



RESEARCH PAPER

CD4 AND LEUCOCYTE COUNTS OF YOUNG APPARENTLY HEALTHY FEMALES DURING THE THREE PHASES OF MENSTRUAL CYCLE IN EKPOMA, NIGERIA

*¹Babatope, I.O., Isabu, P.A.², Eromon, P.A.³, Edosomwan, P.E.¹, Akhigbe, O.I.¹, Asekhome, H.¹

¹Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Nigeria; ²Department of Obstetrics and Gynaecology, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria;

³Department of Family Medicine, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria.

* Correspondence: babatope_olaniyi@yahoo.com

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ABSTRACT

The menstrual cycle is also a window into the general health and well-being of women. This study was aimed at determining the CD4 and leucocyte counts of young apparently healthy females during a single cycle in Ekpoma, Edo State, Nigeria. The study comprised of thirty one (31) subjects aged 17-27 years with a regular 28-day menstrual cycle. The subjects were sampled during a single cycle- that is on the 2nd day, 11th day and 21st day representing menstrual phase (MP), proliferative phase (PP) and secretory phase (SP) respectively. Leucocyte counts were determined using a haematology autoanalyzer while the CD4 counts were done with Partec cyflow machine. The mean of WBC counts were 4.77, 5.10 and 5.10 in the MP, PP and SP respectively. There was no significant difference in both the WBC total and differential counts of the three phases. The mean of CD4 counts were 850.45 cells/ μ l (MP), 939.77 cells/ μ l (PP) and 954.03 cells/ μ l respectively. The CD4 counts in the three phases were also not statistically significant. In this study, we found that the three different phases of menstrual cycle did not have any significant effect on the leucocytes and CD4 counts of the subjects studied.

Key words: Ekpoma, CD4 counts, Leucocyte counts, Menstrual cycle

INTRODUCTION

Menstruation is the physiological shedding of the endometrium and discharge of blood from the endometrial arteries (Raven *et al.*, 2013). Menstrual cycle is a repetitive phenomenon occurring during the reproductive life of a female, involving structural, functional and hormonal changes in the reproductive life (Rajnee *et al.*, 2010). The normal reproductive life of a female are characterized by monthly rhythmical changes in the rates of secretion of the female hormones and corresponding physical changes in the ovaries and other sexual organs. This pattern is called the female monthly sexual cycle or less accurately the menstrual cycle (Guyton and Hall, 2005). The menstrual cycle is due to fluctuations in concentrations of estradiol, progesterone, luteinizing and follicle-stimulating hormones (Agboola, 2006).

The menstrual cycle alternates between two major phases, the follicular phase, typically persisting for 12 to 16 days, characterized by the presence of maturing follicles, and the luteal phase, most commonly persisting for 10-16 days, characterized by the presence of the corpus luteum in the ovary (Begum and Ashwini, 2012). There are three phases of menstrual cycle namely; menstrual phase (MP), proliferative phase (PP) and the secretory phase (SP). The regular cyclic changes may be explained as a phenomenon for periodic preparation for fertilization and pregnancy (Molina, 2010).





It is suggested that a woman's immune responses are influenced by hormones, and since hormone levels change throughout the menstrual cycle, one would expect her immune response to also change (Begum and Ashwini, 2017). Fluctuations in progesterone and estrogen concentration have been reported to influence von Willebrand factor in such a way that platelet function is periodically altered during the menstrual cycle (Drici *et al.*, 1996). Furthermore, granulocyte, granulocyte percentage, mean platelet volume, plateletcrit, platelet width density and platelet larger cell ratio have been shown to be significantly lower in ovulation phase than the luteal phase of the menstrual cycle (Omorogiuwa and Egbeluya, 2014). The sex hormones are known to influence the immune response. The role of sex hormones in the immune response, like the differences in immune response between males and females; alteration in the immune response after gonadectomy and hormone replacement, alteration in response during pregnancy and the presence of receptors for sex hormone of cells of various arms of the immune system (King, 1998) has been studied.

Hence this study was aimed at assessing the CD4 and leucocyte counts of apparently healthy young girls during different phases of menstrual cycle in Ekpoma, Edo State.

MATERIALS AND METHODS

Study Area: This study was carried out in Ekpoma, the administrative headquarters of Esan West Local Government Area of Edo State. Ekpoma is the fourth largest town in Edo State and lies between latitude 6°43'N to 6°45'N of the Equator and longitude 6°6'E to 6°8'E of the Greenwich Meridian with an altitude of about 332 meters above sea level (Aziegbé, 2006). It is made up of several quarters. Ekpoma has a population of 89,628 in 1991 and 127,718 in 2006, majority of which are civil servants, traders, business men/women, transporters, farmers, teachers, lecturers and students by occupation. Ambrose Alli University is situated in this town.

Study Population: The study population comprised of thirty one (31) apparently healthy young female subjects between the age group of 17-27 years studying at Ambrose Alli University, Ekpoma, Nigeria. Only subjects with normal menstrual cycle of twenty eight (28) days during a single menstrual cycle were recruited into this study.

Sample Size: Thirty one (31) apparently healthy young female subjects with regular menstrual period were recruited into this study.

Inclusion Criteria: Only apparently healthy female undergraduate students (Ambrose Alli University, Ekpoma) with regular menstrual cycle of 28 days, aged 17-27 years, free from infections and fevers were recruited into this study.

Exclusion Criteria: Female undergraduate with irregular menstrual flow, history of any gynaecological or endocrinal disorders, history of chronic diseases, anaemia and any other visible ailments were excluded from the study.

Ethical Consideration: Ethical clearance was obtained from the Research and Ethical Committee of Ambrose Alli University, Ekpoma. Inform consent was also obtained from each of the subjects.

Sample Analysis: The thirty one (31) subjects were sampled in three different phases of a single menstrual cycle, that is on the 2nd day, 11th day and 21st day representing menstrual phase (MP), proliferative phase (PP) and secretory phase (SP) respectively. Blood samples (4ml each) were collected under aseptic conditions from the ante-cubital vein by venepuncture. The blood samples were dispensed into specimen bottles containing Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant. The blood samples were inverted for at least 6-8 times immediately after collection and transported from the field to the laboratory in cold chain boxes for analysis. CD4 count samples were collected between the hours of 9:00am – 12:00noon. All blood samples were analysed within 1-2hours of collection. Leucocyte counts (total and Differential leucocyte counts) were determined with Sysmex KX-21-N haematology autoanalyzer (Sysmex Corporation, Japan) while the CD4 count was determined using Partec cyflow machine (Sysmex Partec GmbH, Görlitz, Germany) according to manufacturer's instructions.

Data Analysis: Data was presented as mean \pm S. E. M. The various parameters were statistically analyzed using SPSS (version 20) and one way analysis of variance (LSD test) was carried out to determine the significant differences at $P \leq 0.05$.





RESULTS

Table 1 revealed the socio-demographic profile of the study subjects. Subjects in the age range of 21-24 years recorded the highest frequency distribution of 48.37% followed by those in the age range of 17-20 years (41.94%). Subjects belonging to the age range of 25 years and above were the least (9.68%). Based on religion, Christian subjects dominated (93.56%) with the Muslims accounting for 6.45% of the study population. All the recruited subjects were single (100%).

Table 2 summarized the mean values of leucocytes and CD4 counts during the different phases of menstrual cycle. The mean values of WBC counts ($\times 10^3/\mu\text{l}$) were 4.77, 5.10 and 5.10 in the menstrual phase (MP), proliferative phase (PP) and secretary phase (SP) respectively. There was no statistical significant difference of the WBC count ($\times 10^3/\mu\text{l}$) in the three different phases. There was also no statistical significant difference in the differential leucocyte counts (both percentage % and absolute numbers (#)) across the three phases of the menstrual cycle in the study population. The CD4 counts (cells/ μl) in the three phase of the menstrual showed some variations but these variations were not statistically significant.

Table 1: Socio-demographic profile of female undergraduates in three phases of menstrual cycle

| Variables | Categories | Frequency | Percentage (%) |
|----------------|------------|-----------|----------------|
| Age | 17-20 | 13 | 41.94 |
| | 21-24 | 15 | 48.37 |
| | 25 & above | 3 | 9.68 |
| Religion | Christians | 29 | 93.56 |
| | Muslims | 3 | 6.45 |
| Marital Status | Single | 31 | 100 |
| | Married | 0 | 0 |

Table 2: Leucocytes and CD4 counts during different phases of menstrual cycle in Ekpoma

| Parameters | MENSTRUATION PHASE (MP) N = 31 | PROLIFERATIV E PHASE (PP) N = 31 | SECRETORY PHASE (SP) N = 31 | F-VALUE | P-VALUE |
|--|--------------------------------------|--|-----------------------------------|---------|---------|
| | Mean \pm SD | Mean \pm SD | Mean \pm SD | | |
| White blood cells WBC ($\times 10^3/\mu\text{l}$) | 4.77 \pm 1.11 ^a | 5.10 \pm 1.47 ^a | 5.10 \pm 1.08 ^a | 0.736 | 0.482 |
| Neutrophil NEUT # | 1.94 \pm 0.64 ^a | 2.07 \pm 0.78 ^a | 1.98 \pm 0.68 ^a | 0.293 | 0.747 |
| Lymphocyte LYM # | 2.30 \pm 0.71 ^a | 2.32 \pm 0.77 ^a | 2.48 \pm 0.70 ^a | 0.579 | 0.563 |
| Mid-sized cells MXD # | 0.54 \pm 0.32 ^a | 0.58 \pm 0.25 ^a | 0.60 \pm 0.26 ^a | 0.365 | 0.695 |
| Neutrophil percentage NEUT % | 40.52 \pm 8.71 ^a | 40.61 \pm 8.77 ^a | 39.88 \pm 10.24 ^a | 0.056 | 0.946 |
| Lymphocyte percentage LYMI' % | 48.05 \pm 6.92 ^a | 47.23 \pm 6.36 ^a | 49.06 \pm 8.39 ^a | 0.495 | 0.611 |
| Mid-sized percentage M&D % | 11.76 \pm 5.21 ^a | 11.88 \pm 3.95 ^a | 11.54 \pm 4.95 ^a | 0.041 | 0.960 |
| CD4 (cells/ μl) | 850.45 \pm 213.64 ^a | 939.77 \pm 302.87 ^a | 954.03 \pm 265.16 ^a | 1.411 | 0.249 |

DISCUSSION

The menstrual cycle serves as a window into the general health and well-being of women, and not just an event in the woman's reproductive life. The hormonal changes occurring during the menstrual cycle not only affect the oocyte maturation and the endometrial and vaginal environment but can also have an effect on a number of physiological and biochemical phenomena (Dullo and Verdi, 2008). It can indicate the status of bone health, heart disease, and ovarian failure as well as long-term fertility. Apart from it being physiological, there are various disorders associated with menstrual cycle





which cause morbidity and mortality. The menstrual cycle is characterized by cyclic fluctuations in the levels of follicle stimulating hormone (FSH), leutinizing hormone (LH), estrogen and progesterone. The hormones are known to have an effect on oxygen carrying capacity, immune response and bleeding (Usha *et al.*, 2014).

In this study, the total white blood cells counts (total WBC) in the three phases (Menstrual phase – MP, Proliferation phase – PP, and Secretory phase - SP) of the menstrual cycle recorded only slight variations which were not statistically significant. Studies on white cell indices are divergent; while some studies showed no significant changes in total white blood cell count during the various phases of menstrual cycle (Pathak *et al.*, 1981., Makinoda *et al.*, 1996., Bagby, 2007). Bagby (2007) in his study reported the total white blood cell count of the subjects he studied to be within the normal range of 4,000 to 11,000 cells/mm³. Others showed increased total white cell count from the menstrual phase to the secretory phase (Bouman *et al.*, 2001), increased leucocyte count during the mid-cycle or proliferative phase and decreases during the secretory phase (Bain and England, 1975). The percentage change increase in total WBC in the secretory phase found in one study corroborated with earlier studies and is due to increase in all subpopulations (lymphocytes, monocytes and granulocytes). The levels of estrogen or progesterone are important factors in regulating the neutrophil count. Estrogen seems to enhance granulocyte proliferation in vitro (Pierre, 2002). The significant increase in the total leucocyte count reported by these authors might also be as a result of the release of tremendous number of leucocytes during menstruation (Guyton and Hall, 2000). As a result of this increase, the body and specifically the genital region (organs) is highly resistant to infections. Since the spiral arteries supplying the endometrium are broken and the endometrial surface denuded as reported by Moore and Persuade (2003), the need for an increase in leucocytes (mainly granulocytes) to fight any occurrence of infections.

Differential leucocyte counts (DLC) (percentage and absolute numbers) revealed variations in the three phases of menstrual cycle but these variations were not statistically significant. Our finding is in line with the previous report of Omorogiuwa and Igeleke (2014) who found a non-significant result like ours. In contrast, Shilpa and Reshma (2014) reported that significant changes were observed in neutrophils (%), and lymphocytes (%) during the three phases of menstrual cycle of young Indian girls in Raichur, India. Neuroendocrine regulation on immune responses is suggested during an ovarian cycle, which may be critically for embryonic implantation and pregnancy (Lee *et al.*, 2010). Agoreyo and Asowata (2011) also observed a statistical significant increase in the total leucocyte and granulocyte counts among the subjects they studied. Furthermore, John (2007) in his study reported a statistical significant increase in the total leucocytes and its differential counts with the different durations of menstruation of the subjects studied and observed a statistical significant increase in the total leucocyte and granulocyte counts in subjects whose menstruation lasted for seven days. John (2007) suggested that this could be due to prolonged exposure of the female genital system to infection during menstruation. This implies that individuals whose menstruation last for seven days are more exposed to infections and thus their body produces a stronger immune system in the form of tremendous number of leucocytes to help attack any form of infections (Agoreyo and Asowata, 2011). Different reproductive processes such as ovulation, menstruation, are influenced by the immune system. This results in sexual dimorphism in the immune response in humans (Bouman *et al.*, 2005). It has also been shown that progesterone enhanced chemotactic activity of neutrophils, while estrogen decreased the activity (Miyagi *et al.*, 1992).

Comparison of CD4 counts in the three phases revealed that there was a progressive variation in the mean CD4 counts of the subjects studied but this increase was not statistically significant. This is in tandem with the previous report of Dapper *et al.* (2011) who found a non-significant variation in the CD4 counts of female undergraduates in Port Harcourt, Nigeria. The progressive variation observed in this study may be attributed to increase in the number of helper T cells, cytotoxic T cells and natural killer cells (Faas *et al.*, 2000).

CONCLUSION

In this study, we found that the three phases of menstrual cycle did not have any significant effect on the leucocytes and CD4 counts of the subjects studied.

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AUTHORS' CONTRIBUTION

The contributions of the authors/co-authors are briefly stated as follows:

Babatope, I.O. – Research idea and design, drafted the work and analyzed the samples

Isabu, P.A. – Medically certified the subjects and critically reviewed the write-up

Eromon, P.A. – Data analysis and also reviewed the write-up

Edosomwan, P.E., Akhigbe, O.I. and Asekhame, H. – administration of questionnaires, field work, sample collection and contributed reagents/materials.

