



ARAŞTIRMA / RESEARCH

Evaluation of simultaneous blood and cerebrospinal fluid culture findings in neonatal meningitis

Yenidoğan menenjitinde eşzamanlı kan ve beyin omurilik sıvısı kültürü bulgularının değerlendirilmesi

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Abstract

Purpose: The aim of our present study was to investigate the simultaneous findings of both blood and cerebrospinal fluid cultures and to determine the etiological pathogens responsible for neonatal sepsis and meningitis in our neonatal intensive care unit.

Materials and Methods: This study was carried out in our newborn department from November 2013 to October 2015. During this period 732 neonates with signs and symptoms of neonatal sepsis were included in our study. Sepsis and meningitis work up were performed and simultaneous blood and cerebrospinal fluid cultures were taken from eligible patients.

Results: Cerebrospinal fluid cultures were positive in 100 (13.7%) of cases. Positive blood cultures were found in 59 (8.1%) neonates with meningitis. Staphylococcus bacteria (48%) was the most frequent organism responsible for confirmed meningitis, followed by klebsiella (19%) and pseudomonas (10%). Simultaneous pathogens isolated from blood cultures were Staphylococcus (%24), klebsiella (13%) and pseudomonas (8%).

Conclusion: In our study group staphylococcus, klebsiella and pseudomonas were the main etiological agents that were responsible for neonatal sepsis and meningitis in our unit.

Keywords: Blood culture, cerebrospinal fluid culture, neonatal meningitis, sepsis.

Öz

Amaç: Bu çalışmanın amacı, yenidoğan yoğun bakım ünitesinde hem kan hem de beyin omurilik sıvısı kültürlerinin eşzamanlı bulgularını araştırmak ve yeni doğan sepsis ve menenjitte sorumlu olan etiyolojik patojenleri saptamaktır.

Gereç ve Yöntem: Bu çalışma Kasım 2013 - Ekim 2015 tarihleri arasında yenidoğan bölümümüzde yapıldı. Bu dönemde neonatal sepsis belirtileri ve semptomları olan 732 yenidoğan çalışmaya dahil edildi. Çalışmaya uygun olan hastalardan eş zamanlı kan ve beyin omurilik sıvısı kültürleri alınarak sepsis ve menenjit çalışması yapıldı.

Bulgular: Yüz vakada (%13,7) beyin omurilik sıvısı kültürü pozitif. Menenjit tanılı 59 yenidoğan hastasında kan kültürü pozitif olarak bulundu. Staflokok bakterisi (%48) menenjitte sorumlu en sık görülen organizmaydı ve bunu klebsilla (%19) ve psodomonas (%10) izledi. Kan kültüründen eş zamanlı olarak izole edilen bakteriler ise staflokok (%24), klebsilla (%13) ve psodomonas (%8).

Sonuç: Çalışmamızda, ünitemizde neonatal sepsis ve menenjitte sorumlu olan ana etiyolojik ajanlar staflokok, klebsilla ve psodomonas idi.

Anahtar kelimeler: Beyin omurilik sıvısı kültürü, kan kültürü, sepsis, yenidoğan menenjit

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INTRODUCTION

Bacterial meningitis is still a major public health problem threatening childhood. It is a devastating infection with a high mortality and morbidity rates¹⁻³, especially in newborn infants and frequently among preterm neonates⁴. The risk of developing permanent neurological sequels among the neonates who survive is more than 50%⁵. The incidence of neonatal meningitis varies from 0.2 to 1.9 cases per 1000 live births⁶, low rates have been reported from western countries⁷, but, much higher rates have been reported from developing countries⁸. Bacterial pathogens are commonly associated with newborn meningitis^{1,8-13}. The microorganism spectrum responsible for neonatal meningitis are different geographically, and also show temporal changes within the same country^{4,6,9}. The main causative agents for neonatal bacterial meningitis isolated from cerebrospinal fluids (CSF) from developed countries are: *Group B streptococcus*, *Escherichia coli (E.coli)*, *listeria monocytogenes* and *staphylococcus pneumoniae*^{7,9,14-16}. The etiologic agents responsible for neonatal meningitis in developing countries are different^{4,7-9}. Neonatal and maternal risk factors including prematurity, low birth weight, maternal low socioeconomic status, premature ruptures of membranes and maternal infections play important role in developing neonatal bacterial meningitis^{9,14-16}.

The aim of this study was to identify the etiological agents that cause bacterial neonatal meningitis in our neonatal intensive care unit, and to assess the association between blood and cerebrospinal fluids culture results. The results of our present study will help us to approach correctly to the coming cases of neonatal sepsis and meningitis in order to initiate prompt antibiotic therapy.

MATERIALS AND METHODS

This study was carried out at the neonatology unit of the Zekai Tahir Burak Maternity Teaching Hospital between November 2013 and October 2015. This study was approved by the ethics committee at Zekai Tahir Burak Maternal Health Teaching Hospital (Ankara, Turkey), and informed consents were taken from parents. To ensure confidentiality, inpatient registration number was used to identify the participants and not names. Patients eligible for this study were live born infants delivered between 26 and 41 gestational weeks with

signs and symptoms of neonatal sepsis. We evaluated the hospital records of all patients. Maternal age, maternal medical diseases and infections like: chorioamnionitis, and urinary tract infection. Antenatal and postnatal medical problems were obtained from obstetrics and gynecology department records. Modes of delivery and birth weight, were recorded. Natal and postnatal medical history of eligible infants, demographic and clinical symptoms were recorded.

Defining sepsis and meningitis

Irregular body temperature, hypotension, lethargy, apnea, tachypnea, increased need for oxygen, bradycardia, cyanosis, abdominal distention, vomiting, feeding problems were considered as non-specific symptoms of neonatal sepsis. Neonatal sepsis was defined as clinical sepsis according to the signs, symptoms and laboratory findings. Proven sepsis was based upon the positive blood culture for bacterial growth¹⁷. Neonatal sepsis may be categorized as early-onset if signs and symptoms occur within the first three days of life or late-onset after the third day of life⁸⁻⁹. At the first day of sepsis diagnosis, blood samples of hemogram, C reactive protein (CRP), interleukin-6 (IL-6) and blood culture were taken and lumbar puncture were performed for cerebrospinal fluid biochemistry and culture. Prompt antibiotic treatment was started at the same day. The laboratory findings of neonatal sepsis were recognized significant as follows; leukocytosis was defined as leukocyte count more than 20000 /mm³, leukopenia was identified as leukocyte count less than 5000/mm³ and the absolute neutrophil count less than 1800/mm³. Thrombocytopenia was defined as platelet count less than 100000/mm³. Blood smear of band/total neutrophil ratio more than 0.2 were considered significant. Neonatal meningitis was defined according to the common practice criteria's; instability of body temperature, respiratory distress, apnea, tachypnea, increased need for oxygen, hypotension, lethargy, seizures, abdominal distention, vomiting, feeding problems, irritability, bradycardia or cyanosis. Clinical symptoms and signs of meningitis plus either a positive culture of the cerebrospinal fluid or CSF leukocyte count more than 20/ mm³ were suggestive of bacterial meningitis^{1,4}.

Samples and biochemical analysis

Blood samples were taken from a peripheral vein.

Blood samples were centrifuged at 5.000 rpm for 10 minutes. All blood samples were of good quality without hemolysis, grossly hemolyzed samples were not included in the analysis. CRP serum levels were measured with nephelometric method (sensitive value = 0.8 mg/dl) (CRP kit, Roche, Germany) (IMMAGE device, the Beckman-Coulter, USA). Serum IL-6 levels were measured with solid phase enzyme labelled chemiluminescent immunometric assay (IL-6 kit, Siemens Healthcare Products Ltd, Hanbers, USA) (sensitivity value = 2pg/ml) (Immulate 2000 device, USA). Lumbar puncture was performed in all neonates diagnosed as neonatal sepsis. CSF samples were analyzed for leukocyte count, protein, glucose and culture. Before starting antimicrobial therapy for neonatal sepsis, samples for blood and CSF cultures were taken. Cultures were analyzed using fully automated BACTEC method by BACTEC 9240 device (Becton Dickinson, Heidelberg, Germany). Infants who had positive blood culture were treated according to the antibiogram results of the cultures. The therapy was ceased after clinical and laboratory improvement were achieved.

Statistical analysis

Statistical Package for Social Sciences (SPSS 18.0, Chicago, IL, USA) for Windows programme was used for statistical analysis. The normal distribution of variables was tested with the Kolmogorov-Smirnov test. Descriptive statistics were given as mean and standard deviation (SD) or median and 25-75 percentiles, categorical variables were given as values and percentage. Statistical significance was set at $p < 0.05$.

RESULTS

During the study period, 732 infants were included in the study. Among the 732 patients with neonatal sepsis, 100 patients were diagnosed as CSF culture proved neonatal meningitis. The majority of patients 84.6% were born by cesarean section (CS), and 15.4% neonates were vaginal deliveries, 90.3% of neonates were premature infants born earlier than 37th gestational age, whereas 9.7% were mature infants born at 37th gestational age or later, 76.6% of all patients needed resuscitation at birth. Presence of RDS and the need of surfactant was found in

78.1%. The median birth weight was 1040 gram (gr). Maternal infection was found in 27.3% of cases with positive maternal blood cultures in 25% and maternal antibiotic use in 31.3% of the study group patients. mPositive CSF culture findings were detected in 100 (13.7%) of cases. Simultaneously both blood and CSF cultures were found to be positive in 59 (59%) infants. The demographic features of our study group are depicted in Table 1 and Table 2.

Table 1. Demographic characteristics of the patients.

Variables	
Gestational age < 37weeks (%)	90.3 %
Gestational age ≥ 37weeks (%)	9.7 %
	Mean ± SD
Gestational age (week)	28.79±5.86
Birth head circumference	28.02±5.10
Patients WBC	16819±8834.84
Maternal CRP	26.00±3.08
Maternal WBC	14145±4.40
	Median(25P-75P)
Birth weight	1040.00(730.00-1760.00)
Patients CRP	40.00(10.00-88.00)
Patients IL-6	625.00(250.00-4133.00)
CSF protein	123.00(101.00-181.00)
CSF glucose	67.00(41.00-97.00)
CSF WBC	7.00(0.00-14.00)

WBC: White blood cell, CRP: c reactive protein, IL-6: interleukin-6CSF: cerebro spinal fluid

In our study, neonatal meningitis was associated with early neonatal sepsis in 28.6% and with late neonatal sepsis in 71.4% of infants. The median CRP and IL-6 serum levels at the time of diagnosis were 40.00 and 625.00 respectively. As showed in Table 3, staphylococcus bacteria (48%) was the most frequent organism isolated from CSF culture followed by klebsiella (19%) and pseudomonas (10%). Simultaneous pathogens isolated from blood cultures were Staphylococcus (%24), klebsiella (13%) and pseudomonas (8%). Enterococcus 7 (7%), 3 (5.1%), E.coli 4 (4%), 4 (4.8%), candida 4 (4%), 3 (5.1%) and serrata marcescens 3 (3%), 3 (5.1%) were also other organisms isolated from CSF and blood cultures respectively. There was statistically significant relationship between the CSF and simultaneous blood culture findings with $p < 0.05$ for all microorganisms isolated from both cultures (Table 3).

Table 2. Risk factors for neonatal meningitis.

Variables	%
Mode of delivery	
NVB	15.4%
C/S	84.6%
Maternal infection	27.3%
Positive maternal blood culture	25%
Maternal Antibiotic use	31.3%
Resuscitation at birth	76.6%
Presence of RDS	78.1%
Need of surfactant	78.1%
Early neonatal sepsis	28.6%
Late neonatal sepsis	71.4%
Mortality	65%

NVB: normal vaginal birth, C/S: cesarian section, RDS: respiratory distress syndrome.

Table 3. Simultaneous CSF and blood cultures of neonatal meningitis.

Microorganism isolated from CSF n (%)	Positive blood culture n (%)	Negative blood culture n (%)	P
Staphylococcus bacteria	48 (48%)	24 (50%)	0.0001
Klebsiella	19 (19%)	13 (68.4%)	0.0001
pseudomonas	10 (10%)	8 (80%)	0.0001
Enterococcus	7 (7%)	3 (42.9%)	0.0001
E.coli	4 (4%)	4 (100%)	0.0001
Candida	4 (4%)	3 (75%)	0.0001
Serratia Marcescens	3 (3%)	3 (100%)	0.0001

CSF: cerebrospinal fluid

DISCUSSION

Meningitis is the acute inflammation of the meninges, brain vasculature and subarachnoid space. The infection is defined as the clinical signs and symptoms plus a positive culture¹. There is a great difficulty in predicting the diagnosis of meningitis solely based on the clinical features because the signs and symptoms are mostly subtle, and could not help to establish an exact diagnosis of neonatal meningitis, suggesting that CSF culture continues to be the gold standard for diagnosis, demonstrating the pathogens responsible for meningitis and helping antibiotic initiation¹⁻³.

In our present study we included 732 premature infants with clinically signs and symptoms of neonatal sepsis. One hundred infants (13.7%) were diagnosed as CSF culture proved bacterial meningitis, 59 patients had simultaneously positive blood and CSF cultures. We also found that neonatal meningitis was associated with late

neonatal sepsis (71.4%), staphylococcus bacteria, klebsiella and pseudomonas were the most frequent organisms isolated from CSF and blood cultures of infants diagnosed as neonatal sepsis. In consistent with our study, Stoll et al⁸, Cohen-Wolkowicz et al⁹ and Softic et al¹⁵ reported that the vast majority of neonatal sepsis and meningitis episodes were associated with late neonatal sepsis, the most frequent organism isolated from CSF and blood cultures were Gram-positive organisms^{8-9,15} with staphylococci accounting for 48% of infections⁸. Stoll et al⁸ also found that the most common gram-negative causes of late neonatal infections were E. coli (5%) and Klebsiella (4%). Gestational age and birth weight are important risk factors for acquisition of neonatal infections. Neonatal sepsis and meningitis are predominantly seen in premature infants, and are inversely related to their birth weight and gestational age⁴. In our study, the majority of neonatal infections (90.3%) occurred in very low birth weight infants with mean gestational age of 28.79 weeks, and median birth weight of 1040

gr. Similar to our study, Fanaroff et al⁴ reported that prematurity increases the probability of late onset neonatal infections, and septicemia was commonly seen in very low birth weight infants, and the rate was inversely proportional to their birth weight and gestational age.

In our present study, we aimed to identify the profile of the commonly observed etiological agents that usually cause bacterial neonatal sepsis and meningitis in our neonatal intensive care unit, and to determine the association between blood and cerebrospinal fluid culture results. As shown in Table 3, we found that there was a statistical significant association between meningitis and sepsis with respect to the microorganisms isolated from positive CSF and blood cultures with p value less than 0.05. We also determined that Gram positive bacteria were the dominant organisms isolated from CSF and blood cultures and staphylococcus bacteria was the most frequent gram positive organism. Klebsiella, Pseudomonas and Ecoli were the most Gram negative pathogens isolated from CSF and blood cultures. These findings will help us in the future approach to the neonatal sepsis and meningitis in order to demonstrate the etiological pathogens responsible for infection and to help prompt and correct management.

Data of our retrospective study depended upon information collected from hospital records. Patients with incomplete information about clinical aspects and laboratory test results were excluded from our study. Incomplete data like early and late neurological consequences of neonatal meningitis were not convenient for any statistical analysis. This is why we didnot include these data to our study.

Neonatal meningitis is a dangerous disease that requires a high index of suspicion and early diagnosis, and rapid treatment. CSF culture is critical for confirming the diagnosis, determining the pathogen, and initiating antimicrobial therapy. We found that there was a strong association of bacterial meningitis and late neonatal sepsis with positive CSF and blood cultures.

Yazar Katkıları: Çalışma konsepti/Tasarımı: HH, CT; Veri toplama: HH, ŞİÖ; Veri analizi ve yorumlama: ŞSO; Yazı taslağı: HH; İçeriğin eleştirel incelenmesi: HH, CT; Son onay ve sorumluluk: HH, ŞİÖ, SS, ŞSO, CT; Teknik ve malzeme desteği: SS; Süpervizyon: CT; Fon sağlama (mevcut ise): yok.

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