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Effect of maternal cervical bacterial colonization on neonatal outcome in high-risk pregnancies: results from a tertiary maternity center in Turkey

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Summary

Purpose: To evaluate and compare the morbidity and mortality of neonates born to pregnant women with positive and negative cervical cultures. *Materials and Methods:* The demographic and clinical features of mothers included in this study, along with details of the microorganisms isolated on maternal cervical cultures and the number of days between a positive cervical culture and delivery were recorded. Neonates were stratified into two groups based on cervical culture results of their mothers - Group 1, positive cervical culture; Group 2, negative cervical culture. *Results:* A total of 216 women who delivered 242 infants were included in the study. Group 1 consisted of 90 neonates while Group 2 had 152 newborns. The difference between the groups with demographic characteristics was statistically insignificant. Mean levels of the acute phase reactants, CRP, and IL-6, obtained six hours after delivery were significantly higher in Group 1 compared to Group 2 ($p < 0.05$ for C-reactive protein (CRP) and $p < 0.001$ for IL-6). Although there was no difference between groups in terms of duration of respiratory support, mean duration of hospitalization, as well as mortality rate were significantly higher in Group 1 ($p < 0.001$, $p < 0.05$, respectively). *Conclusions:* Women diagnosed with a high-risk pregnancy should be treated with antibiotics immediately after a positive cervical culture result, and delivery should be delayed until the success of antibiotic treatment can be evaluated. Early initiation of maternal antibiotic therapy is associated with shorter durations of hospital stay for newborns. Close follow-up of mothers with high-risk pregnancies and extension of treatment duration are critical for determining prognosis in newborn infants.

Key words: Cervical bacterial colonization; Maternal antibiotic therapy; Neonatal outcome.

Introduction

Cervical bacterial colonization in pregnancy is a predisposing factor for maternal serious infections such as vaginitis, cervicitis, intra-amniotic infection, endometritis, or septicemia [1]. Fetus is at greater risk of cervical bacterial colonization and short cervix related morbidity and mortality than the mother [2]. Fetal infections may appear as early neonatal infections such as pneumonia, meningitis, and sepsis and are associated with a serious increase in mortality and morbidity in preterm neonates [3]. Preterm neonates who are developing early infections, commonly have subtle and non-specific clinical symptoms. Increasing use of antenatal and intrapartum antibiotics for the prevention of neonatal infection may result in false negative cultures of blood and cerebrospinal fluid, making the diagnosis of sepsis difficult [4]. Neonatal infections should be diagnosed as soon as possible by laboratory tests rather than cultures that result in longer period of time. Cervical culture positivity may have an effect on neonatal morbidity and mortality. When the duration between culture positivity and delivery increases and antibiotic therapy prolongs, neonatal morbidity may be decreased or affected [5].

This retrospective study was aimed to evaluate and compare the morbidity and mortality of the neonates of cervical culture positive and negative pregnant women and to determine the predictive value of cervical cultures for the prognosis of newborns.

Materials and Methods

Study patients

This retrospective study included 216 pregnant women who were admitted to the high-risk pregnancy service of the present tertiary maternity teaching hospital from June 2010 to February 2011 with symptoms of preterm delivery, early membrane rupture or vaginal discharge. This retrospective study was undertaken at Zekai Tahir Burak Maternity Teaching Hospital with the approval of the local ethics committee.

The demographic and clinical features of mothers included in this study, along with details of the microorganisms isolated on maternal cervical cultures, and the number of days between a pos-

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itive cervical culture and delivery were recorded. The cervical cultures of those women were obtained during hospitalization. Neonates were stratified into two groups based on cervical culture results of their mothers - Group 1, positive cervical culture; Group 2, negative cervical culture. If the culture was positive antibiotic therapy was begun.

With the multipregnancies a total of 242 newborns (22 twin, two triplet pregnancies) delivered by those mothers. The demographic features, laboratory evaluation for sepsis, duration of respiratory support, duration of hospitalization, sepsis and prematurity related complications, like respiratory distress syndrome (RDS), patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), intraventricular hemorrhage (IVH) ≥ grade III, and mortality were noted.

Suggested diagnostic criteria for sepsis in neonates (two or more of the following clinical features) were used to identify patients for sepsis evaluations [6]: (1) respiratory compromise includes following tachypnea, apnea, increased ventilatory support, or desaturation; (2) cardiovascular compromise, including bradycardia, pallor, decreased perfusion, or hypotension; (3) metabolic changes including hypothermia, hyper- thermia, feeding intolerance, glucose instability, or metabolic acidosis; or (4) neurologic changes consisting in lethargy, hypotonia, or decreased activity. In addition to laboratory results showing elevated levels of C-reactive protein (CRP) or interleukin-6 (IL-6). Patients with culture positivity were accepted as proven sepsis [6]. Patients diagnosed within the first 72 hours of life were considered to have early onset neonatal sepsis (EOS) [7]. RDS diagnosis made by typical clinical (grunting, cyanosis, tacypnea), and radiologic finding [8]. NEC was defined according to modified Bell's criteria (≥ Grade 2), and PDA was defined as clinical diagnosis plus treatment with ibuprofen, surgical ligation, or both [9, 10]. BPD was defined as requirement for oxygen at 36 weeks' corrected gestational age or at discharge from the participating unit [11]. ROP was diagnosed according to the international classification of retinopathy of prematurity [12]. Diagnosis and severity of IVH were based on the criteria of Papile [13]. Clinical chorioamniotis diagnosis was made by maternal fever (>38°C), leukocytosis (>15,000/mm^3), CRP positivity, vaginal discharge, and abdominal tenderness [14].

Laboratory analyses

Routine venous blood sampling was performed within the first 24 hours after delivery. Blood for complete blood counts was obtained either by venipuncture, by arterial puncture or through a central catheter. Complete blood counts determinations were performed using a twice-daily calibrated automated hemocytometer. Serum concentrations of CRP were measured by a Tinaquant CRP high sensitive immune turbidimetric assay on an analyzer according to manufacturer instructions. Plasma levels of IL-6 were determined by IL-6 solid phase, enzyme labeled, chemiluminescent sequential immunometric assay on an analyzer, as per manufacturer instructions.

Blood cultures

Blood cultures were performed in newborns when neonatal sepsis was suspected in first 72 hours. Blood culture depended on clinical condition and increase in acute phase reactants (IL-6 and CRP), not taken from healthy controls. A microbial detection system was used to detect positive blood cultures.

Cervical culture

Vaginal discharge was collected from the posterior vaginal fornix with a sterilized cotton wool swab. Immediately after collection, the swab specimen was suspended and transported to the laboratory. Collected samples were immediately inoculated on 5% sheep blood agar and eosin-methylene blue agar plates. After 24-48 hours of aerobic incubation at 37°C, the plates were interpreted. Identification and antimicrobial susceptibility testing of microorganisms was performed according to the Clinical and Laboratory Standards Institute Guidelines with conventional microbiological methods and confirmed by an automated microbiology system.

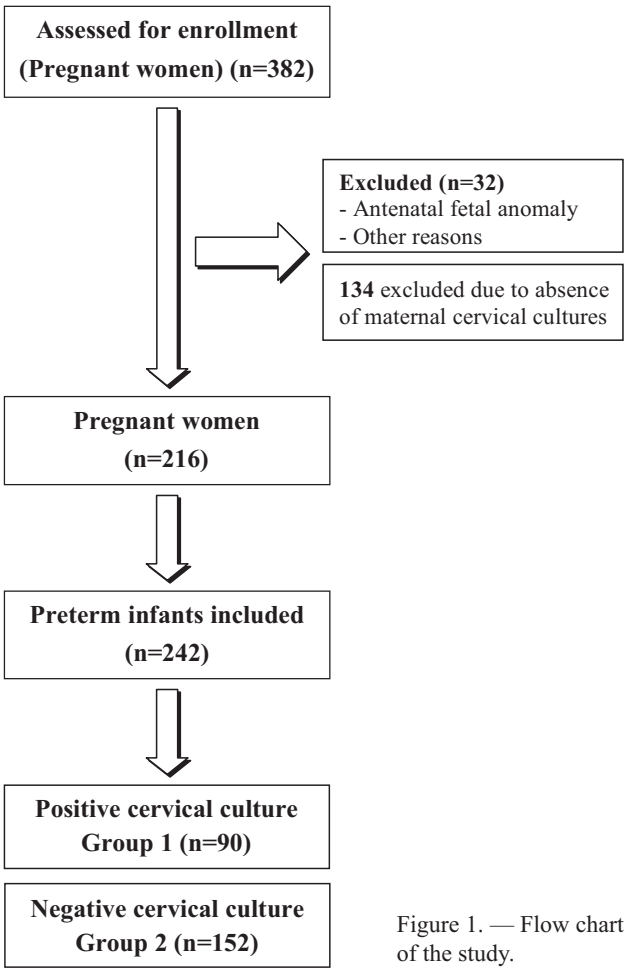

Figure 1. — Flow chart of the study.

Statistical analysis

Statistical analyses were performed using the SPSS for windows (ver. 17.0) statistical package. *Chi-square test* was used to compare categorical variables between groups. Difference between two groups was examined by independent samples *t*-test for normally distributed variables and *Mann Whitney U* test for non-normally distributed variables. Correlation made by *Spearman test*. A *p*-value <0.05 was considered statistically significant.

Results

Of all the medical records reviewed, the records of 216 pregnant women fulfilled the criteria necessary for inclusion in the final analysis (Figure 1). Group 1 (positive cervical culture) consisted of 90 neonates while the remaining

Table 1. — *Demographic characteristics and laboratory parameters of patients in Groups 1 and 2.*

Parameters	Group 1 (n=90)	Group 2 (n=152)	*p*-value
Maternal age (years)[a]	27.3 ± 5.2	27.5 ± 5.8	NS
Gestational age (weeks)[a]	30.6 ± 3.7	31.1 ± 3.3	NS
Birthweight (g)[a]	1595 ± 637	1653 ± 516	NS
Gender (female/male)[b]	38/52 (0.73)	66/86 (0.76)	NS
Mode of delivery (vaginal delivery/caesarean)[b]	34/56 (0.6)	55/97 (0.56)	NS
Time from cervical culture taken to delivery, days[c]	3 (2-14)	3 (2-12)	NS
PROM[b]	32 (35.6)	48 (31.6)	NS
WBC on first day (/µl)[a]	16450 ± 15874	16491 ± 12713	NS
CRP on first day (mg/l)[a]	17.4 ± 11.2	13.9 ± 6.7	<0.05
IL-6 on first day (pg/ml)[a]	169.7 ± 59.6	65.1 ± 45.5	<0.001
EOS[b]	25 (27.7)	38 (25)	NS
Proven EOS[b]	11 (12.2)	15 (9.8)	NS
RDS[b]	31 (34.4)	46 (30.2)	NS
PDA[b]	10 (11.1)	14 (9.2)	NS
NEC[b]	7 (7.7)	15 (9.8)	NS
BPD[b]	5 (5.5)	7 (4.6)	NS
ROP[b]	8 (8.8)	9 (5.9)	NS
IVH (Grade III-IV)[b]	6 (6.6)	7 (4.6)	NS
Mechanical ventilation (days)[a]	1.3 ± 2.3	1.1 ± 2.9	NS
O_2 transport time (days)[a]	5.5 ± 9.7	6.1 ± 13.2	NS
Duration of hospitalization (days)[a]	23.4 ± 16.5	16.5 ± 13.7	<0.001
Mortality[b]	20 (22.2)	13 (8.6)	<0.05

[a] Values are given as mean ± standard deviation,
[b] Values are given as percentage, [c] Values are given median (min-max)
PROM: premature rupture of membranes; WBC: white blood cells;
CRP: C-reactive protein; IL-6: interleukin-6; EOS: early onset neonatal sepsis;
RDS: respiratory distress syndrome; PDA: patent ductus arteriosus;
NEC: necrotizing enterocolitis; BPD; bronchopulmonary dysplasia;
ROP: retinopathy of prematurity; IVH: intraventricular hemorrhage;
NS: not significant.

152 preterm infants made up Group 2 (negative cervical culture).

The demographic, maternal, and clinical characteristics as well as the laboratory findings of the study population are summarized in Table 1. There was no statistically difference between groups in terms of maternal age, gestational age, birthweight, gender, and mode of delivery. Although the frequency of premature rupture of membranes (PROM) was higher in Group 1, the difference was not statistically significant ($p > 0.05$). Cervical culture were taken a median of five days [2-14] prior to delivery. The parameters of complete blood count as hemoglobin, white blood cell, and platelets were not different between groups, but the values of acute phase reactants CRP and IL-6 were higher in Group 1, and the difference was statistically significant (for CRP $p < 0.05$ and for IL-6 $p < 0.001$, Table 1).

Of the 25 (27.7%) newborns in Group 1 who developed EOS, only 11 (12.2%) had positive blood cultures. In Group 2, 38 (25%) of the newborns were diagnosed with EOS, 15 (9.8%) of which had a positive blood culture. The difference between groups in this regard was statistically insignificant ($p > 0.05$).

Table 2. — *The list of microorganism isolated from cervical cultures.*

Microorganism	n (%)
E.coli	51 (56.6)
K. pneumonia	12 (13.3)
S. agalactiae	7 (7.7)
S. aureus	7 (7.7)
E. aerogenes	6 (6.6)
Enterococcus spp	4 (4.4)
P. aeruginosa	2 (2.2)
Serratia fonticola	1 (1.1)
Total	90 (100)

Prematurity associated complications were also evaluated. Despite RDS, PDA, BPD, ROP, and IVH (Grade III-IV) were observed more commonly in Group 1 and NEC in Group 2, the differences were not statistically significant between groups ($p > 0.05$).

Duration of respiratory support was similar but duration of hospitalization and mortality ratio were statistically higher in Group 1; 23.4 ± 16.5 vs 16.5 ± 13.7 days and 20 (22.2%) vs 13 (8.6%), respectively ($p < 0.001$, $p < 0.05$, respectively, Table 1).

Ten of the patients who had positive cervical culture showed histological signs of chorioamnionitis. The subgroup analysis of the patients with positive cervical culture with choriomnionitis or without chorioamnionitis revealed that the complications as BPD, ROP, NEC, and duration of hospitalization were higher in chorioamnionitis cases ($p < 0.05$).

The microorganisms that were isolated from cervical culture of mother were *Escherichia coli* (n. 51, 56.6%), *Klebsiella pneumonia* (n. 12, 13.3%), *Staphylococcus aureus* (n. 7, 7.7%), *Streptococcus agalactiae* (n. 7, 7.7%), *Enterobacter aerogenes* (n. 6, 6.6%), and the others *Enterococcus spp, Pseudomonas aeruginosa* and *Serratia fonticola* (Table 2).

In five of the infants in Group 1, the bacteria detected in blood cultures were the same pathogens which were isolated in the respective maternal cervical cultures, namely *Escherichia coli in* three infants, *Klebsiella pneumonia* in two infants, and *Streptococcus agalactiae* in one infant. In the remaining five infants with a positive blood culture, the microorganism isolated was different from that observed in the respective maternal cervical culture.

The mean duration of maternal antibiotic therapy up to the time of delivery was 7.35 ± 5.2 days. The most frequently preferred antibiotics for mothers with positive cervical cultures were ampicillin-sulbactam (85%), clarithromycin (8.5%), and cefamezin (6.5%).

The duration of the cervical cultures obtained before the delivery were noted. The authors noticed that as the period between the day of positive cervical culture and the delivery shortened, the hospitalization duration was prolonged ($p = 0.017$, r = - 0.285).

Discussion

The lower genital tract is a source of ascending infections in pregnancy and in most cases with positive amniotic fluid cultures, the same organisms are recovered from vaginal swabs. Preterm delivery was shown to have a relationship with bacterial vaginosis [15-18]. McDonald *et al*. pointed out that the women with bacterial vaginosis have increased risk of PROM and preterm labor [19]. The causative microorganisms shown in cervical colonization were different in different countries, *Group B streptoccus* is the predominant microorganism in western countries whereas *E. Coli* is more prevalent in others. Lajos *et al.* evaluated 212 pregnant women with preterm labor or PROM and reported the prevalence of endocervical colonization as 14.2% [20]. *Group B streptococcus* was the most common organism and endocervical colonization was associated with a higher incidence of EOS and neonatal mortality compared with negative cultures. In the present report, *E.coli* was the most common organism of cervical colonization with 56.6%; the others were *Klebsiella pneumonia* (13.3%), *S. aureus* (7.7%), *S. agalactiae* (7.7%), and *E. aerogenes* (6.6%).

In the present study, sepsis was more prevalent in cervical culture positive group but this was not statistically significant. The remarkable feature was as the period between the day of positive cervical culture taken and the delivery shortened, the hospitalization duration of the neonate was prolonged ($p = 0.017$, r = - 0.285). Ovalle *et al.* reported that delivery in culture positivity and high-risk pregnancy should be as soon as possible [21]. On the other hand, King *et al.* showed that waiting for antibiotic therapy for mother and infant would be better than earlier delivery [5]. The present authors think that antibiotic therapy was begun to the mothers immediately when positive cervical cultures had been detected and as the period of antibiotic therapy prolonged and the risk of neonatal complications decreased.

The low rates of postpartum blood culture growths in neonates from Group 1, who were born to mothers with known cervical bacterial colonization, could be attributable to maternal antibiotic use and low growth rates of the causative microorganisms. Therefore neonates born from culture positively mothers may have more morbities and mortality than other infants.

Chorioamnionitis, a common complication of pregnancy, is not only associated with adverse maternal outcomes, such as postpartum infections and sepsis, but has also been implicated in the development of severe fetal and neonatal complications, including still birth, premature birth, neonatal sepsis, and BPD [22]. As would be expected, the presence of chorioamnionitis in the present study population was associated with a higher rate of fetal complications such as BPD, ROP, and NEC, as well as with longer durations of hospital stay.

Since the mothers that cervical cultures taken were in high-risk group of pregnants, the associated risk factors other than cervical colonization might lead to premature birth. To make a better comparison, the pregnant women who had no known medical risk factors for preterm delivery should be studied and this was the limitation of the present study. Further prospective studies will shed light on this subject.

Fichorova *et al.* reported that their data clearly demonstrated that maternal microorganisms associated with systemic inflammatory patterns detectable after birth placental colonization with vaginal microorganisms can induce a systemic inflammatory response in the fetus and newborn [23]. As a result of this important finding, independently from microorganism isolated from maternal cervix, neonates are affected from intrauterine inflammation. Although pregnancy is widely considered to be state of "relative immune compromise", several studies have reported on the contrary. Beigi *et al.* managed to demonstrate up to to two-fold elevations in levels of endocervical cytokines in pregnant women compared to non-pregnant ones [24). They suggested that the systemic inflammatory response associated with pregnancy itself, as well as with cervical colonization leads to elevations in levels of proinflammatory cytokines which have a negative impact on neonatal morbidity and mortality. In the present study, the authors observed that babies born to mothers in Group 1 had higher baseline (within first six hours) levels of the acute phase reactants CRP and IL-6 compared to those in Group 2 ($p < 0.05$ and $p < 0.001$, respectively). Furthermore, the mean duration of hospital stay and mortality rate in Group 1 were significant lower than those observed in Group 2 ($p < 0.001$, $p < 0.05$, respectively). These findings could be attributed to maternal systemic inflammation and to the "cytokine storm" associated with pregnancy.

In the present study, the authors observed a higher albeit insignificant, rate of sepsis in infants born to mothers with positive cervical cultures compared to culture-negative mothers. The authors recommend that all mothers with high-risk pregnancies be screened with cervical cultures, and that antibiotic treatment be initiated immediately after a positive culture result. Delivery should be delayed until the success of antibiotic treatment can be evaluated. Early initiation of maternal antibiotic therapy is associated with shorter durations of hospital stay for newborns. Close follow-up of mothers with high-risk pregnancies and extension of disease duration is critical for determining prognosis in newborn infants.

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