Histologic Evidence of Inflammation and Risk of Placental Abruption

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Histologic evidence of inflammation and risk of placental abruption

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OBJECTIVE: The objective of the study was to determine whether placental abruption is associated with an increased incidence of histologic chorioamnionitis among singleton gestations and whether this association is dependent on its severity.

STUDY DESIGN: Data were derived from the New Jersey–Placental Abruption Study, an ongoing, multicenter, case-control study conducted in New Jersey since August 2002. Subjects were women with a clinical diagnosis of abruption, and controls were matched to cases based on parity and maternal race/ethnicity. Two perinatal pathologists, blinded to the case-control status, performed all histologic examination based on standardized protocol. The association between chorioamnionitis and abruption was quantified based on odds ratio (OR) with 95% confidence interval (CI), after adjustment for potential confounders, and all analyses were stratified based on preterm birth (less than 37 weeks) status.

RESULTS: At preterm gestations (n = 141), chorioamnionitis was present in 30.8% and 12.5% of abruption cases and controls, respectively (OR 3.6, 95% CI 1.7 to 10.5). At term gestations (n = 205), the corresponding rates were 34.6% and 20.4%, respectively (OR 2.8, 95% CI 1.3 to 6.1). Severe chorioamnionitis was 7.2 (95% CI 1.6 to 20.1) and 18.3 (95% CI 2.2 to 150.4) times more common in abruption patients at preterm and term gestations, respectively.

CONCLUSION: Histologic chorioamnionitis is associated with placental abruption. The association was strongest in the presence of severe chorioamnionitis at term and, to a lesser extent, at preterm gestations. These observations suggest that the histologic findings in abruption are accompanied by severe inflammation, in both preterm and term gestations.

Key words: chorioamnionitis, histology, inflammation, placental abruption, preterm birth

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Placental abruption is potentially devastating for both mother and fetus and has been associated with stillbirth, preterm delivery, and fetal growth restriction.[1] It has been estimated to occur in 0.8% to 1.5% of all pregnancies, with a marked increase in the rates of abruption of 92% for black women and 15% for white women during the period from 1979 to 2001.[2] Several pathways leading to placental abruption have been proposed,[3] but the etiology remains elusive. Epidemiologic studies have characterized several associations with abruption including advanced maternal age, multiparity, smoking, cocaine use, black maternal race, poor socioeconomic status, hypertensive disorders, preterm premature rupture of membranes, intrauterine infection, and prior placental abruption.[4-10] The pathways by which these characteristics contribute to placental abruption can be categorized as either chronic processes or acute inflammation–associated processes.[3]

We have previously proposed that acute and chronic inflammatory processes that activate cytokines, such as interleukin (IL)-1β and tumor necrosis factor-α, may lead to placental abruption.[10] These cytokines have been shown to up-regulate the production and activity of matrix metalloproteinases in the trophoblast.[11] The result is destruction of the extracellular matrices and cell-cell interactions, which may lead to disruption of the normal placental attachment and lead to the premature separation of the placenta. Nevertheless, evidence that supports the mechanisms by which inflammatory lesions lead to placental abruption remains sparse.

To better define how inflammation may be linked to abruption, we tested the hypothesis that acute and severe inflammatory processes defined by histologic

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examination of the placenta are associated with placental abruption. Specifically, we evaluated the severity of infiltration of neutrophils into the subchorion, the chorion, and the amnion.

Materials and Methods

The New Jersey–Placental Abruption Study (NJ-PAS) is an ongoing, matched case-control study conducted at 2 hospitals in New Brunswick, New Jersey, since August 2002. Both hospitals, Robert Wood Johnson University Hospital and Saint Peter's University Hospital, are tertiary, level III, regional perinatal centers. The original study was undertaken to evaluate the association of thrombophilia status and placental abruption. Each study participant had a structured in-person interview questionnaire to collect details regarding maternal and paternal demographic characteristics, lifestyle, and behavioral and general health conditions. In addition, each participant gave consent to abstract the medical and prenatal care records from the index and all previous pregnancies and outcomes.

Placental abruption cases were identified, with gestational age greater than or equal to 20 weeks' gestation, based on a clinical diagnosis. Women with placental abruption were identified using 1 of 2 criteria. The criteria for diagnosis of abruption included the classical signs and symptoms of painful vaginal bleeding or hemorrhage, uterine pain or tenderness, uterine hypertonicity, retroplacental clot, or hematoma on the placental surface or on the basis of prenatal sonographic diagnosis.[12] Control patients were women that delivered at gestation of 20 weeks or longer and had no evidence of abruption and were matched to subjects on the basis of parity (0, 1, or 2 or greater), and maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other). Women with placenta previa in the present pregnancy or with a history of abruption were excluded from the control pool. Abruption cases were enrolled during the delivery hospitalization after informed consent. Data from medical records and interviews were obtained prior to discharge. Controls were enrolled on an ongoing basis following the recruitment of a subject. Further details of the NJ-PAS is described elsewhere.[13]

One (S.S.-S.) of 2 perinatal pathologists, who were blinded to abruption status, performed the histologic evaluation of the placentas, and each placenta was reviewed for gross findings and histologic lesions. The histologic classification of lesions was standardized through a protocol and tested in a pilot study for reproducibility prior to initiation of the study (unpublished data). Chorioamnionitis was defined by the presence of inflammatory infiltrates of neutrophils at 2 or more sites on the chorionic plate and extraplacental membranes. The degree of chorioamnionitis was then subclassified into the following categories: none, mild, moderate, and severe. Mild chorioamnionitis was defined by the presence of a few scattered (5-10 per high-powered field) neutrophils in the subchorionic space and adjacent chorion; moderate chorioamnionitis by many (11-30 per high powered field) neutrophils in the lower half of the chorionic plate; and severe chorioamnionitis by dense infiltrates of neutrophils (more than 30 per high powered field) throughout the chorionic plate into the amnion.[6,14]

Statistical analysis

Distributions of maternal sociodemographic and behavioral characteristics were examined according to preterm and term birth status for both cases and controls. The association between chorioamnionitis and case-control status was based on odds ratio with 95% confidence interval derived from logistic regression models before and after adjusting for potential confounding factors. The confounders considered for adjustment included maternal age (grouped as younger than 20, 20-24, 25-34, and 35 years old or older), maternal education (coded as below or above high school), marital status (single or married), maternal smoking during pregnancy (nonsmoker or smoker), and prepregnancy body mass index. Body mass index was derived as the ratio between prepregnancy weight (in kilograms) to squared height (in inches). Confounders were retained in the regression models for adjustment if their presence changed the odds ratio (between chorioamnionitis and placental abruption) by 10% or more. In addition, we further adjusted all analyses for parity (parity 0, parity 1, and parity 2 or greater) and maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other race/ethnicity).

We further examined the association between chorioamnionitis and abruption based on severity of chorioamnionitis (grouped as mild, moderate, and severe grades as well as mild/moderate and severe. All analyses were further stratified based on preterm birth status (20-36 completed weeks). All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

Ethics approval was received by the institutional review boards of both hospitals as well as by the Institutional Review Board of University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, New Brunswick, NJ.

Results

There were 170 placental abruption cases during the study period, of which 68.8% were preterm and 31.2% were term. Preterm and term subjects and controls were examined for differences in clinical and maternal sociodemographic and behavioral characteristics (Table 1). Premature rupture of membranes approached statistical significance for preterm births, with a higher incidence in subjects (24.1%) than controls (8.3%). There were no differences between subjects and controls based on maternal age, parity, marital status. For term births, several differences were observed, including maternal race, education, and smoking.

The overall frequency of chorioamnionitis in the study group was 32%, and 69% of abruption cases occurred at less than 37 weeks' gestation. Figure 1 shows the overall frequency of severity of chorioamnionitis for abruption subjects and

TABLE 1
Demographic characteristics of placental abruption cases and controls delivered at preterm and term gestations

	Preterm birth (20-36 wks)			Term birth (37 wks or longer)		
	Cases (%) (n = 117)	Controls (%) (n = 24)	*P* value	Cases (%) (n = 53)	Controls (%) (n = 152)	*P* value
Maternal age (y)			.81			.61
Younger than 20	3.4	0.0		1.9	3.3	
20-24	18.8	25.0			17.0	13.8
25-34	52.1	45.8		54.7	50.7	
35 or older	25.6	29.2		26.4	32.2	
Maternal race/ethnicity			.70			.03
Caucasian	22.2	20.8		3.8	15.1	
African American	35.0	29.2		37.7	40.1	
Hispanic	29.9	37.5		35.9	29.6	
Other	12.8	12.5		22.6	15.1	
Parity			.77			.11
Parity 0	38.5	45.8		22.6	33.6	
Parity 1	36.8	16.7		41.5	39.5	
Parity 2 or greater	24.8	37.5		35.9	27.0	
Education below high school	41.0	37.5	.75	47.2	29.6	.03
Single marital status	37.6	33.3	.69	26.4	25.0	.84
Maternal smoking	12.0	8.3	.61	11.3	2.0	.01
Prepregnancy body mass index	25.4 ± 5.7	25.0 ± 3.4	.63	23.8 ± 4.3	24.7 ± 5.6	.23
PROM	24.1	8.3	.09	1.9	3.3	.61
Acute deciduitis	8.6	0	.21	11.5	4.6	.31
Gestational age (wks)*	30.8 ± 3.8	32.7 ± 3.1	.01	38.7 ± 1.3	38.8 ± 1.0	.41

* Data represented as mean ± SD.

controls. The frequency of chorioamnionitis was greater for subjects in comparison with controls for the overall chorioamnionitis-abruption relationship and was most striking for severe chorioamnionitis ($P < .05$).

The comparison of histologic findings of chorioamnionitis between abruption cases and controls is shown in Table 2. The associations between chorioamnionitis and placental abruption at preterm (odds ratio [OR] 3.6, 95% confidence interval [CI] 1.7 to 10.5) and term (OR 2.8, 95% CI 1.3 to 6.1) gestations were significant. Severe chorioamnionitis was equally strongly associated with placental abruption at both preterm (OR 7.2, 95% CI 1.6 to 20.1) and term (OR 18.3, 95% CI 2.2 to 150.4). However, mild and moderate chorioamnionitis was not statistically significant.

Because premature rupture of membranes has been associated with increased risk of histologic chorioamnionitis,[7] we performed a separate analysis of abruption and premature rupture of membranes (PROM) (Table 3). PROM was more frequent in abruption cases as the severity of chorioamnionitis increased, with PROM present in 35.3% of abruption cases with severe chorioamnionitis. Among abruption cases, there was an apparent trend for earlier delivery in PROM abruption cases vs non-PROM cases. This pattern of earlier delivery was most striking for severe chorioamnionitis, with delivery occurring on average 4.5 weeks earlier in PROM cases ($P <$

FIGURE 1
Frequency of severity of chorioamnionitis among placental abruption cases and controls

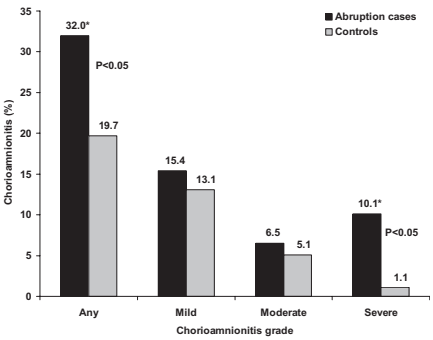

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TABLE 2
Association between histologic chorioamnionitis and placental abruption at preterm and term gestations

	Preterm birth (20-36 wks)			Term birth (37 wks or longer)		
Chorioamnionitis	Cases (%) (n = 117)	Controls (%) (n = 24)	Odds ratio* (95% CI)	Cases (%) (n = 53)	Controls (%) (n = 152)	Odds ratio* (95% CI)
None	69.2	87.5	1.0 (reference)	65.4	79.6	1.0 (reference)
Any	30.8	12.5	3.6 (1.7 to 10.5)	34.6	20.4	2.8 (1.3 to 6.1)
Mild	12.0	8.3	1.6 (0.3 to 8.4)	23.1	13.8	2.3 (1.0 to 5.8)
Moderate	7.7	4.2	1.5 (0.2 to 14.7)	3.8	5.3	1.5 (0.3 to 9.4)
Mild/moderate	19.7	12.5	1.6 (0.4 to 6.3)	26.9	19.1	2.1 (0.9 to 4.9)
Severe	11.1	0.0	7.2 (1.6 to 20.1)	7.7	1.3	18.3 (2.2 to 150.4)

* Odds ratios are adjusted for the confounding effects of maternal age, parity, maternal race/ethnicity, maternal education, marital status, and maternal smoking during pregnancy through multivariable logistic regression.

.05). These data suggest that inflammation-mediated abruption is not determined by PROM status but that PROM is likely a contributor to the association of inflammation and abruption.

Comment

Although many risk factors have been identified in association with placental abruption, causal pathways remain largely speculative. We report on the first prospectively collected series of placental abruption cases, with a complete histological evaluation of the placenta using standardized methodology with well-defined pathologic criteria for inflammatory lesions. Our findings corroborate previous findings that placental abruption is associated with inflammatory lesions of the placenta, especially at preterm gestations. Moreover, a novel finding was the strong association between more severe chorioamnionitis and abruption at term. This finding opens new insight into the importance of inflammatory processes in abruption by suggesting that perhaps inflammatory processes, when severe, may be involved in the pathway that leads to abruption irrespective of gestational age.

Recent evidence has also linked neutrophil infiltration into the decidua with preterm PROM and placental abruption. It has been demonstrated that the risk of abruption is 3.6-fold higher among women with preterm PROM, compared with women with intact membranes. When preterm PROM is accompanied by intrauterine infection, the risk of abruption is 9.0-fold higher, compared with women with intact membranes and no infection.[10] It is known that abruptions are associated with a thrombin-enhanced expression of IL-8, a potent neutrophil chemoattractant, which leads to a marked infiltration of decidual neutrophils.[15,16] This influx of neutrophils into the decidua is a rich source of proteases that can degrade extracellular matrix, leading to premature rupture of the fetal membranes.[17] Therefore, it is difficult to judge definitively whether neutrophil infiltration into the decidua is secondary to vascular disruption or whether it is the primary cause of abruption through inflammation. In our study, the frequency of acute deciduitis was higher in subjects than controls at both preterm (8.6% vs 0%, respectively) and term (11.5% vs 4.6%) gestations, supporting the role of chronic inflammatory environment associated with increased risk of placental abruption.

We have previously demonstrated that neutrophils are increased in the chorion of placentas in women with placental abruption in comparison with controls.[18] Furthermore, the histologic finding of neutrophil infiltration into the decidua was 1.6-fold more common with vaginal bleeding during the pregnancy. In addition, we have reported that rates of inflammation-associated processes were, in general, higher in preterm than term births.[18] Our current study further demonstrates that severe acute inflammatory processes are indeed important in term abruption as well.

We speculate that the placental lesions that manifest as severe chorioamnionitis are indicative of an intense inflammatory process at the interface of the decidua and chorion, which stimulate inflammatory cytokines and chemokines. The result of this cascade of events is destabilization of the uteroplacental interface, culminating in placental abruption, premature rupture of membranes, and preterm labor. Because the development

TABLE 3
Incidence of PROM and mean gestational age at delivery by severity of histologic chorioamnionitis among placental abruption cases

		Gestational age (wks)*		
Chorioamnionitis	PROM n (%)	Non-PROM	PROM*	*P* value
None	17 (15.6)	34.5 ± 4.0	31.7 ± 3.4	.008
Mild	2 (7.7)	33.2 ± 6.6	28.0 ± 4.2	.301
Moderate	3 (27.3)	32.1 ± 5.2	30.0 ± 5.3	.586
Severe	6 (35.3)	31.0 ± 6.0	26.5 ± 2.8	.020

* Table entries denote mean (± SD).

of severe histologic chorioamnionitis takes time, this further provides evidence for abruption as a more chronic process.

One potential explanation for our findings is that abruptions elicit an intense production of thrombin from the decidua that in turn leads to a massive recruitment of neutrophils.[15,16] Therefore, the presence of neutrophils in the chorion may be a manifestation of a pathway that begins with abruption-related hemorrhage, leading to decidual cell production of tissue factor and eventually conversion of prothrombin to thrombin.

Others have described apoptotic cell death in the placentae of patients with histologic evidence of chorioamnionitis.[19,20] Apoptotic nuclei are twice as common in the chorion of subjects with histologic chorioamnionitis, compared with those without chorioamnionitis.[19] Chorioamnionitis may induce hypoxia with production of cytokines and other bioactive mediators, such as nitric oxide, superoxide, and peroxynitrite. These mediators may lead to cell death and subsequent placental abruption. In the study by Nakatsuka et al,[20] placentas of patients with chorioamnionitis and abruption demonstrated a similar up-regulation of inducible nitric oxide synthase and other molecules involved in the apoptotic cascade.

Our study has several strengths: First, the data came from a well-designed prospective, matched case-control study. Furthermore, the collection of data was of very high quality (carried out by patient interview and critical evaluation of medical records), and detailed pathologic examination of the placentas was available for all recruited patients.

A few limitations of this study remain despite the prospective study design. Patients were matched according to parity and maternal race/ethnicity; however, there were several factors such as smoking and cocaine status, which may have been related in the causal pathway that leads to abruption. Specifically, it was tempting to control for preterm PROM and evaluating the latency period to delivery. However, earlier studies have suggested preterm PROM to be implicated in the causal pathway of the exposure-disease relationship.[17] Therefore, PROM cannot be used as a variable to adjust the analysis. In addition, the specific demographic characteristics of our population may limit our ability to generalize the results of our study; however, our population was diverse. In addition, the association between chorioamnionitis and placental abruption has previously been described in other populations, making it more likely the results will be generalizable.

In conclusion, our findings suggest that more severe inflammatory lesions of the placenta, characterized by neutrophil infiltration of the chorion and amnion, are associated with placental abruption in both preterm and term gestations. However, a cause-and-effect relationship could not be established. These findings should stimulate further research, including animal models, into the histological evaluation of the placenta to elucidate the inflammatory changes that occur at the level of the uteroplacental interface, culminating in placental abruption. ■

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Appendix

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