PHAGOCYTIC ACTIVITIES OF NEUTROPHILIC LEUKOCYTES IN WOMEN IN VARIOUS PHASES OF MENSTRUAL CYCLE, AND IN PREGNANCY

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Abstract. This study evaluated the phagocytic activities of neutrophilic leukocytes in both sexes during reproductive age and in women in different phases of the menstrual cycle and in pregnancy. In this study, 9 male and 12 female medical students, 17 to 20 years of age, 23 pregnant women and 9 women after delivery were evaluated. Heparinized blood was incubated with heat killed bacteria *Staphylococcus aureus* at 37°C for 20 minutes. The blood films were stained with Leishman stain and the phagocytic index was found out by the number of neutrophilic leukocytes ingested with organisms in 100 cells and the total number of organisms counted therein. At the interval of 14 days, on 3 consecutive occasions, the phagocytic index was studied on the same male and female subject. The phagocytic activities of neutrophilic leukocytes were found slightly higher in females than in males; more so in pregnancy than in non-pregnant women, woman controls and after delivery. During the expected ovulation period, the phagocytic activities were observed more than during the menstrual and premenstrual phases. The phagocytic activities were found more in pregnancy and during the ovulatory period in women.

INTRODUCTION

The phagocytic activities of neutrophilic leukocytes *in* both *vivo* and *vitro* have been studied in normal and in certain disease conditions. A number of factors have been reported as capable of modifying phagocytosis. In pregnancy, the attachment, ingestion and digestion of *Candida albicans* in neutrophilic leukocytes were found to be increased over non-pregnant females of reproductive age (Barriga *et al*, 1994). Human chorionic gonadotropin (hCG) was implicated for increasing the absorption capacity, bactericidal potential, as well as the enhanced activity of the complement system. The Luminol dependent chemiluminescence (CL) response of whole blood supplemented with exogenous hCG was found to be significantly higher in men, women, and pregnant women (Shmagel, 1994; Shepard *et al*, 1996). The expression of a relative number of Fc and C3b receptors on the surface of polymorphonuclear leukocytes in pregnant women was also found to be increased (Duff *et al*, 1986).

There is a large variation of hormonal secretion and interactions during the menstrual cycle in women, similar to that during pregnancy. There is LH surge just before ovulation. The functions of LH are similar to that of hCG. At the termination of pregnancy hCG secretion ceases. If hCG stimulates the phagocytic activities in pregnancy, there may be variation in phagocytic activity during the different phases of the menstrual cycle and also at the end of pregnancy (Shibuya *et al*, 1991).

MATERIALS AND METHODS

The phagocytic activities were observed in an *in vitro* experiment using whole blood. The subjects were taken from 21 healthy medical students (9 male, 12 female) 17-20 years of age at Nepal Medical College, 23 healthy pregnant women of all trimesters, and 9 women who had a normal delivery at the college hospital.

Preparation of suspension of organisms

In this study, the Gram-positive bacterium, *Staphylococcus aureus*, was used for the phagocytic experiment. A suspension of this organism was prepared in nutrient broth and incubating it for 24 hours at 37°C. The suspension was centrifuged and washed with normal saline; then the

suspension of organism was matched to get the desired concentration. A heat killed suspension of organisms was prepared by keeping it in a water bath at 56°C for one hour. The same suspension was preserved in refrigerator and used for all the experiments. In order to detect proper heat killing of organisms, one drop of suspension was cultured on nutrient agar and growth was not detected after 48 hours of incubation at 37°C.

Determination of the phagocytic index

Two parameters were considered for the phagocytic index: a) the number of neutrophilic leukocytes with engulfed bacteria in 100 cells, and b) total number of organism present therein. The incubation for 20 minutes was preferred after evaluating results from 5, 10, 15, 20, 25 and 30 minutes incubation. Both parameters were found highest after 20 minutes incubation. On three occasions; at the interval of 14 days, studies were done on the same male and female medical student (with a regular menstrual cycle). Pregnant women with normal pregnancy, without any history of drug intake, were selected irrespective of para and trimester. At the termination of pregnancy, during the first week, but two days after normal delivery, blood was collected for study.

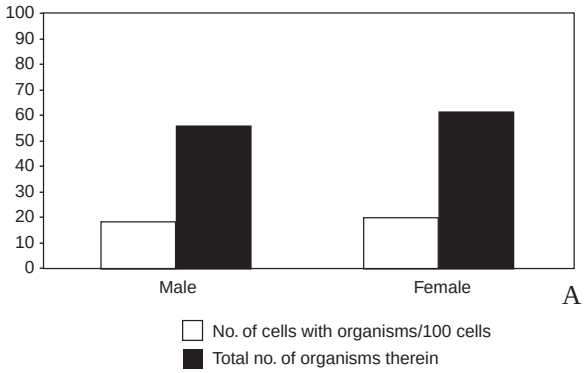
One milliliter of blood was collected in a heparinized vial and 50 µl of saline suspension of heat killed *Staphylococcus aureus* was added having the concentration of 500 million per ml. (ratio of neutrophil: organism is 1:4). The sample was thoroughly mixed and incubated for 20 minutes at 37°C. Immediately after incubation, two smears of blood were prepared from each sample and stained with Leishman stain.

Counting of ingested cells and organisms

From each film, 200 neutrophils were observed by two independent observers. The number of neutrophils with ingested organisms among the two hundred neutrophils and the total numbers of organisms in them were counted. The average number of cells with ingested organisms and the number of organisms per cell, from 400 cells, were evaluated in two independent experiments. The organisms took the Leishman stain nicely and were easy to count. Occasional monocytes were found with ingested organisms but these were not included in the results.

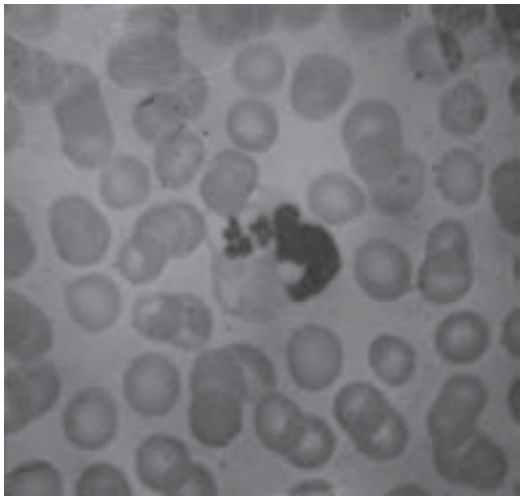
RESULTS

The results of the phagocytic index: the number of neutrophilic leukocytes engulfed with bacteria among 100 cells and the total number of bacteria therein are summarized and presented in Figs 1, 2, 3 and 4. The mean value with standard deviation of the numbers of leukocytes marked with organisms among 100 cells and the total number of organisms therein, between the male and female reproductive age groups were observed and found to be 18.18 ± 4.58; 56 ± 14. 86 and 19.6 ± 6.28; 61.33 ± 28.21 respectively. The higher value in females than in males is not statistically sig-



A

Fig 1a–Mean values of phagocytic index in male and female showing: 1) percentage of neutrophils engulfed with bacteria; 2) total numbers of organisms present therein.



B

Fig 1b–Blood films stained with Leishman stain: neutrophilic leukocytes with engulfed bacteria.

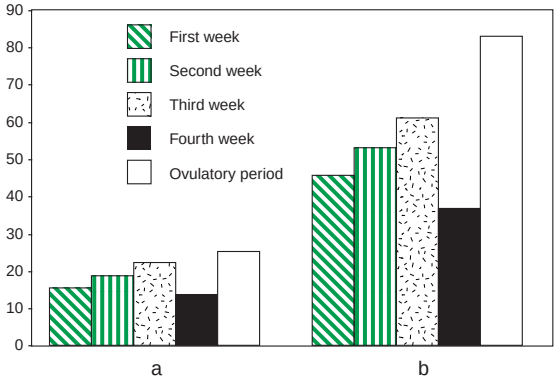


Fig 2–Mean values of the phagocytic index in female at different weeks of menstrual cycle and in the expected period of ovulation.

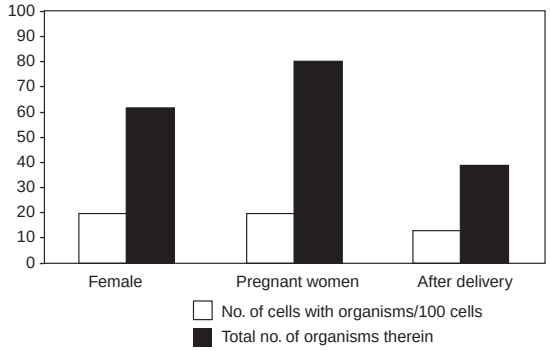


Fig 3–Mean values of the Phagocytic index in: female, pregnant women, and after delivery.

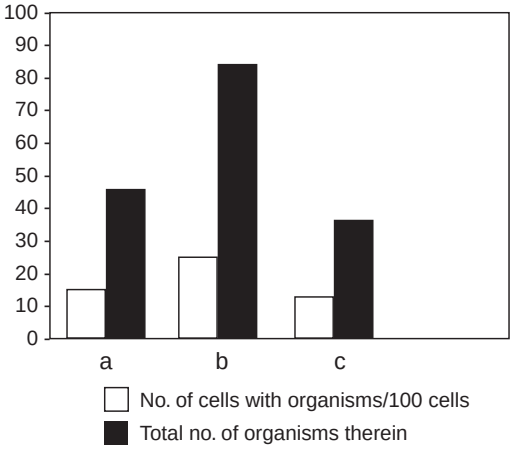


Fig 4–Mean values of the phagocytic index in: a) menstrual period, b) ovulatory period, c) premenstrual period.

nificant. The individual variation of phagocytic index, on three occasions, of the same person, was minimal in males but showed wide variation in females. When the results in females were plotted in relation to their individual menstrual cycle, the mean value was found progressively rising from the first week of menstral cycle to the third week. During the fourth week, both the parameters fell. The mean value during expected period of ovulation was found to be significantly higher ($p<0.01$) than the menstrual and premenstrual phases.

The total number of neutrophilic leukocytes marked with organisms did not vary between pregnant and nonpregnant controls, but the total number of organisms were observed to be significantly higher in pregnancy ($p<0.05$). The data after delivery were found to be significantly lower ($p<0.01$) than in the pregnant women. The average value of the phagocytic index during the expected ovulation period and in the three trimesters of pregnancy were found to be similar, but in the second trimester the total number of organisms engulfed by neutrophils were recorded as slightly higher.

DISCUSSION

Our results of increased phagocytic index during pregnancy are found to be similar to the observations of Barriga *et al* (1994) and Shmagel (1994), though the organisms used were different. In their study, they found an increase in the neutrophilic absorption capacity and bactericidal potential with an increased activity of complement in pregnancy. The Luminol dependent chemiluminescence (CL) response of whole blood supplemented with exogenous hCG, increased significantly in all the male and female subjects and in pregnant women. The relative number of Fc and C3b receptors of neutrophils increased significantly during pregnancy (Shepard *et al*, 1996). These observations suggest that the higher nonspecific immunity in pregnancy is due to the stimulating effect of endogenous hCG. This seems to be a compensatory mechanism, in part, for the weakened specific immunity of the maternal host. Following delivery the hCG and LH levels in blood are insignificant. The decrease in the

phagocytic index after delivery is similar to the premenstrual period, where the LH level is also very low. No significant difference was observed during labor than with the controls,except at the end of the gestational period, where the phagocytic activities of the polymorphonuclear leukocytes in the amniotic fluid were found to be decreased (Stefanovic *et al*, 1993).

In our study, we found decreased phagocytic activities of neutrophils at the termination of pregnancy. All these observations are corroborative. Our observation of higher phagocytic activities during ovulation similar to that of pregnancy, is likely to be due to the stimulating effect of LH, as there is a LH surge preceding ovulation. The physiological functions of LH and hCG are similar. These results suggest that LH and hCG possibly increase non-specific immunity in humans during ovulation and pregnancy.

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