J Membrane Biol (2013) 246:525–528
DOI 10.1007/s00232-013-9562-7

# Spectrophotometric Analysis in Umbilical Cords of Infants with Meconium Aspiration Syndrome

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Received: 14 February 2013 / Accepted: 13 May 2013 / Published online: 7 June 2013


**Abstract** We compared spectrophotometric analysis of the umbilical cords of infants with meconium aspiration syndrome (MAS) or with meconium-stained amniotic fluid (MSAF) and healthy infants. In a prospective study, 15 infants with MAS and 37 infants with MSAF were enrolled. Twenty healthy infants formed a control group. The absorption peak of umbilical cords with meconium was significantly higher in the infants with MAS or MSAF than in controls. Spectrophotometric analysis of the umbilical cords with meconium may be useful to identify developed neonates with MAS or MSAF.

**Keywords** Spectrophotometric analysis · Meconium aspiration syndrome · Newborn · Umbilical cord

## Introduction

The aspiration of meconium-stained amniotic fluid (MSAF) in the newborn can lead to meconium aspiration syndrome (MAS), which is a clinical syndrome with significant morbidity and mortality. Its presence is an indication for continuous fetal heart rate monitoring for reassurance of fetal well-being. Considerable controversy exists regarding the cause of passage of meconium in utero by the fetus. In utero hypoxia leading to relaxation of the anal sphincter has been considered a reason for meconium passage. Adverse intrauterine environment compromising fetal well-being can lead to MSAF (Castellheim et al. 2004; Mollnes et al. 2008; Berkus et al. 1994).

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Swallowing of infected amniotic fluid followed by premature defecation by the fetus could also be a likely explanation for MSAF (Wen et al. 1993; Cleary and Wiswell 1998; Schrama et al. 2001). We noticed that newborn infants with signs of respiratory distress due to meconium aspiration have frequently a meconium-stained umbilical cord soon after birth. In the most striking cases the umbilical cord has a yellowish green color. As an explanation for this, a water-soluble substance derived from meconium might be absorbed through the lungs and stained in the umbilical cord.

The aim of this study was to compare spectrophotometric analyses of the umbilical cords of infants with MAS or MSAF and healthy infants.

## Method

In a prospective study, 15 infants with MAS and 37 infants with MSAF followed in the Neonatal Intensive Care Unit (NICU), Medical School, Yuzuncu Yil University, were enrolled. Twenty healthy infants formed a control group.

Group 1 patients were infants with MAS. Patients with MSAF comprised group 2. Twenty newborns who had normal clinical and laboratory findings were included as a control group. Obstetric and neonatal data for deliveries in groups 1 and 2 and controls were reviewed for the following characteristics: presence of MAS or MSAF, presence of fetal distress as evidenced by late decelerations, birth weight, gestational age, Apgar scores at the first and fifth minutes, score for the neonatal acute physiology (NAP), spectrophotometric evaluations of umbilical cords and MAS with positive chest X-ray.

Diagnosis of meconium aspiration was made when all of the following signs were present: evidence of fetal distress,

passage of MSAF before delivery, an Apgar score of 5 or less within the first 5 min of life, respiratory distress with signs of airway obstruction, presence of meconium or meconium-stained liquid in the trachea and a characteristic X-ray appearance as described by previous studies (Dehan et al. 1978; Gooding and Gregory 1971).

### Biochemical Analysis

Heel blood was obtained using the standard lancet for capillarized gas analysis. The blood was transferred to the standard heparinized capillary tubes, and the samples were studied in an ABL-500 (Radiometer, Brønshøj, Denmark) device in the first 5 min. Samples with clot or hemolysis were not studied within the first 5 min and were excluded from the study.

Umbilical cord samples from all infants were obtained, stored in saline and sent to the biochemistry laboratory. Samples were stored in a deep freezer at −20 °C. Firstly, samples were washed with saline. KCI solution (1.5 %, 3 ml for 1 g of tissue) was added to the samples, and then they underwent homogenization in the Ultra-Turrax T25a (IKA-Labortechnik, Stauffen, Germany). They were centrifuged at 4,000 rpm for 20 min (Nuve 800 R). The sample was then extracted from the top of the supernatant and measured in the spectrophotometer (Novarpec II; Pharmacia Biotech, Cambridge, UK), particularly at 325, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440 and 450 nm wavelengths. This procedure is applicable when using the Pharmacia Biotech Nova Spec II to measure the absorbance of visible light in a sample in a consistent manner, and quality controls are also performed. The biochemist was not aware of the clinical course or neonatal morbidity. The study was approved by the local ethical committee of Yuzuncu Yil University, Faculty of Medicine; and informed consent was obtained from the parents.

### Statistical Analyses

The data were statistically tested by one-way ANOVA, Dunnett's (two-sided) $t$ test, student's $t$ test and Pearson's correlation analysis using SPSS 12.0 (SPSS, Inc., Chicago, IL). $p < 0.05$ was considered to indicate significance. Data were presented as means ± SD.

## Results

The study included 72 neonates: 15 infants with MAS, 37 infants with MSAF and 20 healthy infants. Clinical and laboratory characteristics of the neonates are shown in Table 1. Mean birth weights and gestational ages of infants in all groups were similar. Apgar scores at the first and fifth minutes of infants in groups 1 and 2 were significantly higher ($p < 0.05$) than those in control infants. Acidosis (pH < 7.25 of blood) was significantly higher in newborns with MAS or MSAF compared to controls ($p < 0.05$).

As shown in Table 2, umbilical cord samples of neonates with MAS showed an absorption peak at 410 nm. The peak absorption of umbilical cord samples from normal neonates was at 325 nm. NAP scores were significantly higher in group 1 infants compared to group 2 (average 11.93 vs. 8.08).

## Discussion

To the best of our knowledge, this is the first study presenting the spectrophotometric changes in the umbilical cords of infants with MAS and MSAF in the literature. The Apgar score is very important in infants with meconium in order to assess the prognosis. It was reported that Apgar scores of infants with thick meconium and with MAS were lower (Khazardoost et al. 2007; Bhat and Rao 2008). Karatekin et al. (1999) compared Apgar scores at the first and fifth minutes in 1,243 infants with MSAF and 65 infants with MAS and found a significant difference. Liu and Harrington (2002) stated that in meconium deliveries infants with thick meconium, fetal distress and Apgar scores <7 at 1 and 5 min are at high risk for development of respiratory symptoms. Infants delivered in the absence

**Table 1** Clinical and laboratory characteristics of the study population (group 1 with MAS, group 2 with MSAF and group 3 controls)

| Characteristics | Group 1 ($n = 15$) | Group 2 ($n = 37$) | Group 3 ($n = 20$) |
|---|---|---|---|
| Gestational age (weeks) | 39.53 ± 0.99 | 39.16 ± 2.02 | 38.85 ± 1.84 |
| Birth weight (g) | 2,893.33 ± 523.63 | 2,928.11 ± 929.43 | 2,950.50 ± 792.17 |
| Apgar score at 1 min | 3.55 ± 1.50* | 5.81 ± 1.66 | 6.20 ± 1.10*** |
| Apgar score at 5 min | 6.09 ± 1.81* | 8.45 ± 1.15 | 8.90 ± 0.55*** |
| pH at first hour | 7.13 ± 0.19 | 7.20 ± 0.16** | 7.34 ± 0.06*** |
| SNAP score | 11.93 ± 6.37* | 8.08 ± 3.26 | |

Continuous data are expressed as means ± SD

*$p < 0.05$ vs. group 2, ** $p < 0.05$ vs. control, *** $p < 0.05$ vs. group 1 (student's $t$ test)

**Table 2** Spectrum of aqueous extract of meconium of the study population (group 1 = with MAS, group 2 with MSAF and group 3 controls)

| Optical density (nm) | Group 1 ($n = 15$) | Group 2 ($n = 37$) | Group 3 ($n = 20$) |
|---|---|---|---|
| 325 | 0.95 ± 0.29 | 0.85 ± 0.28 | 0.61 ± 0.25 |
| 330 | 1.03 ± 0.31 | 0.85 ± 0.32* | 0.56 ± 0.20** |
| 340 | 1.04 ± 0.42 | 0.90 ± 0.35* | 0.58 ± 0.28** |
| 350 | 1.05 ± 0.43 | 0.95 ± 0.43* | 0.56 ± 0.31** |
| 360 | 1.03 ± 0.57 | 0.88 ± 0.48* | 0.47 ± 0.11 |
| 370 | 1.08 ± 0.58 | 0.99 ± 0.49* | 0.47 ± 0.20** |
| 380 | 1.15 ± 0.48 | 0.84 ± 0.30* | 0.51 ± 0.22** |
| 390 | 1.09 ± 0.47 | 0.93 ± 0.37* | 0.52 ± 0.23** |
| 400 | 1.14 ± 0.45 | 1.07 ± 0.43* | 0.54 ± 0.28** |
| 410 | 1.23 ± 0.46 | 1.15 ± 0.45* | 0.57 ± 0.29** |
| 420 | 1.21 ± 0.50 | 1.12 ± 0.48* | 0.57 ± 0.31** |
| 430 | 1.13 ± 0.51 | 1.02 ± 0.49* | 0.49 ± 0.25** |
| 440 | 0.76 ± 0.59 | 0.85 ± 0.55 | 0.44 ± 0.25 |
| 450 | 0.86 ± 0.51 | 0.73 ± 0.42* | 0.42 ± 0.23** |

Continuous data are expressed as means ± SD

*$p < 0.05$ vs. control, ** $p < 0.05$ vs. group 1 (student's $t$ test)

of all of these risk factors are at low risk for development of MAS. The mean Apgar score at 1 min was 3.55 and that at 5 min was 6.09 in infants with MAS. These scores were 5.81 and 8.45 in infants with MSAF, respectively. Their findings were significant and consistent with the literature. Similarly, acidemia in these infants may have a severe prognosis parallel to the Apgar score (Grignaffini et al. 2004). It is known that acidosis is a factor affecting the occurrence of MAS. The occurrence rate for MAS increases particularly if fetal pH is lower than 7.25 (Mitchell et al. 1985). Therefore, close monitoring in the intrauterine period and precautions before the development of acidosis will decrease the prevalence of MAS and the severity of clinical signs. We also found significant acidosis in infants with MAS and MSAF compared to the control group. Our findings were compatible with those in the literature.

The NAP is a significant scale for accurately determining the length of stay and mortality in hospitalized infants. Richardson et al. (1993) showed that neonatal mortality could be anticipated with high accuracy by the NAP in 1,643 cases of three NICUs. Maiya et al. (2001) found the sensitivity, specificity, positive predictive value and negative predictive value of NAP score >15 points to be 63, 95, 72 and 92.5 %, respectively, for anticipating mortality in 295 NICU cases. We also assessed NAP score in our study and found mean scores to be 11.9 points in the first group and 8.08 points in the second. In our study, the mean NAP score in five cases who died was 15 points, which was consistent with the literature, noting that NAP score was related to mortality.

Meconium stains fetal membranes, fetal surfaces and umbilical cord a yellowish green color. If the duration of exposure of the fetus and the placenta to meconium is more than 6 h, the color of contact sites turns dark green–dark brown (Yoder 1994). Assessment of umbilical cords with meconium allows confirmation of a diagnosis of meconium aspiration when it is a yellowish green color or elimination of this diagnosis when it is evidently clear (Dehan et al. 1978). When the color of the umbilical cord is not clearly different, basically a spectrophotometric evaluation can make the distinction between normal and abnormal umbilical cords with meconium. The accurate nature of the substance current in the meconium umbilical cords is still indeterminate. Studies of the pigment composition of meconium have shown that several products resulting from the catabolism of hemoglobin could absorb light at about 405 nm, such as mesobilifuchsins, porphyrins and some derivatives of bilirubin (Dehan et al. 1978).

We studied the spectrophotometric characteristics of umbilical cords at various wavelengths. Meconium intensity and duration of exposure are important in umbilical cord staining with meconium (Yoder 1994); we thought that we could make a differential diagnosis of MAS in infants with meconium staining as the light would be refracted to a higher extent in infants with a risk of MAS development.

We determined the degree of refraction at various wavelengths in samples of umbilical cord with meconium from three different groups. The samples of the first group displayed the highest refraction at 410 nm wavelength. The samples of the second group showed the highest refraction at similar wavelength as in the first group. The control group displayed the highest refraction at 325 nm wavelength. There was a significant difference between groups 1 and 3 and between groups 2 and 3 ($p < 0.05$). We noticed that the differences were more strongly significant at 400, 410, 420 and 430 nm wavelengths. There was no significant difference between groups 1 and 2, in contrast to what we had expected ($p > 0.05$). We could not compare our

findings with those of other studies as we could not find any data in the English literature. This could be a limitation in our study.

In conclusion, we suggest that spectrophotometric analysis of umbilical cords with meconium may be useful to identify developed neonates with MAS or MSAF. A similar study with a larger sample is needed to conclusively prove the clinical usefulness of this issue.

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