Comparison of changes in etiologic microorganisms causing early-onset neonatal sepsis between preterm labor and preterm premature rupture of membranes

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Abstract

Objective: To investigate changes in the etiologic microorganisms causing early-onset neonatal sepsis (EONS) in preterm labor (PTL) or preterm premature rupture of membranes (pPROM) cases over the past 16 years and to analyze the associated factors.

Methods: We included consecutive singleton pregnancies delivered before 34 weeks due to PTL or pPROM. The etiologic microorganisms causing EONS in PTL and pPROM cases were compared between period 1 (1996–2004) and period 2 (2005–2012).

Results: There was no difference in the incidence of Gram-positive bacteria causing EONS between period 1 and 2, either in PTL (2.0% versus 2.1%, \(p = 1.0\)) or in pPROM (1.5% versus 1.6%, \(p = 1.0\)). However, the incidence of EONS caused by Gram-negative bacteria was significantly increased in pPROM (0.6% versus 2.7%, \(p = 0.040\)) during period 2, compared to period 1; but not in PTL (0.3% versus 1.2%, \(p = 0.211\)). Multivariable analysis revealed that a prolonged ROM-to-delivery interval (>7 d) was significantly associated with EONS caused by Gram-negative bacteria in pPROM (odds ratio: 6.6, 95% confidence interval: 1.4–31.8, \(p = 0.018\)).

Conclusions: The etiologic microorganisms causing EONS have changed over the past 16 years in pPROM cases but not in PTL cases.

Keywords

Early-onset neonatal sepsis, Gram-negative bacteria, microorganisms, preterm labor, preterm premature rupture of membranes

Introduction

Early-onset neonatal sepsis (EONS) contributes significantly to neonatal morbidity and mortality in preterm infants [1,2]. Although it had been reported that group B Streptococcus (GBS) was one of the important causes of neonatal sepsis, studies in recent decades indicate that Gram-negative bacteria including Escherichia coli (E. coli) have emerged as the major pathogens of neonatal sepsis, especially in preterm infants or very-low-birth-weight (VLBW) infants [3–5]. EONS caused by Gram-negative organisms is reported to be associated with severe morbidity and a higher mortality rate compared with that caused by early-onset GBS [6,7]. Therefore, it is important to analyze obstetrical risk factors for Gram-negative sepsis in order to establish optimal prevention and management strategies.

Meanwhile, as the use of prophylactic antibiotics for preterm premature rupture of membranes (pPROM) became the standard treatment to improve neonatal outcome, gestational age at delivery has been prolonged in clinical practice [8–10]. Also, it is well-known that a prolonged ROM-to-delivery interval may increase neonatal infectious morbidity including EONS [11,12]. Accordingly, it is presumed that these changes in the management of pPROM might affect a change in the microorganism milieu causing EONS.

Interestingly, the majority of reports on the risk factors for EONS and associated factors has been analyzed mainly from the pediatric point of view, and included VLBW infants as the study population. Consequently, little attention has been devoted to differentiating the two critical obstetrical etiologies of preterm birth: PTL and pPROM. In fact, from the obstetrical point of view, there are some distinct differences with respect to the management of PTL and pPROM. For example, prophylactic antibiotics are recommended in the expectant management of pPROM, as stated above, but they are not recommended in patients with PTL unless for use as a GBS prophylaxis [13–15]. Furthermore, it has recently been
suggested that PTL and pPROM differ in the severity of the intra-amniotic inflammatory response and the pattern of antimicrobial peptides expressed in the fetal membrane [16,17].

Given this background, in the present study we aimed to investigate changes that may have occurred over the past 16 years in the etiologic microorganisms causing EONS in preterm births at less than 34 weeks of gestation. We analyzed the microorganisms and associated factors relating to preterm birth caused by either PTL or pPROM.

Methods

Study population

This retrospective study was conducted at Samsung Medical Center, a tertiary academic hospital in the Republic of Korea. The study was approved by the Institutional Review Board in our institution. We included consecutive cases of singleton pregnancy delivered at 24–34 weeks of gestation due to PTL or pPROM between January 1996 and July 2012. In order to evaluate changes in the etiology of EONS during different time periods, the study period was divided into period 1 (January 1996 to December 2004) and period 2 (January 2005 to July 2012). PTL was defined as the presence of regular uterine contractions with a frequency of at least 8 every 60 min along with progressive cervical changes [18]. We also included all cases which delivered preterm despite tocolytics. pPROM was diagnosed as the finding of gross pooling of amniotic fluid in the vaginal and an alkaline vaginal fluid pH as determined by a nitrazine test [19]. The diagnosis of EONS was assigned when microorganisms were isolated from blood cultures or cerebrospinal fluid (CSF) within 3 d of birth [1,12]. Microorganisms were classified as Gram-positive, Gram-negative, or yeast species.

Clinical characteristics of the study population

All maternal and neonatal medical records were reviewed to collect necessary data including maternal age, parity, history of previous preterm labor, gestational age at pPROM, gestational age at delivery, ROM-to-delivery interval, incompetent internal os of cervix (IIOC) status (regardless of the performance of cerclage), neonatal birth weight and gender, delivery mode, and administration of antenatal corticosteroids or tocolytics. Antenatal corticosteroids, as well as tocolytics, were given as recommended. Broad spectrum prophylactic antibiotics were administered if indicated, and antibiotic susceptibility data for the microorganisms causing EONS were collected.

Statistical analysis

We assessed potential associations between EONS and various maternal and neonatal characteristics. The comparisons between the two different time periods (period 1 and period 2) were analyzed using a Mann–Whitney U-test for continuous variables and a Fisher’s exact test for categorical variables. We also performed multivariable analysis adjusting for maternal age, parity, treatment with antenatal corticosteroid and tocolytics, use of antibiotics, gestational age at delivery, and neonatal birth weight to identify the independent risk factors for changes in the microorganisms associated with EONS in both PTL and pPROM.

Results

Clinical characteristics of the study population with PTL and pPROM during each study period

There were 45,159 deliveries in our institution during the study period, from January 1996 to July 2012. Of the total study population, 6153 (13.6%) were gestational age of less than 37 weeks at delivery and 2944 (6.5%) were of less than 34 weeks at delivery. The rates of PTL in preterm birth population were 25.3% (348/1375) and 21.6% (339/1569) during period 1 and period 2. Moreover the rate of pPROM were, respectively, 24.2% (333/1375) and 23.7% (372/1569). Overall, the incidence of EONS was 2.8% (19/687) in PTL and 3.3% (23/705) in pPROM cases during the study period. There was no significant difference between the incidence of EONS in PTL (2.3% versus 3.2%, p = 0.301) and that of pPROM (2.1% versus 4.3% p = 0.136) cases between period 1 and period 2. Period 2 was associated with a higher maternal age and an increased number of primiparous women, both in PTL and pPROM cases (Table 1). Median gestational age at delivery was significantly less in period 2 both in PTL and in pPROM cases. Notably, the ROM-to-delivery interval was significantly prolonged in period 2 in pPROM (4.0 [0–68] versus 4.5 [0–74], p < 0.001) cases. Moreover, the use of antibiotics was also significantly increased in period 2 in pPROM cases. Tocolytics and antenatal corticosteroid were more frequently administered in period 2 compared to period 1 in both PTL and pPROM cases.

Distribution and incidence of microorganisms causing EONS in PTL and pPROM during each study period

We analyzed the distribution of microorganisms causing EONS in PTL and pPROM cases during the two study periods. As shown in Table 2, in PTL, Gram-positive bacteria isolated from neonatal blood accounted for more than half of the etiologic microorganisms causing EONS in period 1 (87.5%) and period 2 (63.6%). Similar results were observed in cases of pPROM during period 1, in that 71.4% of EONS was caused by Gram-positive organisms. However, in cases of pPROM during period 2, Gram-negative organisms accounted for the major etiologic microorganisms (62.5%), with E. coli and Klebsiella being the most commonly isolated pathogens.

Table 1 also indicates the incidence of microorganisms causing EONS in PTL and pPROM during each study period. The results demonstrate that there was no change in the rate of Gram-positive bacteria causing EONS in either PTL (2.0% versus 2.1%, p = 1.0) or pPROM (1.5% versus 1.6%, p = 1.0). However, compared to period 1, the EONS rate caused by Gram-negative bacteria during period 2 was significantly increased in pPROM (0.6% versus 2.7%, p = 0.040), but not in PTL (0.3% versus 1.2%, p = 0.211). No EONS cases within our study were found to have a fungal infection.

Independent risk factors for the notable increase in Gram-negative sepsis in pPROM

Since there were significant changes in the clinical characteristics of the parturient women with PTL and pPROM between the two periods evaluated, as shown in Table 1, we
performed multivariable analysis to ascertain the independent risk factors for the notable increase in Gram-negative sepsis in pPROM. After adjusting for maternal age, parity, treatment with antenatal corticosteroid and tocolytics, use of antibiotics, gestational age at delivery, and neonatal birth weight, the result revealed that a prolonged ROM-to-delivery interval (>7 d) was significantly associated with the development of EONS caused by Gram-negative bacteria in pPROM (odds ratio: 6.6, 95% confidence interval: 1.4–31.8, \( p = 0.018 \)).

Susceptibility of microorganism causing EONS to antibiotics

We also evaluated the antibiotic susceptibility of individual microorganisms causing EONS in PTL (Supplemental Table 1) and in pPROM (Table 3). These tables present data for clinical variables including maternal age, parity, gestational age at delivery, and neonatal birth weight; which were significantly different between period 1 and period 2. Overall, \( E. \) coli was the most common isolate throughout the
### Table 3. Cases of early-onset neonatal sepsis in pPROM: clinical variables, microorganisms, and susceptibility to antibiotics.

| Case No. | Age (y) | Parity | GAD (wks) | Bwt (kg) | Antenatal antibiotics | ROM to delivery interval (d) | Microorganisms of EONS | Susceptibility to antibiotics | Neutonal outcomes | Cause of death |
|----------|---------|--------|-----------|----------|-----------------------|-----------------------------|------------------------|-------------------------------|-----------------|---------------|
|          |         |        |           |          |                       |                             |                        |                               |                 |               |
| **Period 1 (1996–2004)** |         |        |           |          |                       |                             |                        |                               |                 |               |
| 20       | 38      | M      | 24.0      | 0.58     | cefazolin + EM        | 3                           | *Enterococcus faecalis*   | −                | S              | S             | Expired    |
| 21       | 32      | P      | 26.5      | 1.04     | cefazolin + EM        | 10                          | MSSA                   | −                | −              | −             | Survived    |
| 22       | 30      | M      | 27.3      | 1.15     | cefazolin             | 0                           | *Group B Streptococcus*  | S                | S              | −             | Survived    |
| 23       | 36      | M      | 27.6      | 1.18     | cefazolin             | 4                           | Corynebacterium          | S                | S              | −             | Survived    |
| 24       | 29      | M      | 31.0      | 1.98     | cefazolin + EM        | 68                          | CoNS                   | −                | −              | −             | Survived    |
| 25       | 28      | P      | 31.1      | 1.73     | cefazolin + EM        | 8                           | *Pseudomonas aeruginosa* | −                | R              | R             | Expired    |
| 26       | 33      | M      | 31.2      | 1.65     | cefazolin + EM        | 25                          | *Escherichia coli/ESBL−* | R                | S              | S             | Survived    |
| **Period 2 (2005–2012)** |         |        |           |          |                       |                             |                        |                               |                 |               |
| 27       | 30      | P      | 31.0      | 2.05     | cefazolin + CM        | 24                          | *Serratia marcescens*     | R                | S              | S             | Survived    |
| 28       | 30      | P      | 31.0      | 2.05     | cefazolin + CM        | 24                          | *K. pneumonia/ESBL−*      | R                | S              | S             | Survived    |
| 29       | 32      | P      | 24.0      | 0.63     | cefazolin + EM        | 5                           | *Enterococcus faecalis*   | −                | −              | −             | Survived    |
| 30       | 27      | P      | 25.1      | 0.86     | cefazolin            | 0                           | MSSA                   | −                | −              | −             | Survived    |
| 31       | 33      | M      | 25.3      | 0.81     | cefazolin + EM        | 15                          | *Escherichia coli/ESBL−* | R                | S              | R             | Survived    |
| 32       | 30      | M      | 26.3      | 0.94     | cefazolin + EM        | 12                          | *Enterococcus faecalis*   | S                | −              | R             | Expired    |
| 33       | 38      | P      | 28.1      | 1.16     | cefazolin + CM        | 16                          | CoNS                   | −                | −              | S             | Survived    |
| 34       | 31      | M      | 28.3      | 0.98     | cefazolin             | 13                          | Proteus penneri          | R                | S              | S             | Survived    |
| 35       | 29      | P      | 28.6      | 1.37     | cefazolin             | 34                          | *K. pneumonia/ESBL−*      | R                | S              | S             | Expired    |
| 36       | 29      | M      | 28.6      | 1.37     | cefazolin + EM        | 16                          | *Enterococcus faecalis*   | S                | −              | R             | Survived    |
| 37       | 36      | M      | 29.0      | 1.42     | cefazolin             | 18                          | *K. pneumonia/ESBL−*      | R                | R              | S             | Survived    |
| 38       | 28      | P      | 31.4      | 1.58     | cefazolin             | 2                           | *Escherichia coli/ESBL+*  | R                | R              | S             | Survived    |
| 39       | 33      | M      | 32.0      | 1.73     | cefazolin + EM        | 10                          | *Escherichia coli/ESBL−*  | S                | S              | S             | Survived    |
| 40       | 23      | P      | 32.2      | 1.67     | cefazolin             | 1                           | *Streptococcus mitis/oralis* | S         | S              | −             | Survived    |
| 41       | 33      | P      | 32.6      | 2.11     | cefazolin + CM        | 21                          | *K. pneumonia/ESBL−*      | R                | S              | S             | Survived    |
| 42       | 33      | P      | 32.6      | 2.11     | cefazolin + CM        | 21                          | *Escherichia coli/ESBL−*  | S                | S              | S             | Survived    |

pPROM, preterm premature rupture of membranes; GAD, gestational age at delivery; Wks, weeks; Bwt, neonatal birth weight; ROM, rupture of membranes; EONS, early-onset neonatal sepsis; M, multipara; P, primipara; EM, erythromycin; CM, clarithromycin; MSSA, methicillin-susceptible *Staphylococcus aureus*; CoNS, coagulase-negative Staphylococci; *K. pneumonia, Klebsiella pneumonia*; ESBL, expended spectrum beta lactamase; R, Resistant; S, Sensitive.
study period (9/42), with the majority (8 of 9) being isolated in period 2. Among these eight E. coli isolates, four were isolated from PTL and four from pPROM. Furthermore, half of these eight E. coli isolates were resistant to ampicillin. Notably, there were four cases of EONS associated with Klebsiella pneumonia infection, which were all derived from the pPROM group in period 2 and all were found to be completely resistant to ampicillin. All cases with Klebsiella pneumonia were associated with a prolonged ROM-to-delivery interval (>7 d), with the median interval time being 22.5 d (range, 18–34 d).

Discussion

Our data revealed a significant increase in the rate of Gram-negative sepsis in preterm neonates delivered before 34 weeks of gestation in pPROM, but not in PTL, over the past 16 years. Interestingly, multiple logistic regression analysis, adjusting for variables (maternal age, parity, gestational age at delivery, and neonatal birth weight) revealed that a prolonged ROM-to-delivery interval (>7 d) was the sole independent variable affecting the development of Gram-negative sepsis in preterm neonates delivered after pPROM.

Our study results concur with other recent studies which have reported an increase in Gram-negative and a concomitant decrease in Gram-positive bacteria among VLBW infants [2,5]. In fact, the proportion of Gram-negative bacteria in VLBW infants in the USA during the period spanning 1991 to 1993 was reported to be 43% [20]; however, a substantial increase to 61% was observed for the period spanning 1998 to 2000 [1]. Among Gram-negative bacteria, E. coli was reported to be the most prevalent, accounting for 41% of VLBW cases between 2002 and 2003 in the USA [2]. A Norwegian study reported that 60% of very early-onset septicemia (defined as septicemia diagnosed within 24 h of delivery) cases in extreme preterm infants (gestational age <28 weeks or birth weight <1000 g) were caused by E. coli [21]. Similarly, a study from Israel demonstrated that E. coli accounts for 26.8% of the pathogens associated with cases of VLBW with EONS [12]. The next most prevalent pathogen in that study was Klebsiella, which was found in 6.8% of cases. In our results, the overall proportion of Gram-negative bacteria in preterm births (24–34 weeks of gestation) increased from 20% (3/15) to 52% (14/27) during the periods spanning 1996–2004 and 2005–2012, respectively. E. coli accounted for 30% (8/27) of all pathogens causing EONS during 2005–2012. During this same period, Klebsiella comprised 15% (4/27) of all isolated pathogens. Among the cases attributable to Klebsiella, the underlying cause of preterm birth was always pPROM.

Studies evaluating the potential risk factors for EONS have reported diverse and conflicting results depending on the study population and the inclusion criteria for the study. A report by Klinger et al. involving VLBW infants (<1500 g) indicated that the development of EONS was associated independently with a lack of prenatal care, PROM and amnionitis, but showed no association with gestational age at delivery. In this study, it was reported that cases of prolonged rupture of membranes (≥24 h) with the presence of amnionitis had about an 8-fold increased risk for developing EONS [12]. Using multivariable analysis, Salem et al. showed that the use of tocolytic drugs is an independent risk factor for EONS in VLBW infants (<1.500 g), with an odds ratio of 4.8 [22]. With respect to studies that have allowed preterm birth as an inclusion criteria, it has been demonstrated that the administration of multiple courses of antenatal corticosteroids and gestational age at delivery are significantly associated with EONS in infants delivered between 24 and 34 weeks of gestation due to pPROM [23]. This same study did not find any association with latency. These findings contrast with the results of our multivariable analysis. Such contradictory results with respect to the risk factors for EONS may result from differences in the inclusion criteria for each study. In fact, this study has excluded any cases with clinical chorioamnionitis and tocolytics exposure.

Our study found that the incidence of ampicillin resistant E. coli among EONS cases with E. coli was 55.6% (5 of 9 isolates). When only the recent period 2 (2005–2012) was evaluated, there was no difference in the rate of ampicillin-resistant E. coli between PTL and pPROM (each for 50%) cases. This rate is quite similar with that reported by a French multicenter study in which 50% of E. coli was ampicillin resistant in cases evaluated between 2004 and 2005 [24]. Likewise, Bizzarro et al. (USA) reported a 54% E. coli resistance rate during the period spanning 1997–2006 [3]. However, higher rates of EONS caused by ampicillin resistant E. coli have been reported by others, with rates being as high as 69% to 79% [25,26]. Meanwhile, it is noteworthy that all cases of EONS caused by Klebsiella in our study were resistant to ampicillin. However, 100% of these ampicillin-resistant strains of Klebsiella were sensitive to gentamycin, while other reports have indicated very high rates of gentamycin-resistant Klebsiella in EONS [27].

There are several limitations to our study. First is the relatively small sample size of our study, which hinders the statistical analysis of the change in frequency of Gram-negative sepsis especially in PTL and the detailed statistical assessment regarding resistant strains of microorganisms causing EONS over the study period. Second, this study is derived from a single tertiary center rather than a multicenter study which is generally considered to provide greater power. However, as the purpose of this study was to investigate the changes in microorganisms causing EONS in preterm birth after PTL or pPROM over 16 years, the use of single center data minimized the diversity of clinical practice in the management of PTL and pPROM, and may have lessened the impact of confounding variables. Third, our data may be somewhat limited as we did not correlate the severity of intra-amniotic infection or inflammation with neonatal EONS, which was done in several previous studies [28,29].

To the best of our knowledge, this is the first study to ever examine the etiologic microorganisms in EONS in preterm birth with respect to the two important causes of preterm birth: PTL and pPROM. Herein, we demonstrated that over the past 16 years, an increase in the rate of Gram-negative sepsis occurred in pPROM cases, but not in PTL cases, at our institution. Furthermore, we observed that a prolonged ROM-to-delivery interval (>7 d) was associated with Gram-negative sepsis in pPROM.
A recent Cochrane database review concluded that there is insufficient evidence to support the use of tocolytic therapy for management of pPROM [30]. Within this context, our study indicates the need for very prudent expectant management in cases with pPROM and a prolonged ROM-to-delivery interval (>7 d), particularly in cases of extreme preterm birth due to the high mortality rate attributable to EONS. In this respect, we hope our results will be recapitulated by studies performed with data from other high-risk units.

**Declaration of interest**

The authors report no declaration of interest.

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Supplementary information available online

Supplemental Table 1.