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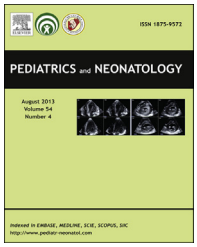

ORIGINAL ARTICLE

Histological Chorioamnionitis: Effects on Premature Delivery and Neonatal Prognosis

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Key Words
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Background: Chorioamnionitis is closely related to premature birth and has negative effects on neonatal morbidity and mortality.
Methods: In this prospective study, 43 mothers who delivered earlier than 35 gestational weeks and their 57 infants were evaluated clinically and with laboratory findings. Placentas and umbilical cords were investigated histopathologically for chorioamnionitis and funisitis.
Results: The overall frequency of clinical and histological chorioamnionitis (HCA) was 8.3% and 23.2%, respectively. The frequency of HCA was 47.3% and 83.3% in mothers delivered <32 weeks and <30 weeks, respectively. Maternal demographic and clinical findings and also leukocyte and C-reactive protein values were not indicative of HCA. Infants of mothers with HCA had significantly lower Apgar scores together with higher SNAP-PE-II and CRIB scores. These infants had increased mechanical ventilator and surfactant requirements, higher incidences of patent ductus arteriosus, early sepsis, and bronchopulmonary dysplasia, and higher mortality rates. The effect of HCA on neonatal morbidity and mortality was more prominent than the effect of low birthweight and lower gestational age.
Conclusion: Chorioamnionitis not only causes premature deliveries, but is also associated with neonatal complications and increased mortality. Clinical findings and infectious markers in mother or infant do not predict the diagnosis of histological chorioamnionitis.

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Therefore, placental histopathology may have a role in predicting neonatal outcome in premature deliveries, especially those below 30 weeks.

1. Introduction

Chorioamnionitis (CA)—infection of the uterine cavity, fetal membranes, and placenta—is seen in 2–4% of term deliveries and in 25% of premature deliveries. Bacterial invasion of the placenta generally results in abortus in early pregnancy. The presence of infection could trigger premature delivery at the end of the second trimester. However, due to the recognition and eradication of bacterial antigens by the maternal immune system, the frequency of CA decreases in the final phases of pregnancy, and CA is more frequent in premature deliveries below 30 weeks.[1]

Premature rupture of membranes (PROM) refers to rupture of membranes (ROM) prior to the onset of labor but beyond 37 weeks' gestation. ROM prior to 37 weeks' gestation is called preterm PROM (PPROM). PPROM is responsible for one-third of premature deliveries.[2] The incidence of CA increases up to 48% with PPROM.[3] Prolonged ROM is any ROM that persists for more than 24 hours prior to the onset of labor; it increases neonatal sepsis incidence 2–10-fold because of the facilitated transmission of infectious agents to the uterine cavity via ascending pathways, and this risk becomes four times higher when ROM is accompanied by CA.[4] CA has been proposed to affect neonatal outcome adversely by increasing the risk of bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), patent ductus arteriosus (PDA), neonatal sepsis, and neurodevelopmental sequelae.[5,6]

The reported incidence and possible effects of CA vary widely among studies. These variations may be due to several factors including the different prevalences of risk factors in the studied populations, the use of different diagnostic criteria, and variations in obstetric practices. CA may be grouped as clinical or subclinical CA (CCA) and histological CA (HCA) based on the presence or absence of clinical signs and laboratory confirmation. The inflammation of the chorioamnion (HCA) and umbilical cord (funisitis) are the manifestations of the maternal and fetal immune responses in case of intra-amniotic infection.[7,8]

Histological and microbiological evidence of inflammation may not always accompany each other or clinical signs in the mother. The frequency of HCA is higher than that of CCA with positive bacterial cultures. The administration of antibiotics and difficulty of growing fastidious organisms may result in negative cultures even in the presence of histological evidence of placental inflammation. For this reason, pathological evaluation of the placenta is essential for diagnosis.

Our study was conducted to show the effects of HCA on neonatal outcome in preterm infants and to determine whether it can be foreseen using clinical findings and laboratory tests in mothers and newborns.

2. Methods

The study was prospectively performed in the Medical Faculty of Ege University between October 2004 and May 2005 after approval from the Local Ethics Committee. Informed consent was obtained from every mother recruited to the study.

2.1. Patients

Forty-three mothers who had delivered earlier than 35 weeks and their 57 newborn infants were recruited. Pregnant women with genital pathology (uterine anomaly or cervical incompetence), chronic diseases (i.e., renal, cardiac, hepatic, or endocrinological) or pregnancy problems (i.e., eclampsia, HELLP syndrome, or rhesus/ABO incompatibility) that could lead to preterm delivery were excluded from the study. Detailed maternal histories including previous premature delivery, infertility treatment, diabetes, fever during labor, vaginal discharge with foul odor, and uterine sensitivity were taken. The socioeconomic status of the pregnant women was evaluated using the Hollingshead score.[9]

2.2. Diagnosis of CA

2.2.1. Clinical diagnosis of CA

Levels of the acute-phase marker C-reactive protein (CRP) and complete blood counts during labor were determined for all mothers. CCA was diagnosed based on the presence of a fever of 38.3°C or higher during labor as well as the presence of at least two of the following criteria: leukocytosis ($>18{,}000/mm^3$), vaginal discharge with foul odor, uterine sensitivity, and tachycardia (maternal >100/min, fetal >180/min).

2.2.2. Histological diagnosis of CA

Placentas and umbilical cords were evaluated by the same pathologist. HCA was defined as the infiltration of the amnion, chorion, and parietal deciduas with maternal neutrophils. Placentas were fixed in formalin for at least 24 hours; subsequently, a minimum of four samples were taken from each placenta (two from the maternal and two from the fetal side, as well as additional samples from regions determined by macroscopic evaluation of the placenta). In addition, samples were obtained from four different parts of the umbilical cord and placental membranes. These samples were treated with alcohol–xylene and paraffin and thus transformed into paraffin blocks. Sections of 3 μm were cut using a microtome device. These were stained with hematoxylin–eosin and examined under

a light microscope for histological signs of neutrophil infiltration and CA.

2.3. Neonatal evaluation

For infants, the following data were recorded: gestational age, sex, birthweight, height, head circumference, mode of delivery, intrauterine growth retardation (IUGR), CRIB (Clinical Risk Index for Babies),[10] SNAP-PE-II (Score for Neonatal Acute Physiology—Perinatal Extension-II),[11] and Töllner sepsis scores,[12] admission to the neonatal intensive care unit (NICU), early and late sepsis, mechanical ventilator (MV) requirement, and the occurrence of BPD, IVH, NEC, and mortality in the neonatal period. Infants with a deteriorating general condition and a Töllner sepsis score over 5 were accepted as cases of suspected sepsis, and antibiotic treatment was started.[12] Definitive cases of sepsis were those infants with positive blood cultures. The sepsis cases in the first 7 days were defined as early sepsis, and sepsis cases after the seventh day were accepted as late sepsis. Infants who still needed oxygen support after the 36th postconceptional week were diagnosed with BPD.[13] Diagnosis and grading of IVH was done by the same radiologist with serial transfontanel ultrasonography examinations using Toshiba Nemio 20 (Tokyo, Japan) ultrasound.[14] Bell's criteria were used for the diagnosis of NEC.[15]

2.4. Statistical analysis

All the data obtained were evaluated using SPSS (IBM, Chicago, Illionis, USA) Windows 14.0 statistical software. The relationship between pairwise ordinal variables was analyzed by Chi-square and Fisher's exact tests. Differences between numeric variables in pairwise groups were investigated by the Student *t* test, while one-way analysis of variance was used to investigate the difference between numeric variables in multiple groups. Correlation between numeric variables was examined by Pearson correlation score. Statistical significance was set to $p < 0.05$.

3. Results

3.1. Maternal characteristics

Demographic and clinical characteristics and the risk factors of the mothers are given in Table 1. Twenty-seven out of 43 mothers involved in this study had singleton pregnancies, 15 mothers had twin pregnancies, and one mother had a triplet pregnancy. Only one twin was included in the study from the three twin pregnancies due to intrauterine loss of the other twin mate. The mean age of healthy mothers (30.1 ± 4.7 years) was similar to the mean age of the mothers who had HCA (30.1 ± 6.9 years; $p = 0.81$). A good socioeconomic status was reported in 66.7% of the healthy group and 50% of the HCA group ($p = 0.45$). There was no patient with a low socioeconomic status in the study group, and the frequency of HCA was not found to be related to socioeconomic status.

3.2. Incidence of HCA

HCA was detected in 10 women who delivered before 35 weeks (23.25%). It was detected in 47.3% of mothers who

Table 1 Demographic and clinical characteristics and risk factors of mothers.

	All mothers ($n = 43$)	Healthy mothers ($n = 33$)	Mothers with HCA ($n = 10$)	p*
Maternal age (years)	30.2 ± 5.2	30.1 ± 4.7	30.1 ± 6.9	0.81
Gestational age (weeks)	30.8 ± 2.9	32.0 ± 2.0	27.5 ± 2.5	<0.01
Socioeconomic status				
Good	27 (62.8%)	22 (66.7%)	5 (50%)	0.45
Middle	16 (37.2%)	11 (33.3%)	5 (50%)	
Multiple pregnancy	16 (37.2%)	14 (42.2%)	2 (20%)	0.26
Infertility	15 (34.8%)	12 (36.3%)	3 (30%)	0.72
History of premature birth	2 (4.6%)	1 (3%)	1 (10%)	0.43
Pre-eclampsia	4 (9.3%)	3 (9%)	1(10%)	1.00
Gestational diabetes	7 (16.2%)	7 (21%)	—	—
Pyuria	18 (41.8%)	12 (36.3%)	6 (60%)	0.46
Bacterial vaginosis	9 (20.9%)	7 (21.2%)	2 (20%)	1.00
Antenatal steroid	28 (65%)	23 (69.6%)	5 (50%)	0.41
Tocolysis	29 (67.4%)	23 (69.6%)	6 (60%)	0.71
Antibiotics use	16 (37.2%)	11 (33.3%)	5 (50%)	0.50
PPROM	12 (27.9%)	9 (27.2%)	3 (30%)	1.00
Antibiotics use at PPROM	9 (75%)	6 (66.7%)	3 (100%)	0.509
Diagnosis of clinical chorioamnionitis	2 (4.6%)	1 (3%)	1 (10%)	0.433
White blood cell count (/mm^3)	13,710 ± 440	13,710 ± 664	13,730 ± 425	0.844
C-reactive protein (mg/dL)	2.9 ± 1.8	2.4 ± 1.2	4.4 ± 3.0	0.114

Data are presented as mean ± standard deviation or *n* (%) as appropriate.
*p values for healthy mothers and mothers with HCA.
HCA = histological chorioamnionitis; PPROM = preterm premature rupture of membranes.

delivered before 32 weeks and in 83.3% of mothers who delivered before 30 weeks ($p < 0.01$). The mean gestational age was 30.8 ± 2.9 weeks for all mothers in this study group, but 32.0 ± 2.0 weeks for mothers without HCA and 27.5 ± 2.5 weeks in HCA group ($p < 0.01$). HCA was detected in one of the two women who were considered to have CCA.

3.3. Perinatal variables and HCA relationship

Mothers with or without HCA were not significantly different when compared for age, incidence of multiple pregnancy, PPROM, history of previous premature delivery, pre-eclampsia, gestational diabetes, pyuria, bacterial vaginosis (BV), administration of antenatal steroids, tocolytics, and antibiotics, diagnosis of CCA, leukocyte count, and CRP values ($p > 0.05$) (Table 1). The mean duration of PPROM was 66.1 ± 42.0 hours in the mothers ($n = 9$) who had PPROM without HCA, and 192.0 ± 168.1 hours in the PPROM with HCA group ($n = 3$; $p = 0.94$). PPROM lasting longer than 72 hours was detected in 100% of mothers with HCA and in 44.4% of mothers without HCA ($p = 0.20$). Tocolysis was administered to 67.4% of all mothers, of whom 60% were detected to have HCA. The mean duration of tocolysis was 15.4 ± 18.1 days for the healthy group and 8.6 ± 15.3 days for HCA group ($p = 0.59$). The frequency of prenatal antibiotic administration was 37.2% in the entire study group and 50% in the HCA group; antibiotic treatment was given to 66.7% of cases with PPROM without HCA and to 100% of cases with PPROM and HCA ($p = 0.509$).

3.4. Neonatal characteristics

The demographic, clinical, and laboratory findings for the infants are given in Table 2. A total of 57 babies were included in the evaluations. All infants were delivered by cesarean section. Forty-five (78.94%) out of 57 infants (30.8 ± 2.9 weeks, 1675.27 ± 584.28 g, for all infants) were delivered by healthy mothers, and 12 (21.06 %) were delivered by mothers with HCA. Preterm deliveries earlier than 30 weeks were observed in 83.3% of mothers with HCA.

Infants born to healthy mothers or to mothers with HCA showed no significant differences in terms of duration of hospitalization in NICU, multiple pregnancy rate, IUGR, sex, oxygen use, late sepsis, NEC, IVH, cord blood CRP values, hemoglobin values, and platelet counts. However, the mean gestational age was significantly lower, and the early sepsis rate, need for and duration of MV, surfactant use, BPD, PDA, and neonatal mortality rates were significantly higher in infants delivered by mothers with HCA, although funisitis was not detected in any of the infants (Table 2). Significantly lower 1- and 5-minute Apgar scores and higher

Table 2 The clinical and laboratory features of infants.

	All infants ($n = 57$)	Infants of healthy mothers ($n = 45$)	Infants of mothers with HCA ($n = 12$)	p*
Gestational age (wk)	30.8 ± 2.9	32.0 ± 2.0	27.5 ± 2.5	<0.01
Mean birthweight (g)	1675.27 ± 584.28	1849.07 ± 522.73	1073.63 ± 307.64	0.04
Hospitalization in NICU	45 (78.9%)	33 (73%)	12 (100%)	0.97
IUGR	4 (7%)	3 (6.6%)	1 (8.3%)	0.87
Multiple pregnancies	33 (57.8%)	28 (62.2%)	5 (41.6%)	0.11
Male sex	26 (45.6%)	20 (44.4%)	6 (50%)	0.83
Early sepsis	32 (56.1%)	22 (48.8%)	10 (83.3%)	0.01
Late sepsis	8 (14%)	5 (11.1%)	3 (25%)	0.52
Mechanical ventilator treatment	19 (33.3%)	9 (20%)	10 (83.3%)	<0.01
Mechanical ventilation duration (days)	3.0 ± 1.0	1.3 ± 0.5	9.4 ± 1.4	<0.01
Surfactant therapy	18 (31.2%)	9 (20%)	9 (75%)	<0.01
Use of oxygen	23 (40.3%)	15 (33.3%)	8 (66.6%)	0.07
Bronchopulmonary dysplasia	8 (14%)	2 (4.4%)	6 (50%)	<0.01
Patent ductus arteriosus	7 (12.2%)	2 (4.4%)	5 (41.6%)	0.03
Necrotizing enterocolitis	1 (1.7%)	—	1 (8.3%)	0.21
Intraventricular hemorrhage	7 (12.2%)	4 (8.8%)	3 (25%)	0.14
Neonatal mortality	9 (15.7%)	2 (4.4%)	7 (58.3%)	<0.01
Apgar 1 min	6.35 ± 1.5	6.84 ± 1.4	4.5 ± 1.1	0.02
Apgar 5 min	8.71 ± 1.1	9.09 ± 0.2	7.3 ± 1.0	0.01
SNAP-PE II score	6.5 ± 3.3	2.4 ± 0,8	21.4 ± 4.5	<0.01
CRIB score	5.13 ± 3.2	3.72 ± 0.9	10.1 ± 5.3	<0.01
White blood cell count (/mm^3)	10,110.2 ± 2110.8	9,870.0 ± 3667.1	11,000.9 ± 4473.3	0.58
Hemoglobin (g/dL)	15.0 ± 3.3	15.1 ± 2.9	14.4 ± 3.5	0.23
Platelets (/mm^3)	242,000 ± 71,080	247,000 ± 74,510	224,000 ± 90,120	0.67
C-reactive protein (mg/dL)	0.48 ± 0.42	0.37 ± 0.30	0,98 ± 0.91	0.99

Data are presented as mean ± standard deviation or n (%) as appropriate.
*p values for infants of healthy mothers and of mothers with histological chorioamnionitis.
HCA = histological chorioamnionitis; IUGR = intrauterine growth retardation; NICU = neonatal intensive care unit.

CRIB and SNAP-PE II scores showing the increased severity of clinical problems were observed in the preterm infants of mothers with HCA. In fact, the frequency of early sepsis rose to be as high as 83.3% (10/12) in infants born to mothers with HCA, compared to 48.8% (22/45) in the healthy mothers group ($p = 0.01$).

3.5. Antenatal factors related to HCA and neonatal morbidity

The effects of antenatal steroids, antibiotics, and PPROM are summarized in Table 3. A full dose of antenatal steroids was given to 50% of the HCA group and 69.6% of the healthy group. Antenatal steroids had no effect on surfactant and oxygen needs, IVH, PDA, early sepsis frequency, or development of BPD.

Steroid administration was not related to the incidence of HCA, since HCA was detected in 33% versus 17% of the mothers with and without use of antenatal steroids, respectively ($p = 0.41$). The duration of MV (2.0 ± 2.2 vs. 5.7 ± 4.9 days) and oxygen treatment (3.2 ± 3.7 vs. 6.6 ± 6.2 days) were similar with or without antenatal steroids ($p = 0.19$ and $p = 0.25$, respectively) (not shown in Table 3).

Antenatal antibiotic use had no significant effect on respiratory distress syndrome (RDS) development, oxygen need, development of early neonatal sepsis, or PDA, IVH, and BPD development, while the surfactant requirement was significantly higher in the infants whose mothers had received antibiotics (Table 3).

Early neonatal morbidity, in terms of surfactant and oxygen requirement, frequency of IVH, PDA, early sepsis, and BPD, was not affected by the presence of PPROM in the mother (Table 3).

We explored the relationship between HCA, antenatal steroid use and RDS. RDS was diagnosed in four out of five infants (80%) born to mothers with HCA, but without antenatal steroid administration. Among the nine infants delivered to healthy mothers who did not receive antenatal steroids, only one infant (11.1%) had RDS. HCA increased the risk of RDS in the absence of antenatal steroid use ($p = 0.02$). In total, seven infants were delivered from mothers with HCA after antenatal steroid administration, and all had RDS. Among the 36 infants born to healthy mothers who received antenatal steroids, only eight infants (22.2%) showed RDS. HCA also increased the frequency of RDS in the presence of antenatal steroids, but this result did not reach statistical significance ($p = 0.31$).

Multiple regression analysis for the evaluation of multiple risk factors for neonatal mortality and morbidity could not be computed due to the low number of patients in the subgroups. Therefore, we determined gestational age and birthweight cut-off values of 28 weeks and 1000 g, and explored the effects of HCA in these high-risk groups. In order to evaluate the importance of low birthweight compared with the presence of HCA, 12 infants born to mothers with HCA were compared for prematurity complications classifying for birthweights over or below 1000 g (ELBW). Surfactant use, MV duration, and oxygen requirement, as well as the incidences of PDA, IVH, NEC, and BPD, and mortality rates, were similar in both groups, which showed that the effects of maternal HCA are more prominent than being born ELBW (Table 4). Similarly, when infants born to mothers with HCA were grouped as below and over 28 weeks of gestation, the frequencies of these neonatal morbidities and mortality rates were similar in these two groups (Table 5).

4. Discussion

Intrauterine infection is one of the frequent causes of preterm birth.[16] Prostaglandins that stimulate uterine contractility are thought to be produced by the microbial endotoxins and proinflammatory cytokines.[16–18] Intrauterine infection/inflammation not only causes preterm labor, but is also associated with PROM and decreased response to tocolytics.[19,20] In addition, exposure to intrauterine inflammation has a negative impact on the morbidity and mortality of the prematurely born infant.

The incidence of HCA is reported as 60—80%, 40—50%, and 5—30% in deliveries at gestations less than 28 weeks, between 29 and 34 weeks, and greater than 34 weeks, respectively.[21] In the present study, HCA rate was also found to be inversely related to week of delivery. This rate was especially noteworthy in deliveries below 30 weeks, at 83.3%. With regard to deliveries below 35 weeks, mothers without HCA gave birth at the 31st gestational week on average, while the delivery week decreased to 27 weeks in the HCA group (Table 1).

CCA was suspected in only two infants, of whom only one was proven to have HCA. Therefore, clinical findings are not

Table 3 The effects of antenatal steroids, antenatal antibiotics and preterm premature rupture of membranes (PPROM) on neonatal prognosis.

	AS(+) ($N = 43$)	AS(−) ($N = 14$)	p	Aab(+) ($N = 14$)	Aab(−) ($N = 43$)	p	PPROM(+) ($N = 16$)	PPROM(−) ($N = 41$)	p
	n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
Early sepsis	23 (53.4)	9 (64.2)	0.52	12 (85.7)	20 (46.5)	0.09	14 (87.5)	18 (43.9)	0.07
Surfactant therapy	14 (32.5)	4 (28.5)	1.00	8 (57.1)	10 (23.2)	0.03	8 (50)	10 (24.3)	0.11
Oxygen requirement	18 (41.8)	5 (35.7)	0.74	7 (50)	16 (37.2)	0.36	8 (50)	15 (36.5)	0.52
Bronchopulmonay dysplasia	4 (9.3)	4 (28.3)	0.17	3 (21.4)	5 (11.6)	0.24	3 (18.7)	5 (12.2)	1.00
Patent ductus arteriosis	5 (11.6)	2 (14.2)	1.00	2 (14.2)	5 (11.6)	0.68	2 (12.5)	5 (12.2)	1.00
Intraventricular hemorrhage	5 (11.6)	2 (14.2)	1.00	2 (14.2)	5 (11.6)	0.68	2 (12.5)	5 (12.2)	1.00

Aab = antenatal antibiotics; AS = antenatal steroids.

Table 4 Assesment of extremely low birthweight (ELBW) and larger preterm infants who are born from mothers with histological chorioamnionitis (HCA).

	Infants of mothers with HCA (n = 12)	HCA(+) <1000 g (n = 6)	HCA(+) ≥1000 g (n = 6)	p*
Mean birthweight (g)	1073.63 ± 307.64	821.6 ± 141.9	1283.3 ± 230.3	0.04
Mean gestational age (wk)	27.58 ± 2.53	26.50 ± 2.66	28.67 ± 2.06	0.147
Mechanical ventilation duration (d)	9.4 ± 1.4	13.6 ± 9.8	6.0 ± 4.9	0.17
Surfactant therapy	9 (75%)	5 (83.3%)	4 (66.6%)	0.48
Oxygen duration (d)	13.8 ± 9.8	20.0 ± 9.6	9.0 ± 7.4	0.08
Bronchopulmonary dysplasia	6 (50%)	3 (50%)	3 (50%)	0.65
Patent ductus arteriosus	5 (41.6%)	2 (33.3%)	3 (50%)	0.75
Necrotizing enterocolitis	1 (8.3%)	—	1 (16.7%)	0.36
Intraventricular hemorrhage	3 (25%)	1 (16.7%)	2 (33.3%)	0.56
Neonatal mortality	7 (58.3%)	5 (83.3%)	3 (50%)	0.52

Data are presented as mean ± standard deviation or n (%) as appropriate.
*p values for birthweight <1000 g and ≥1000 g infants of mothers with HCA.

sensitive for the diagnosis of CA. Maternal blood leukocyte count, CRP and IL-6 values, and glucose and cytokines in the amniotic fluid are biochemical parameters suggested for the diagnosis of CA, but their use in clinical evaluation is quite limited. Sensitivity of maternal CRP value for intrauterine infection was found to be 27%, with a specificity of 80%, and maternal serum CRP level is not affected by antenatal steroids.[22,23] Yoon et al reported that maternal CRP and leukocyte count were significantly higher in CA, but amniotic fluid leukocyte count was more sensitive.[24] The results of the present study demonstrated that intrapartum leukocyte counts and CRP values are not indicative of CA, showing the importance of amnion fluid culture and histological evaluation of the placenta (Table 1).

Placental infection is mostly related to PPROM in pregnancy, and BV is known to cause PPROM.[25] BV is observed in 15–40% of pregnancies and is related to a lack of prenatal care being more commonly observed in pregnant women at younger ages, who are single and of low socioeconomic status.[26,27] Since vaginal cultures were not taken regularly, foul odorous vaginal discharge was evaluated as BV in around 20% of both the HCA and healthy groups. Although these numbers are close to the rates reported in the literature, this diagnostic limitation might have caused the failure to show the relation between BV and the presence of HCA (Table 1). We also could not detect any significant relationship between the socioeconomic status of the mothers and HCA in our study (Table 1).

PROM is observed in 2–3.5% of term but in up to 30–40% of preterm deliveries.[2, 28] PPROM either forms an entrance route for infectious factors to reach the sterile amniotic cavity, or is caused by a primary subclinical infection. Both opinions support the view that inflammatory cytokines start labor.[19] In the present study, PPROM was present in 27.9% of pregnant woman who delivered earlier than 35 weeks, and 30% of these women had HCA. Only one (8.3%) pregnant women with PPROM had clinical signs of CA, showing the lack of sensitivity of the clinical findings. In the comparison of pregnant women with and without HCA, there was no significant difference in terms of the presence and duration of PPROM (Table 1). This shows that, even in the absence of clinical signs and PPROM, HCA may still be present in case of premature labor.

Underlying CA is considered to cause labor and delivery that cannot be stopped by the tocolytics.[20] However, in our study, the efficacy of tocolytics was similar in mothers who

Table 5 Assesment of effects of histological chorioamnionitis (HCA) below and over 28 weeks of gestation.

	Infants of mothers with HCA (n = 12)	HCA(+) <28 wk (n = 7)	HCA(+) ≥28 wk (n = 5)	p*
Mean birthweight (g)	1052.5 ± 302.3	881.43 ± 157.77	1292.0 ± 301.77	0.011
Mean gestational age (wk)	27.58 ± 2.53	25.8 ± 1.46	30.0 ± 1.41	0.001
Mechanical ventilation duration (d)	9.4 ± 8.1	10.00 ± 8.48	8.80 ± 8.70	0.823
Surfactant therapy	9 (75%)	6 (85.7%)	3 (60%)	1.00
Oxygen duration (d)	13.8 ± 9.8	17.50 ± 10.21	11.00 ± 9.53	0.357
Bronchopulmonary dysplasia	6 (50%)	3 (75%)	3 (60%)	1.00
Patent ductus arteriosus	5 (41.6%)	3 (42.8%)	2 (40%)	1.00
Necrotizing enterocolitis	1 (8.3%)	1 (14.2%)	—	—
Intraventricular hemorrhage	3 (25%)	2 (28.5%)	1 (20%)	1.00
Neonatal mortality	7 (58.3%)	4 (57.1%)	3 (60%)	1.00

Data are presented as mean ± standard deviation or n (%) as appropriate.
*p values for gestational age <28 weeks and ≥28 weeks infants of mothers with HCA.

delivered prematurely due to HCA or other reasons, since the presence and mean durations of tocolysis were similar between groups (Table 1).

Antibiotic use in the perinatal period is only suggested in the presence of PPROM or indicators of infection to reduce neonatal mortality and complications of prematurity in infants.[28] However, some studies report that PPROM is based on the underlying subclinical infection, and that antibiotics cannot stop the ongoing inflammatory process and reduce neonatal sepsis risk.[29] In the present study, neither the presence nor the duration of PPROM and antibiotic treatment were related to the frequency of CA, suggesting either that resistant strains were active or that the ongoing inflammatory process could not be stopped by antibiotics. The benefits of antenatal antibiotics shown in some short-term outcomes should be balanced against the lack of evidence for the benefit of others, including perinatal mortality and longer term outcomes.[28] In our study, group antibiotic treatment was given to all pregnant women with PPROM upon clinical and laboratory infectious findings. We observed that although 50% of mothers with HCA had received antibiotics in the perinatal period, the incidence of early sepsis, BPD, PDA, NEC, and IVH, as well as mortality, was not reduced.

Antenatal steroids were previously thought to increase the frequency of perinatal infection in mothers and infants; however, it was later reported that steroids reduced certain negative effects of CA such as IVH and RDS, as well as mortality by suppression of inflammation.[30] In the present study, antenatal steroids did not alter the frequency of HCA in pregnant women (Table 1). No protective effects of antenatal corticosteroids were observed for any of the neonatal morbidities in our group of infants (Table 3). HCA increased RDS frequency significantly unrelated to antenatal steroid use.

CA both causes preterm birth and aggravates problems and complications of prematurity. Intrauterine bacterial infections in newborns present with early neonatal sepsis. Intrauterine infection accelerates fetal lung maturation similar to the stress response, but inflammatory cytokines cause alveolarization to deteriorate, and this leads to the development of chronic lung disease.[31] Cytokines have direct effects on the central nervous system, cause apoptosis by increasing the activity of caspase and excitatory amino acid synthesis, and negatively affect neurological development by causing fetal hypotension. Inflammation increases the sensitivity of neurons to damage by hypoxia and thus aggravates hypoxic—ischemic damage.[32]

Ogunyemi et al reported that pregnant women with HCA give birth earlier (at approximately 28.4 weeks); neonatal morbidities including BPD, PDA, IVH, and early neonatal sepsis are more frequent; and neonatal mortality is also higher in these infants.[5] Studies performed in recent years have reported that mortality, morbidity, early neonatal sepsis, and frequencies of RDS, BPD, and IVH increased in the infants of pregnant women with CA.[5,6] In the present study, early neonatal sepsis, RDS, surfactant use, BPD, and PDA were found to be more frequent, and neonatal mortality was significantly increased in the preterm infants of mothers with HCA compared to preterm infants born to otherwise healthy mothers. Although IVH was observed in 25% and 8.8% of the respective groups, the difference was not statistically significant, which could be due to the small number in the study group (Table 2).

A significant relationship has been reported between HCA and low Apgar scores/severity of illness in different studies.[6,33] In the present study, the mean 1- and 5-minutes Apgar scores were also significantly lower, and the CRIB and SNAP-PE II scores were significantly higher, in the HCA group, showing the severity of illness and the increased need for intensive care support (Table 2).

Leukocyte count in the cord blood has low sensitivity (74%) and specificity for the diagnosis of early neonatal sepsis, since it is affected by many other factors such as difficult delivery and induction with oxytocin (56%).[34] CRP can be indicative of intrauterine infection as it crosses the placenta and rapidly increases in inflammatory events. However, high cord blood CRP level is related not only to infection, but also to PROM longer than 24 hours and maternal fever, prolonged labor, and the presence of meconium in the amnion. Therefore, negative CRP values are more important for ruling out infection.[4,35] In the present study, the mean leukocyte count and CRP values seemed to be higher in the infants of mothers with HCA than those of healthy group, but this difference was not statistically significant (Table 2).

Morbidity and mortality rates are reported to be much higher in ELBW infants of mothers with HCA.[36] ELBW infants in our study group had similar surfactant, MV, and oxygen needs and mortality rates compared to larger preterms born to mothers with HCA. Moreover, infants born to mothers with HCA before or after 28 weeks had similar outcomes, which suggested that the relationship between CA and poor neonatal prognosis was not solely related to early gestational age or extremely low birthweight, but also to HCA (Tables 4 and 5).

As a whole, the incidence of HCA increased remarkably with decreasing gestational age at delivery, and the delivery week fell to the 27th gestational week in the HCA group in the present study. Antenatal steroid administration neither increased HCA frequency nor reduced the neonatal complications due to HCA. Clinical characteristics and acute-phase indicators of both mother and infant were not predictive of HCA and early sepsis. Apgar scores at 1 and 5 minutes were significantly lower in infants born to mothers with HCA, and the neonatal scoring systems yielded higher scores, indicating clinical severity. HCA not only causes preterm delivery, but may also have significant negative effects on neonatal prognosis independent of lower gestational age and low birthweight.

In conclusion, HCA is an important cause of neonatal mortality and morbidity, but it remains difficult to predict by clinical or laboratory methods. Therefore, routine histopathological examination of the placenta, also known as the "black box" of pregnancy, can provide valuable information to the clinician.

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