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Original Article

Associations of viscosity, stercobilin and bilirubin levels in meconium stained amniotic fluid to meconium aspiration syndrome

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Abstract

Background Meconium-stained amniotic fluid (MSAF) increases morbidity and mortality in neonates. Meconium aspiration syndrome (MAS) occurs in 2-9% of neonates with MSAF. Viscosity of MSAF is associated with the amount of the meconium release.

Objective To determine the associations between viscosity and the presence of stercobilin and bilirubin in MSAF with MAS in neonates.

Methods This observational cohort study was performed with term babies who were born with MSAF in Kariadi Hospital from August 2009 to May 2010. Amniotic fluid specimens were taken at birth and neonates were observed for respiratory symptoms until the 5th day of life. Analysis was done by chi-square test, Fisher's exact test and relative risk.

Results The majority of the 48 subjects were male, with mean gestational age of 39.9 (SD 1.73) weeks. Classification of MSAF as thick or thin was done by macroscopic examination with Kappa test 0.741. The MSAF tested positively for stercobilin and bilirubin in 12/48 and 17/48 subjects, respectively. Thick MSAF correlated significantly to MAS (P=0.03) with a relative risk of 10.1 (95% CI=1.2-87.6), while stercobilin and bilirubin presence did not.

Conclusion Thick MSAF was associated with MAS and was a risk factor for MAS. Stercobilin and bilirubin presence in MSAF were not associated with MAS. **[Paediatr Indones. 2011;51:101-6]**.

Keywords: *meconium-stained amniotic fluid, stercobilin, bilirubin, meconium aspiration syndrome*

Neonatal respiratory distress is defined as breathing difficulties in neonates with prior normal respiration or in those with asphyxia, but who recovered following resucitation.[1] In preterm babies, neonatal respiratory distress is usually caused by pneumonia, hyaline membrane disease, or immaturity of central nervous system. In term babies, meconium aspiration syndrome (MAS), transient tachypnea of the newborn, pneumonia, and congenital malformations are likely causes.[1,2]

Meconium aspiration syndrome (MAS) remains one of the principal causes of neonatal respiratory distress, frequently leading to respiratory failure and death.[3] Meconium-stained amniotic fluid (MSAF) occurs in 8 to 20% of all pregnancies and is usually associated with term fetuses.[4] MAS occurs in 2 to 9% of infants born with MSAF and has a mortality rate of 40%. Meconium has been found below vocal cords in 20 to 45% of infants born with MSAF.[5] The passage of

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meconium in utero indicates acute or chronic hypoxia and is observed primarily in cases of advanced fetal maturity.[6,7]

The quality of meconium in amniotic fluid has been classified as thick, medium or thin.[4] Rossi *et al.*[9] reported that MAS in newborns with thick, moderate and thin meconium occured in 19%, 4.6% and 2.9%, respectively. MAS has been associated with the amount of meconium released in amniotic fluid or constituents of meconium. It is unclear which constituents of meconium cause MAS. Meconium is the first newborn stool stored in the baby's intestines until birth. It consists of undigested portions of swallowed debris, various products of secretion, excretion and desquamation, such as bile pigments, bile salts, lanugo, squamous cells, mucopolysaccharides and cholesterol. Urobilin, bilirubin and stercobilin are bile salts in adult stool.[6]

The purpose of this study was to determine the associations of MSAF viscosity, and the presence of stercobilin and bilirubin in MSAF to MAS.

Methods

This observational, cohort study, was carried out from August 2009 to May 2010 at Kariadi Hospital, Semarang. We included neonates with MSAF who were delivered from singleton pregnancies with gestation of 37 weeks or more, had cephalic presentation, and no history of mother's illness during pregnancy. We excluded neonates with febrile history, heart failure, congenital heart disease, aspiration pneumonia, history of premature rupture of membranes, and those with diabetic mothers. Informed consent was obtained from mothers. This research was approved by the Ethics Committee, Diponegoro University/ Kariadi Hospital.

We collected data from mothers on baby's gender, mother's age, route of delivery, birth weight, gestational age, history of maternal illness, and maternal respiratory problems. We measured Apgar scores at 1 minute and 5 minutes after birth. We collected MSAF specimens at labour, to be clinically and macroscopically assessed by the physician and laboratory technicians. Kappa test was also performed. MSAF was further categorized on the basis of meconium consistency (or viscosity) into thick and thin. Thick meconium was defined as turbid and viscous or particulate. Thin meconium was defined as watery and thinly stained. Stercobilin in MSAF was examined using Schlessinger methods and bilirubin by Fouchet methods.[9]

All babies were followed-up to 5 days after delivery and admitted to the neonatal unit. We examined subjects for signs of respiratory problems and MAS daily. Chest X-rays were taken at day 5 (unless there was a respiratory problem earlier), and analyzed by radiologist. A diagnosis of MAS was made based on the presence of MSAF, clinical signs of respiratory distress, and the presence of irregularities or coarse and patchy infiltrate in the chest X-ray.

All data were analyzed using SPSS for Windows. Chi-square test or Fisher's exact test was used to compare categorical variables. Univariate and multivariate analysis of risk factors associated with quality of MSAF and maternal factors were analyzed with 95% confidence interval (95% CI). Forward logistic regression analysis was used to estimate the risk of MAS. $P < 0.05$ was considered statistically significant.

Results

Forty-eight term neonates were enrolled in our study. The majority were male and born of primiparous mothers. Mean gestational age was 39.9 (SD 1.73) weeks. Kappa test was 0.74. **Table 1** shows the characteristics of subjects, divided into 2 groups, thick MSAF and thin MSAF.

The influence of fetal and maternal factors on MSAF viscosity are shown in **Table 2**. There were no statistically significant differences between the two groups in the following fetal and maternal factors: fetal distress, gestational age, history of taking herbal medicine, pre-eclampsia, eclampsia and hypertension.

Respiratory problems were found in 11 babies. Chest X-ray showed 4 neonates had MAS and 7 had pneumonia. MAS was observed in 3 neonates with thick MSAF and 1 neonate with thin MSAF. **Table 3** also shows the association between MSAF viscosities with MAS. We found thick MSAF was associated with MAS (RR 10.1, 95% CI 1.2 to 87.6).

Table 1. Characteristics of subjects

Characteristic	Meconium-stained amniotic fluid	
	Thick n = 11	Thin n = 37
Male gender	8	18
Mean birth weight, g (SD)	3000 (569.6)	3104.1 (444)
Mean mother's age, yrs (SD)	33.1 (6.2)	27.6 (6.8)
Mean gestational age, weeks (SD)	39.6 (2.3)	40 (1.6)
Mean gravida	2	1
Route of delivery		
spontaneous	5	10
vacuum extraction	2	7
caesarean	4	20
Stercobilin		
- positive	4	8
- negative	7	29
Bilirubin		
- positive	6	11
- negative	5	26

Associations among confounding factors such as maternal hypertension, history of herbal medicine, Apgar score and neonatal infection status are shown in **Table 4**.

Using binary logistic regression analysis to identify risk factors that may predict MAS occurrence, we found the 5 minute Apgar score to be such a factor. In addition, we found neonatal infection to be statistically significant (adjusted OR 9.9, 95% CI 2.0 to 47.9).

Discussion

MSAF is a serious sign of fetal compromise and is associated with increased prenatal morbidity and mortality.[10] In our study, the mean gestational ages of subjects in the thick and thin meconium groups were 39.6 (SD 2.3) weeks and 40 (SD 1.6) weeks, respectively. Narli *et al*. found a significantly increased

Table 2. Influence of fetal and maternal factors on MSAF viscosity.

Fetal and maternal factors	Meconium-stained amniotic fluid		P
	Thick, n	Thin, n	
Fetal distress	5	7	0.1
Gestational age			
post term	2	2	
term	9	35	0.2
History of herbal medicine	4	4	0.07
Pre-eclampsia	2	5	0.6
Eclampsia	0	2	1.0
Hypertension	0	4	0.6

Table 3. Association between viscosity of MSAF with MAS

Viscosity of MSAF	MAS		RR	95% CI
	Yes	No		
Thick	3	8	10.1	1.2 to 87.6
Thin	1	36		

Table 4. Association between maternal/neonatal factors and MAS

Confounding factors	MAS		RR	95% CI
	Yes	No		
Maternal factors:				
History of herbal medicine	2	6	5.0	0.8 to 30.5
Hypertension	0	4	-	-
Neonatal factors				
Moderate-severe asphyxia	4	5	-	-
Neonatal infection	4	6	5.0	1.7 to 14.7

MSAF in neonates of 39 weeks gestation.[4] We also found that MSAF increased with gestational age. One explanation is that the hormone motilin is secreted in increasing quantities by the fetus as gestational age advances and most meconium discharge is thought to occur in postdated gestations since motilin levels are highest at that time.[11,12] MSAF may be a fetal response to hypoxia, vagal stimulation from transient umbilical cord entrapment, or decreased sympathetic nervous system activity with loss of sphincter tone. In Southern Africa, it was reported that 30% of labouring women exhibit MSAF due to the use of herbal medicines with oxytocin properties.[13]

We observed increased mean birth weight. Sedaghatian *et al*. observed similar results in their study.[14] The observations of Naven *et al*. support the idea that meconium staining is more common in growth-retarded babies subjected to chronic intrauterine hypoxia, depending on the site of placenta, cord length, depth of pelvis and tightness of cord.[12]

Primiparity, known as a risk factor for adverse perinatal outcome was significantly associated with MSAF. We observed that the majority of mothers was primiparous, in keeping with the finding by Saunders in Zimbabwe. Primiparity places the fetus under stress due to longer duration of labour and rupture of membranes, as well as obstructed labour, all of which were shown to be significantly associated with MSAF.[15]

Viscosity of MSAF was classified as thick or thin, 23% and 77% of samples, respectively, in our study. Sheiner *et al*.[16] showed most (78%) of their cases had thin MSAF, however, Khazardoost *et al*.[17] found a higher percentage of thick meconium (90%) than thin meconium. MSAF viscosity depends on the constituents of meconium, the relationship between asphyxia and meconium release, the time and cause of the passage of meconium into amniotic fluid, and the amount of amniotic fluid.[18]

The presence of stercobilin was not significantly different in thick and thin meconium specimens. Since we performed a qualitative examination, a positive test result was given if the value exceeded a threshold, but if it was under the threshold value, the result was considered negative. Poggi *et al*. reported that determining the presence of meconium in amniotic fluid typically rests on visual observation of greenish fluid coloration. Unfortunately, there is no definitive test to confirm the clinical impression of meconium presence in amniotic fluid.[19] Many efforts have been made to chemically break down the constituents of meconium, but its variable composition and similarity to other breakdown products make identification difficult.

Fetal distress rates were also not significantly different between the thick and thin meconium groups. Strict monitoring of fetal heart rates was performed at Kariadi Hospital, a referral hospital. Abnormal fetal heart rates would be followed by appropriate medical intervention. Also, meconium aspiration is not predicted by fetal heart decelerations. However, true fetal distress may result in severe hypoxia and permanent brain damage.[13] Berkus *et al*. observed significantly higher risk of abnormal fetal heart rate and arterial pH < 7.2 in an MSAF group.[11,12] In our study, arterial blood gas analysis was not routinely done. Mode of delivery was influenced by the presence of MSAF with 42% Caesarean deliveries. In agreement, Dargavilee *et al*. found caesarean delivery to be associated with a higher risk of subsequent MAS in the presence of MSAF.[20]

Maternal factors such as pre-eclampsia, eclampsia, hypertension and history of herbal medicine use were similar in the two groups. Narli *et al*. found that maternal eclampsia was significantly higher with thick meconium.[4] The presence of thick MSAF may be due to limited amniotic fluid diluting the passed meconium.[21]

We found that thick MSAF was a risk factor for MAS. Ziadeh *et al*. found the incidence of MAS and respiratory distress were significantly increased in those with MSAF.[22] MAS was primarily associated with acute hypoxic events late in labour or chronic prenatal diseases related to acute events occurring late in labour or after birth. MAS also depends on increasing consistency of meconium.[11] Meconium aspiration can occur either during labour or at the time of baby's first breath.[23] The mechanisms of MAS may be explained as (1) atelectasis due to complete obstruction of the small and large airways by large particles of meconium, (2) emphysema due to partial obstruction of the small and large airways by small particles, (3) chemical pneumonitis due to bile salts and proteolytic enzymes in the meconium, or (4) bronchoconstriction due to airway irritation.[6,20] The evidence that chemical

pneumonitis is caused by meconium was first documented in rabbits by the precence of infiltration of polymorphonuclear leukocytes in alveolar septae within 6 hours of instillation of meconium. Other study on pigs documented increased counts and chemotactic activity of neutrophils in lung lavage fluid. Leukocytes are important sources for three main inflammatory branches that are induced or activated by meconium: cytokines (such as IL-6, IL-8, TNF-α, and IL-1β), arachidonic acid metabolites and reactive oxygen species.[24] Ghidini and Spong postulated that most cases of severe MAS are caused by pathological processes occuring during pregnancy, mainly chronic asphyxia and infection.[25]

A limitation of this study was that we did not perform amniocentesis to rule out patients with MSAF in early labor and those who developed the condition intrapartum. In conclusion, MSAF is associated with MAS. Furthermore, thick MSAF is a risk factor for MAS. Stercobilin and bilirubin in MSAF are not risk factors for MAS. Proportion of MAS was higher in thick MSAF. Further research is needed to determine cytokine changes in MSAF.

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