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The Effect of the Mode of Delivery on Neonatal Immunity

Yenidoğan Doğum Şeklinin Lenfosit Alt Gruplarına Etkisi

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ABSTRACT

Objective: The mode of delivery is important for the development of the immune system in newborns. We aimed to investigate whether the changes in the activity and numbers of immune system cells are related to their birth pattern.

Materials and Methods: In this study, peripheral blood leukocyte, monocyte, granulocyte, and lymphocyte counts as well as lymphocyte subpopulations were investigated in the umblical cord blood samples of 74 normal pregnancies, delivered either vaginally (n=34) or by elective cesarean section (n=40). A laser-based flow cytometer was used to analyze surface markers on the mononuclear cells. Statistical analysis was performed by using SPSS analysis package program (Microsoftware version 17.0). P≤0.05 was considered significant.

Results: We found that mean total leukocyte, lymphocyte, granulocyte, and monocyte counts were observed to be lower in the cesarean delivery group than in the normal delivery group. However, no statistically significant difference was found between two groups. There were higher percentage values of CD3+, CD4+, and CD8+, lower percentage and absolute values of CD16+, and percentage values of CD19+ in the cesarean delivery group. The percentage values of CD3+, CD4+, CD8+, and percentage and absolute values of CD16+ were significantly different between the two groups. However, there was no significant difference in CD19+ values.

Conclusion: With this study, we tried to show possible effects of delivery methods on the lymphocyte subgroups and immune system of the newborns. However, future studies with larger groups are needed to enlighten this issue.

Keywords: Immunity, vaginal delivery, obstetric, cesarean section, lymphocyte subpopulation

ÖZ

Amaç: Çalışmamızda, doğum şeklinin lenfosit alt gruplarına etkisinin araştırılması hedeflenmektedir.

Gereç ve Yöntem: Bu prospektif çalısma; 37. haftadan büyük gebeliği olan, İstanbul Medeniyet Üniversitesi Göztepe E.A.H. Kadın Doğum Servisine başvuran 40'ı Sezaryen ve 34'ü Normal doğum yapan toplam 74 gebe analiz edildi. Gebelerin umblikal kord kanı örneklemesinde total lökosit, monosit, granulosit, lenfosit sayıları ve lenfosit subpopulasyonları ile oranları araştırılmıştır. Lazer bazlı flow sitometri, mononükleer hücrelerin yüzey belirteçlerinin analizi için kullanılmıştır. Çalışmada SPSS 17.0 paket programından faydalanılmış olup; p≤0,05 anlamlı olarak kabul edilmiştir.

Bulgular: Bu çalışmada sezaryen ile doğum yapanlarda total lökosit, lenfosit, granülosit ve monosit sayıları daha düşük gözlemlenmesine rağmen, her iki grup arasında anlamlı istatistiksel bir fark izlenmemiştir. Lenfosit subpopülasyonunda sezaryen ile doğum yapanlarda, CD3+, CD4+, CD8+ yüzde değerlerinin daha yüksek, CD16+ yüzde ve mutlak değerlerinin ve CD19+ yüzde değerlerin daha düşük olduğu görülmüştür. CD3+, CD4+, CD8+ yüzde değerlerinin ve CD16+ yüzde ve mutlak değerlerinde her iki grup arasında anlamlı fark oluşurken; CD19+ değerlerinde anlamlı fark görülmemiştir.

Sonuç: Bu çalışma ile doğum şeklinin lenfosit alt gruplarına ve yenidoğanın immun sistemi üzerine olası etkilerini göstermeye çalıştık. Bu konudaki detaylı araştırmalar için, hasta sayısının arttırılması öngörülmektedir.

Anahtar Kelimeler: İmmunite, vajinal doğum, obstetrik; sezaryen ile doğum, lenfosit alt grupları

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INTRODUCTION

Normal birth and cesarean delivery are the forms of birth that have certain advantages and disadvantages in terms of mother and baby. Cesarean birth is defined as birth of a fetus with incisions made in the wall of the abdomen and wall of the uterus (1). When examining the literature on Obstetrics and Gynecology and Pediatrics, we found that both birth methods have positive and/or negative effects on both mother and infant (2). One of these effects is whether the mode of delivery affects neonatal immunity.

There are many studies showing that the way of delivery is important for the development of the immune system in newborns. The newborn period includes the first 28 days after birth, and the newborn's immunity system is susceptible to many effects during this period. Because the immune system cells are considered immature and inexperienced during the newborn period, it is even more important whether the changes in the activity and numbers of these cells are related to their birth pattern. In particular, lymphocyte cells may be more prominent in the immune system of newborns due to sensitivity to post-natal infection. Because the baby's blood is circulating in the umbilical cord, the parameters examined in the cord blood reflect the value of the baby (3).

In this study, we aimed to investigate the effect of the mode of delivery on lymphocyte subgroups and the comparison of lymphocyte subgroups. For this, lymphocyte subgroups were studied in the cord blood, the changes in the cord blood of the lymphocyte subgroups were compared, and the effect of the birth pattern was tried to be determined.

MATERIALS and METHODS

Seventy-four pregnant women, 40 of whom had cesarean delivery and 34 had normal delivery, admitted to the Ministry of Health, Istanbul Medeniyet University Göztepe Training and Research Hospital, Maternity and Women's Services between May 2014 and July 2014 were examined in our study. The ethics committee approval and permission for the study were obtained from the Ministry of Health, Istanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethics Committee with the decision number 2014/0075 and date 17/06/2014. Informed consent was received from all the patients and control group.

Lymphocyte subsets were analyzed by means of flow cytometry. Blood samples were collected in tubes containing Etilen diamin tetraasetik asit (EDTA) as the anticoagulant.

Samples were transferred to the laboratory for the assessment of surface markers on the mononuclear cells by flow cytometry.

The samples were run in a Full Blood Count autoanalyzer (Abbott, Cell Dyn 3700/USA), which was run in both the laser and impedance methods, for the analysis of leukocyte numbers in the hemogram.

To analyze lymphocyte surface expression of CD antigens, the following fluorophore-conjugated monoclonal antibodies were used: CD45+ (PerCP-Cy5.5), CD3+ and CD16/56+ (FITC), CD8+ (PE), and CD4+ and CD19+ (APC). Samples were hemolyzed and fixed with a lysing reagent (FACSTM lysing solution; Becton Dickinson, Mountain View, CA). They were then washed once and subjected to two-color flow cytometry in a FACSCalibur Analyzer flow cytometer (BD Biosciences/US) and analyzed using BD CellQuest™ Pro software ver. 6 to determine the percentage of each lymphocyte in total lymphocytes.

Statistical Analysis

Statistical analysis of the data was performed using the IBM Statistical Package for the Social Sciences 17.0 package program (IBM SPSS Corp.; Armonk, NY, USA). In comparison of continuous variables between groups, the Shapiro–Wilk test was used to determine whether they were parametric or non-parametric. Student's *t*-test was then performed for parametric subjects, whereas Mann–Whitney U test was performed for non-parametric subjects. The analysis of dependent and independent variables was performed by regression analysis. Spearman's rho correlation analysis was used for the normal distribution matching parameters and Pearson's correlation analysis was used for non-normal distribution parameters. $P \leq 0.05$ was considered significant.

RESULTS

Of the 74 newborns involved in the study, 41 were boys (55%) and 33 were girls (45%). Sixteen of the boys were born with normal delivery (39%) and 25 with cesarean section (61%). Eighteen (54%) of the girl babies were born with normal delivery and 15 (46%) with cesarean delivery. In other words, 34 of 74 newborns were born with normal delivery (46%) and 40 with cesarean section (54%) (Table 1).

Distribution of lymphocyte subgroups (LAGs) was investigated by percent number (%) and absolute number (#).

Absolute (#) values of lymphocyte subgroups were calculated using the formula:

Lymphocyte (#)=% lymphocyte×WBC×10

Parameter (for example, CD4) (#)=Lymphocyte(#)×% parameter/10

According to LAG evaluation, percent (%) and absolute value (#) of NK cells (CD16+/CD56+) were found to be lower in the cesarean-born group than in the normal-born babies ($p<0.01$) (Table 2).

Also, percent (%) values of (CD3+/CD4+) helper T lymphocytes (Th) and (CD3+/CD8+) cytotoxic T lymphocytes (Tc) were found to be lower in the group born with normal delivery than in the group born with cesarean section ($p<0.05$) (Table 2).

Percent values of (CD3+) T lymphocytes were found to be lower in the group born with cesarean section than in the group born with normal delivery ($p<0.05$) (Table 2).

The erythrocyte and Hgb values were found to be lower in the cesarean delivery group. The MCHC (mean red blood cell hemoglobin concentration) parameter was observed

Table 1. Type of delivery distribution by gender

Gender	Normal birth	Cesarean birth	Total
Boy	16	25	41
Girl	18	15	33
Total	34	40	74

Table 2. Lymphocyte subgroup analysis

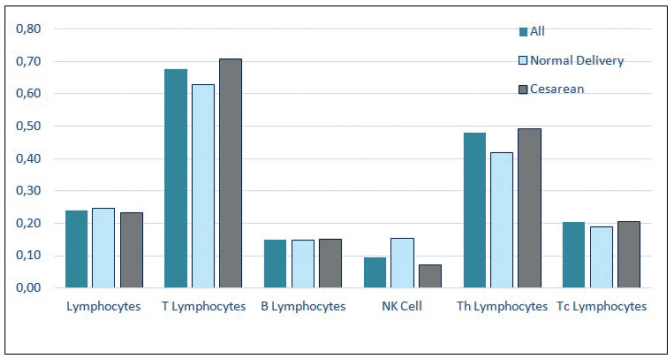
Parameter	Normal delivery	Cesarean delivery	All	p
LAG_% lymphocytes (CD45/SSC)	23,03±5,99	22,81±7,71	22,91±6,93	0,893
LAG_% T lymphocytes (CD3+)	63,34±10,3	70,12±7,59	67,01±9,5	0,002
LAG_% B lymphocytes (CD19+)*	15,84±7,95	15,05±5,1	15,41±6,53	0,799
LAG_% NK Cell (CD16/56+)*	16,65±9,89	8,81±5,18	12,42±8,61	0
LAG_% helper T lymphocytes (CD3+/CD4+)	43,63±10,81	48,92±8,04	46,49±9,72	0,022
LAG_% cytotoxic T lymphocyte (CD3+/CD8+)	18,84±4,78	21,61±5,57	20,34±5,37	0,026
LAG_# lymphocytes (CD45/SSC)*	3,21±1,13	2,81±1,15	2,99±1,15	0,116
LAG_# T lymphocytes (CD3+)	2±0,72	1,95±0,69	1,97±0,7	0,746
LAG_# B lymphocytes (CD19+)*	0,51±0,26	0,42±0,22	0,46±0,24	0,116
LAG_# NK cell (CD16/56+)*	0,57±0,39	0,27±0,32	0,41±0,38	0
LAG_# helper T lymphocytes (CD3+/CD4+)	1,39±0,59	1,35±0,5	1,37±0,54	0,764
LAG_# cytotoxic T lymphocyte (CD3+/CD8+)*	0,6±0,26	0,61±0,29	0,6±0,27	0,666

The results of the LAG evaluation. Results in this study are given in mean±SD (standard deviation). The values found statistically significant between the two groups were written in bold type and $p\leq0.05$ was considered significant. NK: Natural Killer

Table 3. Complete blood count parameters in cord blood

Parameter	Normal delivery	Cesarean delivery	All	p
WBC	14,28±3,86	12,69±3,56	13,41±3,76	0,073
NEU*	7,51±2,48	6,74±2,66	7,09±2,59	0,1
LYM	4,95±1,54	4,3±1,2	4,6±1,39	0,051
EOS*	0,45±0,26	0,41±0,28	0,43±0,27	0,542
BAS*	0,22±0,09	0,21±0,13	0,21±0,11	0,253
MONO	1,15±0,43	1,04±0,51	1,09±0,47	0,34
RBC*	4,41±0,61	4,09±0,41	4,23±0,53	0,001
HGB*	15,45±2,24	14,55±1,53	14,96±1,92	0,004
HCT	47,24±6,72	43,61±4,61	45,25±5,9	0,008
MCV*	107,13±5,18	106,65±4,41	106,87±4,74	0,96
MCH*	35,1±2,51	35,62±1,65	35,38±2,08	0,305
MCHC*	32,75±2,09	33,42±1	33,12±1,61	0,04
PLT	208,99±88,37	225,52±61,07	218,05±74,56	0,349
MPV*	7,63±2,02	8,11±1,47	7,91±1,72	0,405
PDW*	17,95±1,13	17,8±0,93	17,86±9,5	0,534

The results of the LAG evaluation. Results in this study are given in mean±SD (standard deviation). The values found statistically significant between the two groups were written in bold type and $p\leq0.05$ was considered significant.
WBC: white blood cell; NEU: neutrophil; LYM: lymphocyte; EOS: eosinophil; BAS: basophil; MONO: monocyte; RBC: red blood cell; HGB: hemoglobine; HCT: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet; MPV: mean platelet volume; PDW: platelet distribution width



	All	Normal Delivery	Cesarean
Lymphocytes	0,24	0,25	0,23
T Lymphocytes	0,67	0,63	0,71
B Lymphocytes	0,15	0,15	0,15
NK Cell	0,09	0,15	0,07
T_h Lymphocytes	0,48	0,42	0,49
T_c Lymphocytes	0,20	0,19	0,21

Figure 1. Percent distribution of lymphocyte subpopulation according to birth pattern

to be slightly higher in babies born by cesarean delivery. Accordingly, a statistically significant difference has been shown ($p<0.05$) (Table 3).

In addition, the graphical representation of the percent distribution of lymphocyte subpopulation by birth pattern is shown in detail in Figure 1.

DISCUSSION

In our study, there was no statistically significant difference between normal births and cesarean births in terms of total leukocyte, lymphocyte, eosinophil, basophil, neutrophil, and monocyte values in cord blood.

For the first time, the effect of the mode of delivery on the immune system of the newborns has been evaluated by Frazier et al. (4) They showed that the pattern of delivery changes the polymorphonuclear cell numbers and functions of the newborn. They suggested that this change might be due to physiological changes associated with stress during labor.

Chirico et al. (5) showed that leukocyte and neutrophil counts were higher in vaginally born babies than in babies born with cesarean section and this lasted for twelve hours.

Gessler and Dahinden (6) showed that neutrophils of normal-born babies have more respiratory burst products and surface adhesion molecules and therefore these have a higher functional capacity.

However, in the studies of Pittard et al. (7) and Minareci et al. (3), total leukocyte, lymphocyte, granulocyte, and monocyte values were not statistically different between the two groups. They suggested that this may be due to mitogen-induced lymphocyte proliferation responses and pokeweed mitogen-induced antibody-secreting cells are more numerous in cesarean-born babies than in babies born with normal delivery (7).

Hemogram parameters in the studies of Pittard et al. (7) and Minareci et al. (3) were not statistically significant in terms of birth pattern. Our results were also consistent with these two studies.

In our study, absolute numbers of (CD3+), (CD4+), and (CD8+) lymphocytes are not changed in both groups; there was a statistically significant increase in the percentages of (CD3+), (CD4+), and (CD8+) lymphocytes in the group born with cesarean section. The reason why the difference in absolute values is not observed can be that absolute lymphocyte counts can be affected by changes in lymphocyte and leukocyte counts in the hemogram, regardless of the birth pattern.

Pittard et al. (8) investigated the effect of labor pains on mononuclear cell subpopulations and found that the absolute numbers of CD3+ and CD4+ lymphocytes were significantly higher. These differences are explained by the increased neonatal levels of circulating catecholamines or cortisone in cord blood.

In our study, percent (%) changes in lymphocyte subgroups, consistent with the work of Pittard et al. (8), and increased lymphocyte responses in cesarean delivery may be associated with the absence of labor pain and may be due to increased neonatal levels of catecholamines or cortisone.

In our study, both definite and percentage values of CD16+/56+ NK cells were significantly higher in the group with normal birth than in the group with cesarean birth. We think that it may be the effect of labor pain or stress-related hormonal changes. In addition, we can say that anesthetics given to the mother also affect NK cell activity.

The results of our study are consistent with those of Frazier et al. and De Amici et al. (9-11). There were certain limitations in this study in which the effect of the delivery methods on lymphocyte subgroups was investigated. The small number of patients and examination of these cells only from the cord blood taken at birth provide only a perspective for the lymphocyte subpopulations on the immune system of the newborn. Accordingly, this study can only refer to the effect of delivery methods on lymphocyte subpopulations. With more detailed tests for both

mother and baby after delivery, more information about the condition of post-natal immune system cells can be obtained.

As a result, in this study, we evaluated the effects of delivery methods on the immunity of newborns; we found an increase in the percentages (%) of Th lymphocytes and Tc lymphocytes in the cesarean delivery and both percent (%) and absolute (#) values of NK cells in normal delivery. In this study, we tried to show that both the birth patterns might have effects on the immune system of the newborns. Future studies with larger groups are needed to enlighten this issue.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul Medeniyet University (2014/0075).

Informed Consent: Informed consent was received from all the patients and control group.

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