Innate Immunity in Mothers and Their Newborn Infants During Preterm Premature Rupture of Membranes

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ABSTRACT

Background: Many aspects of the innate immunity of mothers and their preterm infants in association with preterm premature rupture of membranes (PPROM) are unknown.

Objective: To investigate the complement system and circulating immunoglobulin (Ig) in mothers and their preterm neonates in association with the duration of PPROM.

Design: A convenience sample of 96 mother-infant pairs from singleton vaginal deliveries at gestational age 28 to 37 weeks with PPROM was studied. The total complement functional activity (CH50), components of the classical pathway (C1q-C5), and IgG, IgA, IgM, and IgE levels were measured in maternal, cord, and neonatal blood on day 3 to 4 of life and analyzed with respect to the duration of PPROM: less than 6 hours (n = 39); 6 to 24 hours (n = 38), and more than 24 hours (n = 21).

Results: There was no association between PPROM and complement or Ig level in maternal and cord blood. However, 3 to 4 days after birth, neonates with PPROM more than 24 hours demonstrated significantly higher C5 ($95.7 \pm 4.1 \times 10^{12}$) and IgM ($0.77 \pm 0.14$ g/L) levels.

Conclusions: PPROM of more than 24 hours duration leads to the activation of anti-inflammatory immune reactions in neonates that may be beneficial for the prevention of infection-related morbidity, but could potentially place them at risk for complement-induced organ damage.

INTRODUCTION

Preterm premature rupture of membranes (PPROM) accounts for 25% to 33% of all preterm deliveries,[1,2] and is associated with infection-related neonatal and/or maternal morbidity.[3,4] The histological counterpart of the fetal inflammatory response syndrome (funisitis) is twice as common in PPROM-complicated preterm deliveries as compared to labor with intact membranes.[5] The likelihood of preterm neonatal sepsis increases with the duration of rupture of membranes.[6]

Table 1. Clinical Characteristics of the PPROM Groups (mean ± standard deviation)

PPROM Group	GA (wks)	BW (g)	Apgar		PPROM (h)
			1 min	5 min	
< 6 hours (n = 39)	33.5 ± 2.1	2149 ± 462	5.5 ± 1.3	6.6 ± 1.1	3.0 ± 1.5
6-24 hour (n = 36)	33.7 ± 2.1	2202 ± 395	5.7 ± 1.0	6.8 ± 1.1	10.7 ± 5.2
> 24 hours (n = 21)	32.9 ± 2.0	2098 ± 375	5.5 ± 1.4	6.5 ± 1.0	57.6 ± 37.2

Immunological changes pertaining to the complement system and circulating immunoglobulin (Ig) levels that occur in the mother and her fetus during the latency period of PPROM are not well studied. Prolonged rupture of membranes is associated with the up-regulation of gene expression for complement factors.[7] Nevertheless, the total complement hemolytic activity of classical pathway (CH50) in the maternal and cord blood is unrelated to PPROM.[8,9] There is an association between PPROM of more than 72 hours and increased cord serum levels of IgA and IgM.[10] To date, no study has investigated the complement system and circulating Ig levels in the maternal–fetal-neonatal interface in relationship to the duration of PPROM. We hypothesized that activation of the complement cascade and synthesis of circulating Ig is associated with prolongation of the latency period between the rupture of membranes and preterm delivery. To test this hypothesis we compared total complement functional activity (CH50), the complement components (C1q-C5), and circulating IgG, IgA, IgM, and IgE in the maternal, cord, and infant blood (on day 3 to 4 of life) with respect to the duration of PPROM (< 6 hours, 6-24 hours and > 24 hours).

MATERIALS AND METHODS

We conducted a prospective study using a convenience sample of 96 mothers and their preterm infants with gestational age between 28 and 37 completed weeks as estimated by the maternal menstrual dates and confirmed by the clinical assessment of maturity. After obtaining maternal permission, approximately 1 to 1.5 mL of maternal, cord, and neonatal blood (on day 3 to 4 of life) was collected when other blood tests were ordered by the primary care physician, thereby avoiding additional venopuncture. Within 25 to 30 minutes of collection, the blood was centrifuged and the serum stored at –80˚C until analysis.

Data that was recorded for the mothers and their infants included gestational age (weeks), birth weight (grams), parity (nuliparity, multiparity), mode of delivery (vaginal, cesarean section), complications during pregnancy (genital and urinary tract infections, diabetes, pregnancy induced hypertension, and asthma), intrapartum clinical signs of chorioamnionitis,[11] Apgar score (at 1 and 5 minutes), and the duration of PPROM (hours). The duration of PPROM was determined by the interval between the documented leakage of amniotic fluid and the onset of labor. Additionally, we collected information regarding any complications or treatments in the infants during the first three days of life.

We identified 39 mother-infants pairs with PPROM of less than 6 hours, 38 pairs with PPROM of 6 to 24 hours, and 19 with PPROM of more than 24 hours before the onset of labor. A previously published definition of PPROM was used to categorize the study groups.[12]

The sera obtained from the maternal, cord, and neonatal blood (day 3-4 of life) was tested for total hemolytic activity of the classical pathway (CH50) and functional activity of complement com-

Table 2. Total Hemolytic (CH50) and Complement Components (C1q-C5) Activity of the Classical Pathway, and Circulating IgM, IgA, IgG and IgE in Maternal, Cord, and Neonatal Blood on Day 3-4 of Life Based on Duration of PPROM

		Tested Parameter			
Blood Sample	PPROM	C1X 10^{12}	C2 x 10^{12}	C3 x 10^{12}	C4 x 10^{12}
Maternal	< 6 hours	113.2 ± 11.7	101.3 ± 10.6	136.9 ± 12.8	110.4 ± 14.6
	6-24 hours	113.9 ± 9.7	93.4 ± 6.9	127.4 ± 7.8	100.3 ± 9.9
	> 24 hours	95.5 ± 13.5	94.1 ± 13.2	130.3 ± 17.9	97.4 ± 13.9
	P value	NS	NS	NS	NS
Cord	< 6 hours	80.3 ± 8.4	48.0 ± 5.2	85.0 ± 7.7	81.5 ± 10.1
	6-24 hours	81.0 ± 7.6	53.3 ± 6.6	93.6 ± 9.2	75.7 ± 10.4
	> 24 hours	80.2 ± 11.0	57.6 ± 6.7	86.8 ± 7.9	90.7 ± 15.5
	P value	NS	NS	NS	NS
Neonatal Day 3-4	< 6 hours	82.8 ± 6.1	69.9 ± 4.5	99.1 ± 7.6	87.7 ± 8.8
	6-24 hours	80.3 ± 7.6	60.3 ± 7.2	97.9 ± 8.2	76.6 ± 9.6
	> 24 hours	93.7 ± 7.2	84.1 ± 8.7	109.6 ± 8.9	89.6 ± 7.5
	P value	NS	NS	NS	NS

ponents (C1q-C5), and circulating IgM, IgA, IgG, and IgE. The CH50 and C1q-C5 was measured using specific antibody/hemolysin coated sheep erythrocytes.[13,14,15] The CH50 was expressed as the titer of CH50 U/mL that was a reciprocal of the dilution of complement, which lysed 50% of the sheep erythrocytes (U/mL). Activity of C1q-C5 was expressed by the quantitative determination of the number of effective molecules/mL (x 10^{12}) of the complement components in the human serum. This was estimated by multiplication of the number of erythrocytes with the number of lyzed erythrocytes using the equation $z = -ln\ (1-y)$, where y was the number of lyzed erythrocytes. The concentration of the three major classes of IgA, IgM, and IgG was determined using commercially available monospecific antiserum by the single radial immunoassay method.[16] The ABBOTT solid-phase enzyme immunoassay was used to measure total serum IgE in human serum and plasma (IU/mL) in dynamic range of 0.5 to 200 IU/mL.[17]

Statistical analysis was performed using "STATISTICA" software (Statistica 6.0 for Windows, Stat Soft, Inc.). Continuous data was presented as mean ± standard deviation. One-way ANOVA with post-hoc comparisons and multiple regressions were used to analyze the data. Two-sided P value < 0.05 was considered statistically significant.

RESULTS

All preterm neonates included in this study were singleton, born vaginally without asphyxia, and were appropriate for gestational age. The study groups were comparable by gestational age, birth weight, and Apgar scores at 1 and 5 minutes (Table 1). Genital and/or urinary tract infection during pregnancy was diagnosed in 16 mothers (41.0%) with PPROM of less than 6 hours, 16 (42.1%) with PPROM of 6 to 24 hours, and 8 (42.1%) with PPROM of more than 24 hours. None of the mothers had a history of asthma, diabetes, or hypertension. The clinical presentation of chorioamnionitis (ie, maternal fever associated with fetal or maternal tachycardia, uterine tenderness, or foul-smelling amniotic fluid) was recorded in three mothers with rupture of mem-

C5 x 10^{12}	CH50 U/mL	IgMg/L	IgGg/L	IgAg/L	IgEIU/mL
97.4 ± 5.9	136.1 ± 6.8	1.11 ± 0.13	10.3 ± 0.72	1.3 ± 0.14	33.6 ± 4.4
94.2 ± 5.3	126.9 ± 7.8	1.13 ± 0.13	9.8 ± 0.69	0.99 ± 0.10	36.2 ± 5.8
82.2 ± 7.1	119.4 ± 10.7	1.17 ± 0.18	10.9 ± 1.03	0.98 ± 0.10	29.5 ± 6.2
NS	NS	NS	NS	NS	NS
68.5 ± 4.6	81.9 ± 8.0	0.29 ± 0.03	7.5 ± 0.47	0.46 ± 0.20	1.8 ± 0.38
72.8 ± 5.4	72.9 ± 8.4	0.25 ± 0.03	8.4 ± 0.77	0.27 ± 0.04	1.7 ± 0.39
71.2 ± 5.8	71.6 ± 9.8	0.21 ± 0.03	7.0 ± 0.38	0.28 ± 0.05	1.6 ± 0.47
NS	NS	NS	NS	NS	NS
82.6 ± 4.6	87.2 ± 4.9	0.41 ± 0.04	7.0 ± 0.37	0.46 ± 0.04	3.3 ± 0.62
71.2 ± 4.5	72.3 ± 5.9	0.49 ± 0.09	6.9 ± 0.32	0.59 ±0.08	2.9 ± 0.55
95.7 ± 4.1	88.7 ± 9.6	0.77 ± 0.14	6.8 ± 0.57	0.57 ± 0.09	4.5 ± 1.09
0.01	NS	0.02	NS	NS	NS

branes for more than 24 hours (14.3%). After birth, the majority of neonates in each PPROM group (74.4%, 71.1%, and 73.7%, respectively) received antibiotic prophylactic therapy and none developed signs or symptoms of sepsis.

There was no significant variation in the complement components and Ig level in the maternal and cord blood in relation to PPROM (Table 2). However, on day 3 to 4 of life, the neonates with PPROM of more than 24 hours had significantly increased number of effective molecules of C5 and elevated IgM levels. The variation in the gestational age-adjusted C5 and IgM values in the neonatal blood (day 3 to 4 of life) was significantly associated with the duration of PPROM (C5 $\beta = 0.241 \pm 0.110$, $P = 0.024$ and IgM $\beta = 0.245 \pm 0.111$, $P = 0.023$). The regression analysis-derived predictive values of C5 and IgM showed significant and similar correlation with the duration of PPROM (Figures 1 and 2).

DISCUSSION

We found that PPROM of more than 24 hours was associated with increased C5 and IgM levels in neonatal blood on day 3 to 4 of life. However, the duration of PPROM was not associated with changes in complement activity or Ig level in the maternal and cord blood. Therefore, it is not surprisingly that previous studies did not find an association between PPROM duration and CH50 in cord blood.[8,9] Our study was unable to confirm the previously reported association between PPROM of more than 72 hours and increased levels of IgA and IgM in the cord blood,[10] perhaps due to the small sample size. However, our findings of increased IgM level on day 3 to 4 of life in preterm infants with PPROM of more than 24 hours indicates an immunological response to an antigenic stimulus. The ability of preterm infants to synthesize IgM antibodies in response to intrauterine infection has been previously reported.[18,19] The IgM antibodies are capable of activating the complement cascade,[20] which could be of relevance to our findings of increased C5 levels. The complement system is activated directly by bacterial endotoxin[21] and other factors such as lactic acidosis, especially in neonates.[22]

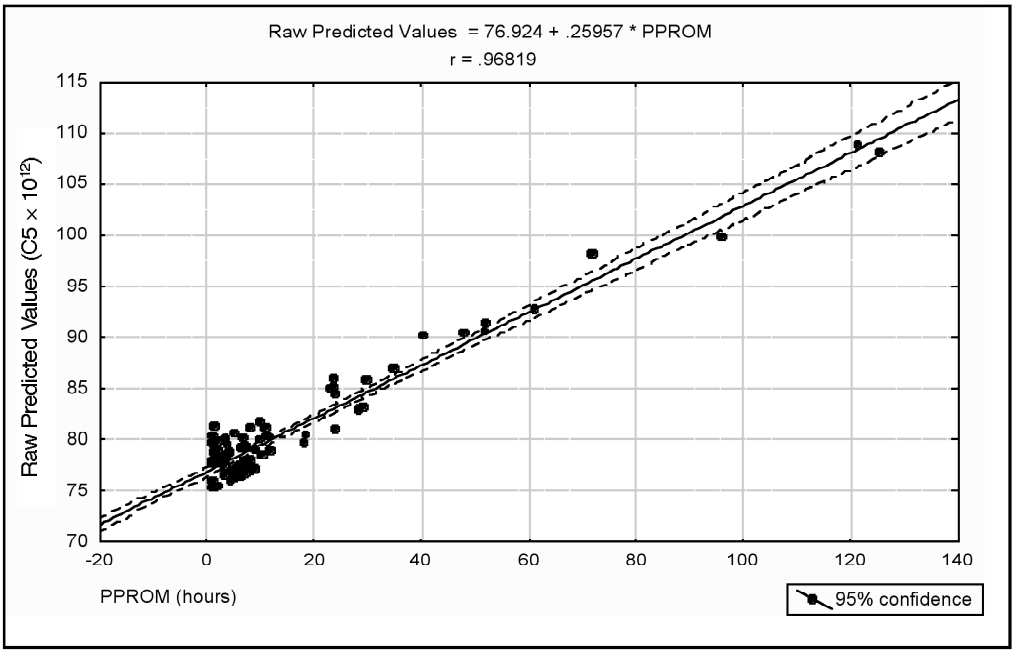

Figure 1. Predictive ability of PPROM for the estimation of C5 in neonatal blood.

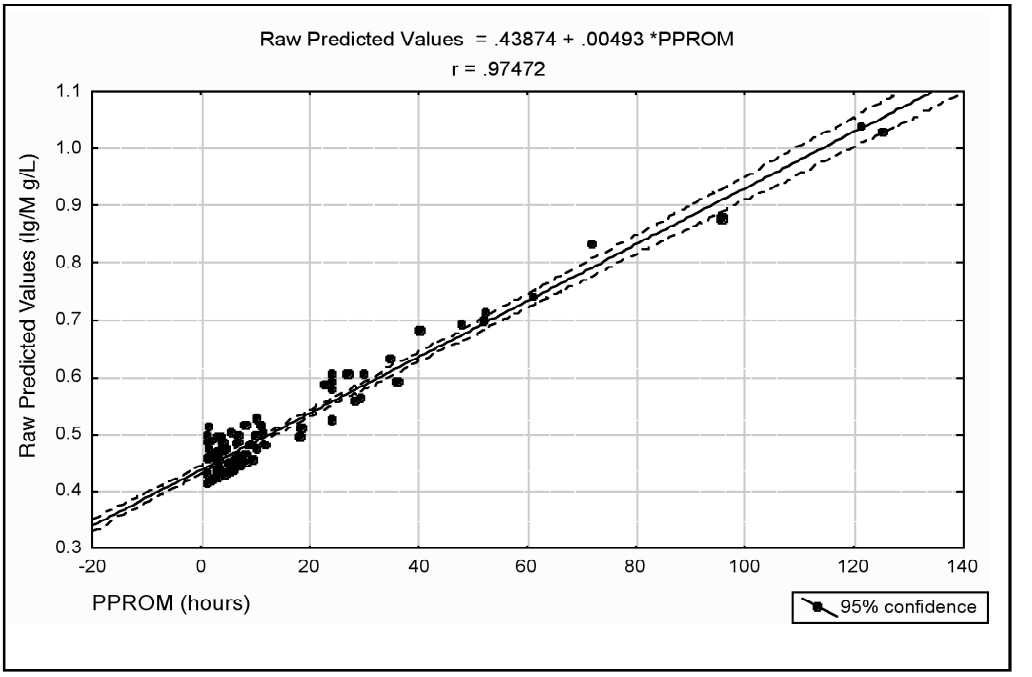

Figure 2. Predictive ability of PPROM for the estimation of IgM in neonatal blood.

The increased numbers of effective molecules of C5 in neonatal blood represent the terminal pathway of complement activation, which may be important for the recruitment of polymorphonuclear leukocyte and bacterial cell lysis.[23,24,25]

PPROM of more than 24 hours leads to the activation of anti-inflamma-

tory immune reactions in preterm neonates that protect against infection-related morbidity, but could potentially place them at risk for complement-mediated tissue damage[27,28] due to their poor capacity to control complement activation.[26] We speculated that the serious morbidity in preterm neonates exposed to PPROM [3,29] may caused in part by uncontrolled complement activation rather than as a consequence of direct bacterial invasion, and certainly merits further research.

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