



Original Research

The Effects of Antenatal Steroid Treatment on Preterm Infants' Early Laboratory Analysis

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Abstract

Objectives: We assessed the effects of antenatal steroid treatment on preterm laboratory analysis conducted in the first 24 hours of life.

Methods: Medical records of inborn preterm infants whose gestational age was ≤ 32 weeks were retrospectively reviewed in this study. Preterm infants whose mothers received antenatal betamethasone treatment of either 12 mg or 24 mg and who did not were divided into two groups. Maternal and neonatal demographic characteristics, all preterm morbidities and mortality rates, early laboratory examinations were compared between the two groups.

Results: Medical records of 603 infants between 2008 and 2013 were retrospectively reviewed. Data from 515 infants were analyzed. Three hundred and four infants ($n=304$) were in the antenatal steroid treatment (AST) group and 211 infants were in the group that did not receive the treatment. The incidence of preeclampsia and oligohydramnios was significantly higher in the AST group. Intubation in the delivery room rates decreased in the AST group. APGAR scores at five minutes were significantly higher in the AST group. White blood counts (WBC) significantly decreased, whereas the platelet counts were higher in the AST group. Serum C-reactive protein (CRP) and Interleukin-6 (IL-6) levels did not differ between groups.

Conclusion: We did not demonstrate any relationship between inflammatory markers and antenatal steroid treatment in preterm infants.

Keywords: Antenatal steroids; interleukin-6; prematurity; thrombocyte; white blood count.

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Today, premature births cannot be prevented due to the widespread use of assisted reproductive techniques, resulting in increased frequency of multiple pregnancies and the increase in maternal age. With the continuation of premature births, the importance of antenatal steroid treatment (AST), which accelerates the maturation of the baby, is gradually increasing. Neonatal mortality, respiratory distress syndrome (RDS), intracranial hemorrhage (ICH),

necrotizing enterocolitis (NEC), need for mechanical ventilation and sepsis rates in the first 48 hours decrease with AST.^[1] The American Association of Obstetrics and Gynecology (ACOG) and Turkish Neonatal Society Respiratory Distress Syndrome and Surfactant Treatment Guideline 2018 Update recommends antenatal steroid administration to all pregnant women between 231/7 weeks and 346/7 weeks with the threat of preterm labor.^[2, 3] Accordingly, 12

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mg betamethasone (Celestone chronodose ampoule®) i.m. twice with an interval of 24 hours, or 6 mg dexamethasone (Dekort ampoule®) i.m. applied four times with an interval of 12 hours, are administered.

Antenatal steroids have become a part of standard care today; apart from its proven benefits, different physiological or clinical effects are also subject to research. Studies investigating the effects of AST on the laboratory findings of newborns in the literature are mostly associated with blood gas parameters, which are frequently used to determine the severity of respiratory morbidity.^[4] In an old animal study, AST has been shown to increase umbilical cord hemoglobin levels and mean corpuscular hemoglobin concentration (MCHC), but there is not enough clinical data yet.^[5] In a recent study, maternal betamethasone administration has been shown to increase CRP RNA in the liver of preterm lambs.^[6] There are conflicting results in the limited number of studies conducted on the effects of antenatal steroid treatment on interleukin levels and lymphocyte and leukocyte counts in preterm babies.^[7-10] We aimed to investigate the effects of AST on hemogram, blood gas parameters, CRP and IL-6 levels obtained in the first hours of life in a cohort of preterm babies with a relatively high number of patients.

Methods

The records of babies born in our hospital between 2008 and 2013, with a gestational week of ≥ 24 and ≤ 32 , and whose file information can be accessed, were analyzed retrospectively. Babies with major congenital anomalies, who were admitted after birth from an external center, and babies with a pH of < 7.00 in the neonatal blood gas were not included in this study. The patients were divided into two groups as babies who received AST (betamethasone single dose (12 mg) or two (24 mg) doses) and babies born without AST administration. In the perinatology clinic, steroid treatment was applied to all pregnant women with threatened preterm labor, in accordance with ACOG recommendations. Steroid treatment could not be applied in cases that applied to the emergency department with full cervical dilation and in cases where the pregnancy was terminated urgently because it threatens the life of the fetus or mother. As a unit policy, hemogram and blood gas parameters are obtained while vascular access is established as soon as the newborn is admitted to intensive care in all preterm babies. Again, as a unit policy, CRP and IL-6 levels are routinely examined at the postnatal 6th hour in order to exclude early neonatal sepsis. In both groups, the neonatal blood gas, hemogram, CRP and IL-6 parameters obtained in the first six postnatal hours were compared. Frequently followed-up morbidities and mortality of the preterm babies

were recorded. The diagnosis of intracranial hemorrhage was made according to the Papille classification, the NEC staging was made according to the Bell classification, and the diagnosis of bronchopulmonary dysplasia (BPD) according to the National Institute of Child Health and Human Development (NICHD) criteria. International Classification was used for the diagnosis and staging of Retinopathy of Prematurity (ROP). Before this study, the ethical approval of the Zekai Tahir Burak Maternity Teaching Hospital Education Planning Committee and Ethics Committee (No: 50/2019) were obtained.

Statistical Analysis

SPSS 19 (SPSS Inc., Chicago, IL) software package was used for data recording and statistical analysis. $P < 0.05$ value was considered statistically significant. X^2 and Fisher's exact tests were used to compare categorical variables. Parametric tests were used for the analysis of continuous variables with normal distribution, and nonparametric tests were used for non-normal distribution. Descriptive statistics with normal distribution were given as mean \pm SD. Descriptive statistics with non-normal distribution were given as median, IQR. Kruskal-Wallis analysis and, when necessary, Mann-Whitney U tests for paired comparisons were used to evaluate the significance. Spearman correlation test was used for correlation analysis.

Results

The data of 515 babies, whose laboratory markers and preterm morbidities were available in their file records, were analyzed. Three hundred four babies in the group receiving AST and 211 babies in the group not receiving antenatal steroid treatment were examined. Characteristics and frequency of neonatal morbidities are summarized in Table 1. The frequency of preeclampsia (25.3%-7%, $p < 0.01$) and oligohydramnios (19%-8%, $p < 0.01$) was significantly increased in the group receiving antenatal steroids compared to those who did not. The rate of intubation in the delivery room was significantly lower (31.2%-46.4%, $p = 0.01$) and the 5th minute APGAR scores (median 8 and 7, $p = 0.02$) were statistically significantly higher in the group receiving steroid therapy (Table 1). According to the laboratory evaluations, the WBC count was significantly lower (14.095 ± 10.309 and 20.311 ± 15.815 mm³, $p = 0.01$), and the platelet count was significantly higher (205622 ± 82.402 - 185.576 ± 76126 mm³, $p = 0.023$) in the group who received AST (Table 2). Median IL-6 (23.1 and 23.5 pg/ml $p = 0.92$) and CRP levels (0.35 and 0.37 mg/L, $p = 0.84$) were similar in both groups (Table 2). No significant correlation was found between preeclampsia and WBC and IL-6 ($r = -0.49$, $p = 0.34$ and $r = -0.44$, $p = 0.49$, respectively). No significant correlation

Table 1. Basic characteristics, comparison of neonatal morbidity and mortality

	Antenatal steroid administered	Antenatal steroid not administered	p
	(n=304)	(n=211)	
BW, g mean±SD	961±186	946±201	0.31
GA, week mean±SD	27.6 ±1.9	27.1±2.1	0.09
APGAR 5.min median	8 (2-9)	7 (1-9)	0.02
Gender, M, n (%)	154 (50.6)	111 (52.6)	0.37
SGA, n (%)	44 (14.4)	26 (12.3)	0.25
Preeclampsia, n (%)	77 (25.3)	15 (7)	<0.01
IUGR, n (%)	37 (12.1)	19 (9)	0.16
Chorioamnionitis, n (%)	8 (2.6)	5 (2.3)	0.54
Oligohydramnios, n (%)	58 (19)	17 (8)	<0.01
PPROM, n (%)	57 (18.7)	32 (15.1)	0.17
DR intubation, n (%)	95 (31.2)	98 (46.4)	0.01
RDS, n (%)	207 (68)	147 (69.6)	0.38
Severe ICH, n (%)	32 (10.5)	24 (11.3)	0.50
hsPDA, n (%)	129 (42.4)	80 (37.9)	0.40
Early Sepsis, n (%)	79 (25.9)	53 (25.1)	0.32
Stage ≥2 NEC, n (%)	49 (16)	30 (14.2)	0.49
ROP, n (%)	149 (49)	74 (35)	0.36
Mortality, n (%)	78 (24.3)	101 (47.8)	<0.01

BW: Birth weight; GA: Gestational age; SGA: Small for gestational age; IUGR: Intrauterine growth restriction; PPRM: Prolonged premature rupture of membranes; DR: Delivery room; RDS: Respiratory Distress Syndrome; ICH: Intracranial hemorrhage; hsPDA: Hemodynamically significant PDA; NEC: Necrotizing Enterocolitis; ROP: Retinopathy of Prematurity.

Table 2. Comparison of laboratory values between groups

	Antenatal Steroid Use		p
	Yes (n=211)	No (n=304)	
pH, (mean±SD)	7.20±0.11	7.22±0.11	0.47
pCO ₂ , mmHg (mean±SD)	59.7±28.1	54.3±14.7	0.09
HCO ₃ , mmol/L (mean±SD)	22.1±6.2	21.4±3.8	0.39
BE, mmol/L (mean±SD)	-6.7±4.7	-7±6.9	0.37
SpO ₂ , % (mean±SD)	90.6±10	92.3±5	0.19
Hb, g/dL (mean±SD)	15.7±3	16.8±9.9	0.26
Hematocrit, % (mean±SD)	49.9±10	50 ±8.4	0.95
Platelet Count, /mm ³ (mean±SD)	185576±76126	205622±82402	0.03
White blood cell count, /mm ³ (mean±SD)	20311±15815	14095±10309	0.01
CRP, mg/L Median (IQR)	0.35 (0.16-1.21)	0.37 (0.14-1)	0.84
Interleukin -6, pg/mL Median (IQR)	23.1 (10-138)	23.5 (9-75)	0.92

was found between oligohydramnios and WBC and IL-6 ($r=0.32$, $p=0.56$ and $r=-0.6$, $p=0.33$).

Discussion

Antenatal steroids have been known and used for nearly 30 years to protect preterm infants from respiratory problems. Many published randomized controlled studies have demonstrated the beneficial effects of antenatal steroids on the respiratory, nervous system and gastrointestinal system.^[1-4] Antenatal steroids have different effects on many organs and tissues. The immune system is an important target of antenatal steroids. Although its general effect on the immune system is immunosuppressive, positive or negative effects on different immune system responses have been shown.^[11, 12] Although the effects of exogenous steroid administration on the developing fetal immune system are not known in detail, there are clinical studies and animal studies with very conflicting and limited data in the literature. There are studies showing that antenatal steroids cause a decrease in lymphocyte count and increase in neutrophil and total leukocyte count in infants.^[7, 9, 13] In our study, contrary to these findings, the total leukocyte count decreased in the group which was exposed to steroid, but the effects on leukocyte subgroups could not be evaluated due to technical impossibilities.

In a study conducted by Kumar et al.,^[10] the findings showed that antenatal steroids did not alter the cytokine production capacity of leukocytes in cord blood. Similar to these findings, there was no significant effect on CRP and IL-6 values in our patient group. In a study, it was shown that antenatal steroids cause leukocytosis in the mother but also cause lymphopenia by increasing lymphocyte apoptosis.^[14] Maternal data were not included in our study because there was no fixed time to obtain maternal hemogram data and some data were taken before AST. The inadequate presentation of maternal data constitutes one of the important limitations of this study.

In a comprehensive study in which 1216 babies were included, 21 pro-inflammatory cytokines were studied and data indicating that steroids had both anti- and pro-inflammatory effects were obtained.^[15] According to the results of this study, it was found that CRP and IL-1 β were low in the first week, IL-8 was high on the 21st day, TNF- β was high on the 28th day, and there was no difference in IL-6.^[15] In a study involving preterms who were administered full dose antenatal steroids, with a birth weight of less than 1500 grams, IL-6 levels in cord blood were low, unlike our study, and this effect was concluded to be beneficial for preterm births.^[8] IL-6 levels were measured on the 15th hour, 1st day, 2nd day and 7th day after i.m. betamethasone in preterm lambs, and

they found that the levels measured at the 15th hour were significantly low.^[16] Because of the retrospective design of our study, only one value obtained in the first 24 hours was examined, and since markers, such as CRP, IL-6 were examined in the following postnatal days and hours not routinely but as needed, the next values were not included in this study. The duration of the low level of WBC shown in this study and how this effect is reflected in the clinical findings is unknown since this value is not controlled periodically and this study has a retrospective design. The absence of consecutive values makes it difficult to interpret the clinical significance of low WBC.

Intrauterine inflammation is one of the major causes of preterm births and causes immune activation and cytokine production.^[13, 14] In our study, the frequency of morbidities, such as PPROM, chorioamnionitis, which were predicted to affect the laboratory values, was similar between the groups. Again, conditions, such as hypoxia and asphyxia, that may affect these values were excluded from the analysis. Because it is a retrospective analysis of patients hospitalized for a certain time period, the incidence of pre-eclampsia and oligohydramnios increased in the group receiving incidentally steroid therapy. However, the effects of these morbidities on laboratory values were examined by correlation studies and no significant relationship was found between them.

According to the literature, antenatal steroid administration decreases the frequency of moderate/severe RDS.^[1-3] However, in our study, no difference was shown between the groups in RDS frequency and surfactant requirement. This finding may be due to the retrospective design of our study, applications that may show differences in five years of data acquisition period, and during which primary caregivers might change. Since it is a retrospective analysis, unlike other morbidities, standard criteria were not used for the diagnosis of RDS and the decision of surfactant administration. In addition, in a study evaluating the effects of AST on RDS according to the weeks of gestation, RDS was not found to show the difference in patients between 25-28 weeks of gestation in the group receiving and not receiving corticosteroids, while RDS was significantly lower in the steroid receiving group in patients born older than 29 weeks.^[17, 18] Less positive effects of steroids on RDS in early gestational weeks is explained in literature with different responses of fetuses to drugs, with the increase in steroid receptors in type 2 alveolar cells with the week of gestation, with the tubular structure of the lung and less alveolar structure, resulting in inadequate response to steroids.^[5, 6] Since the mean week of gestation of babies included in our study was <29 weeks, RDS frequency may not be affected based on the above hypothesis. The primary aim

of this study was not to investigate the effects of steroid therapy on neonatal morbidity. Since we aimed to investigate the effects on laboratory values, we think that the equal frequency of RDS strengthens the results obtained for the primary purpose. Antenatal steroid treatment may reduce mortality by reducing respiratory morbidity. In our study, in accordance with the literature data, antenatal steroid therapy decreased mortality statistically significantly.^[3] Although there was no decrease in the frequency of RDS, the high 5th minute APGAR scores of the babies who received steroid treatment and the low rate of intubation in the delivery room suggest that they experienced a better transition period and therefore, the mortality was less.

The most important limitation of our study is its retrospective design. As a result, the effects of AST on inflammatory biomarkers could not be revealed in our study. Clarifying the relationship of the preterm immune system with perinatal factors may allow for lifelong immunological improvements in neonatal care. There is still a need for strong prospective studies on this subject.

Disclosures

Ethics Committee Approval: Zekai Tahir Burak Maternity Teaching Hospital (No: 50/2019).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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References

1. Roberts D, Brown J, Medley N, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev* 2017;3:CD004454.
2. The American College of Obstetricians and Gynecologists (ACOG). Committee Opinion on Antenatal Steroids. Available at: www.acog.org/More-Info/AntenatalCorticosteroids. Accessed Oct 26, 2020.
3. Özkan H, Erdevi Ö, Kutman HGK. Turkish Neonatal Society guideline on the management of respiratory distress syndrome and surfactant treatment. *Turk Pediatri Ars* 2018;53:S45–54.
4. Zhang H, Liu J, Liu T, Wang Y, Dai W. Antenatal maternal medication administration in preventing respiratory distress syndrome of premature infants: A network meta-analysis. *Clin Respir J* 2018;12:2480–90.
5. Truog WE, Kessler DL, Murphy J, Standaert TA, Woodrum DE, Hodson WA. Antenatal glucocorticoid administration: effects on oxygen-hemoglobin affinity and hemoglobin levels in experimental hyaline membrane disease. *Gynecol Obstet Invest* 1983;15:251–7.

6. Visconti K, Senthamarai Kannan P, Kemp MW, Saito M, Kramer BW, Newnham JP, et al. Extremely preterm fetal sheep lung responses to antenatal steroids and inflammation. *Am J Obstet Gynecol* 2018;218:349.e1–349.e10.
7. Barak M, Cohen A, Herschkowitz S. Total leukocyte and neutrophil count changes associated with antenatal betamethasone administration in premature infants. *Acta Paediatr* 1992;81:760–3.
8. Caldas JP, Vilela MM, Braghini CA, Mazzola TN, Marba ST. Antenatal maternal corticosteroid administration and markers of oxidative stress and inflammation in umbilical cord blood from very low birth weight preterm newborn infants. *J Pediatr (Rio J)* 2012;88:61–6.
9. Correa-Rocha R, Pérez A, Lorente R, Ferrando-Martínez S, Leal M, Gurbindo D, et al. Preterm neonates show marked leukopenia and lymphopenia that are associated with increased regulatory T-cell values and diminished IL-7. *Pediatr Res* 2012;71:590–7.
10. Kumar P, Venners SA, Fu L, Pearson C, Ortiz K, Wang X. Association of antenatal steroid use with cord blood immune biomarkers in preterm births. *Early Hum Dev* 2011;87:559–64.
11. Agakidis C, Sarafidis K, Tzimouli V, Agakidou E, Taparkou A, Kanakoudi-Tsakalidou F, et al. Antenatal betamethasone does not influence lymphocyte apoptosis in preterm neonates. *Am J Perinatol* 2009;26:485–90.
12. Kavelaars A, van der Pompe G, Bakker JM, van Hasselt PM, Cats B, Visser GH, et al. Altered immune function in human newborns after prenatal administration of betamethasone: enhanced natural killer cell activity and decreased T cell proliferation in cord blood. *Pediatr Res* 1999;45:306–12.
13. Fuenfer MM, Herson VC, Raye JR, Woronick CL, Eisenfeld L, Ingardia CJ, et al. The effect of betamethasone on neonatal neutrophil chemotaxis. *Pediatr Res* 1987;22:150–3.
14. Romejko-Wolniewicz E, Oleszczuk L, Zaręba-Szczudlik J, Czajkowski K. Dosage regimen of antenatal steroids prior to preterm delivery and effects on maternal and neonatal outcomes. *J Matern Fetal Neonatal Med* 2013;26:237–41.
15. Faden M, Holm M, Allred E, Fichorova R, Dammann O, Leviton A; ELGAN Study Investigators. Antenatal glucocorticoids and neonatal inflammation-associated proteins. *Cytokine* 2016;88:199–208.
16. Kramer BW, Ikegami M, Moss TJ, Nitsos I, Newnham JP, Jobe AH. Antenatal betamethasone changes cord blood monocyte responses to endotoxin in preterm lambs. *Pediatr Res* 2004;55:764–8.
17. Melville JM, Moss TJ. The immune consequences of preterm birth. *Front Neurosci* 2013;7:79.
18. Ballard PL, Ballard RA. Cytoplasmic receptor for glucocorticoids in lung of the human fetus and neonate. *J Clin Invest* 1974;53:477–86.