different effect on immune response between male and females, as is said that women are having more vigorous humoral and cell mediated immunity compared men ⁴, even Clinicians have noted that women were more resistant to variety of infections, which correlated with their greater longevity. These observations implicates that the influence of sex hormones which has received relatively scant attention ⁵, There are studies which have concentrated on effect of gender on WBCs count ^{6.7}. but these studies have not mentioned which phase of womens cycle was used to compare with men. So second aim was to compare total WBCs and differential count in each phase of women with men. This will be the first study which compared each menstrual phase of women with men.

2. Material and methods

2.1 This is a descriptive study

The ethical committee of Institution approved the study protocol. The study was conducted on MBBS students (both boys and girls) of Amala medical college, Kerala. Before initiating study we explained the study protocol to all the students and written, informed consent was taken. Subjects were selected on based on inclusion and exclusion criteria,

Exclusion criteria- those who are suffering from both acute and chronic infections, anemia, gynecological disorders endocrine diseases, hemostatic disorders, subjects with diabetes were excluded. Athletes were also excluded.

Inclusion criteria- both boys and girls between the age of 18 to 24 years and girls with regular menstrual cycles were included.

2.2 Protocol for boys

A total of 74 boys who were willing to participate in the project were asked to come to the physiology lab in the afternoon, we took height and weight, allowed them to take rest for 10 minutes, Then Blood pressure(BP), Heart rate (HR) and Respiratory rate (RR) was taken. Left ring finger was pricked with all aseptic precautions, wiped out the first drop and the second drop was pipetted in the WBC pipette, immediately the WBC diluting fluid was pipetted and mixed well and kept for 5 to 10 minutes, which was used to read Total WBC count, then we took three drops of blood on three different slides to make smear which was used for differential count after fixation and staining with leishman stain. Then finger was cleaned with cotton and spirit. Total WBC was counted with help of Neubauer's chamber under low power lens, and differential count under oil immersion lens. Counting was followed as in the manual ⁸.

2.3 Protocol for girls

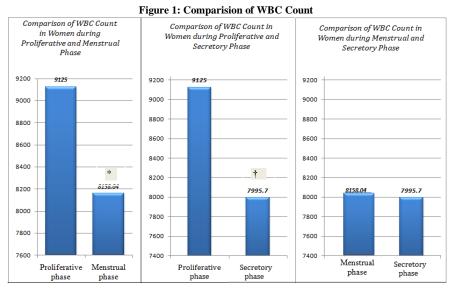
A total of 56 girls were participated, we took blood sample for three times during different phases of their menstrual cycle. First sample was on 2^{nd} or 3^{rd} day of menstruation, second during proliferative phase (around 8^{th} to 10^{th} day) and third during secretory phase(around 22^{nd} to 24^{th} day). We had taken detailed menstrual history of all the girls regarding their regular cycles (at least three consecutive regular cycles for the last three months). Then we

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asked all the girls to come to the lab when they had their 2^{nd} or 3^{nd} day of menstruation, according to that we have calculated their date of proliferative, secretory phases and asked them to come for second and third sample on those days respectively, remaining protocols were same as that of boys protocol. **2.4 Statistical Analysis**

Normality of variables was checked by using Shapiro Wilk test of normality. Since none of the variables does not follows normal distribution (P<0.05), Non parametric tests was used for analysis. Mann Whitney U test was used for comparing two independent groups. Comparison of study variables between different phases in girls was done by using Kruskal Wallis test. A p value of less than 0.05 was considered as statistically significant results.

3. Results



0.002* 0.000† **Table1: Comparison between Men and Women with menstruation phase** Physiological variation of WBC count

	Group	Number of subjects	Median WBC count	P value		
	Men	74	6250(4150-10500)	0.000*		
	Women	56	8158.04(4400-11650)	(Z= 5.232)		
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* P value based on Mann Whitney U test.

The average WBC count was comparably high among women during menstruation phase and this difference was statistically significant with a P value less than 0.05.

Table2: Comparison between Men and Women with Proliferative phase

Physiological variation of WBC count					
Group	Number of subjects	Median WBC count	P value		
Men	74	6250(4150-10500)	0.000*		
Women with Proliferative phase	56	9125 (5950-15550)	(Z= 6.9)		

*P value based on Mann Whitney U test.

The average WBC count was comparably high among women during proliferative phase and this difference was statistically significant with a P value less than 0.05.

Table3: Comparison between Men and Women with secretory phase

	Physiological variation of WBC count			
	Group	Number of subjects	Median WBC count	P value
	Men	74	6250(4150-10500)	0.000*
	Women with Proliferative phase	56	7995.7(5750-11500)	(Z= 6.9)
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The average WBC count was comparably high among women during secretory phase and this difference was statistically significant with a P value less than 0.05.

Table 4: Lymphocyte Comparison between Men and Women with proliferative phase

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	Group	Median	P value		
	Men	29.5 (2-45)	0.004*		
	Women	33.25 (21-45)	(Z= 2.86)		
Table 5: Lymphocyte Comparison between Men and Women with menstruation phase					
	Group	Median	P value		
	Men	29.5 (2-45)	0.002*		
	Women	34.16 (22-51)	(Z= 3.05)		
Table 6: Lymphocyte Comparison between Men and Women with secretory phase					
	Group	Median	P value		
	Men	29.5 (2-45)	0.000*		
	Women	34.5(25-47)	(Z=4.12)		

*Significant P values

Among women proliferative phase shows significantly more total WBC count compared to menstruation and secretory phase. Women are having significantly more Total WBC count and lymphocytes in all phases compared to men.

4. Observations

5. Discussion

According to our results discussion will be divided in to two heading.

5.1 Among women there is a physiological variation of total WBC in different phases of their menstrual cycle

In our study there was a significant increase in total WBC count during proliferative phase compared to menstrual and secretory phase. These results are comparable other studies as they showed WBCs start rising from 8th day onwards^{3,9}, some showed similar results but not significant². One of the underlying mechanism could be that a 10 times increased production of Granulocyte colony stimulating factor (G-CSF) from the follicular cells of ovarian follicles specially at late follicular phase which might have increased the WBC count in proliferative phase^{10,11}. As there are studies which have mentioned that estrogen could be the reason for increased WBC as it increases the granulocyte proliferation^{6,9}, inhibits the apoptosis of granulocytes (functions of neutrophils will be intact in spite of delayed apoptosis)¹², in different clinical scenario like as a treatment for prostatic cancer in males^{13,14}, others like Hormonal replacement therapy(HRT) in case of postmenopausal women there was increased in total WBC count^{15,16}.

But other studies have shown different results like total WBC count was increased in luteal phase compared to follicular phase of ovarian cycle^{17,18}, more during secretory phase compared to proliferative phase of menstrual cycle^{19,20}, only absolute lymphocyte count will get increased in secretory phase compared to menstrual phase²¹.

5.2 Comparison of all the phase of women with men

In our study compared to men women are having significantly more total WBC count and Lymphocytes in all the phases, our results are comparable with others but they have not mentioned which phase of women cycle was compared with men^{6,7}. Study done by Allen *et al* showed up to the age of 50 years WBCs are more in women and after 50 years starts declining and becomes less in women compared to men, indicating that female hormonal effect²². One of reason could be estrogen, as mentioned in earlier discussion. Other studies have confirmed the presence of an immunoreactive gene product for GM-CSF in human endometrium throughout the menstrual cycle. These findings indicate that human endometrium synthesizes GM-CSF and that epithelial cells are a major contributor to its production²³.

Significant increase in lymphocytes in women compared to men which is comparable to other studies^{17,20}. The decreased lymphocyte counts in males as compared to females may be due to the increased testosterone concentrations, since testosterone may increase apoptosis in T cells²⁴. Also studies in animals have shown an effect of estrogens on B cell development: estrogens increase bone marrow progenitor B cells in mice by protecting the progenitor cells from apoptosis²⁵.

6. Conclusion

We here by conclude that there is a effect of different phases of menstrual cycle on total WBC count. And also our study showed that significant variation of total WBC count among men and women can be attributed to the female sex hormones.

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