**Meconium stained amniotic fluid and offspring long-term neurological health- a population based chort analysis**

Ron MATALONa\* BSc, Tamar WAINSTOCKb PhD, Asnat WALFISCHd MD, and Eyal SHEINERC MD PhD

Author affiliations

*aThe Goldman Medical School at the Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.*

*bDepartment of Public Health, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel*

*c Department of Obstetrics and Gynecology, Soroka University Medical Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel.*

*dDepartment of Obstetrics and Gynecology, Hadassah Mt, Scopus Medical Center, Jerusalem, Israel.*

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***Authors e-mail addresses:***

Tamar Wainstock: wainstoc@bgu.ac.il

Eyal Sheiner: [sheiner@bgu.ac.il](mailto:sheiner@bgu.ac.il)

Asnat Walfisch: [asnatw@bgu.ac.il](mailto:asnatw@bgu.ac.il)

***Corresponding author:***

Ron Matalon, BSc

The Goldman Medical School at the Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Ben-Gurion University Medical School, Soroka University Medical Center, 151 Izak Rager Ave., Beer-Sheva 84101, Israel.

Phone: +972-54-2204085

Email: ron.matalon@gmail.com

**ABSTRACT**

**Objective:**

The study was aimed to investigate the possible impact of meconium stained amniotic fluid (MSAF), recently found not to be sterile as previously thought, on the occurrence of neurological related hospitalizations in the offspring throughout childhood and adolescence.

**Methods:** In this population-based cohort analysis, all singleton deliveries occurring between 1991-2014 at a single tertiary medical center were included. Fetuses with congenital malformations were excluded. A Kaplan-Meier survival analysis was constructed for evaluation of cumulative hospitalization rate due to neurological morbidity (such as autism, cerebral palsy, developmental disorders, etc.) over the 18 years of follow-up, and a Cox proportional hazards model was used to study the independent association between MSAF and neurological morbidity while controlling for potential confounders.

**Results:** During the study period 242,342 deliveries met the inclusion criteria. A total of 7,543 hospitalizations due to neurological related morbidity were documented with a rate of 3.2% (1,152) in children exposed to MSAF as compared with 3.1% (6,391) in the unexposed group (odds ratio 1.05, 95% CI 0.98-1.12, *p*=0.149). The survival curve showed comparable cumulative hospitalization rate in the MSAF exposed group as compared to the unexposed group (log rank p=0.349). The Cox analysis, controlled for maternal diabetes and hypertension as well as for gestational age and maternal age, demonstrated MSAF exposure ***not to be*** an independent risk factor for neurological related hospitalizations during childhood in the offspring (adjusted HR=1.025, 0.962-1.092). This was also true when analyzed for preterm deliveries (<37 weeks) only (adjusted HR=1.194, 0.906-1.572).

**Conclusion:** Fetal exposure to MSAF, whether at term or preterm, does not appear to be an independent risk factor for neurological related hospitalizations in the offspring throughout childhood and adolescence.

**Introduction:**

Meconium passage is a normal non-pathological event usually occurring within 24-48 hours after birth (1). However, one of the severe signs of fetal compromise is exposure to meconium in amniotic fluid (MSAF), observe in 5%-20% of labors, which may lead to significant morbidity in offspring (2). Despite previous assumptions that the first pass meconium is sterile (3), MSAF was recently found not to be sterile as previously thought (4).

MSAF is significantly more common in the post-term deliveries as compared to term deliveries (14.9% and 25.9% respectively) (5). It was also found that the longer the duration of labor, the higher the frequency of MSAF (6). Other risk factors associated with MSAF are older maternal age, multiparty and lack of prenatal (7).

Studies found that MSAF can lead to several pregnancy complications, not only in high-risk pregnancies but also in low-risk pregnancies (8). MSAF was found as a risk factor for intrauterine fetal death (9), intrapartum fetal death (10), abnormal fetal heart rate tracings and low Apgar scores at 1 minute (9). MSAF was also found as a risk factor for meconium aspiration syndrome, neonatal hypoxic-ischemic encephalopathy, neonatal sepsis and seizures (11).

The long-term implications of MSAF exposure are varied. Meconium aspiration syndrome, one of the major impacts of MSAF, has long-term pulmonary sequelae that including airway obstruction, hyperinflation, elevated closing volumes, and airway hyperreactivity (12). Interestingly, the presence of meconium during labor showed lower rates of long-term infectious (13) and dermatological morbidity in the offspring (14), emphasizing a possible role of meconium in the maturation of the immune and microbial systems of the newborn.

Another critical outcome that, to the best of our knowledge, was not investigated thoroughly is the association between exposure to MSAF and long-term neurological morbidity. We aimed to determine whether MSAF exposure during labor may adversely affect the long-term neurological health of the offspring.

**Materials and methods**:

In this retrospective population-based chort analysis, we aimed to investigate the impact of meconium-stained amniotic fluid on the occurrence of neurological related hospitalizations of the offspring throughout childhood and adolescence. The study population consisted of all singleton deliveries occurring between the years 1991-2014 at the Soroka University Medical Center (SUMC), a tertiary hospital in the Negev (southern part of Israel). This hospital serves approximately 1,272,100 residents living in the Negev region, the fourth largest in Israel in terms of population size, and very diverse in terms of population characteristics and forms of settlements (15).

The study protocol was approved by the SUMC institutional review board and informed consent was exempt due to the nature of the study design.

The study population consisted of all singleton deliveries, divided into two groups by the independent variable- the exposure to MSAF, a comparison performed between offspring born with or without exposure to MSAF. Fetuses with congenital malformations were excluded.

Data were collected from two datasets. The first is a computerized perinatal database of the obstetrics and gynecology department at SUMC, included obstetrical and general maternal, fetal, and neonatal data which documented during the delivery by the attending physician and hospital staff.

The second, a dataset of all pediatric hospitalizations at SUMC which includes medical diagnosis and demographic characteristics according to ICD-9 Codes. Long-term neurological morbidity was pre-defined by a pediatrician as one or more of the ICD-9 code list of diagnoses detailed in the Supplement Table, which includes various neurological pathologies.. The two datasets were cross-linked and merged. It should be noted that the information was routinely approved and tested by experience medical secretaries before entering the database to ensure data accuracy. Follow up time defined as time to event (first neurological related hospitalization) or until censoring occurred (death child, end of the study period or 18 years old).

Background and dependent variables were compared between the two study groups in univariable analysis. The tests that were used include t-test or Man-Whitney U tests for continuous variables according to their distribution and chi-square test for categorical variables. Calculating cumulative incidence rates were performed with Kaplan-Meier, using the log-rank test to determine significant differences between the groups.

A Cox regression analysis was used to control for confounders. Potential confounders were taken into account based on the univariable analysis, as well as on the clinical significance of the variables. The final model was chosen based on the best fit and the minimal -2log likelihood. All the models and statistical analysis were conducted and performed using STATA (version 12 or higher) or SPSS (version 23 or higher) software.

**Results:**

During the study period, 243,725 deliveries met the inclusion criteria; 35,897 of the cohort (14%) constituted the exposed group (MSAF exposed infants), while the rest of the cohort (n=207,828) constituted the comparison, or 'no MSAF', group.

Table 1 shows the demographic characteristics and immediate perinatal outcomes of the exposed and unexposed group. The exposed MSAF group was characterized by slightly older mothers (28.65±5.9 years vs. 28.08±5.8 years, p<0.001), longer gestational age (mean 39 5/7±2 vs. 38 6/7±2 weeks of gestation) and a higher mean birth weight (3,281 grs±486 vs. 3,192±514 gr).

Total neurological morbidity up to the age of 18 years was similar among children exposed to MSAF (3.2% vs. 3.1%, OR=1.048; 95%CI 0.98-1.12; table 2). No significant difference was found on the specific neurological pathologies (CP, PDD, etc.) between the two groups. Moreover, no difference in cumulative incidence of neurological hospitalizations was between the exposed and unexposed groups, as demonstrated in the survival curves (figure 1).

The Cox analysis, controlled for maternal diabetes and hypertension as well as for gestational age and maternal age, demonstrated MSAF exposure ***not to be*** an independent risk factor for neurological related hospitalizations during childhood in the offspring (adjusted HR=1.025, 0.962-1.092; Table 3). This was also true when analyzed for preterm deliveries (<37 weeks) only (adjusted HR=1.194, 0.906-1.572).

**Discussion:**

Intrauterine extra-alimentary exposure to the infantile gut microbiome is frequently associated with microbial invasion of the amniotic cavity, resulting in increased cytokine production. Meconium can stimulate vasoconstriction of placental veins, thereby precipitating brain-damaging hypoxemia. Therefore, we assumed that MSAF will be associated with an increased occurrence of neurological related hospitalizations in the offspring throughout childhood and adolescence. However, in this large population-based chort study, MSAF exposure was not found to be an independent risk factor for neurological related hospitalizations during childhood in the offspring.

Although our study did not find any long-term impact on neurological morbidity, other studies found that MSAF has a significant short-term impact on the offspring such as low Apgar scores, intrapartum fever (16), operative vaginal and cesarean (17) and meconium aspiration syndrome (MAS) (18). The fact that MSAF has no impact on long-term neurological morbidity of offspring can be due to several reasons:

Studies found an association between the gut microflora and the body's ability to deal with pathogens (19, 20). Early exposure to gut microbiota was found to reduce the risk of long-term inflammatory disease, which is reinforced by the fact that children who were born by cesarean and not in vaginal delivery, were more likely to suffer from asthma, celiac disease, diabetes type 1 disease and neurological morbidity (21-24).

Though MSAF is assumed to be an adverse reactant, recent studies have questioned this assumption and presented the meconium as a protecting factor to a long-term infectious (13) and dermatological morbidity (14). Therefore, it can be assumed that meconium has a positive association with the newborn, and the stigma on meconium as a negative long-term cause is not necessarily accurate.

Also, although neurological development is an ongoing process which continues long after birth, it can be assumed that the fast and effective treatment in offspring that were exposed to MSAF helped prevent neurological morbidity and future damage. An adequate treatment which include supportive therapy like oxygen supplementation, mechanical ventilation and intravenous fluids, availability of surfactant, inhaled nitric oxide, high frequency ventilators and extracorporeal membrane oxygenation, reduce the morbidity and mortality of meconium aspiration syndrome (25).

Another hypothesis that may explain the lack of association between MSAF and long- neurological morbidity is the fact that MSAF is associated with peripartum stress such as hypoxia (26, 27). The peripartum stress through MSAF delivery causes an activating of the hypothalamic-pituitary-adrenal (HPA) axis and producing stress hormones like cortisol (28). The stress hormone production helps the compensatory mechanism to control the stress situation by supplying energy via protein catabolism, gluconeogenesis and glucogenesis. It can cause hyperglycemia, hyperlipidemia, and blood, bones, muscles, cardiovascular, gastrointestinal, endocrinal and central nervous system changes (29, 30). Studies have shown that corticosteroid treatment can reduce and even improve neurological morbidity in offspring (31, 32). Thus, the increase of the stress-related hormones may reduce adverse neurological outcomes.

The main strength of our study is the population on which it's conducted. The study has consisted of a large number of patients (more the 200,000) in SUMC, which is the only tertiary medical center treating the providing comprehensive care for the entire population of the Negev region. This fact prevented loss of information and follow- up data and allowed long-term follow up of offspring health and hospitalizations that occurred during childhood and adolescence. Furthermore we were able to control for many parameters and potential confounders regarding pregnancy and delivery.

However, our study has some limitations. First, immigration outside the Negev region or health care in a different hospital, are a reasonable possibility of a loss of follow-up. However, it is unlikely to assume that differences in immigration will be based on exposure (or not) to MSAF.

Another important limitation is the fact that we do not have data on the density of meconium within the amniotic fluid. Additionally, we could not distinguish the onset of meconium passage during labor (i.e. primary meconium, that was already present at the time of membranes rupture, or secondary).

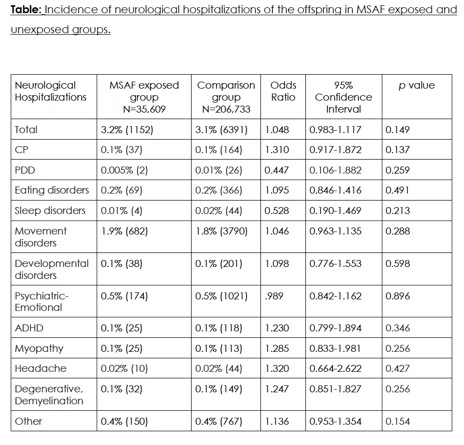
In conclusion, in our study, we did not find MSAF exposure as an adverse reactant on neurological morbidity, and it does not appear to be an independent risk factor for long-term neurological hospitalizations in the offspring throughout childhood and adolescence.

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**Table 1:** Maternal characteristics of the study population.

**Table 2:** incidence of neurological hospitalizations of the offspring in MSAF exposed and unexposed groups.



**Table 3**: Cox model to predict offspring long-term neuropsychiatric morbidity.

**Figure 1:** Kaplan Meier survival curve for cumulative incidence of neurological morbidity in offspring of patients with and without MSAF; Log rank p=0.349

