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초극소저체중출생아에서 조직학적 융모양막염 및 유레아플라즈마와 만성기계환기 의존적 기관지폐이형성증의 연관성

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의학과

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Correlation of Histologic Chorioamnionitis with *Ureaplasma* species and Chronic Ventilator-Dependent Bronchopulmonary Dysplasia in Extremely Low Birth Weight Infants

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by

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ABSTRACT

Correlation of Histologic Chorioamnionitis with *Ureaplasma* species and Chronic Ventilator-Dependent Bronchopulmonary Dysplasia in Extremely Low Birth Weight Infants

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Purpose: Histologic chorioamnionitis (HCAM) and *Ureaplasma* have been considered independent risk factors for bronchopulmonary dysplasia (BPD). This study tested the hypothesis that extremely low birth weight (ELBW) infants with coexisting HCAM and *Ureaplasma* are at an increased risk for more severe BPD necessitating chronic ventilator support.

Materials and Methods: A retrospective cohort study was performed. Pathologic examination of the placenta was carried out, and gastric fluids of infants were analyzed with real-time PCR and culture to detect *Ureaplasma* at birth. The demographic and clinical characteristics were compared according to the presence of HCAM and *Ureaplasma* (HCAM+U+, HCAM+U-, HCAM-U+, HCAM-U-), and the risk factors for chronic ventilator-dependent BPD were searched.

Results: Twenty (19.6%) of 102 infants were in the HCAM+U+ group. Although there were no significant differences in the clinical characteristics

including the incidence of BPD, the rate of chronic ventilator dependency was higher in the HCAM+U+ group compared with the other groups: HCAM+U+ (27.8%), HCAM+U- (0%), HCAM-U+ (0%), and HCAM-U- (2.3%) (p=0.005). In the multivariate logistic regression analysis, coexistence of HCAM and *Ureaplasma* was independently a significant risk factor for chronic ventilator dependent BPD (odds ratio 14.5, 95% confidence interval 1.1 – 188.9)

Conclusion: Coexistence of HCAM and *Ureaplasma* is a significant risk factor for chronic ventilator-dependent BPD in ELBW infants.

Keywords : chorioamnionitis; ureaplasma; bronchopulmonary dysplasia

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INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a multifactorial disease that develops in premature infants as a result of various antenatal and postnatal injuries to an immature lung [1]. Both histologic chorioamnionitis (HCAM) and *Ureaplasma* are considered important contributors in antenatal lung injury. Antenatal infections and inflammation are known to be not only etiologic factors for BPD, but also priming factors that accentuate postnatal lung injury [2]. However, interpretations concerning the impact of HCAM on lung development have varied in many studies, and current opinions suggest that isolated HCAM is questionable as a risk factor for BPD in premature infants [3-5]. HCAM is a non-specific maternal response to a variety of stimuli. Most cases of HCAM are associated with intrauterine infections caused by microorganisms such as *Ureaplasma* species [6]. *Ureaplasma* species, *Ureaplasma urealyticum*, and *Ureaplasma parvum* are commensal flora in the genital tract of 40-80% of women, and are the most common perinatally acquired pathogens in premature infants [7, 8]. *Ureaplasma* is likely to contribute to the development of BPD by dysregulating the inflammatory response in immature lungs, thus impairing alveolarization with early fibrosis [9]. A recent meta-analysis of 39 studies found that *Ureaplasma* increased the risk for BPD despite controversy on their role in the development of BPD [4, 10]. While HCAM and *Ureaplasma* may interact intricately in developing

BPD, there are insufficient studies on the impact of the coexistence of HCAM and *Ureaplasma* on BPD.

The current NICHD criteria for BPD [11] lack consideration of the conception of chronic ventilator-dependent BPD, where invasive mechanical ventilation is needed beyond 36 weeks of postmenstrual age (PMA). Although this chronic ventilator-dependent BPD fits under severe BPD in the current criteria, it can be distinguished from the ordinary severe BPD in regards to clinical manifestations, pathology, and prognosis [12–14]. More importantly, chronic ventilator-dependent BPD is a major cause of severe morbidity and mortality of extremely premature infants [13]. Mortality from severe BPD requiring prolonged mechanical ventilator support was reported to be up to 90% [14] and all survivors who were ventilated over 120 days had adverse neurodevelopmental impairment including cerebral palsy, blindness, and deafness [13].

Coexistence of HCAM and *Ureaplasma* in premature infants was assumed to be more injurious to the immature lung than single exposure, thus contributing to the development of chronic ventilator-dependent BPD. Therefore, this study aimed to test the hypothesis that extremely low birth weight (ELBW) infants with coexisting HCAM and *Ureaplasma* are at an increased risk for more severe BPD necessitating chronic ventilator support.

MATERIALS AND METHODS

Study design. This was a retrospective cohort study on preterm infants with ELBW (< 1,000 g) born at Seoul National University Bundang Hospital and admitted to the neonatal intensive care unit between January 2008 and December 2013. Perinatal and neonatal information on each infant was collected from the medical records. Patients with major congenital malformation or died within 7 days of birth were excluded from the analysis to reduce the confounding effect of congenital malformation, early onset sepsis, or perinatal asphyxia on the development of BPD. To assess the impact of the coexistence of HCAM and *Ureaplasma*, all subjects were divided into four groups according to the presence of HCAM and *Ureaplasma*: HCAM+*Ureaplasma* group (HCAM+U+); HCAM+No *Ureaplasma* group (HCAM+U-); No HCAM+*Ureaplasma* group (HCAM-U+); No HCAM+No *Ureaplasma* group (HCAM-U-). Data on maternal placental pathology and *Ureaplasma* were retrieved from the medical records. At our institution, maternal placenta biopsy and identification of *Ureaplasma* are performed routinely in preterm infants under the gestational age (GA) of 34 weeks, and the corresponding data are recorded in the medical records. The demographic characteristics on GA, birth weight, sex, multiple births, preeclampsia, type of delivery, antenatal steroid use, Apgar score at 1 and 5 min and the major morbidities including BPD, respiratory distress syndrome (RDS), patent ductus arteriosus (PDA), significant neurologic injury (SNI), necrotizing

enterocolitis (NEC), retinopathy of prematurity (ROP), late onset sepsis, and mortality were collected for each subject. Treatment with macrolides for *Ureaplasma* was not a routine practice and was done on a case by case basis with various protocols during the study period: Erythromycin 40 mg/kg/day for 10 days; Clarithromycin 20 mg/kg/day for 2 weeks; Azithromycin 10 mg/kg/day for 7 days followed by 5 mg/kg/day for a maximum of 6 weeks.

The Institutional Review Board of Seoul National University Bundang Hospital approved the collection and use of clinical information for research purposes before the investigation was started and waived the requirement for informed consent (B–1410/271–110).

Detection of Ureaplasma. Immediately after birth, 1 mL of gastric fluid was collected via sterile orogastric tube. The specimens were analyzed using real-time PCR and cultured with MYCOFAST EvolutioN2 (International Microbio, Signes, France) to detect *Ureaplasma*. Real-time PCR for the detection of *Ureaplasma* was done as follows: DNA was extracted using the automated system, EasyMag (bioMérieux, Durham, UK). Melting curve analysis was performed in adjunct with real-time PCR. To detect *Ureaplasma*, DNA extracts were amplified using '*Ureaplasma* primer and probe' targeting *Ureaplasma* specific sequence of the 16S rRNA, which detects *Ureaplasma parvum* and *Ureaplasma urealyticum* including all 14 *Ureaplasma* serovars. The sequence of forward primer was 5′-CTC CCC ACA CTT TCG AGC CTA AGC-3′, and reverse primer was 5′-TCA GTG ATA GTC CAA GTT GGC GCC T-3′. The hybridization detector probe sequence was 5'-LightCycler

Red 640-TCA GTG ATA GTC CAA GTT GGC GCC T-phosphate-3', and the hybridization anchor probe sequence was 5'-CTC CCC ACA CTT TCG AGC CTA AGC-fluorescein-3' [15]. *Ureaplasma* exposure was defined by isolating *Ureaplasma* through culture or PCR.

Histologic chorioamnionitis. A single pathologist performed histopathologic examinations of the maternal placentas obtained by obstetricians; tissue sections included the amnion, chorio-decidua, umbilical cord, and chorionic plate. Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections of tissue blocks were stained with hematoxylin and eosin. HCAM was defined as the presence of inflammatory changes with polymorphonuclear leukocytes infiltrating any of amnion, chorio-decidua, umbilical cord, or chorionic plate according to the grading system suggested by Salafia *et al* [16].

Clinical outcomes. The diagnosis of BPD was determined at PMA 36 weeks as follows: breathing air – mild BPD; need for supplementary oxygen (less than 30%) – moderate BPD; ≥30% supplementary oxygen and/or continuous positive airway pressure or on mechanical ventilator – severe BPD [12]. Chronic ventilator dependency was defined as being supported by invasive mechanical ventilator at PMA 36 weeks, excluding cases on short-term (< 7 days) ventilator support for operation or acute illness. RDS was diagnosed by the presence of respiratory distress, increased oxygen requirement (≥40%), and corresponding chest radiographic findings. Significant PDA was defined

when pharmacological and/or surgical treatment was required to mitigate hemodynamic disturbance. SNI included intraventricular hemorrhage grade ≥2 or periventricular leukomalacia. NEC was defined according to the criteria suggested by Bell *et al* [17]. ROP was diagnosed by a single ophthalmologist based on the international classification of ROP. Late onset sepsis was defined as culture-proven bacterial infection documented by a positive blood or cerebrospinal fluid culture after 7 days of birth.

Statistical Analysis. Group comparisons were carried out with χ^2 test or Fisher's exact test for categorical data and one-way ANOVA or Kruskal-Wallis test for continuous data. Logistic regression analyses were conducted to estimate the effects of the main explanatory variables of interest. All statistical analyses were performed with SPSS Version 18.0 (SPSS, Inc., Chicago, IL) with the statistical significance set at a p value of <0.05.

RESULTS

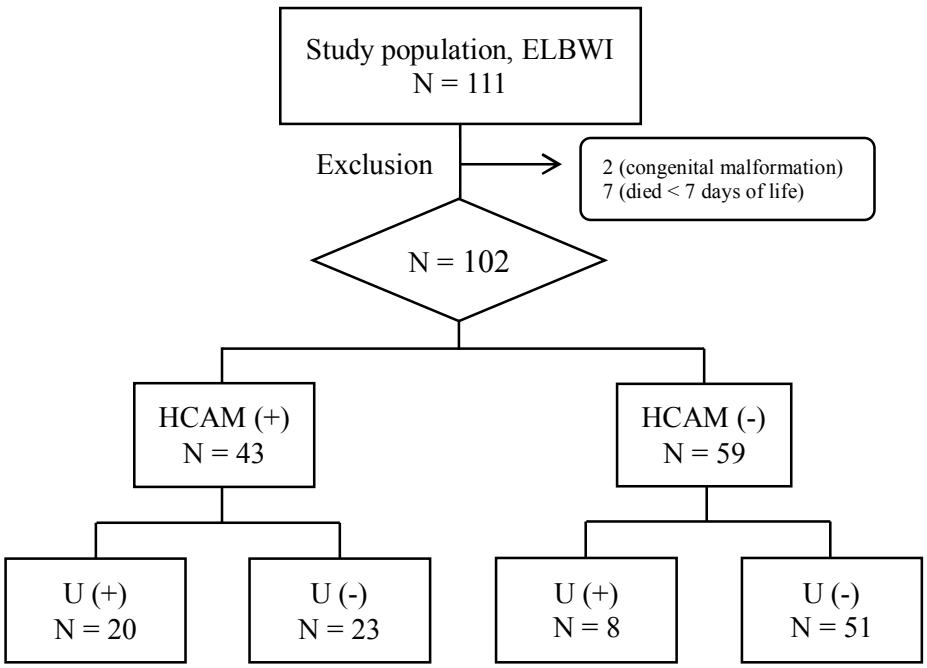
During the study period, a total of 111 infants were enrolled. Two infants with major congenital malformations and 7 who died within 7 days of birth were excluded. For the remaining 102 infants, the mean (SD) GA was 26^{+4} (2^{+1}) weeks, and the mean (SD) birth weight was 766.4 (146.3) g. Females composed 46.1%. Twenty infants (19.6%) were both HCAM and *Ureaplasma* positive (HCAM+U+), 23 were only HCAM positive (HCAM+U-), and 8 were only *Ureaplasma* positive (HCAM-U+). The remaining 51 were not positive for HCAM or *Ureaplasma* (HCAM-U-) (Figure 1).

Clinical characteristics were compared according to the presence of HCAM and/or *Ureaplasma* (Table 1). There were no significant differences in GA, birth weight, sex, multiple births, preeclampsia, and the proportion of very preterm infants with GA <28 weeks. Cesarean section was less frequent in HCAM+U+ and HCAM+U- groups compared with the HCAM-U- group (p=0.005 and p=0.035, respectively). There were no significant differences in the incidences of moderate and severe BPD or antenatal and postnatal corticosteroid therapies among the groups. However, the rate of ventilator dependency at PMA 36 weeks was significantly higher in the HCAM+U+ group (27.8%) compared with the other three groups; HCAM+U- (0%), HCAM-U+ (0%) and HCAM-U- (2.3%) (p=0.005). Treatment with macrolides was more frequent in the HCAM+U+ and HCAM-U+ groups than the HCAM-U- group (p<0.001 and p=0.004, respectively).

The number of ventilator-dependent infants was six out of 88 survived ELBW infants at PMA 36 weeks (6.8%). Upon comparing ELBW infants with chronic ventilator-dependent BPD to those without, treatment with macrolides, exposure to *Ureaplasma*, and coexistence of HCAM and *Ureaplasma* were significantly associated with chronic ventilator dependency (Table 2).

In a multivariate logistic regression analysis including GA, birth weight, sex, mode of delivery, and treatment with macrolides, the only significant risk factor for chronic ventilator-dependent BPD was coexistence of HCAM and *Ureaplasma* (odds ratio, 14.5; 95% confidence interval, 1.1 to 188.9). Neither HCAM nor *Ureaplasma,* alone, was found to be significant (Table 3).

Figure 1. Study population. A total of 102 infants were divided into four groups depending on the presence of histologic chorioamnionitis and *Ureaplasma.*



Abbreviations: ELBWI, extremely low birth weight infants; HCAM, histologic chorioamnionitis; U, *Ureaplasma*.

Table 1. A comparison of the demographic and clinical characteristics of extremely low birth weight infants among the groups

	HCAM+U+ (N = 20)	HCAM+U- (N = 23)	HCAM-U+ (N = 8)	HCAM-U- (N = 51)	*P* value
Gestational age, weeks	25^{+6} (1^{+3})	26^{+1} (2^{+1})	26^{+2} (2^{+2})	26^{+6} (2^{+2})	0.279
Infants with GA <28 weeks	18 (90.0)	18 (78.3)	7 (87.5)	31 (60.8)	0.054
Birth weight, g	785.4 (153.8)	771.7 (130.4)	775.0 (116.2)	755.2 (156.8)	0.878
Female	8 (40.0)	12 (52.2)	3 (37.5)	24 (47.1)	0.854
Multiple births	6 (30.0)	8 (34.8)	2 (25.0)	19 (37.3)	0.976
Preeclampsia	1 (5.0)	7 (30.4)	2 (25.0)	19 (37.3)	0.056
Cesarean section	10 (50.0)*	14 (60.9)†	6 (75.0)	43 (84.3)	0.019
Antenatal corticosteroid use	16 (80.0)	19 (82.6)	8 (100)	39 (76.5)	0.579
Apgar score at 1min	3 (1 - 5)	3 (2 - 4)	3 (1 - 5)	4 (2 - 5)	0.873
Apgar score at 5min	6 (5 - 7)	6 (4 - 7)	6 (4 - 7)	6 (5 - 7)	0.719
Moderate or severe BPD	12/18 (66.7)	8/20 (40.0)	6/8 (75.0)	23/43 (53.5)	0.267
Severe BPD	7/18 (38.9)	6/20 (30.0)	3/8 (37.5)	10/43 (23.3)	0.647
Ventilator dependency at PMA 36 weeks	5/18 (27.8)‡	0/20 (0.0)	0/8 (0.0)	1/43 (2.3)	0.005
Postnatal corticosteroid use	9/18 (50.0)	6/20 (30.0)	3/8 (37.5)	11/43 (25.6)	0.327
RDS	18 (90.0)	22 (95.7)	8 (100.0)	40 (78.4)	0.168
Treatment with macrolides	11 (55.0)§	2 (8.7)	3 (37.5)‖	1 (2.0)	<0.001
NEC stage ≥ 2	2 (10.0)	2 (8.7)	2 (25.0)	3 (5.9)	0.328
SNI	0 (0.0)	5 (21.7)	3 (37.5)	9 (19.6)	0.081
PDA	17 (85.0)	20 (87.0)	7 (87.5)	40/51 (82.4)	0.877
ROP stage ≥ 3	10/18 (55.6)	13/20 (65.0)	6/8 (75.0)	16/42 (38.1)	0.104
Late onset sepsis	3 (15.0)	3 (13.0)	2 (25.0)	8 (17.6)	0.869
Mortality	2 (10.0)	3 (13.0)	0 (0)	9 (17.6)	0.727

Data are presented as numbers (%), numbers/survivors (%), mean (SD), and medians (IQR). *$P = 0.005$, †$P = 0.037$, ‡$P = 0.008$, §$P < 0.001$ and ‖$P = 0.004$ *versus* HCAM-U- group

Abbreviations: HCAM, histologic chorioamnionitis; U, *Ureaplasma*; GA, gestational age; BPD, bronchopulmonary dysplasia; PMA, postmenstrual age; RDS, respiratory distress syndrome; NEC, necrotizing enterocolitis; SNI, significant neurologic injury; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity; SD, standard deviation; IQR, interquartile range.

Table 2. Factors associated with chronic ventilator-dependency at PMA 36 weeks in extremely low birth weight infants

	Ventilator-dependent at PMA 36 weeks (N = 6)	Not ventilator-dependent at PMA 36 weeks (N = 82)	*P* value
Gestational age, week	26^{+0} (1^{+3})	27^{+1} (2^{+1})	0.213
Birth weight, g	792.5 (108.4)	789.2 (141.2)	0.955
Female	2 (33.3)	42 (51.2)	0.676
Multiple births	0 (0.0)	29 (35.4)	0.227
Cesarean section	3 (50.0)	63 (76.8)	0.162
Antenatal corticosteroid use	6 (100.0)	67 (81.7)	0.921
RDS	5 (83.3)	70 (85.4)	0.892
Treatment with macrolides	4 (66.7)	13 (15.9)	0.012
SNI	1 (16.7)	13 (15.9)	0.958
PDA	5 (83.3)	68 (82.9)	0.980
Presence of HCAM	5 (83.3)	33 (40.2)	0.081
Exposure to *Ureaplasma*	5 (83.3)	21 (25.6)	0.008
Coexistence of HCAM and *Ureaplasma*	5 (83.3)	13 (15.9)	0.001

Data are presented as numbers (%) and mean (SD).

Abbreviations: PMA, postmenstrual age; RDS, respiratory distress syndrome; SNI, significant neurologic injury; PDA, patent ductus arteriosus; HCAM, histologic chorioamnionitis; SD, standard deviation;

Table 3. Risk factors associated with chronic ventilator-dependent BPD in logistic regression analysis in extremely low birth weight infants

Risk factors	OR (95% CI)	
	unadjusted	adjusted*
Presence of HCAM	7.4 (0.8 - 66.5)	3.7 (0.3 - 41.1)
Exposure to *Ureaplasma*	14.5 (1.6 - 131.5)	6.6 (0.5 – 90.9)
Coexistence of HCAM and *Ureaplasma*	26.5 (2.9 - 246.1)	14.5 (1.1 - 188.9)

*Adjusted for gestational age, birth weight, sex, the mode of delivery and the treatment with macrolides

Abbreviations: BPD, bronchopulmonary dysplasia; OR, odds ratio; CI, confidence interval; HCAM, histologic chorioamnionitis

DISCUSSION

We have shown that coexistence of HCAM and *Ureaplasma* in ELBW infants results in high chances of chronic ventilator dependency at 36th postmenstrual weeks. The presence of HCAM or *Ureaplasma* alone did not significantly increase the rate of chronic ventilator dependency at 36th postmenstrual weeks in ELBW infants.

Chronic ventilator dependency is a serious problem in preterm infants. Prolonged invasive ventilation results in alveolar distortion and pulmonary microvascular alteration, which are related with increased mortality and long term morbidities [12, 18, 19]. A recent study reported that antenatal exposure to *Ureaplasma* and subsequent prolonged ventilator support increases the risk of moderate to severe BPD [20]. In our study, prolonged ventilator support was the primary outcome variable and whether coexistence of HCAM and *Ureaplasma* increased the risk of prolonged ventilator support beyond PMA 36 weeks was investigated. Results show that prolonged ventilator support increases the risk of severe BPD, but ventilator dependency at PMA 36 weeks, alone, is already a hallmark of very severe BPD.

HCAM has long been investigated as a risk factor for BPD [3, 4]. Currently, the independent association of HCAM with the development of BPD is questioned; although, controversy on this issue remains [5]. The simple associations of HCAM with BPD can be confounded by the microorganisms [21]. Although a majority of HCAMs are thought to be associated with

intrauterine infections, microorganisms are isolated only in approximately 75% of the placentas evident with HCAM [6]. *Ureaplasma* species are the most common pathogen, and are also associated with the severity of HCAM [22]. In our study, 46.5% of HCAM cases had evidence of *Ureaplasma* exposure documented by real-time PCR or culture, and 71.4% of *Ureaplasma*-positive cases had HCAM.

Although there was no consistent protocol for macrolide therapy to prevent BPD during the study period, preterm infants who received macrolides developed chronic ventilator-dependent BPD more frequently than those who did not (66.7% *versus* 15.9%). Meanwhile, a majority of the infants who were given macrolides were positive for *Ureaplasama* (14/17, 82.3%). Thus a trend for a higher occurrence of chronic ventilator-dependent BPD in infants who were given macrolides may have been confounded by the positivity for *Ureaplasma*. The loss of statistical significance of macrolides treatment as an independent risk factor for chronic ventilator dependency in the logistic regression analysis supports this supposition. A recent meta-analysis demonstrated that prophylactic azithromycin prevented BPD contrary to other macrolides [23]. In our study, there were no significant differences in the rate of BPD or chronic ventilator dependency by macrolide type among preterm infants who were given macrolides.

It is not practical to detect *Ureaplasma* in the amniotic fluid via amniocentesis in all mothers. Moreover it is not feasible to detect *Ureaplasma* via tracheal aspiration in preterm infants supported by non-invasive ventilation. We detected *Ureaplasma* from the gastric fluids of newborn

infants, as previously reported [24]. Assuming that the gastric fluid at birth has been in contact with both amniotic and lung fluid, the presence of *Ureaplasma* in the gastric fluid may reflect antenatal exposure of the fetal lung to *Ureaplasma*. In our study, the PCR and culture studies for *Ureaplasma* via amniocentesis were performed in 26 of the 102 enrolled cases. All maternal amniotic fluid and neonatal gastric fluid were consistent in the detection of *Ureaplasma*: All twelve cases positive for *Ureaplasma* in the amniotic fluid were also all positive in the gastric fluid, whereas all 14 cases negative for *Ureaplasma* in the amniotic fluid were also all negative in the gastric fluid. Therefore, detection of *Ureaplasma* from the gastric fluids of newborn infants may be regarded as a feasible and reliable method to examine in-utero exposure to *Ureaplasma* in preterm infants. Furthermore, a combination of PCR and culture used in our study increased the reliability for detecting *Ureaplasma* [25]. As presented in Table 4, PCR was more sensitive than culture study in detecting *Ureaplasma* from the gastric fluids of infants.

Several limitations exist in this study; first, *Ureaplasma* was not directly detected from the placental tissue, and second, there is no long-term data on the respiratory and neurodevelopmental outcomes of our subjects. However, we adopted a feasible and reliable method to identify *Ureaplasma,* which can be an option to replace direct placental isolation. Although long-term outcome data at the corrected age of 18-24 months were unavailable in all subjects, we were able to assess 6 preterm infants at the corrected age of 12 months, who had ventilator dependency at PMA 36 weeks. All six infants had developmental delay in the form of motor, cognitive, or behavior, however

none had definite cerebral palsy. Three infants received tracheostomy and were on home ventilator after discharge. There was no mortality among these 6 infants at the corrected age of 12 months.

We presume that the HCAM-U+ group is at a state of simple colonization with *Ureaplasma* whereas HCAM+U+ group has actual intra-amniotic and/or fetal inflammation provoked by *Ureaplasma*. Demonstrated by the multivariate logistic regression analysis, exposure to *Ureaplama* alone did not reach statistical significance whereas coexistence of HCAM and *Ureaplama* was an independent risk factor for chronic ventilator dependency. Actual inflammation rather than simple colonization would be more injurious to the developing lungs. Our presumption may be relevant in this context. However, as mentioned as a limitation in our study, we did not identify *Ureaplasma* directly from the placentas or umbilical cords; therefore, our presumption needs to be verified in subsequent studies.

Although antenetal exposure of *Ureaplasma* is significantly related to HCAM, the presence of *Ureaplasma* does not always overlap with HCAM. Furthermore, all HCAM is not associated with *Ureaplasma*. These notions may have been the origin of debate on the impact of HCAM on the development of BPD.

In conclusion, our study indicates that coexistence of HCAM and *Ureaplasma* is significantly associated with chronic ventilator dependency in ELBW infants. Larger studies to validate our results and a prospective study to observe long term respiratory and neurodevelopmental outcome are needed.

REFERENCES

1. Jobe AH. Antenatal associations with lung maturation and infection. *J Perinatol*. 2005;25 Suppl 2:S31-5.
2. Speer CP. Inflammation and bronchopulmonary dysplasia: A continuing story. *Semin Fetal Neonatal Med*. 2006;11(5):354-62.
3. Watterberg KL, Demers LM, Scott SM, Murphy S. Chorioamnionitis and early lung inflammation in infants in whom bronchopulmonary dysplasia develops. *Pediatrics*. 1996; 97: 210–215.
4. Speer CP. Chorioamnionitis, Postnatal Factors and Proinflammatory Response in the Pathogenetic Sequence of Bronchopulmonary Dysplasia. *Neonatology*. 2009;95:353–361.
5. Hartling L, Liang Y, Lacaze-Masmonteil T. Chorioamnionitis as a risk factor for bronchopulmonary dysplasia: a systematic review and meta-analysis. *Arch Dis Child Fetal Neonatal Ed*. 2012;97(1):F8-F17.
6. Hermansen MC, Hermansen MG. Perinatal infections and cerebral palsy. *Clin Perinatol*. 2006;33(2):315-33.
7. Waites KB, Katz B, Schelonka RL. Mycoplasmas and ureaplasmas as neonatal pathogens. *Clin Microbiol Rev*. 2005;18(4):757-89.
8. Viscardi RM. Ureaplasma species: role in neonatal morbidities and outcomes. *Arch Dis Child Fetal Neonatal Ed*. 2014;99(1):F87-92.
9. Viscardi RM, Hasday JD. Role of Ureaplasma species in neonatal chronic lung disease: epidemiologic and experimental evidence. *Pediatr Res*.

2009;65(5 Pt 2):84R-90R.

10. Lowe J, Watkins WJ, Edwards MO, et al. Association between pulmonary ureaplasma colonization and bronchopulmonary dysplasia in preterm infants: updated systematic review and meta-analysis. *Pediatr Infect Dis J*. 2014;33(7):697-702.
11. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2001;163:1723-1729.
12. De Paepe ME, Mao Q, Powell J, et al. Growth of pulmonary microvasculature in ventilated preterm infants. *Am J Respir Crit Care Med*. 2006;173(2):204-11.
13. Walsh MC, Morris BH, Wrage LA. Extremely low birthweight neonates with protracted ventilation: mortality and 18-month neurodevelopmental outcomes. *J Pediatr*. 2005;146(6):798-804.
14. Overstreet DW, Jackson JC, van Belle G. Truoq WE. Estimation of mortality risk in chronically ventilated infants with bronchopulmonary dysplasia. *Pediatrics*. 1991;88(6):1153-60
15. Jacobsson B, Aaltonen R, Rantakokko-Jalava K, Morken NH, Alanen A. Quantification of Ureaplasma urealyticum DNA in the amniotic fluid from patients in PTL and pPROM and its relation to inflammatory cytokine levels. *Acta Obstet Gynecol Scand*. 2009;88:63-70.
16. Salafia CM, Weigl C, Silberman L. The prevalence and distribution of acute placental inflammation in uncomplicated term pregnancies. *Obstet Gynecol*. 1989;73:383-389.
17. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing

enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg*. 1978;187:1-7.

18. Thibeault DW, Mabry SM, Ekekezie II, Zhang X, Truog WE. Collagen scaffolding during development and its deformation with chronic lung disease. *Pediatrics*. 2003;111(4 Pt 1):766-76.
19. Apisarnthanarak A, Holzmann-Pazgal G, Hamvas A, Olsen MA, Fraser VJ. Ventilator-associated pneumonia in extremely preterm neonates in a neonatal intensive care unit: characteristics, risk factors, and outcomes. *Pediatrics*. 2003;112(6 Pt 1):1283-9.
20. Inatomi T, Oue S, Ogihara T, et al. Antenatal exposure to Ureaplasma species exacerbates bronchopulmonary dysplasia synergistically with subsequent prolonged mechanical ventilation in preterm infants. *Pediatr Res*. 2012;71(3):267-73.
21. Jobe AH. The new bronchopulmonary dysplasia. *Curr Opin Pediatr*. 2011;23(2):167-72.
22. Namba F, Hasegawa T, Nakayama M, et al. Placental features of chorioamnionitis colonized with Ureaplasma species in preterm delivery. *Pediatr Res*. 2010;67(2):166-72.
23. Nair V, Loganathan P, Soraisham AS. Azithromycin and other macrolides for prevention of bronchopulmonary dysplasia: a systematic review and meta-analysis. *Neonatology*. 2014;106(4):337-47.
24. Oue S, Hiroi M, Ogawa S, et al. Association of gastric fluid microbes at birth with severe bronchopulmonary dysplasia. *Arch Dis Child Fetal Neonatal Ed*. 2009;94(1):F17-22.

25. Gerber S, Vial Y, Hohlfeld P, Witkin SS. Detection of Ureaplasma urealyticum in second-trimester amniotic fluid by polymerase chain reaction correlates with subsequent preterm labor and delivery. *J Infect Dis*. 2003;187(3):518-21.

국문 초록

목적 : 조직학적 융모양막염과 유레아플라즈마는 오랫동안 기관지폐이형성증의 위험인자로 간주되어왔다. 본 연구에서는 초극소저체중출생아에서 조직학적 융모양막염과 유레아플라즈마 동시 존재와 만성기계환기의존적 기관지폐이형성증의 연관성에 대해 확인하고자 하였다.

방법: 본 연구는 후향적 코호트 연구이다. 태반의 조직학적 검사가 시행되었으며, 유레아플라즈마를 검출하기 위해 출생 직후 신생아의 위액을 이용하여 PCR 및 배양검사를 시행하였다. 조직학적 융모양막염과 유레아플라즈마의 존재 여부에 따라 각 군의 임상적 특성들을 비교하였으며, 만성기계환기의존적 기관지폐이형성증의 위험인자를 분석하였다.

결과: 연구에 등록된 102명 중에서 20명 (19.6%)이 조직학적 융모양막염과 유레아플라즈마가 동시 존재한 군이었다. 각 군은 기관지폐이형성증의 발생률을 포함한 임상적 특성에서 유의미한 차이를 보이지 않았으나, 조직학적 융모양막염과 유레아플라즈마가 동시 존재한 군에서 기타 군들에 비해 만성기계환기의존도의 비율이 높았다 (P =0.005). 다중 로지스틱 회귀분석에서 조직학적 융모양막염과 유레아플라즈마의 동시 존재가 만성기계환기의존적 기관지폐이형성증의 유일한 위험인자로 확인되었다

(OR 14.5, CI 1.1 – 188.9) .

결론: 조직학적 융모양막염과 유레아플라즈마의 동시 확인은 초극소저체중출생아에서 만성기계환기의존적 기관지폐이형성증과 연관이 있다 .

주요어: 융모양막염; 유레아플라즈마; 기관지폐이형성증

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