Number, maturity and phagocytic activity of neutrophils in the three trimesters of pregnancy

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عدد العَدِلات ونضجها وفعَّاليتها البلعميَّة في الأثاليث الحملية الثلاثة

سانشيتا برَامَنيكْ، تاباس بْرَامَنيكْ، سوبخاس مَنْ دَلْ، راتنا شَنْ دَا

الخلاصة: تتناقص المناعة النوعية تناقصاً ملحوظاً أثناء الحمل. وقد صُمِّمت هذه الدراسة لمعرفة كون المناعة اللانوعية التي تتواسَطُها الكريات البِيض العَدِلات neutrophils ذات شأن في التأقلم مع حالة الكَبْت المناعي هذه. وقد ازداد عدد الكريات البيضاء والعَدِلات ولاسيَّما عدد العَدِلات الفتية المأطورة band، ازدياداً تدريجياً مع تقدُّم الحمل. كما ازدادت الفعَّالية البلعميَّة للعَدِلات في الأُثلوثَيْن trimesters الثاني والثالث. مـمَّا يدل على أن العَدِلات قد تعوّض جزئياً عن ضعف المناعة النوعية للحامل.

ABSTRACT Specific immunity decreases markedly in pregnancy. This study was designed to determine whether the non-specific immunity mediated by neutrophils plays any role in coping with this immunosuppressed condition. The number of leukocytes and neutrophils, especially the number of young band neutrophils, increased gradually with the advancement of pregnancy. The phagocytic activity of neutrophils increased in the 2nd and 3rd trimesters. Neutrophils may compensate in part for the weakened specific immunity of pregnant women.

Nombre, maturité et activité phagocytaire des polynucléaires neutrophiles au cours des trois trimestres de la grossesse

RÉSUMÉ Au cours de la grossesse, l'immunité spécifique diminue sévèrement. Cette étude avait pour objectif de déterminer si l'immunité non spécifique médiée par les neutrophiles joue un rôle quelconque pour palier cette immunodépression. Le nombre de leucocytes et de neutrophiles, en particulier celui des neutrophiles non segmentés immatures, augmente progressivement au fil de la grossesse. L'activité phagocytaire des neutrophiles s'intensifie au deuxième et au troisième trimestre. Les neutrophiles sont capables de compenser partiellement l'affaiblissement de l'immunité spécifique qui caractérise la femme enceinte.

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Introduction

Specific immunity decreases during pregnancy [*1*], and this is marked by a fall in IgG in the 2nd and 3rd trimesters [*2*]. There is a marked decrease in the number of helper T-lymphocytes between the 14th and 28th weeks, resulting in impairment in maternal immunity [*3*]. As specific immunity decreases in pregnancy, non-specific immunity, mediated by neutrophils and migratory phagocytes, plays an important role in the body's defences. The phagocytic cells use primitive, non-specific recognition systems which allow them to bind to a variety of microbial products. These cells are responsible for innate immune responses and are the first line of defence against infection [*4*]. Neutrophils are involved in the body's defence against microorganisms, especially against pyogenic bacterial infections [*5*].

It has been shown that the phagocytic activity of neutrophils increases in pregnancy [*2,6*] but how it alters during each trimester has not been clearly determined. One study demonstrated that the phagocytic activity of neutrophils was greater in the 2nd trimester in comparison with non-pregnant women of reproductive age [*1*]. On the other hand, another study reported that the phagocytic activity of neutrophils was similar in women in the 1st and 2nd trimesters of pregnancy and non-pregnant women, but significantly decreased during the 3rd trimester [*7*]. It is also clear from the existing literature that the numbers of Fc and C3b receptors on the neutrophil surface increase significantly in the 3rd trimester compared with levels in non-pregnant women of reproductive age, indicating that the neutrophils have a greater capacity for attachment to microorganisms, and thus have increased phagocytic activity [*8*].

These contradictory reports prompted our interest in studying what exactly happens with regard to neutrophil status in the different trimesters of pregnancy. This study was therefore designed to explore the variation in number, maturity and phagocytic activity of neutrophils in the 3 trimesters of pregnancy.

Methods

The study was carried out over a period of about 1 year starting February 2003. We approached pregnant women (aged 20–35 years) in different trimesters who were attending the outpatient department of J.B. Roy State Ayurvedic Hospital, Kolkata, India for routine antenatal check-up and invited them to participate in the study. Women who had any bacterial or viral infection, cold, fever, chest congestion, stomach upset or loose bowel motions were excluded. Only a few of the women refused to participate as they were apprehensive.

A sample of venous blood was drawn into a heparinized syringe from each participant and transferred to the laboratory of the Department of Physiology at Vidyasagar College for Women in Kolkata for testing. Total leukocyte count was estimated manually using a haemocytometer [*9*]. Absolute neutrophil count was calculated as follows: absolute value (neutrophils/mm^3) = total leukocyte count (cells/mm^3) × relative value (%) obtained from the differential count [*10*].

A blood smear was stained with Leishman stain and was examined to determine the differential count and the proportion of band forms among the neutrophilic leukocytes. Neutrophils which had no distinct fine filament (length but no breadth) connecting the nuclear lobes were considered band forms [*10,11*], young immature forms of neutrophils [*11*]. Band neutrophils were counted per 100 neutrophils.

The phagocytic activity of the neutrophils was studied by noting phagocytosis

[*12*]. Heat-killed *Candida albicans* was added to a leukocyte suspension obtained from the buffy coat of 0.5 mL heparinized blood. The mixture was incubated at 37 °C for 15 minutes then centrifuged. Smears were made of the precipitate and stained by Leishman stain. Intracellular *C. albicans* cells stain intensely and can be identified and counted inside the neutrophils under oil immersion. The number of neutrophils positive for *C. albicans* ingestion per 100 neutrophils gives the phagocytic index and the total number of *C. albicans* cells counted per 100 positive cells divided by 100 gives the avidity index [*13*].

Healthy volunteers, students and staff of the same age group (having no infection: bacterial, viral or parasitic) of Vidyasagar College for Women, Kolkata and J.B. Roy Ayurvedic College, Kolkata, served as non-pregnant controls. Informed consent was given. Blood samples were taken and tested in the same way as for the women in the test group.

All the parameters derived from both groups of women were compared and analysed by Student *t*-test.

Results

The mean total leukocyte count increased in pregnancy. Mean absolute neutrophil count, which was 3054/mm^3 blood in non-pregnant women, increased throughout pregnancy and was 4518/mm^3 blood in the 3rd trimester. The proportion of young neutrophils also increased with the advancement of pregnancy, 29% in non-pregnant women compared with 57% in women the in the 2nd and 3rd trimesters. Avidity index gradually increased from 2.03 in non-pregnant women to 2.56 in women in the 3rd trimester.

For all parameters except phagocytic index in the 1st trimester, the increases were statistically significant (Table 1).

Discussion

When stimulated by oestrogen, the adrenal cortex produces increasing levels of total and free plasma cortisol and other corticosteroids from the 12th week of pregnancy to term [*14*]. Glucocorticoid increases the total

Table 1 **Number, maturity and phagocytic activity of neutrophils in pregnant and non-pregnant women**

Parameter	Non-pregnant women (*n* = 25) Mean (SD)	Pregnant women 1st trimester (*n* = 25) Mean (SD)	2nd trimester (*n* = 25) Mean (SD)	3rd trimester (*n* = 25) Mean (SD)
Total leukocyte count (cells/mm^3)	5039 (919)	5958* (1013)	5765* (1512)	6169* (1316)
Absolute neutrophil count (cells/mm^3)	3054 (706)	4035* (727)	4102* (1073)	4518* (1112)
Young (band) neutrophils/ 100 neutrophils	29 (4)	44* (11)	57* (4)	57* (5)
Phagocytic index	41 (9)	41 (6)	49* (7)	58* (8)
Avidity index	2.03 (0.27)	2.28* (0.43)	2.43* (0.42)	2.56* (0.45)

**P < 0.05 (values compared with those of non-pregnant women).*
SD = standard deviation.

number of leukocytes and the neutrophil count, and decreases the lymphocyte count by decreasing lymphocyte mitotic activity [*15–17*]. In our study also it was noted that, with the advancement of pregnancy, lymphocyte count decreased whereas there was a significant rise in the number of neutrophils.

Moderate leukocytosis of up to 15×10^9 cells/L is common during pregnancy [*18*], with a peak about 8 weeks before parturition, i.e. in the 3rd trimester [*19*]. The cause of leukocytosis is neutrophilia, which may be a result of increased cortisol level during pregnancy as corticosteroids produce a delayed but sustained rise in blood neutrophil count. It may also be due to promotion of the release of neutrophils from the bone marrow under the influence of corticosteroids, which accounts for neutrophil leukocytosis [*20*]. In our study, the number of leukocytes and neutrophils increased with the advancement of pregnancy, corroborating the findings of previous researchers [*21–23*].

Clinically, it is recognized that an increase in younger forms (band cells, metamyelocytes, etc.) suggests increased release of young neutrophils from the bone marrow [*24*]. We observed an increase in the number of band cells among the neutrophils with the advancement of pregnancy, which is consistent with the findings of previous studies [*23,24*]. Tsakonas et al. also reported a significant decrease in lobularity index in pregnancy compared to controls (indicating the production of immature neutrophils during pregnancy) but mean lobularity index did not change significantly with gestation [*25*].

In our study, we found greater production of band (immature) neutrophils with the advancement of pregnancy. These are younger forms which contain more myeloperoxidase [*10*]. The myeloperoxidase system is the most competent bactericidal system present in neutrophils. The number of younger neutrophils increased in the 2nd and 3rd trimesters, indicating that bactericidal activities of neutrophils may be greater in women in the last 2 trimesters compared to non-pregnant women and those in the 1st trimester.

The phagocytic activity of neutrophils, indicated by phagocytic index and avidity index, increased in the 2nd and 3rd trimesters. Previous studies also showed an increase in the activity of neutrophils in pregnancy [*2,6*], but in which trimester and to what extent had not been demonstrated. The phagocytic activity of neutrophils is increased with stress, when glucocorticoid levels are high [*13*]. In our study, the increased levels of corticosteroids in the 2nd and 3rd trimesters may mediate increased activity of neutrophils.

It has also been reported that human chorionic gonadotrophin has a stimulatory effect on the phagocytic activity of neutrophils [*8*]. This placental hormone is secreted in large amounts during the first few weeks of pregnancy, after which it declines slowly, reaching a nadir of approximately 20 000 IU/L serum at approximately 120 days gestation that persists until delivery [*26*]. The increased phagocytic activity of neutrophils in the 2nd and 3rd trimesters may be a result of the synergistic action of human chorionic gonadotrophin and cortisol present in higher concentrations at that time.

It is likely that the nonspecific immunity offered by increased numbers of neutrophils and increased phagocytic activity may compensate in part for the weakened specific immunity of pregnant women.

Although our study gives an indication of the number, maturity and phagocytic activity of neutrophils in the 3 trimesters of pregnancy, the bactericidal activity in each trimester is yet to be determined.

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Corrections

Visceral leishmaniasis control in Thi Qar Governorate, Iraq, 2003
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The name of the fourth author was spelled incorrectly and should read: B. Budosan.

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The order of the authors was incorrect and should read: N. Sofaer,[1] A. Jafarey,[2] R.P Lei,[3] X. Zhang[4] and D. Wikler[5].
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