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Original Article

Coagulation function and placental pathology in neonates with placental abruption

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Abstract: This study aimed to investigate the coagulation function and placental pathology in neonates with placental abruption (PA). A total of 60 neonates with PA and 60 neonates without PA were recruited. Neonates with PA were further subdivided into mild and severe groups. Neonates with PA had a higher incidence of anemia and hypovolemic shock ($P<0.05$), and were more likely to develop metabolic acidosis, asphyxia, intracranial hemorrhage, hypoxic-ischemic myocardial injury, hypoxic-ischemic encephalopathy, and disseminated intravascular coagulation ($P<0.05$). They also had longer hospital stay ($P=0.033$). At 6 h, the prothrombin time (PT), activated partial thromboplastin time (APTT), and D-dimer (D-D) were higher ($P<0.05$), but fibrinogen (FIB) was lower ($P=0.000$) in PA neonates than in controls. Significant differences were observed in tissue factor (TF), tissue factor pathway inhibitor (TFPI), and TF/TFPI among the mild PA group, whereas the severe PA and control groups were observed among different time points ($P<0.05$), and during time-group interaction ($P<0.05$). Thus, PA may cause coagulation dysfunction in neonates. The larger the separation area, the more evident the coagulation dysfunction was. TF and TFPI are involved in the pathogenesis of coagulation dysfunction in PA neonates and early heparin intervention is effective to imporve coagulation function and prognosis.

Keywords: Placental abruption, prothrombin time, activated partial thromboplastin time, coagulation

Introduction

PA may cause stillbirth, premature delivery, neonatal asphyxia, neonatal anemia, neurological sequelae, and other complications [1-6]. PA related coagulation dysfunction is closely related to some complications in neonates such as early disseminated intravascular coagulation (DIC), intracranial hemorrhage, and pulmonary hemorrhage [7, 8]. Clinicians have recognized the harm of PA in pregnant women, but little is known about the adverse influence of PA on neonates, especially coagulation. Although studies have emphasized the influence of PA on the prognosis of newborn twins [9, 10], prediction of coagulation dysfunction in these neonates is still a challenge, and clinicians usually fail to recognize and monitor coagulation function in neonates with PA, which presents difficulty for early identification, prevention, and treatment. Thus, measures are often taken only when clinical symptoms (such as bloody stool and subcutaneous bleeding) are present. Under this condition, the optical timing of treatment is missed, which significantly increases the mortality and disability. To date, no study has been reported about the relationship between coagulation dysfunction and placental pathology in neonates with PA. Thus, better understanding the clinical characteristics of PA neonates and to investigate the characteristics and pathogenesis of coagulation dysfunction are critical for the prevention and treatment of coagulation dysfunction. This prospective study was undertaken to investigate the clinical characteristics, coagulation dysfunction, and the pathological characteristics of PA, in which the regulatory effects of tissue factor (TF) and tissue factor pathway inhibitor (TFPI) were also evaluated. This study aimed to provide evidence for early recognition of PA as an approach to reduce mortality and neurological sequela in these neonates.

Materials and methods

Subjects

This was a prospective, case-control study. PA was diagnosed according to the diagnostic cri-

teria for PA in the Obstetrics and Gynecology (7th edition) and Practical Neonatology (4th edition). Hospitalized PA neonates (n=60) were recruited from the Neonatal Intensive Care Unit (NICU) between July 2015 and June 2016 from the Bayi Children's Hospital of Army General Hospital. These patients were subdivided into a mild PA group (n=35) and a severe PA group (n=25). In addition, 60 neonates without PA were also recruited as controls in the same period. There were no significant differences in gender, gestational age, and birth weight between PA neonates and controls. Inclusion criteria: (1) Patients in PA group were diagnosed with PA, had no coagulation disorders, and no family history of hereditary bleeding disorders; anal atresia, intestinal atresia, hypospadias, cleft palate and congenital laryngeal stridor were allowable in controls; (2) Mild PA: mild PA was characterized by external bleeding, the area of PA was ≤1/3 of the whole placenta and patients had no evident clinical signs; severe PA: severe PA was characterized by internal or mixed bleeding, and the area of PA was >1/3 of the whole placenta or patients had complications such as uteroplacental apoplexy and hemorrhagic shock. Exclusion criteria: Subjects did not meet above inclusion criteria; the mother had gestational hypertension and/or amniotic fluid embolism at delivery; patients had bleeding related blood diseases including vitamin K_1 deficiency, hemophilia, immune thrombocytopenia, liver diseases, and congenital fibrin deficiency; patients had septic shock; patients had incomplete medical information; patients withdrew before the end of study. The methods were carried out in accordance with the approved guidelines. All experimental protocols were approved by the Ethics Committee of The Army General Hospital of People's Liberation Army (PLA). Written informed consent was obtained from all subjects.

Clinical information

The following clinical information was recorded: factors related to mother: age, multiple fetuses, stillbirth, method of delivery, severity of PA, abnormal amniotic fluid (excess, insufficient and contaminant), anemia and DIC. Neonate-related factors: gestational age, gender, birth weight, small for gestational age, asphyxia, intracranial hemorrhage, acidosis, anemia, aspiration syndrome, hypovolemic shock, hypoxic ischemic myocardial injury, respiratory distress syndrome, DIC, hypoxic-ischemic encephalopathy, necrotizing enterocolitis, hyperbilirubinemia, patent ductus arteriosus, retinopathy of premature, bronchopulmonary dysplasia, prognosis, hospital stay and body weight on discharge. Prognosis: complete remission (absence of characteristics of hypotension, shock, organ dysfunction, and coagulation dysfunction; absence of systemic signs of bleeding; normal laboratory parameters related to coagulation function), partial remission (2 of above items in complete remission); non-remission (absence of remission or deterioration after treatment).

Methods

Blood sampling and detection of coagulation related factors: Venous blood (1 ml) was collected at 6 h, 24 h, and 72 h after birth and processed for the detection of prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FIB), D-dimer (D-D), fibrin degradation product (FDP), platelets (PLT), TF, TFPI and TF/TFPI.

Evaluation of coagulation function and criteria for DIC: Coagulation function was evaluated with PT, APTT, FIB, D-D, PLT, FDP, TF, TFPI and TF/TFPI ratio. DIC was diagnosed according to previously reported [11]: score of >5 is indicative of dominant DIC.

Pathological examination of the placenta: The placenta was collected after delivery or surgery and processed for pathological examination. For placenta from PA neonates, both maternal and fetal sides were observed, and full thickness tissues (1.0 cm×1.0 cm×1.0 cm) were collected at the sites of PA and non-PA. The calcification area was avoided, blood and adipose tissues were removed, and only lesioned areas were preserved, washed in normal saline, and fixed in 4% paraformaldehyde. In control group, placental tissues were collected at 3 and 9 o'clock positions. Tissues were routinely processed for HE staining.

Main treatments: Pre-existing diseases were treated with corresponding strategies: red blood cells transfusion was done for the treatment of anemia; hypovolemic shock was treated with 0.9% sodium chloride, dopamine, and other drugs; asphyxia was managed according to standardized procedures; 1.4% sodium bicarbonate was used to treat acidosis. In the

Table 1. Clinical characteristics of mothers in two groups

Parameters	PA group (n=60)	Control group (n=60)	t/χ^2	*P*
Age of mother (yr)	29.32±5.35	28.13±3.68	1.412	0.161
Multiple fetuses	32 (53.3%)	23 (38.3%)	2.719	0.099
Stillbirth	2 (3.3%)	0.000	2.034	0.154
Cesarean section	51 (85.0%)	44 (73.3%)	2.476	0.116
Amniotic contamination	15 (25.0%)	17 (28.3%)	0.170	0.680
Abnormal volume of amniotic fluid	13 (21.7%)	11 (18.3%)	0.208	0.648
Maternal anemia	5 (8.3%)	1 (1.7%)	1.579	0.209
Maternal DIC	2 (3.3%)	0 (0%)	0.508	0.476

PA group, transfusion of plasma and coagulation factors was performed after admission, and additional low molecular weight heparin was administered at 12.5-25 U/kg once every 6 h for consecutive 3 days for preventing DIC. In DIC neonates of both groups, low molecular weight heparin was intravenously administered continuously at 10-15 U/kg/h in hypercoagulable stage; in the fibrinolysis stage, low molecular weight heparin was intravenously administered continuously at 5-10 U/kg/h in the supplement of intravenous fresh frozen plasma, coagulation factors, cold precipitates and/or platelets. The dose and treatment of heparin was determined according to the coagulation function.

Statistical analysis: Statistical analysis was performed with SPSS version 13.0 (SPSS, Inc., Chigago, IL, USA). Quantitative data are expressed as mean ± standard deviation ($\bar{x}$ ± SD) and compared with independent t test between two groups or repeated measures analysis of variance among groups. Categorical data are expressed as proportion and compared with Chi square test. A value of $P<0.05$ was considered statistically significant. F represents the statistical main effect or interaction effect.

Results

Clinical characteristics of patients

The clinical characteristics of patients in PA group and control group are shown in **Tables 1** and **2**. The characteristics of mothers were comparable between two groups ($P>0.05$). The PA neonates were older and had higher incidences of anemia and hypovolemic shock as compared to controls (26.7% vs 11.7%, $\chi^2=4.357$, $P=0.037$; 48.3% vs 25%, $\chi^2=7.033$, $P=0.008$; 35% vs 16.7%, $\chi^2=5.263$, $P=0.022$; respectively). In addition, PA neonates were more likely develop metabolic acidosis, asphyxia, intracranial hemorrhage, hypoxic-ischemic myocardial injury, hypoxic-ischemic encephalopathy, and DIC (35% vs 16.7%, $\chi^2=5.263$, $P=0.022$; 31.7% vs 13.3%, $\chi^2=5.783$, $P=0.016$; 13.3% vs 3.3%, $\chi^2=3.927$, $P=0.048$; 21.7% vs 15%, $\chi^2=4.658$, $P=0.031$; 21.7% vs 8.3%, $\chi^2=4.183$, $P=0.041$; 8.3% vs 0%, $\chi^2=5.217$, $P=0.022$; respectively). The hospital stay in PA group was significantly longer than in control group (18.05±4.40 days vs 15.17±2.82 days, $t=4.271$, $P=0.033$).

Coagulation function

The parameters of coagulation function are shown in **Table 3**. There were only 5 neonates with DIC in control group and FDP has been used as a marker of DIC. Thus, FDP was not compared in this study. At 6 h, the PT, APTT, and D-D were significantly higher than in control group (14.11±2.98 s vs 12.15±1.85 s, t=4.316, $P=0.000$; 73.55±27.26 s vs 44.43±8.04 s, $t=7.932$, $P=0.000$; 3.18±2.86 s vs 0.23±0.11 s, $t=7.977$, $P=0.000$; respectively), but FIB in PA group was markedly lower than in control group (1.88±0.70 g/L vs 2.67±0.50 g/L, $t=6.16$, $P=0.000$). After comprehensive therapy, the PT, APTT, FIB, D-D, and PLT were comparable between two groups at 72 h and in the normal ranges.

TF, TFPI, and TF/TFPI

TF is a potent pro-coagulation factor and TFPI is an anti-coagulation factor. The TF, TFPI, and TF/TFPI ratio were compared among the mild PA group, the severe PA group, and the control group (**Tables 4**-**6**). TF was significantly different among groups ($F=49.836$, $P=0.000$); TF also changed significantly over time ($F=20.666$, $P=0.000$) and there was a significant interaction between group and time ($F=19.978$, $P=0.000$). TFPI was significantly different among groups ($F=34.951$, $P=0.000$) and also changed markedly over time ($F=16.595$, $P=0.000$), and there was interaction between group and time ($F=13.800$, $P=0.000$). TF/TFPI

Table 2. Clinical characteristics of neonates in two groups

Parameters	PA group (n=60)	Control group (n=60)	t/χ^2	*P*
Gestational age (w)	33.95±2.92	34.08±2.90	0.252	0.802
Male	34 (56.7%)	36 (60%)	0.137	0.711
Birth weight (g)	2235.73±620.17	2357.47±652.42	1.048	0.297
Small gestational age (SGA)	16 (26.7%)	7 (11.7%)	4.357	0.037
Asphyxia	19 (31.7%)	8 (13.3%)	5.783	0.016
Intracranial hemorrhage	8 (13.3%)	2 (3.3%)	3.927	0.048
Metabolic acidosis	21 (35.0%)	10 (16.7%)	5.263	0.022
Anemia	29 (48.3%)	15 (25.0%)	7.033	0.008
Aspiration Syndrome	19 (31.7%)	11 (18.3%)	2.844	0.092
Hypovolemic shock	21 (35.0%)	10 (16.7%)	5.263	0.022
Hypoxic-ischemic myocardial injury	19 (21.7%)	9 (15.0%)	4.658	0.031
Disseminated intravascular coagulation (DIC)	5 (8.3%)	0 (0%)	5.217	0.022
Hypoxic-ischemic encephalopathy (HIE)	13 (21.7%)	5 (8.3%)	4.183	0.041
Respiratory distress syndrome (RDS)	20 (33.3%)	13 (21.7%)	2.048	0.152
Necrotizing enterocolitis (NEC)	2 (3.3%)	0 (0%)	0.508	0.154
Hyperbilirubinemia (HI-BIL)	11 (18.3%)	9 (15.0%)	0.240	0.624
Patent ductus arteriosus (PDA)	24 (40.0%)	15 (25.0%)	3.077	0.079
Retinopathy of premature children (ROP)	5 (8.3%)	3 (5.0%)	0.134	0.714
Bronchopulmonary dysplasia (BPD)	4 (6.7%)	2 (3.3%)	0.175	0.675
Hospital stay (d)	18.05±4.40	15.17±2.82	4.271	0.033
Body weight on discharge (g)	2967.38±322.52	3022.00±263.21	1.016	0.312
Complete remission	49 (81.7%)	53 (88.3%)	1.046	0.306
Partial remission	10 (16.7%)	7 (11.7%)	0.617	0.432
Mortality	1 (1.6%)	0 (0%)	0	1.000

Table 3. Coagulation function of patients in two groups at 6 h and 72 h after birth

Parameters	Group (6 h)		*t*	*P*	Group (72 h)		*t*	*P*
	PA	Control			PA	Control		
PT (s)	14.11±2.98	12.15±1.85	4.316	0.000	14.30±8.26	14.07±8.29	0.149	0.882
APTT (s)	73.55±27.26	44.43±8.04	7.932	0.000	43.50±8.74	42.68±6.89	0.567	0.572
FIB (g/L)	1.88±0.70	2.67±0.50	6.160	0.000	2.67±0.50	2.74±0.64	0.687	0.493
D-D (mg/L)	3.18±2.86	0.23±0.11	7.977	0.000	0.26±0.11	0.24±0.10	1.004	0.317
PLT ($\times 10^9$)	238.92±77.72	280.43±71.94	0.316	0.753	280.43±71.94	28.75±56.5	0.704	0.483

Table 4. TF in different groups at different time points (ng/L)

Group	Time points			Sum	*F*	*P*
	6 h	24 h	72 h			
Severe PA (n=25)	278.93±38.62	420.16±83.75	217.31±71.34	285.33±100.73	15.660	0.000
Mild PA (n=35)	121.85±70.20	201.92±56.38	259.14±95.71	168.48±95.37	30.062	0.000
Control (n=60)	61.61±41.12	52.31±32.55	66.26±27.41	53.23±36.03	1.189	0.332
Sum	133.19±95.99	195.70±143.16	86.48±113.78	156.76*±113.71*	20.666*	0.000*
F	55.008	70.853	32.939	49.836*	(F=19.978; P=0.000)#	
P	0.000	0.000	0.000	0.000*		

Notes: *main effect: *F* and *P*; #interaction: *F* and *P*.

was significantly different among groups (*F*=85.429, *P*=0.000) and also changed markedly over time (*F*=33.836, *P*=0.000), and there was interaction between group and time

Table 5. TFPI in different groups at different time points (μg/L)

Group	Time points			Sum	*F*	*P*
	6 h	24 h	72 h			
Severe PA (n=25)	97.30±44.97	114.80±20.62	69.92±52.27	15.44±2.67	12.458	0.000
Mild PA (n=35)	62.16±27.58	69.05±34.64	86.41±31.00	18.02±2.26	66.423	0.000
Control (n=60)	22.73±2.65	23.247±1.89	23.54±2.89	23.14±2.61	0.502	0.684
Sum	19.45±3.33	18.80±4.04	18.17±4.36	19.21*±3.84*	16.595*	0.000*
F	24.583	65.131	49.860	34.951*	(F=13.800; P=0.000)#	
P	0.000	0.000	0.000	0.000*		

Notes: *main effect: F and P; #interaction: F and P.

Table 6. TF/TFPI in different groups at different time points (μg/L)

Group	Time points			Sum	*F*	*P*
	6 h	24 h	72 h			
Severe PA (n=25)	12.88±1.47	31.87±6.43	14.31±5.15	19.32±8.73	19.537	0.000
Mild PA (n=35)	4.62±2.43	11.31±3.31	16.63±16.63	9.76±6.19	39.969	0.000
Control (n=60)	3.37±2.45	2.32±1.58	6.13±1.50	2.86±1.87	1.317	0.289
Sum	7.60±6.42	12.43±11.26	11.60±7.85	9.38*±8.27*	33.836*	0.000*
F	55.464	86.706	41.089	85.429*	(F=29.063; P=0.000)#	
P	0.000	0.000	0.000	0.000*		

Notes: *main effect: *F* and *P*; #interaction: *F* and *P*.

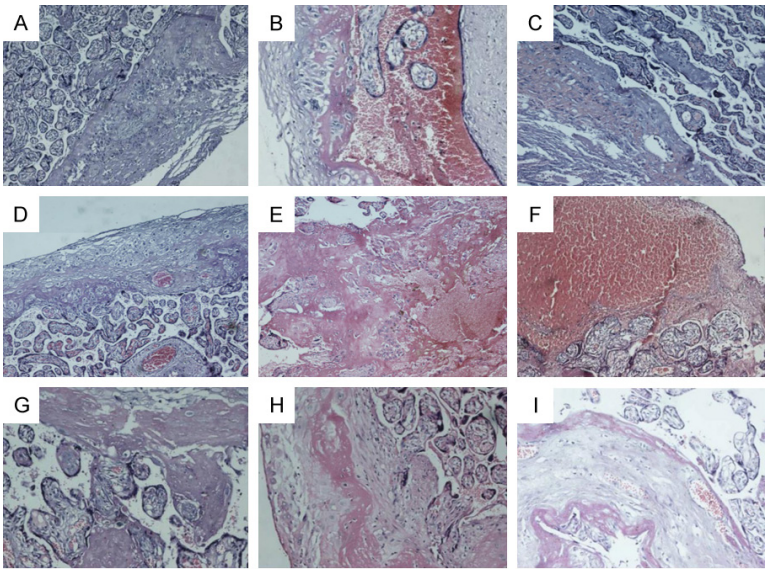

Figure 1. Pathological changes of the placenta in PA of different severities (HE staining, 100×). A: Fetal side of the normal placenta; B: Fetal side of the lesioned placenta in mild PA; C: Maternal side of the lesioned placenta in mild PA; D: Fetal side of the non-lesioned placenta in mild PA; E: Maternal side of the non-lesioned placenta in severe PA; F: Fetal side of the lesioned placenta in severe PA; G: Maternal side of the lesioned placenta in severe PA; H: Fetal side of the non-lesioned placenta in severe PA; I: Maternal side of the non-lesioned placenta in severe PA.

(*F*=29.063, *P*=0.000). These findings suggest that TF and TFPI are involved in the coagulation dysfunction of PA neonates.

Pathological features of the placenta in PA neonates

In case of PA, blood cells infiltrate into the placenta to form a hematoma, which compresses the surrounding tissues. Hemorrhage was observed at both maternal and fetal sides. In severe PA neonates, more blood loss was observed as compared to mild PA neonates (**Figure 1A-I**). In cases of PA, the placenta uterine spiral arteries became dilated and congestive, and thrombosis was observed. Continuous production of microthrombosis and plasmin may result in the consumption of fibrin and fibrinogen, which leads to production of FDP and subsequent deterioration of bleeding (**Figure 2A-C**). In severe PA, there was swelling of microvilli, the space between microvilli was widened, villous vessels became dilated and congestive, micro-thromboi were found in some microvilli, and fibrinoid necrosis was noted in a fraction of

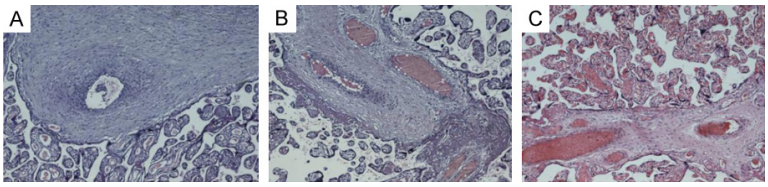

Figure 2. Uterine spiral arteries of the placenta in PA of different severities (HE staining, 100×). A: Normal placenta; B: Mild PA; C: Severe PA.

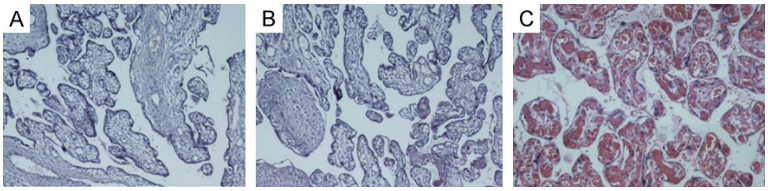

Figure 3. Microvilli of the placenta in PA of different severities (HE staining, 200×). A: Normal placenta; B: Mild PA; C: Severe PA.

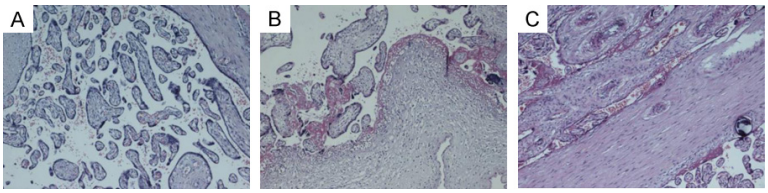

Figure 4. Special pathological changes of the placenta in PA of different severities (HE staining, 200×). A: Normal placenta; B: Scattered necrosis in the placenta with mild PA; C: Calcification foci and patchy necrosis in the placenta with severe PA.

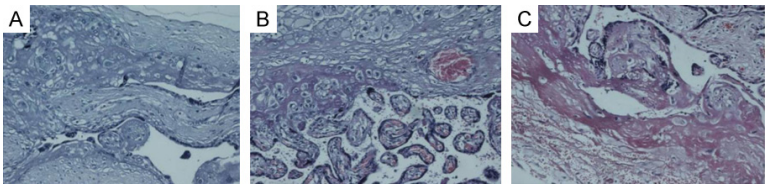

Figure 5. Trophoblastic pathology of the placenta with PA of different severities (HE staining, 100×). A: Normal placenta; B: Mild PA; C: Severe PA.

microvilli. In mild PA, fibrinoid necrosis was observed in a small amount of microvilli, there was no thrombosis, and the pathological changes were mild (**Figure 3A-C**). In the severe PA group, there was patchy fibrinoid necrosis in the placental matrix and villi and calcification foci were also observed. In mild PA neonates, there was scattered fibrinoid necrosis (**Figure 4A-C**). In the severe PA group, there was evident trophoblastic hyperplasia, with cells that were irregularly organized, the cell layer was thickened and had uneven thickness, and syncytiotrophoblast cells were hyperplastic and had formation of syncytial cell nodules. In the mild PA group, these pathological changes were milder (**Figure 5A-C**).

Discussion

The placenta consists of the amnion, leafy chorion, and decidua basalis and is an indispensable organ for normal fetal development. PA will affect the normal growth and development of the fetus and cause intrauterine growth retardation, or even fetal death. In addition, PA will increase the susceptibility of neonates surviving PA after birth to other diseases and the risk for long-term neurological sequelae. In the Department of Obstetrics, antithrombotic therapy with heparin is often employed to prevent pregnancy complications, which may attenuate the adverse consequences of PA to the mother and fetus [12]. Despite this, PA still significantly threatens the health of fetus and neonates in clinical practice. In our previous retrospective study, we investigated coagulation dysfunction of different severities in neonates with PA [13]. In this prospective, case-control study, the clinical characteristics of neonates with and without PA were reviewed, and the results showed that PA neonates were older, had high incidences of anemia and hypovolemic shock, were more likely to develop metabolic acidosis, asphyxia, intracranial hemorrhage, hypoxic-ischemic myocardial injury, hypoxic-ischemic encephalopathy, DIC and had longer hospital stays. These may be explained as follows: in cases of PA, the nutrient transport is disordered in the placenta, the blood flow to the uterine and placenta reduces, the nutrient supply to the fetus is also insufficient, and there is chronic hypoxia in the fetus, which may affect the absorption of nutrients in the fetus [14]. In addition, PA may directly cause disruption of

blood flow to the placenta, which may cause acute hypoxia in the uterine and asphyxia after birth, leading to hypoxemia, metabolic acidosis, vascular endothelial injury, and coagulation dysfunction. Moreover, hemorrhage in cases of PA may cause anemia and hypovolemic shock in neonates [15, 16]. Taken together, PA may cause abnormalities in gas exchange and nutrient supply in the fetus, and then the placenta releases some TFs [15]. The lack of early recognition of PA in neonates may result in missing the optimal time of treatment and accumulation of coagulation dysfunction, which further deteriorates the disease condition, causing a vicious cycle and resulting in a poor outcome to the fetus or neonate.

In this study, coagulation function was also investigated in neonates with and without PA. PT mainly reflects the contents and activities of coagulation factors in the extrinsic coagulation pathway. The TFs involved in coagulation are released from tissues. When vascular endothelial injury and slow blood flow are present, damage to the structure and function of endothelial cells may cause a series of changes at the molecular level, leading to a prothrombotic state and then causing thrombosis. APTT mainly reflects the contents and activities of coagulation factors involved in intrinsic coagulation pathway. These coagulation factors are from the blood and are a key factor involved in the deep vein thrombosis. FIB is a central protein in the coagulation system and reflects the structure and content of the protein shared by both extrinsic and intrinsic coagulation pathways. FIB is the final substrate of the coagulation pathway and may be degraded into fibrin via catalysis of thrombin, which may promote coagulation and increase the intercellular bridge. At 6 h after birth, the PT, APTT, and D-D in the PA group were significantly higher than in the control group. At 72 h after comprehensive therapy, the PT, APTT, FIB, D-D, and PLT were comparable between the control and PA groups and were in the normal ranges. These findings indicate that splacental injury in cases of PA may cause release of tissue thromboplastin (factor III) in the placental trophoblast into the fetal circulation, which then activates the extrinsic coagulation system, leading to the coagulation dysfunction. This may be the cause of coagulation dysfunction at 6 h after admission when neonates are in the hypercoagulable state and susceptible to DIC [4, 10]. In the intravenous transfusion of plasma, early application of low molecular weight heparin is helpful for improvement of coagulation dysfunction and prevention of DIC. After 72 h, coagulation function became normal, and no PA neonates developed DIC. This indicates that early use of low molecular weight heparin may improve coagulation dysfunction in PA neonates and prevent against DIC. Of note, low molecular weight heparin has potent anti-coagulation activity and may inhibit the coagulation and fibrinolysis at different stages. In the fibrinolysis stage of DIC, low molecular weight heparin should be administered to supplement coagulation factors (such as fresh frozen plasma, cold precipitate, and platelets), its dose should be reduced, and the coagulation function should be closely monitored during the heparin treatment [15].

Generally, TF increases in normal pregnancy, and TFPI is also involved in this regulation, leading to an adaptive hypercoagulable state in pregnant women [17]. To investigate the role of TF and TFPI from the placenta and myometrium in the coagulation in case of PA, the TF, TFPI, and TF/TFPI were determined in the mild PA group, the severe PA group and the control group. There were significant differences in TF, TFPI, and TF/TFPI at different time points and among groups, and interaction was also observed between time and group. In the severe PA group, the TF, TFPI, and TF/TFPI were also significantly different at different time points and peaked at 24 h. In the mild PA group, the TF, TFPI, and TF/TFPI were also markedly different at different time points and peaked at 72 h. These findings indicate that TF and TFPI are involved in the pathogenesis of coagulation dysfunction in PA neonates, and that coagulation dysfunction may be explained as follows: PA may cause the rupture of the uterine spiral arteries as well as the damage to surrounding tissues; then a large amount of TF can be released from the placental villi and decidua into the fetal circulation, resulting in a coagulation cascade and micro-thrombosis, which finally causes DIC. However, TFPI is able to inhibit TF induced coagulation. Of note, natural TFPI is insufficient to inhibit TF induced coagulation dysfunction [18, 19]. In addition, PA may also cause the rupture of placental vessels, which exposes collagens and vWF, leading to surface activation and activation of the intrin-

sic coagulation system. Moreover, PA induced hypoxia in the fetus and acidosis may deteriorate coagulation function [15, 20]. On the basis of clinical characteristics, our results indicate that the larger the lesioned area of the placenta, the more severe the coagulation dysfunction, which can result in DIC.

Pathological examination showed blood cell infiltrrate in the placenta in cases of PA, causing hematoma which compressed the surrounding tissues. There was hemorrhage at both the placental and maternal sides. In severe PA, more blood loss was observed as compared to mild PA. The uterine spiral arteries became dilated and congestive, and throbosis was observed in these arteries. In the severe PA group, there was swelling of microvilli, the space between villi was narrowed, the villous vessels were dilated, and congestive, micro-thrombosis was observed in a fraction of villi, and fibrinoid necrosis was noted in some villi. In the mild PA group, fibrinoid necrosis was observed in only a small amount of villi, thrombosis was not observed, and the pathological changes were mild. In the severe PA group, the placental matrix and villi displayed massive fibrinoid necrosis and clacification foci. In the mild PA group, there was scattered fibrinoid necrosis. In the severe PA group, there was evident trophoblastic hyperplasia, cells were irregularly arranged, the cell layer was thickened and had uneven thickness, and syncytiotrophoblast cell hyperplasia and formation of syncytial cell nodules were found. In the mild PA group, the pathological changes were attenuated. Our findings were consistent with previously reported [16, 21].

The above pathological changes in the placenta after PA may explain the different poor outcomes in the fetuses and neonates. In cases of PA, hemorrhage at the placenta is not only a cause of early anemia and hypovolemic shock, and may also cause fetal hypoxia and neonatal asphyxia after birth. The uterine spiral arteries become dilated and have thrombosis in case of PA, which may cause fetal hypoxia, nutrient insufficiency, and release of pro-coagulation factors, leading to activation of the fetal coaguation system. Swelling of microvilli and narrowing of inter-villous spaces may affect the substance interchange, which may cause fetal hypoxia, acidosis, and fetal distress and lead to secretion of stress related hormones, causing the chemotaxis and infiltration of immune cells, the production of a large amount of pre-inflammatory cytokines, and activation of coagulation system [22]. On the other hand, fibrinoid necrosis may cause entry of pro-coagulation substances such as cellular debris into the circulation, resulting in coagulation. In severe PA neonates, fibrinoid necrosis was most evident and involved more placental tissue, leading to the release of a large amount of pro-coagulation factors, which is a basic cause of coagulation dysfunction in neonates with severe PA. In addition, the patchy necrosis and calcification foci in the placental tissues indicate functional aging of the placenta, which may deteriorate with ischemia and hypoxia of the fetus, resulting in chronic or acute interuterine distress, increasing the release of pro-coagulation factors, and deteriorating the coagulation function.

Studies have reported that PA is a major cause of DIC in neonates with asphyxia, may increase the risk for chronic lung diseases in neonates with gestational age of 22-26 weeks, but fails to increase neonatal mortality and the incidence of cerebral palsy [23, 24]. The present study investigated the influence of PA on the coagulation function, clinical morbidities and outcomes of neonates via clinical epidemiological, labratory, and pathological approaches, and emphasized the close relationship between severity of PA and severity of coagulation dysfunction. Our study indicated the PA induced coagulation dysfunction in neonates. The larger the lesioned placenta, the more severe the coagulation dysfunction. In addition, PA neonates are older, have higher incidences of anemia and hypovolemic shock, and are more likely to develop metabolic acidosis, asphyxia, intracranial hemorrhage, hypoxic-ischemic myocardial injury, hypoxic-ischemic encephalopathy and DIC.

However, there were still limitations in this study. The long-term prognosis (such as neurological sequelae) of neonates with PA is unclear because these patients were not followed up in this study. Our study showed the anti-coagulation effect of heparin was definite, but the standardized dose of heparin and indications to heparin treatment are still poorly understood.

Thus, more studies are required to clarify these issues.

Taken together, this study shows that PA may cause coagulation dysfunction in neonates and that the larger the lesioned area of the placenta, the more severe the coagulation dysfunction. TF and TFPI are involved in the pathogenesis of coagulation dysfunction in neonates after PA. Early heparin treatment is therefore helpful to improve coagulation function and prognosis in PA neonates.

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Disclosure of conflict of interest

None.

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