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CLINICAL STUDY

The association between respiratory tract *Ureaplasma urealyticum* colonization and severe retinopathy of prematurity in preterm infants ≤ 1250 g

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Abstract

Aim **To evaluate the association between respiratory tract *Ureaplasma urealyticum (Uu)* colonization and development of retinopathy of prematurity (ROP) requiring treatment.**

Methods **The infants with birthweight (BW) ≤ 1250 g born in a third-level neonatal intensive care unit between March 2009 and May 2010 were prospectively identified. Nasopharyngeal swabs for *Uu* colonization were taken in postnatal first 3 days. Culture-positive patients were reevaluated on the twelfth day by nasopharyngeal swabs for *Uu*. The primary outcome was to define whether there was an association between respiratory tract *Uu* colonization and severe ROP requiring treatment. Independent sample's *t*-test or Mann–Whitney *U*-test was used to compare continuous variables and Chi-square test or Fisher's exact test for categorical variables. Multivariate (backward) logistic regression analysis was performed to simultaneously measure the influence of the independent variables with ROP as the dependent variable.**

Results **A total of 25 (12.1%) infants developed severe ROP requiring treatment among 206 infants who underwent ROP screening. Mean BW and gestational age of total cohort were 1013 ± 159 g and 27.9 ± 1.6 weeks, respectively. Multivariate analysis demonstrated that BW (OR: 0.64 (95% CI 0.47–0.88); $P = 0.006$), duration of mechanical ventilation (OR: 1.17 (95% CI 1.06–1.28); $P = 0.001$), premature rupture of membrane >18 h (OR: 3.83 (95% CI 1.2–12.2); $P = 0.02$), and *Uu* positivity in both cultures (OR: 5.02 (95% CI 1.8–13.9); $P = 0.002$) were independent risk factors for the development of severe ROP requiring treatment.**

Conclusions **Respiratory tract colonization with *Uu* was independently associated with severe ROP requiring treatment.**

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Keywords: retinopathy of prematurity; *Ureaplasma urealyticum*; laser surgery; premature infant.

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Introduction

Retinopathy of prematurity (ROP), a retinal vascular disease of preterm infants, continues to be a major cause of childhood blindness all over the world.[1,2] The structural characteristics of ROP are an initial cessation of retinal vascularization and subsequent oversprouting retinal vessels.[3] It is widely acknowledged that ROP is a multi-factorial disorder, with low gestational age (GA), low birthweight (BW), oxygen exposure, blood transfusion, necrotizing enterocolitis, intraventricular haemorrhage (IVH) being important risk factors.[4–7]

Growing evidence suggests a role for neonatal infection and perinatal inflammation in ROP pathogenesis. However, the exact mechanism and timing of this inflammatory component of ROP pathogenesis is unclear.[8]

There is a need for prospective studies designed to elucidate the role of antenatal and postnatal infection/inflammation in the occurence and progression of ROP.[9,10]

Infection with ureaplasmas may occur in utero or perinatally and leads to an increased risk of perinatal morbidities including pneumonia, bacteraemia, or meningitis.[11,12] The rate of *Ureaplasma urealyticum (Uu)* in preterm infants seems to be high in contrast to term infants. Two recent studies from developed countries reported *Uu* rates in the neonatal intensive care unit (NICU) as 37.5% and 31%, respectively.[13,14] Moreover, for some infants, infection with these organisms triggers a vigorous inflammatory response in the lungs facilitating development of bronchopulmonary dysplasia.[15] Inflammatory processes associated with ureaplasmas might also interfere with normal retinal vascularization in the most vulnerable retinas.

This prospective study was performed to evaluate the association between respiratory tract *Uu* colonization and the development of ROP requiring treatment in preterm infants with BW $\leq$1250 g.

Patients and methods

Preterm infants with BW $\leq$1250 g admitted to the NICU of Zekai Tahir Burak Maternity Teaching Hospital, who were born between May 2009 and December 2010, were prospectively identified for inclusion into the study. Infants with major congenital abnormalities and intrauterine growth retardation (IUGR), lack of parental consent and those who died before development of ROP requiring treatment or full vascularization of the retina were excluded from the study. The study was approved by the hospital ethics committee and written informed consent was obtained from the parents or guardians before enrollment. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

Ophthalmologic screening for ROP was first done between 4 and 6 weeks of age and subsequent follow-up examinations were done once or twice a week, depending on the severity of the retinopathy at the dicscretion of the ophthalmologist. Stage of ROP was defined according to the International Classification of ROP[16] and the infants were all classified by their most highest grade ROP recorded in any examination. Infants were divided into two groups as having no ROP or mild ROP and severe ROP. In this study, mild ROP was defined as the ROP that did not meet the criteria for treatment and severe ROP was defined as one requiring treatment. Treatment criteria were: zone 1 any stage of ROP with plus disease or zone 1 stage 3 without plus and zone 2 stage 2 or 3 with plus disease as defined by the Early Treatment for Retinopathy of Prematurity Cooperative Group.[17]

Nasopharyngeal swabs for *Uu* colonization were taken in postnatal first 3 days and on the twelfth day only in culture-positive infants, transported to the laboratory and cultured for *Uu* immediately in special medium. *Uu* was detected according to the method defined by Biernat-Sudolska *et al*.[18]

Perinatal characteristics including BW, GA, APGAR scores, prenatal steroid use, the presence of premature rupture of membrane (PROM), chorioamnionitis, culture proven sepsis, respiratory distress syndrome, haemodynamically significant patent ductus arteriosus, necrotizing enterocolitis ($\geq$stage 2), IVH (grade 3 or 4), as well as postnatal clinical parameters including transfusion volumes, duration of mechanical ventilation, and O_2 therapy were recorded. The primary outcome of the study was occurence of severe ROP requiring treatment.

Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Independent sample's *t*-test or Mann–Whitney *U*-test was used to compare continuous variables and Chi-square test or Fisher's exact test for categorical variables. Multivariate (backward) logistic regression analysis was performed to simultaneously measure the influence of the independent variables with ROP as the dependent variable. Variables that have a *P*-value <0.25 in the univariate analysis were used in multivarite analysis as possible risk factors. A *P*-value of <0.05 was considered statistically significant.

Results

During the study period, 272 infants with BW $\leq$1250 g were admitted to our NICU. Of the infants, 48 were excluded because of major congenital abnormalities, lack of parental informed consent, IUGR, and death. There were a total of 224 infants who met the eligibility criteria, 150 of whom were culture-negative and 74 were culture-positive for *Uu* colonization in the first 3 days of life. Among the 74 infants who had positive colonization for *Uu* in the first 3 days, 6 died on follow-up, 34 were culture-negative and 34 were culture-positive for *Uu* colonization on the twelfth day of life. In total, 206 infants underwent ophthalmologic screening for ROP. Mean BW and GA of total cohort were 1013 $\pm$ 159 g and 27.9 $\pm$ 1.6 weeks, respectively. In all, 88 (43%) of the infants were $<$1000 g. Severe ROP requiring treatment occured in 25 (12.1%) infants. Infants with severe ROP had significantly lower BW (902 $\pm$ 15 g, $P<0.01$) and GA (27 $\pm$ 1.9 weeks, $P<0.01$) compared with those with mild ROP and no ROP.

Table 1 Univariate analysis of risk factors for the development of severe retinopathy of prematurity

	*No ROP/mild ROP (*n*: 181)*	*Severe ROP (*n*: 25)*	P
Birthweight, mean ± SD (g)	1029 ± 154	902 ± 15	<0.01
Gestational age, mean ± SD (weeks)	28 ± 1.6	27 ± 1.9	<0.01
Male, *n* (%)	73 (40.3)	14 (56)	NS
Caesarean delivery, *n* (%)	139 (76.8)	17 (68)	NS
Prenatal steroid, *n* (%)	130 (71.8)	16 (64)	NS
PROM >18 h, *n* (%)	24 (13.3)	8 (32)	0.01
Chorioamnionitis, *n* (%)	6 (3.3)	0	NS
RDS, *n* (%)	111 (61.3)	17 (68)	NS
PDA, *n* (%)	61 (33.7)	9 (36)	NS
Days on MV, mean ± SD (days)	1.5 ± 3	6.4 ± 8.7	<0.01
Total days on supplemental oxygen, mean ± SD (days)	14 ± 22.1	33.8 ± 35.1	<0.01
Transfusion of red blood cells volume ± SD (ml)	49.2 ± 31.5	54 ± 23.2	NS
Proven sepsis, *n* (%)	70 (38.7)	13 (52)	NS
NEC (≥ stage 2), *n* (%)	20 (11)	3 (12)	NS
IVH (grade 3 or 4), *n* (%)	25 (13.8)	5 (20)	NS
Uu colonization first 3 days of life (first time), *n* (%)	55 (30.4)	13 (52)	0.03
Uu colonization both in the first 3 days and on twelfth day of life, *n* (%)	23 (12.7)	11 (44)	<0.01

Abbreviations: IVH, intraventricular haemorrhage; MV, mechanical ventilation; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; PROM, premature rupture of membrane; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity; *Uu*, *Ureaplasma urealyticum*.

PROM >18 h was more common in infants with severe ROP ($P=0.01$). Number of days on mechanical ventilation (MV) ($P<0.01$) and supplemental O_2 ($P<0.01$) were significantly higher among infants with severe ROP. Positive colonization for *Uu* in the first 3 days ($P=0.03$) and in combination with positivity on the twelfth day of life ($P<0.01$) were found to be significant risk factors for severe ROP. Risk factors for the development of severe ROP are shown in Table 1.

When the risk factors including BW, GA, gender, days on mechanical ventilation and supplemental O2, PROM >18 h, proven sepsis, and positive culture for *Uu* colonization were put into a logistic regression model, BW (OR: 0.64 (95% Cl 0.47–0.88); $P=0.006$), duration of MV (OR: 1.17 (95% Cl 1.06–1.28); $P=0.001$), PROM >18 h (OR: 3.83 (95% Cl 1.2–12.2); $P=0.02$), and *Uu* positivity in both cultures (OR: 5.02 (95% Cl 1.8–13.9); $P=0.002$) were found to be independent risk factors for the development of severe ROP (Table 2).

Table 2 Independent risk factors for severe retinopathy of prematurity

	Adjusted odds ratio (OR)	*95% confidence interval*	P-*value*
Birthweight[a]	0.64	0.47–0.88	0.006
Days on mechanical ventilation[b]	1.17	1.06–1.28	0.001
Premature rupture of membrane[c]	3.83	1.2–12.2	0.02
Positive culture for *Ureaplasma urealyticum*[d]	5.02	1.8–13.9	0.002

[a] OR for every 100 g.
[b] OR for each day on mechanical ventilation.
[c] >18 h.
[d] *Ureaplasma urealyticum* positivity in both cultures.

Discussion

The incidence of ROP in preterm infants has increased worldwide as a consequence of higher preterm survival rates. Several associated risk factors for ROP have still continued to be focus of extensive researches. In addition to varieties in ROP rates in different populations.[19,20] This study is the first that investigates the association between *Uu* colonization and the development of ROP requiring treatment in preterm infants with BW ≤1250 g. Our results showed that repiratory tract *Uu* colonization in addition to BW, duration of MV, and PROM >18 h was independently associated with severe ROP. We demonstrated that persistent positive colonization for *Uu* was significantly associated with more than five-fold increase in the need for laser surgery.

Although immaturity at birth and exposure to supplemental oxygen unquestionably have important aetiological roles in ROP,[21] the possibility that exposure to infectious and inflammatory stimuli contribute to ROP is gradually receiving attention. Mittal *et al*[22] reported that candida sepsis was independently associated with increased severity of ROP and the need for laser surgery in extremely low BW infants. Neonatal sepsis in general is also associated with ROP.[23] Klinger *et al*[24] showed that among very low BW infants, those who had early sepsis were twice as likely to develop severe ROP as their peers who did not have early sepsis. Although we did not seek *Uu* in blood cultures, we suggest that defined association between *Uu* colonization and severe ROP in our cohort deserves further investigation for the role of *Uu* as a septis agent.

Markers of inflammmation appear to be associated with ROP in human studies. Polam *et al*[25] reported an association between histological chorioamnionitis and ROP based on a univariable data analysis. Additionaly, Dammann *et al*[9] showed that both antenatal and neonatal exposure to inflammmation appeared to

conribute to the increased ROP risk in preterm infants. Antenatal infection-associated variables including preterm labour, PROM >12 h, and chorioamnionitis were more common in cases with ROP and were most common among infants with high-grade ROP. Similarly, we found that PROM >18 h, that was suggested a variable of antenatal infection/inflammmation, was independently associated with severe ROP. In a recent study, it was showed that over the first three postnatal weeks, the systemic levels of eight cytokines and chemokines were significantly different in infants with ROP from those in controls.[26]

Ureaplasma colonization has been associated with neonatal morbidities and perinatal death.[12,27,28] The previously proposed ureaplasmal virulence factors include IgA protease, urease, phospholipases A and C, and production of hydrogen peroxide.[29] We speculate that *Uu* could injure developing blood vessels in the retina through the membrane phospholipid degradation, prostoglandin synthesis, and membrane peroxidation, which makes them more vulnerable to develop severe ROP. Additionally, the stimulatory effect of ureaplasma on cytokine release has been confirmed. Li *et al*[30] demonstrated that human macrophages exposed to *Uu* produce tumour necrosis factor-α and interleukin-6. Furthermore, they found that macrophages exposed to *Uu* antigen release vascular endothelial growth factor (VEGF) and intercellular adhesion molecule-1. We therefore suggest that VEGF, involved in pathogenesis of ROP through modulation of angiogenesis[31] may be also responsible for the development of severe ROP in the present study.

In conclusion, for the first time our study's results confirm a strong association between respiratory tract *Uu* colonization and the development of severe ROP in preterm infants with BW $\leq$1250 g. Nonetheless, questions about the association between *Uu* colonization and the outcomes of preterm infants need to be further addressed. Future researches should be planned to clarify the association between *Uu* colonization and the other short and long-term outcomes of preterm infants in the light of this preliminary study.

Summary

What was known before

- Growing evidence suggests a role for neonatal infection and perinatal inflammation in ROP pathogenesis. However, the exact mechanism and timing of this inflammatory component of ROP pathogenesis is unclear.

What this study adds

- For the first time, our study's results confirm a strong association between respiratory tract *Uu* colonization and the development of severe ROP in preterm infants.

Conflict of interest

The authors declare no conflict of interest.

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