ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

The Effect of Labor Type on the Neonatal Immune System: Leukocyte Counts and Lymphocyte Subpopulations

Doğum Şeklinin Neonatal İmmün Sistem Üzerine Etkisi: Lökosit Sayıları ve Lenfosit Subpopülasyonları

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ABSTRACT Objective: To assess the effect of the route of delivery or the labor over the leukocyte counts and lymphocyte subpopulations on the neonates. **Material and Methods:** This prospective study was administered on the healthy neonates of 68 singleton pregnant women, delivered at 38 to 42 weeks of gestation in the Clinic of Gestation with High Risk, Zekai Tahir Burak Women Health, Education and Research Hospital. Peripheral blood leukocyte, monocyte, granulocyte, lymphocyte counts and lymphocyte subpopulations were investigated in the umblical cord blood samples of 68 normal pregnancies, delivered either vaginally (n=33) (Group I) or by elective cesarean section (n=35) (Group II). A laser based flow cytometer was used to analyze surface markers on the mononuclear cells. Statistical analysis was performed by using SPSS analysis package programme (microsoftware version 11.0). Student's t and Mann-Whitney U tests were used to compare continuous data and chi-square test and Fisher's exact tests were used to compare the ratios and the percentages of independent samples. **Results:** In this study, mean total leukocyte, lymphocyte, granulocyte and monocyte counts were observed as lower in the Group I, delivered by vaginal route. However, no statistically significant difference was found between two groups with regard to these parameters. Mean percentages of lymphocyte, granulocyte, monocyte were found as higher in Group II, delivered by cesarean section. There was no statistically significant difference between two groups, also. Absolute values of CD3+, CD4+, CD8+, CD16+, CD19+ lymphocyte subpopulations were observed as higher in the group delivered by cesarean section without former labor than those delivered vaginally. CD4+/CD8+ lymphocyte subpopulations ratio was recorded high in the group delivered vaginally. Again, there was no significant difference between two groups, with regard to lymphocyte subpopulation counts and ratios. **Conclusion:** These data suggest that route of delivery may have an effect on the number of total leukocytes, monocytes, granulocytes, lymphocytes and different subsets of lymphocytes. However, future studies with larger groups are needed to enlighten this issue.

Key Words: Immunity, maternally-acquired; delivery, obstetric; cesarean section

ÖZET Amaç: Çalışmamızın amacı, doğum şeklinin yenidoğan immün sisteminde lökosit, monosit, granülosit, lenfosit sayıları ve lenfosit subpopülasyonları üzerine etkisini araştırmaktır. **Gereç ve Yöntemler:** Bu prospektif çalışmada gebeliğinin 38-42 haftalarında Zekai Tahir Burak Kadın Sağlığı Eğitim ve Araştırma Hastanesi Yüksek Riskli Gebelik Departmanında sağlıklı doğum yapan 68 gebe kadın analiz edildi. 68 normal gebeliği olan hastada vajinal yolla doğum yapan (n=33)(Grup 1) veya elektif sezaryenle doğum yapan (n=35) (Grup 2) gebelerin umblikal kord kanı örneklemesinde periferal kan lökosit, monosit, granülosit, lenfosit sayıları ve lenfosit subpopülasyonları ile ve oranları araştırıldı. Lazer bazlı flowsitometri mononukleer hücrelerin yüzey belirteçlerinin analizi için kullanıldı. Çalışmada SPSS 11.0 paket programından faydalanılıp Student t test ve Mann-Whitney U testleri ile sürekli değişkenler karşılaştırılırken, ki-kare ve Fisher exact testleri ile bağımsız verilerin yüzde ve oranları ile ilgili istatistiksel analizler yapıldı. **Bulgular:** Bu çalışmada vajinal yolla doğum yapanlarda (Grup 1) ortalama total lökosit, lenfosit, granülosit ve monosit sayıları daha düşük gözlemlendi. Buna rağmen her iki grup arasında anlamlı istatistiksel bir fark izlenmedi. Sezaryen ile doğum yapanlarda (Grup 2) lenfosit, granülosit, monosit yüzdeleri daha yüksekti. Ancak iki grup arasında anlamlı istatistiksel fark bulunmadı. Lenfosit subpopülasyonunda CD3+,CD4+,CD8+,CD16+,CD19+ kesin değerlerinin sezaryen ile doğum yapanlarda daha yüksek olduğu gözlemlendi. CD4+/CD8+ lenfosit subpopülasyonunun oranı da normal vajinal yolla doğum yapan grupta daha yüksek saptandı. Bununla birlikte lenfosit subpopülasyonu sayı ve oranları açısından her iki grup arasında anlamlı fark yoktu. **Sonuç:** Bu çalışma gösteriyor ki; yenidoğan immün sistemi, total lökosit, monosit, granülosit, lenfosit sayıları ve lenfosit alt grupları doğum şeklinden etkilenmektedir. Buna rağmen bu düşünceyi aydınlatmak için daha geniş grupları kapsayan çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: İmmunite, maternal-kazanımlı; doğum, obstetrik; sezaryen ile doğum

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T lymphocytes are the mediators of cellular immunity and essential for induction of humoral immunity to most naturally encountered antigens. They circulate in blood where they make up 60% to 70% of peripheral lymphocytes. Each T cell is genetically programmed to recognize a specific cell-bound antigen by means of an antigen-spesific T cell receptor (TCR). TCRs are non-covalently linked to a cluster of polypeptide chains, referred to as the CD3 molecular complex. In addition to CD3 proteins, T cells express a variety of other non polymorphic function-associated molecules, including CD4 and CD8.[1]

B lymphocytes constitute 10% to 20% of the circulating peripheral lymphocyte population. This includes CD19, CD 20 and CD 21.[1]

Natural killer cells make up approximately 10% to 15% of the peripheral blood lymphocytes. Two cell surface molecules, CD16 and CD56, are widely utilized to identify natural killer cells.[1]

The total white blood cell count has been of little clinical use in the diagnosis of neonatal infection, because of wide variation in white blood cell counts; both in normal and sick infants.[2]

During the neonatal period when adaptive immune responses are still relatively immature, neutrophils, monocytes, and naturel killer cells which do not depend on prior exposure to foreign antigen to mount an immune response may play a vital role in host defense. As the cell-mediated immune response has great importance during this period, factors affecting this response were investigated extensively. Several studies have revealed that labor may have an effect on effector cells of the immune system; leukocytes, monocytes, lymphocytes and studies have also reported that patients delivered by cesarean section have higher lymphocyte proliferative responses.[3,4]

The aim of this study was to assess the effect of labor and route of delivery on the effector cells of the immune system and the lymphocyte subpopulations in the neonates.

MATERIAL AND METHODS

SUBJECTS

This prospective study was carried out on the healthy neonates of 68 singleton pregnant women, delivered at 38 to 42 weeks of gestation. These infants were discriminated in terms of the route of delivery; either by vaginally (Group I) (n=33) or by elective cesarean section without former labor (Group II) (n=35). Physical and laboratory-test evaluations were administered to all of the cases. Cases with pregnancy related obstetric complications and/or systemic diseases were not included in this study. Infants with a gestational age of <38 weeks were also excluded from the study. The indication for elective prelabor cesarean section was the only targeted population among the parturients of this study. Subjects with other cesarean section indications such as primigravid breech presentation or previous cesarean section delivery were not included in the study. The mode of maternal anesthesia used for each cesarean section without former labor was either epidural or general anesthesia. Informed consents were taken from all women who are included in this study and the study received approval from the Ethical Committee of the Institution.

Maternal age, gestational age, gravidity and parity were all recorded (Table 1). Mean maternal age of the subjects in Group I and II were 27.50 ± 4.20 years and 26.03 ± 4.61 years respectively. Two groups were comparable with regard to these parameters ($p < 0.05$).

TABLE 1: Gestational age, maternal age, gravidity and parity of the subjects.

	Group I (n=33)	Group II (n=35)	P
Gestational age (week)	39.75±1.03 (37-41)	39.20±1.10 (37– 41)	P=0.375
Maternal age (year)	27.50±4.20 (21-36)	26.03±4.61 (18-34)	P=0.428
Gravidity	2.03±1.01 (1-4)	2.27±1.29 (1-5)	P=0.565
Parity	0.80±0.78 (0-3)	0.94±1.06 (0-4)	P=0.723

Values are expressed as mean ±SD
Numbers in parentheses represent the range of the values.

SPECIMEN COLLECTION AND CELL ISOLATION

Lymphocyte subsets were analyzed by means of flow cytometry. Blood samples were collected in two tubes containing EDTA as anticoagulant, from umbilical venous blood from the fetal side of the placenta just after clamping the cord. First sample was transferred to the laboratory to analyse leukocyte counts by standard hematological methods by using a counter (coulter micro Diff II).

The second sample was transferred to the laboratory for the assessment of surface markers on the mononuclear cells by flow cytometry. Then the samples were hemolysed and fixed with lysing reagent (FACS™ lysing solution; Becton Dickinson, Mountain View, CA). They were then washed once and subjected to two-color flow cytometry in a FACScan™ with an auto-compensation system (Becton Dickinson, Mountain View, CA) to determine the percentage of each lymphocyte in total lymphocytes. The absolute numbers of these lymphocyte subsets in whole blood were calculated as the products of the percentages of each lymphocyte subset and the absolute lymphocyte number which were obtained with a Total Haematology Management System H-6000 (Techniton, Tarrytown, NY).

STATISTICS

Statistical analysis was performed by using SPSS analysis package programme (Microsoftware-Version 11.0). Student's t-test and Mann-Whitney U tests were used to compare continuous data and χ^2 test and Fisher's exact test were used to compare the ratios and the percentages of independent samples.

RESULTS

Each neonate in the study was clinically normal and appropriately grown for term gestation. There was no significant difference with regard to birth weight and length of the neonates (p> 0,05) between Group I and Group II (Table 2). In the cesarean section group, there were 12 (34%) female neonates, where 22 (66%) female neonates delivered by vaginal route. The groups were also comparable with regard to the gender of the neonates (p> 0.05).

TABLE 2: Birth weight, length and gender of the neonates

	Group I (n=33)	Group II (n=35)	P
Birth weight	3230±400 (2490-4302)	3175±406 (2423-3941)	P^a=0.688
Birth length	50.18±1.52 (49-54)	50.13±1.17 (48-53)	P^b=0.900
Gender female	22 (66 %)	12 (34 %)	P^b<0.5

Values are expressed as mean ±SD
Numbers in parentheses represent the range of the values, or percentages.
a: Student's t test
b: Fisher's Exact test

TABLE 3: Absolute counts of total leukocytes, lymphocyte, granulocyte, monocyte

	Group I (n=33)	Group II (n=35)	P
Total white blood cell count (per microliter)	11628±2015 (7310-15620)	13426±4545 (6340-21720)	P>0.05
Lymphocyte count (per microliter)	4113±980 (2100-6020)	4458±2220 (1080-9370)	P>0.05
Granulocyte count (per microliter)	6723±1558 (4220-10120)	7988±2674 (3540-12638)	P>0.05
Monocyte count (per microliter)	920±210 (610-1410)	1080±390 (403-2018)	P>0.05

Values are expressed as mean ±SD
Numbers in parentheses represent the range of the values

In this study, mean total white blood cells, lymphocytes, granulocytes and monocytes counts were observed as lower in the Group I, delivered by vaginal route. However, no statistically significant difference was found between two groups with regard to these parameters (Table 3).

CD4+/8+ lymphocyte subpopulations ratio was recorded high in the group delivered vaginally and absolute values of CD3+, CD4+, CD8+, CD16+, CD19+ lymphocyte subpopulations were observed to be higher in the group delivered by cesarean section than those delivered vaginally (Table 4). There was no significant difference between two groups, with regard to lymphocyte subpopulation counts and ratios (p>0.05).

DISCUSSION

Bacterial infections in the newborn still account for a considerable rate of morbidity and mortality. This

TABLE 4: Absolute values of lymphocyte subpopulations.

	Group I (n=33)	Group II (n=35)	p
CD3 + lymphocyte	2194±568 (1126-3308)	2504±1120 (495-4839)	P=0.313
CD4+ lymphocyte	1840±450 (939-2439)	1970±960 (570-4096)	P=0.615
CD8+ lymphocyte	810±295 (456-1440)	1085±615 (292-2793)	P=0.141
CD19+ lymphocyte	458±208 (145-940)	587±383 (77-1588)	P=0.277
CD16+ lymphocyte	418±165 (142-665)	538±368 (105-1376)	P=0.223

Values are expressed as mean ±SD
Numbers in parentheses represent the range of the values.

is partly due to the tendency of the newborn and especially the premature to serious infections by various microorganisms, especially Gram-negative microorganisms. Because the signs of these infections both local and general may be absent or minimal and hard to detect fatal septicemia may occur with ambiguous warnings.[2]

In our study, mean total leukocytes, lymphocytes, granulocytes and monocytes counts were observed as lower in the Group I, delivered by vaginal route. However, no statistically significant difference was found between two groups with regard to these parameters (Table 3). Mean percentages of lymphocyte, granulocyte, monocyte were found to be higher in Group II delivered by cesarean section. There was no statistically significant difference between two groups.

Investigations of in vitro neonatal lymphocyte functions have revealed that, patients delivered by cesarean section have greater mitogen-induced lymphocyte proliferative responses and greater numbers of pokeweed mitogen-induced antibody-secreting cells when compared to those delivered vaginally. Further, these increased lymphocyte responses on more in depth analyses were noted to be related to the absence of labor in the majority of cases, delivered by cesarean section. These findings were found to be suggestive for the labor that may have an effect on the effector cells of the immune system leukocytes, monocytes and lymphocytes.[3-7]

However, Fraizer et al.[8] reported that polymorphonuclear leukocytes were significantly higher in cord blood from infants delivered vaginally. In this study, it was found that infants delivered vaginally had lower counts of polymorphonuclear leukocytes in cord blood, but there were no statistically significant correlations between the two groups.

In our study, however, no statistically significant difference was observed between two groups, with regard to the number of CD3 and CD4 cells.

Pittard et al[6] investigated the effect of labor on mononuclear cell subsets and found the absolute number of CD3 and CD4 positive lymphocytes to be significantly higher (P< 0.02). These differences can be attributed to effect of increased neonatal levels of circulating catecholamines or cortisone, associated with labor or the T-cell subpopulation in cord blood.[3]

In our study, absolute values of CD3+, CD4+, CD8+, CD16+, CD19+ lymphocyte subpopulations were observed to be higher in the group delivered by cesarean section without former labor than those delivered by vaginal route (Table 4).

Thilaganathan et al[9] observed that, the number of leukocytes, polymorphonuclear leukocytes, monocytes and natural killer cell counts were higher in the group delivered vaginally. They also found that, there was no significant difference in the number of lymphocytes, CD3+, CD4+, CD8+, CD19+ cells and CD4+/ CD8+ ratio between two groups.[9,10]

In this study CD4+/CD8+ lymphocyte ratio was found low in the group delivered by cesarean section. This can be attributed to labor or stress related hormonal changes in the neonate's blood. There was no significant difference between two groups, with regard to lymphocyte subpopulation counts and ratios (p>0.05).

Fraizer J.P. et al[11] observed that, the method of delivery had an influence on the natural killer cytotoxicity (NKC) and on antibody-dependent cellular-cytotoxicity (ADCC). It wasreported that NKC and ADCC activity of neonates, delivered by cesarean section without labor, was lower than

NKC and ADCC of the neonates delivered vaginally.[11] Further experiments would delineate the cause of these variations, which probably include labor or stress related hormonal changes in the mother or the neonate

In conclusion these data suggest that, route of delivery may have an effect on the number of white blood cells and different subsets of lymphocytes, however, future studies with larger groups are needed to enlighten this issue.

REFERENCES

1. Kumar V, Cotran RS, Robbins S. Basic Pathology 6th ed. Philadelphia: WB Saunders Company; 1997. p.82-131.
2. Sevost'ianova Olu, Teplova SN, Cherdantseva GA, Medvedev BI, Mazurov AD, Chistiakova GN, et al. Characteristics of the population and subpopulation composition of peripheral blood lymphocytes in noncomplicated pregnancy based on flow cytofluorometry data. Zh Mikrobiol Epidemiol Immunobiol 2001;4:97-100.
3. Pittard WB 3rd, Schleich DM, Geddes IK, Sorensen RU. Newborn lymphocyte sub-populations: The influence of labor. Am J Obstet Gynecol 1989; 160:151-4.
4. Sendag F, Itil IM, Terek MC, Yilmaz H. The changes of circulating lymphocyte subpopulations in women with preterm labour: A case-control study. Aust N Z J Obstet Gynaecol 2002;42:358-61.
5. Blidaru I, Zugun F, Cianga C, Carasevici E. Maternal immunophenotypic profile in normal pregnancy and preterm birth. Rev Med Chir Soc Med Nat Iasi 2002;107:343-7.
6. Pittard WB 3rd, Miller KM, Sorensen RU. Perinatal influences on in vitro neonatal B lymphocyte differenciation in human neonates. Pediatr Res 1985;19:655-8.
7. Greenough A, Lagercrantz H, Pool J, Dahlin I. Plasma catecholamine levels in preterm infants. Effect of birth asphyxia and Apgar score. Acta Pediatr Scand 1987;76:54-9.
8. Fraizer JP, Cleary TG, Pickering LK, Kohl S, Ross PJ. Leukocyte function in healthy neonates following vaginal and ceserian section deliveries. J Pediatr 1982;101:269-72.
9. Thilaganathan B, Meher-Homji N, Nicolaides KH. Labor: an immunologically beneficial process for neonate. Am J Obstet Gynecol 1994;171:1271-2.
10. Kinoshita Y, Masuda K, Kobayashi Y. Adherence of cord blood neutrophils: effect of mode of delivery. J Pediatr 1991;118:115-7.
11. Fraizer JP, Kohl S, Pickering LK, Loo LS. The effect of route of delivery on neonatal naturel killer cytotoxicity and antibody-dependent cellular cytotoxicity to herpes simplex virus-infected cells .Pediatr Res 1982;16:558-60.