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Risk Factors for Grade III Meconium-Stained Amniotic Fluid in Term Singleton Pregnancies: A Retrospective Comparative Study

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Abstract: Background: Grade III meconium-stained amniotic fluid (MSAF) is an important intrapartum finding associated with increased perinatal risk. However, risk factors for severe meconium contamination in term singleton pregnancies remain incompletely defined. Objective: This study aimed to identify maternal, obstetric, intrapartum, and infection-related factors associated with grade III MSAF in term singleton pregnancies. Methods: This retrospective comparative study included 200 women with term singleton pregnancies who delivered at Lingnan Hospital, The Third Affiliated Hospital of Sun Yat-sen University, between January 2023 and December 2024. Women were divided into a grade III MSAF group (n=100) and a clear amniotic fluid control group (n=100). Maternal characteristics, obstetric variables, intrapartum factors, laboratory findings, and ultrasound parameters were compared between groups. Variables with P<0.05 in univariate analysis were entered into a multivariable logistic regression model to identify independent risk factors for grade III MSAF. Results: Compared with controls, women with grade III MSAF had a higher proportion of advanced maternal age, higher gestational age at delivery, higher proportion of gravidity ≥2, longer first stage of labor, higher incidence of intrapartum fever, higher frequency of elevated pre-delivery white blood cell count, and higher rate of maternal Group B Streptococcus colonization. Multivariable logistic regression analysis showed that longer duration of the first stage of labor (OR=1.002, 95% CI: 1.000--1.003, P=0.034), higher gestational age at delivery (OR=1.532, 95% CI: 1.002--2.344, P=0.049), gravidity ≥2 (OR=3.377, 95% CI: 1.410--8.085, P=0.006), elevated pre-delivery white blood cell count (OR=4.518, 95% CI: 1.109--18.408, P=0.035), and maternal GBS colonization (OR=17.896, 95% CI: 7.161--44.729, P<0.001) were independently associated with grade III MSAF. Conclusion: Higher gestational age at delivery, gravidity ≥2, prolonged first stage of labor, elevated pre-delivery white blood cell count, and maternal GBS colonization were independent risk factors for grade III MSAF in term singleton pregnancies. These findings suggest that both labor-related and infection-related factors should be considered in intrapartum risk assessment for severe meconium contamination.

Keywords: Meconium-stained amniotic fluid; grade III; term pregnancy; risk factors; Group B Streptococcus

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1. Introduction

Meconium-stained amniotic fluid (MSAF) is a common obstetric finding during labor, particularly among term and post-term pregnancies, with a reported incidence of approximately 7--22% in term deliveries [1,2]. Although meconium passage into the amniotic cavity may reflect physiological maturation of the fetal gastrointestinal tract, it may also be associated with fetal hypoxia, umbilical cord compression, placental insufficiency, or intrauterine stress [1,2]. Therefore, MSAF is generally regarded as an important intrapartum sign requiring careful fetal and neonatal assessment.

The clinical significance of MSAF depends partly on the degree and thickness of meconium contamination. Thick or grade III MSAF has been associated with a higher risk of adverse neonatal outcomes, including low Apgar scores, need for neonatal resuscitation, neonatal intensive care unit admission, meconium aspiration syndrome, and other respiratory complications [3,4]. Previous studies have shown that the severity of meconium staining is positively correlated with unfavorable neonatal outcomes, suggesting that grade III MSAF may represent a clinically important subgroup requiring further investigation [3].

The etiology of MSAF is complex and multifactorial. Reported risk factors include advanced gestational age, prolonged labor, premature rupture of membranes, oligohydramnios, hypertensive disorders of pregnancy, gestational diabetes mellitus, fetal distress, and intra-amniotic infection [2,5]. However, the relative contribution of these factors varies across different study populations, and available evidence regarding risk factors specifically associated with grade III MSAF remains limited. Moreover, most previous studies have focused on overall MSAF or neonatal outcomes, whereas fewer studies have evaluated maternal, obstetric, labor-related, and infection-related indicators together in a single risk-factor model.

Identification of independent risk factors for grade III MSAF is clinically relevant because it may help obstetricians recognize high-risk women during labor and improve intrapartum monitoring and neonatal preparedness. Therefore, the present study retrospectively analyzed clinical data from term singleton pregnancies and compared maternal characteristics, obstetric factors, labor-related variables, and infection-related indicators between women with grade III MSAF and those with clear amniotic fluid. Multivariable logistic regression analysis was further performed to identify independent risk factors associated with grade III meconium-stained amniotic fluid.

2. Materials and Methods

2.1. Study Design and Participants

This retrospective comparative study included women who delivered at the Department of Obstetrics, Lingnan Hospital, The Third Affiliated Hospital of Sun Yat-sen University, between January 2023 and December 2024. The inclusion criteria were as follows: term singleton pregnancy, gestational age ≥37 weeks, documented amniotic fluid characteristics at delivery, and complete medical records. The exclusion criteria were grade I--II meconium-stained amniotic fluid, bloody amniotic fluid, unclear documentation of amniotic fluid characteristics, fetal trauma or hemorrhagic shock, prenatally diagnosed fetal structural anomalies or chromosomal abnormalities, and missing key clinical or laboratory data. A total of 200 women were included and divided into the grade III meconium-stained amniotic fluid group (n=100) and the clear amniotic fluid control group (n=100). Grade III meconium-stained amniotic fluid was defined as thick, opaque, dark-green or brown amniotic fluid observed macroscopically at delivery [6].

2.2. Data Collection and Definitions

Clinical data were extracted from the electronic medical record system, including maternal demographic characteristics, obstetric variables, intrapartum factors, laboratory findings, and ultrasound parameters. Maternal variables included age, educational level, gravidity, pregnancy complications, and Group B Streptococcus (GBS) screening results. Advanced maternal age was defined as maternal age ≥35 years [7]. Gravidity was categorized as 1 or ≥2. GBS positivity was defined as a positive vaginal--rectal culture obtained at 36 0/7--37 6/7 weeks of gestation [8]. Obstetric and intrapartum variables included gestational age at delivery, fetal presentation, duration of the first and second stages of labor, labor epidural analgesia, intrapartum fever, premature rupture of membranes, cervical balloon catheter induction, artificial rupture of membranes, and oxytocin augmentation. Intrapartum fever was defined as a maternal temperature ≥38.0C during labor [9]. Laboratory and ultrasound variables included pre-delivery white blood

cell count, amniotic fluid depth, and placental maturation grade. Elevated pre-delivery white blood cell count was defined as ≥15.0×10⁹/L based on pregnancy-specific reference intervals and clinical practice [10]. Placental maturation grade was categorized as ≤II or >II.

2.3. Statistical Analysis

Statistical analyses were performed using SPSS version 26.0. Continuous variables are presented as mean ± standard deviation and were compared using the independent-samples t test. Categorical variables are presented as number and percentage and were compared using the χ^2 test or Fisher's exact test, as appropriate. Grade III meconium-stained amniotic fluid was used as the dependent variable, with clear amniotic fluid coded as 0 and grade III meconium-stained amniotic fluid coded as 1. Variables with P<0.05 in univariate analysis were entered into a multivariable logistic regression model using a forward stepwise method. Odds ratios and 95% confidence intervals were calculated. A two-sided P<0.05 was considered statistically significant.

3. Results

3.1. Baseline Maternal, Obstetric, and Intrapartum Characteristics

A total of 200 term singleton pregnancies were included in this study, comprising 100 women with grade III meconium-stained amniotic fluid and 100 women with clear amniotic fluid. The comparison of maternal, obstetric, and intrapartum characteristics between the two groups is shown in Table 1. Compared with the control group, women in the grade III meconium-stained amniotic fluid group had a higher proportion of advanced maternal age (17.0% vs. 6.0%, P=0.027), greater gestational age at delivery (39.34 ± 0.95 vs. 38.89 ± 0.97 weeks, P=0.001), and a higher proportion of gravidity ≥2 (47.0% vs. 15.0%, P<0.001). Regarding intrapartum characteristics, the duration of the first stage of labor was significantly longer in the grade III meconium-stained amniotic fluid group than in the control group (631.97 ± 300.50 vs. 524.72 ± 237.31 min, P=0.006). The incidence of intrapartum fever was also higher in the grade III meconium-stained amniotic fluid group (42.0% vs. 27.0%, P=0.027). In addition, elevated pre-delivery white blood cell count was more frequent in the grade III meconium-stained amniotic fluid group than in the control group (29.0% vs. 4.0%, P<0.001), and maternal GBS colonization was also more common in the grade III meconium-stained amniotic fluid group (67.0% vs. 52.0%, P=0.044). No significant differences were observed between the two groups in maternal educational level, fetal presentation, amniotic fluid depth, placental maturation grade, duration of the second stage of labor, labor epidural analgesia, premature rupture of membranes, cervical balloon catheter induction, artificial rupture of membranes, oxytocin augmentation, gestational diabetes mellitus, hypertensive disorders of pregnancy, or maternal cardiac disease (all P>0.05).

Table 1. Maternal, Obstetric, and Intrapartum Characteristics of the Study Population

Variable	Contamination group (n=100)	Control group (n=100)	Statistic	P value
Advanced maternal age (≥35 years)	17 (17.0)	6 (6.0)	$\chi2$=4.913	0.027
Gestational age at delivery (weeks)	39.34 ± 0.95	38.89 ± 0.97	t=-3.317	0.001
Gravidity ≥2	47 (47.0)	15 (15.0)	$\chi2$=16.615	<0.001
Amniotic fluid depth (mm)	47.46 ± 13.13	48.41 ± 11.22	t=-0.550	0.583
Placental maturation grade >2	10 (10.0)	8 (8.0)	$\chi2$=0.244	0.621
Duration of first stage of labor (min)	631.97 ± 300.50	524.72 ± 237.31	t=2.801	0.006
Duration of second stage of labor (min)	63.85 ± 50.27	69.85 ± 42.23	t=-0.914	0.362
Labor epidural analgesia	87 (87.0)	86 (86.0)	$\chi2$=0.000	1.000
Intrapartum fever	42 (42.0)	27 (27.0)	$\chi2$=4.900	0.027
Premature rupture of membranes (PROM)	14 (14.0)	20 (20.0)	$\chi2$=0.886	0.347

Elevated pre-delivery WBC count (>15×109/L)	29 (29.0)	4 (4.0)	$\chi2$=22.656	<0.001
Cervical balloon catheter induction	28 (28.0)	20 (20.0)	$\chi2$=1.343	0.247
Artificial rupture of membranes (AROM)	27 (27.0)	27 (27.0)	$\chi2$=0.000	1.000
Oxytocin augmentation	32 (32.0)	30 (30.0)	$\chi2$=0.023	0.879
Gestational diabetes mellitus (GDM)	19 (19.0)	12 (12.0)	$\chi2$=1.374	0.241
Hypertensive disorders of pregnancy (HDP)	7 (7.0)	1 (1.0)	$\chi2$=3.255	0.071
Maternal cardiac disease	14 (14.0)	11 (11.0)	$\chi2$=0.183	0.669
Maternal GBS colonization	67 (67.0)	52 (52.0)	$\chi2$=4.067	0.044

3.2. Multivariable Logistic Regression Analysis

Variables with P<0.05 in the univariate analysis were entered into the multivariable logistic regression model. As shown in Table 2, duration of the first stage of labor, gestational age at delivery, gravidity ≥2, elevated pre-delivery white blood cell count, and maternal GBS colonization were independently associated with grade III meconium-stained amniotic fluid. Specifically, a longer duration of the first stage of labor was associated with an increased risk of grade III meconium-stained amniotic fluid (OR=1.002, 95% CI: 1.000--1.003, P=0.034). Increased gestational age at delivery was also independently associated with grade III meconium-stained amniotic fluid (OR=1.532, 95% CI: 1.002--2.344, P=0.049). Women with gravidity ≥2 had a higher risk of grade III meconium-stained amniotic fluid than those with gravidity of 1 (OR=3.377, 95% CI: 1.410--8.085, P=0.006). Elevated pre-delivery white blood cell count was independently associated with grade III meconium-stained amniotic fluid (OR=4.518, 95% CI: 1.109--18.408, P=0.035). Maternal GBS colonization was also identified as an independent risk factor (OR=17.896, 95% CI: 7.161--44.729, P<0.001). Advanced maternal age and intrapartum fever were not independently associated with grade III meconium-stained amniotic fluid after adjustment for other variables.

Table 2. Independent Risk Factors for Contamination Identified by Multivariable Logistic Regression Analysis

Variable	β	SE	Wald $\chi2$	OR (95% CI)	P value
Duration of first stage of labor	0.002	0.001	4.493	1.002 (1.000–1.003)	0.034
Gestational age at delivery	0.427	0.217	3.872	1.532 (1.002–2.344)	0.049
Gravidity ≥2	1.217	0.445	7.462	3.377 (1.410–8.085)	0.006
Advanced maternal age	0.319	0.759	0.177	1.376 (0.311–6.093)	0.674
Intrapartum fever	0.755	0.459	2.709	2.128 (0.866–5.231)	0.100
Elevated pre-delivery WBC count	1.508	0.717	4.428	4.518 (1.109–18.408)	0.035
Maternal GBS colonization	2.885	0.467	38.094	17.896 (7.161–44.729)	<0.001

4. Discussion

In this retrospective comparative study of term singleton pregnancies, we found that gestational age at delivery, gravidity ≥2, duration of the first stage of labor, elevated pre-delivery white blood cell count, and maternal GBS colonization were independently associated with grade III meconium-stained amniotic fluid. These findings suggest that severe meconium contamination is related to both labor-related and infection-related factors.

Grade III meconium-stained amniotic fluid is clinically important because thick meconium has been associated with less favorable perinatal outcomes compared with clear amniotic fluid or mild meconium staining [11,12]. Zhu et al. reported that grade III meconium-stained amniotic fluid was associated with increased obstetric intervention and poorer maternal and neonatal outcomes [11]. Similarly, Gluck et al. showed that the thickness of meconium staining was correlated with adverse neonatal outcomes [12].

Therefore, identifying risk factors for grade III meconium contamination may help improve intrapartum risk assessment and neonatal preparedness.

Gestational age at delivery was independently associated with grade III meconium-stained amniotic fluid in our study. This finding is consistent with previous reports indicating that meconium staining becomes more common as gestational age advances [13,14]. The association may be explained by progressive maturation of the fetal gastrointestinal tract and increased intestinal peristalsis at term. Although our study included only term pregnancies, the persistence of this association after adjustment suggests that advancing gestational age within the term period may still contribute to severe meconium contamination.

A longer duration of the first stage of labor was also independently associated with grade III meconium-stained amniotic fluid. Prolonged labor may increase fetal exposure to repetitive uterine contractions, intermittent reductions in uteroplacental perfusion, and intrapartum stress, which may stimulate vagal activity and promote meconium passage. Previous studies have similarly reported associations between prolonged or obstructed labor and meconium-stained amniotic fluid [13,14]. In our model, the odds ratio for first-stage labor duration was close to 1 because the variable was analyzed per minute increase; therefore, its clinical effect should be interpreted cumulatively over a longer labor duration.

Elevated pre-delivery white blood cell count was independently associated with grade III meconium-stained amniotic fluid. Physiological leukocytosis is common during pregnancy; therefore, white blood cell count should be interpreted using pregnancy-specific reference intervals [15]. Nevertheless, a markedly elevated white blood cell count before delivery may indicate maternal inflammatory activation or occult infection. Our findings suggest that maternal inflammatory status may be involved in the occurrence of severe meconium contamination, although white blood cell count alone cannot distinguish physiological leukocytosis from infection-related inflammation.

Maternal GBS colonization was also independently associated with grade III meconium-stained amniotic fluid. GBS colonization is a clinically relevant perinatal infectious factor and has been associated with adverse maternal and neonatal outcomes in recent studies [16]. In the present study, GBS colonization may represent an altered genital tract microbial environment or increased susceptibility to inflammatory responses during labor. However, because this was a retrospective study, the observed association should not be interpreted as evidence of causality. Further studies incorporating quantitative GBS load, inflammatory biomarkers, antibiotic exposure, and placental pathology are needed to clarify this relationship.

Advanced maternal age and intrapartum fever were significant in univariate analysis but did not remain independently associated with grade III meconium-stained amniotic fluid after adjustment. This suggests that their crude associations may have been influenced by other factors, such as gestational age, labor duration, leukocytosis, or GBS colonization. Intrapartum fever is clinically important but nonspecific, as it may be related to infection, epidural analgesia, prolonged labor, or noninfectious inflammatory responses.

This study has several limitations. First, this was a single-center retrospective comparative study, and selection bias cannot be excluded. Second, the sample size was limited, which may have affected the precision of some estimates. Third, grade III meconium-stained amniotic fluid was diagnosed by macroscopic clinical assessment, which may introduce observer variability. Fourth, some potentially relevant variables, including fetal heart rate patterns, umbilical artery blood gas results, duration of membrane rupture, antibiotic exposure, neonatal outcomes, inflammatory biomarkers, and placental histopathology, were not included in the model. Finally, this study can identify associations but cannot establish causal relationships.

5. Conclusion

In conclusion, higher gestational age at delivery, gravidity ≥2, longer duration of the first stage of labor, elevated pre-delivery white blood cell count, and maternal GBS colonization were independently associated with grade III meconium-stained amniotic fluid in term singleton pregnancies. These findings suggest that both labor-related factors and infection-related indicators should be considered in the risk assessment of severe meconium contamination.

Conflict of Interest Statement: The authors have no conflict of interest to declare.

Data Availability Statement: The datasets generated and analyzed during the current study are not publicly available due to patient privacy concerns, but are available from the corresponding author on reasonable request.

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