

Significance Changes in Phagocytes Chemotoxine in Pregnant Women

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الخلاصة:

تتعرض النساء الحوامل للاصابة بالالتهاب الجرثومية بشكل أكبر في الأشهر الأخيرة من الحمل. المعروف أن كريات الدم البيضاء خلال حركتها الذاتية والالتهامية في الأنسجة حيث يعد وجودها بأنه التحفيز المناعي الأبتدائي للجسم. تبين من دراسة حالة (42) امرأة حامل و (15) منهن تمت دراستها بين حالة الوضع من خلال التجارب العملية خارج الجسم عند استخدام مادة الاكاروزالهلامية بأن الحركات الذاتية لكريات الدم لتلك النساء و خلال أية فترة من فترات الحمل كانت ضمن المجال الطبيعي حيث تم دراسة (25) امرأة منهن و التي تتراوح فترة حملهن ما بين (8-33) اسبوعاً كانت طبيعة عند مقارنتها مع (20) نساء آخر غير متزوجات في حالة صحية جيدة جداً .

ثبت من خلال التجارب ان (17) امرأة والتي كانت فترة حملهن ما بين 34 اسبوع ونهاية الحمل (حالة الوضع) بان الحركة الذاتية والالتهامية فيها قليلة خلال تلك الفترة من الحمل، تمت ملاحظة ان النساء بعد الوضع بثلاثة ايام تزداد فيها الحركة الذاتية الالتهامية الى اعلى مجال طبيعي للحركة وعند استخدام الحصول لهذه النسل وحفظها سوية مع كريات الدم البيضاء لاتؤثر على طبيعة الحركة الذاتية سواء زيادة او نقصان في مجال الحركة. لكنها أي الحركة الالتهامية نقل بشكل واضح عندما تكون المرأة الحامل مهينة للاصابة بالالتهابات الجرثومية خلال فترة الحمل.

Abstract:

Pregnant Women have an increased risk for some infections, particularly during the last trimester. Phagocyte emigration from the circulation into tissues is an important aspect of the initial immune response. There for, circulating phagocytes of 42 pregnant and 15 post partum patients were studied in vitro for random and chemotactic (or directional) migration by agarose gel. Random migration of phagocytes for all 42 pregnant patients studied in each trimester was with in normal limits, chemotactic migration of 25 patients who were between 6 and 33 weeks of pregnancy was also similar to values obtained with control leucocytes (20 non-

pregnant normal female). However phagocytes of 17 other women studied between week 34 of pregnancy and term showed marked depressions in chemotaxis during labor and with in 3 days delivery, chemotactic migration increased to supernormal levels in 14 of 15 women studied. Sera from 6 pregnant patients with proven chemotactic defects did not reduce migration when incubated with normal phagocytes. These chemotactic defects appear to be intrinsic and may be important in predisposing to infections during late pregnancy.

Introduction:

Most infections during pregnancy affect neither the fetus nor the course of pregnancy, but some (like rubella) can cause profound teratogenic effect ^[1,2,3] or others (such as small pox and chicken pox) can result in severe natal and maternal infections ^[4,5].

Pregnant women also appear to be hyper susceptible to influenza infections. Limited data exist as to the immune status of pregnant females or their incidence of various infections. One model of uterine infection in pregnant animals indicated an enhanced susceptibility to bacterial infection especially during late gestation ^[6,7]. The intensity of leucocytic infiltration into experimentally infected uteri are also less in pregnant than non pregnant females. Deficiencies in phagocytic chemotaxis could be important in determining the early course of infectious challenges, for these reasons we decided to study phagocytic chemotaxis of healthy pregnant females.

Material and Methods:

Characteristic of patients and control chemotaxis of circulating venous leucocytes from 57 pregnant and postpartum patients was compared with that of 20 non pregnant, age-matched female controls.

These patients were selected for absence of a personal history of diabetes mellitus, inter current infections and family histories of recurrent infections written and informed consent was obtained from all patients and control donors. Patients taking medications other than vitamins or Iron supplements were excluded from study. Gestation in 17 patients ranged between 9 and 20 weeks and in 8 other patients it ranged between 21 and 33 weeks. Seventeen additional patients ranged between 34 weeks and term, and five patients were in active labor. Ten patients were examined within 3 days of parturition. Parity of past partum patients included the present pregnancy controls also were not taking any medications.

Emigrational Assay:

In vitro leucocytes chemotaxis was tested by Nelson, 1975 method ^[8]. This method included pattern of wells used. Agarose was mixed with an equal volume of supplemented 199 medium. Heat inactivated pooled human serum was added to

final concentration of 10% per ml. of agarose medium wells 2.4 mm in diameter and spaced 2.4mm a part were cut in each plate using plexiglass template and stainless steel punch. agarose plugs were picked out using a hypodermic needle leucocytes rich plasma was obtained here 25ml of heparinized venous blood by allowing erythrocyte sedimentation for 1 hour in 6% dextrin. After the plasma rich fraction was washed with 0.87% (w/v) ammonium chloride (pH 7.4) and centrifugal (500xg) the cells were suspended in phosphate buffer solution (pH 7.4). Concentration of segmented and non segmented polymorphonuclear leucocytes and monocytes were then adjusted to 5×10^6 phagocyte cells per ml. one tenth milliliter of this phagocyte suspension was added to 0.25ml of Hanks balanced salt solution (pH 7.4) after peri incubation for 15 minutes with autologous or homologous sera. These cells were then deposited by gravity sedimentation, polymorphonuclear leucocytes were suspended in supplemented 199 medium the center well of each three-well series receiving 10ml of the PMN suspensions, the outer well received 10ml of E. coli suspensions and the inner well received 10ml of heat-inactivated pooled human serum.

The plates were incubated at 37°C in humidified atmosphere for 2 hours, removed and fixed by additions of 3ml. volume of absolute methanol for 30 minutes followed by 3ml. of 47% buffered formalin for 30 minutes after fixation the gel was removed intact and the plates were stained with Wright's stain and air-dried. The stained cell patterns were projected on to white background and magnified using a zone reader (Labrook scientific Inst. Co. England).

The distance the cells had moved from the margin of the well towards the well containing the E. coli cells (distance A) was measured and also the distance (distance B) the cells had moved from the margin of the well towards control medium (heated-inactivated serum) known as spontaneous migration Achemotaxis index A/B and chemotaxis differential $A-B$ were calculated from each eight readings and the mean values were determine for A/B and $A-B$ and asscatterogram was plotted a migration index (MI) was calculated by multiplying the number of leucocytes found in the leading edges this product was then divided by 1.000. All assays were always done in duplicated 3 hours of vein puncture specimens were discarded of this time period was exceeded as in table-1.

Results:

Characteristics of the assay system; migration indices were computed from leading edges so that both the number of cell and their respective distances traveled. These distances were notably consistent in duplicate leucocytes samples run for each patient and control donors and did not vary by more than 15um. The mean migratory distance for control cells was $88\pm 4\mu\text{m}$ (range =75 to 100 um) phagocytes obtained from all pregnant patients showed a mean migratory distance of 84um plus or minus one standard deviation of 13um (range = 50 to 100um). In particular the mean of the ranges of migration was 88um for patients who were 0 to 33 weeks pregnant and 80um for women in the last trimester, see table (1).

Leucocytes from all patients and control were also studied the unstimulated migration probably reflected random migration. Mean distance of random migration for all pregnant patients was $45\pm 10\mu\text{m}$ (range= 20 to 80um) and for control donors it was $49\pm 1\mu\text{m}$ (range equal 30 to 82um) phagocytes of women in the first two trimesters showed a mean distance of 48um and of 42um in the last trimester.

Differential counts of phagocytes (polymorphonuclear leucocytes and monocytes) contained in the phagocytic suspension were simitained in the phagocytic suspension were similar for all patient groups and control subject.

Effect of duration of pregnancy:

Chemotactic activities of phagocytes from pregnant and postpartum patients and non pregnant controls are shown (Fig. I) values of migrations indices are on the vertical axis, where as the abacissa is divided in to groups of females controls and patients who were studied at 0 to 33 and 34 to 40 weeks of gestation, during labor, and within 3 days of delivery. Twenty control patients had phagocytes with a mean migration indices of 20.2 ± 4.7 (range=13.7 to 33.9) phagocytes from one normal control were studied over 4 consecutive days and showed a mean migration indices of 20.1 ± 5.2 (range= 15.0 to 25.7) phagocytes from 25 patients whose gestation was less than 34 weeks showed a mean migration indices of 18.7 ± 5.8 (range = 9.9 to 35.4).These latter values were very similar to those obtained with leucocytes from healthy controls.

Seventeenth other patients who were pregnant between 34 weeks and term had lower phagocytic migration indices with a mean of 11.3 ± 5.6 (range=3.5 to 25.3). Migration indices of their group were significantly lower than migration indices of control subject. Only six of these patients near term had leucocytes with normal values of MIs five patients in active labor (with out Oxytocin administration) had phagocytes which demonstrated a wide range of low, normal and high MIs mean= 28.4 ± 12.3 (range=8.1 to 50.6). Ten postpartum patients were studied with in

3 days of delivery, their leucocytes had a mean MIs of 40.3 ± 14.6 (range=21.7 to 73.0) which was significantly greater than control values.

No association were found by analysis of variance between the degree of chemotactic defect and 1- the number of our patients parity or 2- if the patients had a positive family history for diabetes mellitus ages of our patients and control donors also showed no correlation with the degree of chemotactic defect also no association was seen between the degree of impairment in MI and the number of mature or band-type neutrophils nor the venous leucocytes count before cellular recovery and adjustment.

Effect of Sera from pregnant patients:

Sera from pregnant patients were added to normal phagocytes to observe of values were caused by serum inhibitors which were not removed by cellular isolation techniques sample (0.1ml) of sera from six patients with proven low values of MIs (range=8.1 to 10.0) were added to 0.9ml of the phagocytes suspensions obtained from eight normal donors and 0.25ml of this mixture was placed in agarose wells. Every patient's serum was tested with phagocytes obtained from normal healthy donors. Pregnant sera caused little alteration in MIs of these normal phagocytes when compared to MIs values found with normal sera.

Discussion:

Several alterations in both cellular and humeral immunity during pregnancy have already been described^[9,10] circulating phagocytes of some pregnant women show decreased phagocytosis and killing of *E. coli* and *pseudomonas. Sp.*^[11] and the sera from pregnant women can reduce bacterial ingestion of phagocytes from non pregnant donors, our data indicate that significant decreases occur in phagocytic chemotaxis of pregnant women, particularly between 34 weeks of pregnancy and term. These data confirm the study by Maltzer and Silva 1980^[6] which showed not only an intrinsic depression in chemotaxis during the last half of pregnancy but also decreases in phagocytosis and *E. coli*. These investigators did not separate the period of gestation nor study patients during or after parturition.

We also found that these chemotactic defects appear to reserve during parturition as only one of five patients in labor had depressed chemotaxis postpartum patients demonstrated very significant increases in phagocytic chemotaxis when studied within 3 days of delivery, although there was a wide range of values. No depressions were induced in chemotaxis of control phagocytes after incubation in serum obtained from pregnant patients having known chemotactic defect.

These results suggest that on intrinsic defect occurs in circulating phagocytes between 34 weeks of pregnancy and term.

This observed defect in chemotaxis may be related to circulating steroidal compound which increase during gestation such as estrogens these alterations in

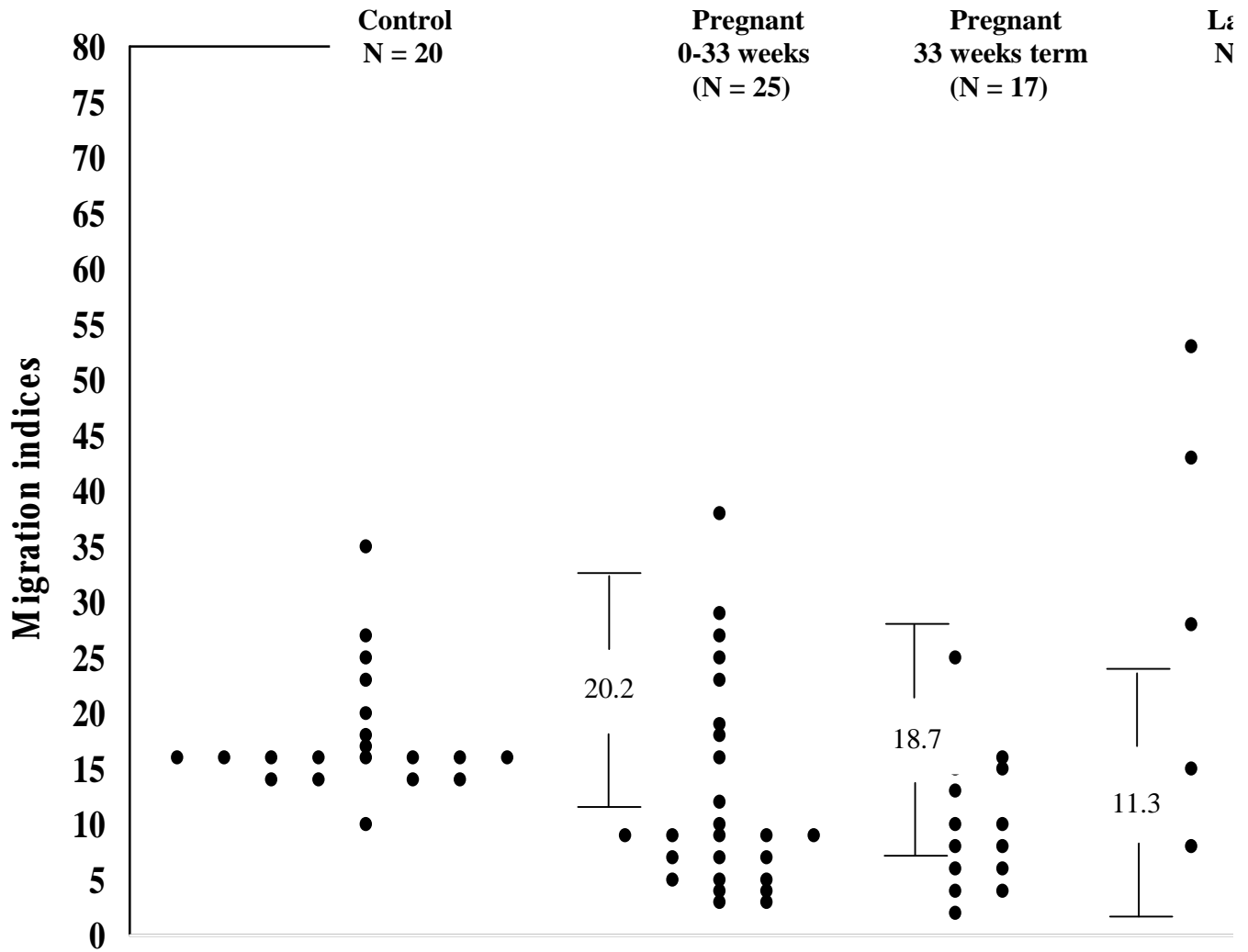
levels of hormones which occur during gestation may alter phagocytes migration toward chemotactic stimuli.

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Control	Pregnant				Labor		Post partum		
	Number	Range	Number	Range	Number	Range	Number	Range	
20	13.7-33.9	25	15-25.7	17	3.5-25.3	5	8.1-50.6	10	21.7-73.0
MI: 20.2±4.7		MI: 20.1±5.2		MI: 11.3±5.6		MI: 28.4±12.3		MI: 40.3±14.6	

Table (1) Migration Indices



(Fig-1) Migration indices during pregnancy, labor and post