CLINICAL USE OF MECONIUM FATTY ACID ETHYL ESTERS FOR IDENTIFYING CHILDREN AT RISK FOR ALCOHOL- RELATED DISABILITIES: THE FIRST REPORTED CASE

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ABSTRACT

Fatty acid ethyl esters (FAEEs) in meconium are validated biomarkers of heavy fetal alcohol exposure that may potentially be used clinically for identifying children at risk for alcohol-related disabilities. However, until now, FAEEs have been largely used anonymously in epidemiological studies, and by child protection authorities in need for verification of heavy alcohol use in pregnancy. Here we describe the first case of a neonate identified as part of a research study on a pilot neonatal screening program for prenatal alcohol exposure. The neonate's meconium tested high for FAEEs (52 nmol/g; positive cut-off ≥2 nmol/g), which prompted active follow-up of the infant's development, identifying early neurocognitive problems and allowing initiation of a remedial program.

Key Words: *Fatty acid ethyl esters, meconium, neonatal screening, fetal alcohol spectrum disorder, developmental follow-up*

Prenatal alcohol exposure (PAE) can result in a wide range of physical anomalies and cognitive and behavioural deficits known collectively as Fetal Alcohol Spectrum Disorders (FASD). In North America, FASD affects an estimated 1 percent of live births[1], making it the leading preventable cause of mental retardation and a significant social and economic burden.[2,3] Although the ethanol-induced damage is irreversible, early diagnosis and management of FASD may decrease the risk of developing secondary disabilities and improve prognosis in affected children.[4] However, identification of children affected by PAE is difficult, with diagnosis often hinging on maternal reports of alcohol use in pregnancy[5]; which are unreliable and difficult to obtain.

Fatty acid ethyl esters (FAEE) are products of non-oxidative ethanol metabolism that are formed when ethanol is conjugated to endogenous free fatty acids or fatty acyl-CoA.[6] During gestation, ethanol ingested by the mother crosses the placenta and undergoes metabolism in the fetal compartment to FAEEs, which then deposit and accumulate in meconium.[7] As a result, elevated levels of FAEEs in neonatal meconium may serve as objective markers of maternal alcohol use in pregnancy, as has been established in numerous studies.[8-11]

Meconium analysis for FAEEs is currently utilized in the context of child protection, and has been used to anonymously obtain epidemiological data on the prevalence of PAE in select populations.[12-14] As of yet, there has been little use of this test in the context of diagnosing FASD but it has been suggested that it can be used as a screening tool to identify children at risk for disabilities.[15] Using meconium testing clinically as a screening tool would not only provide a history of PAE required to make a diagnosis but; if coupled to long-term developmental follow-up, interventions, and social supports; may aid early detection and management of alcohol-related disabilities.

We have conducted a study involving a pilot screening program of this nature in a high-risk obstetric unit in London, Ontario, the objective of which was to determine if women would willingly

participate in screening that aimed to identify and follow-up ethanol-exposed newborns such that intervention efforts could be initiated in a timely manner if developmental delays emerged.[16] During this study, a highly FAEE-positive case was identified and the follow-up of this infant highlights the potential benefits of meconium screening for early identification of at-risk babies.

The Study

An open meconium screening program for prenatal alcohol exposure was piloted in a high-risk obstetric site previously shown to have a high prevalence of FAEE-positive meconium by anonymous testing. This study has been described in detail elsewhere.[16] Briefly, meconium screening with subsequent developmental follow-up of FAEE-positive cases was offered from November 1st, 2008 to May 31st, 2010 to all women from a regional population in Ontario who delivered at St. Joseph's Health Care in London, Ontario. With consent, meconium specimens were collected and shipped to the Motherisk Laboratory at Hospital for Sick Children in Toronto for analysis.

Meconium FAEEs were measured using headspace solid-phase microextraction followed by gas-chromatography with mass spectrometry (HS-SPME GC/MS) according to previously published methodology.[17] A positive cut-off of $\geq$2 nmol/g sum of four FAEEs (ethyl palmitate, linoleate, stearate, and oleate) was considered indicative of heavy PAE.[10]

Study participants whose neonates tested positive for FAEEs were followed-up though the ''Healthy Babies Healthy Children'' (HBHC) program; an existing public health program for families with newborns in Ontario. This voluntary program offers free home-visits by a public health nurse who provides assistance, educates, assesses the family's needs, and devises an appropriate family service plan if ongoing follow-up and support may be of benefit to the family.[18] The family service plan for those identified by the meconium screen included regular developmental assessments of the baby by the public health nurse using *Ages and Stages Questionnaires*® and additional neurodevelopmental testing by a certified clinical psychologist at 3 months and 1-1.5 years of age using the Bayley Scales of Infant and Toddler Development®, Third Edition (Bayley-III). The latter comprehensively assessed cognitive, linguistic, and motor functioning of the infant. Upon detection of developmental delays, the public health nurse made referrals to intervention programs and support services for the baby and, if needed, for the family; all of which were provided at no cost to the family.

The study was approved by the research ethics boards of the Hospital for Sick Children and the University of Western Ontario.

Identification and Follow-up of the First Positive Case

Meconium screening identified a neonate with high FAEE levels in meconium (52 nmol FAEE/g meconium), suggesting heavy *in-utero* alcohol exposure (see Figure 1). Ethyl oleate and ethyl linoleate constituted the largest proportion of the total sum, with levels of 32.87 nmol/g and 17.58 nmol/g, respectively.

This neonate was born full term (40 weeks gestation) to a young, primiparous, single mother after an uncomplicated pregnancy and delivery. No complications or concerns were noted in the chart with respect to poor neonatal outcomes such as growth restriction or low birth weight. The APGAR scores at 1 and 5 minutes were 9, and the infant passed the infant hearing test. On antenatal forms, it was reported that the mother had a history of depression a few years prior to pregnancy. She did not take preconceptual folate, denied use of street drugs, but reported smoking during pregnancy (5 cigarettes/day). She also admitted to daily alcohol consumption (3-4 drinks/day) prior to her knowledge of pregnancy (early first trimester). No other risk factors or concerns were noted.

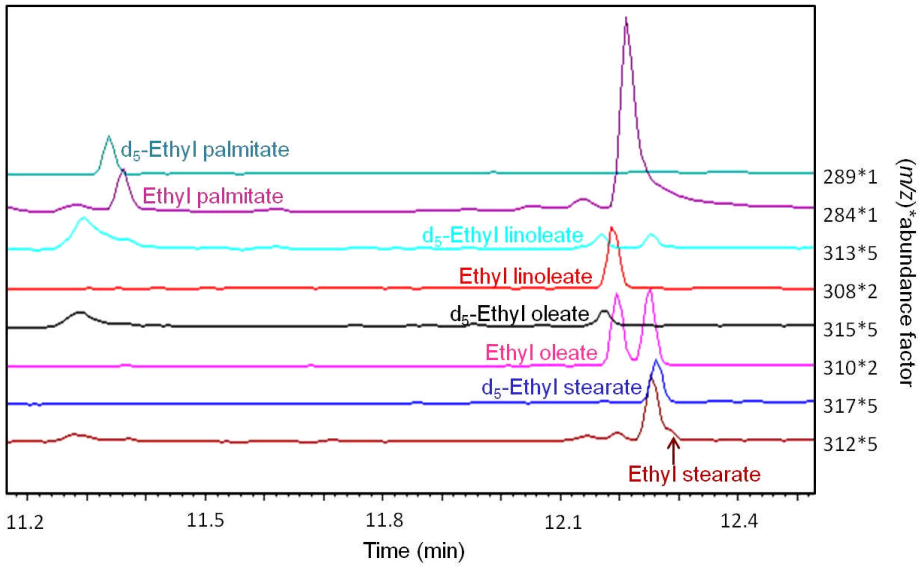

FIG. 1 Gas chromatography-mass spectrometry chromatogram of the positive meconium sample from a neonate identified in a meconium screening program for prenatal alcohol exposure. Four fatty acid ethyl esters (FAEEs) were quantified using their corresponding d_5-FAEEs as internal standards. The *m/z* values and abundance factors are displayed on the right.

As a result of the positive meconium test, follow-up was arranged as per protocol through the HBHC program and a public health nurse was appointed to manage the case. A family service plan was devised to provide support and guidance in consideration with the mother's young age and in-experience, low educational attainment and household income, potential challenges of single parenting, and the available resources/programs in the area of residence. The baby was of generally good health, and no concerns with regard to hearing or growth were reported by the public health nurse assigned to the case throughout the duration of the study.

Neurodevelopmental assessment conducted by a clinical psychologist at 3 months of age (using the BSID-III) did not suggest developmental delays. Specifically, the infant performed in the high average range for a 3-month old infant on the Mental and Motor scales of the Bayley (75^{th} and 79^{th} percentiles, respectively), and in the average range in expressive and receptive language abilities (50^{th} percentile). Slight delays in motor development (initially in fine motor, and later in gross motor) became apparent in the 6-month and 8-month assessments conducted by the public health nurse using the *Ages & Stages Questionnaire*®. At 8 months, the infant would only sometimes perform activities such as rolling from back to the stomach, could not get into the crawling position, and when stood up against furniture, could not hold on without leaning against the furniture or crib wall.

At 14 months of age, the infant was again assessed by a clinical psychologist using the BSID-III, which confirmed the presence of developmental delays. While on the cognitive scale, the infant performed in the average range (63^{rd} percentile), on the Motor Scale of the Bayley, the child performed in the low average range, scoring in the 50^{th} percentile in fine-motor abilities, but in the 9^{th} percentile in gross-motor abilities. Additionally, on the language scales, the infant performed in the low average range, scoring in the 50^{th} percentile in receptive language abilities, but in the 5^{th} percentile in expressive language abilities. The infant has been referred to an infant and child development program and will be enrolled in a language and speech development program in the area.

DISCUSSION

This case, to the best of our knowledge, is the first reported instance of using meconium FAEE in a clinical setting to identify and follow-up infants at risk for alcohol-related disabilities in order to facilitate early intervention. The child identified in our pilot screening program tested high for FAEEs, with ethyl oleate and ethyl linoleate levels comprising the largest proportion of the total sum. Both of these esters have been shown by other groups to be the strongest correlates of maternal alcohol consumption.[8,11,19] Developmental follow-up of this child revealed delays in motor and language abilities, particularly in gross motor and expressive language functioning, which were well below age expectations at 14 months (in the 9th and 5th percentile on the BSID-III, respectively). These delays prompted referral to available intervention programs in the area and special focus on developing the impaired skills.

It should be stressed that the child has not yet been referred for diagnostic assessment and that we cannot make conclusions with regard to the cause of the developmental delays as there may be numerous co-existing risk factor (which will be discussed below). It is of interest, however, that the observed delays in motor and language abilities have been described in children with FASD. Many studies on alcohol's effects on the developing motor system have reported delayed motor development in infants and children with PAE, with both fine and gross-motor dysfunction, as well as, tremors, weak grasp, and poor hand-eye coordination.[20-23] Animal studies have also provided evidence for motor dysfunction, and have consistently found impairments in balance, reflex development, and disturbances in gait following PAE.[24,25] Studies have also described the detrimental effects of PAE on both receptive and expressive language abilities, noting articulation disorders and delays in language acquisition, comprehension, language and speech development, and overall language competence.[20,26-28]

In agreement with the developmental findings in this case report, two studies have found associations between meconium FAEEs and psychomotor development after controlling for other risk-factors. Peterson and colleagues (2008) have found that increasing levels of FAEEs were associated with poorer mental and psychomotor development during the first 2 years of age[29], while Hicks and colleagues (2007) reported that children with elevated levels of FAEE in meconium were found to be delayed on the BSID-II Psychomotor Development Index at 2 years of age.[30]

In the present case, however, we cannot exclude the role that other risk factors; such as maternal psychopathy, IQ (which was not assessed), prenatal care, social history, and smoking in pregnancy; may have played in bringing about or, at least, contributing to the poor developmental outcomes. Furthermore, we cannot rule out prenatal exposure to other drugs and the effect that these may have had. Although the mother denied using illicit drugs, the reliability of such self-reported information on antenatal forms is questionable, especially considering that the claim of drinking cessation early in pregnancy is inconsistent with the positive meconium result as this matrix does not begin to form until the second trimester. Nonetheless, since many of these other risk factors may be associated with maternal drinking in pregnancy, it is reasonable to presume that the positive meconium test for PAE may identify newborns with several risk factors for poor developmental outcomes in addition to PAE.

In summary, the close follow-up of a baby identified as "at-risk" for alcohol-related disabilities by meconium testing, facilitated early detection of developmental delays and initiation of interventions. This reported case was identified as part of a larger study investigating the potential utility and logistics of offering meconium screening in a clinical setting, and was meant to exemplify how the piloted screening program functioned and the potential benefits that such programs may offer if implemented in clinical practice. In our pilot program, follow-up and interventions were integrated into existing community health programs, which is likely the most logistically and economically feasible option. Some of these existing programs, particularly those focusing on infant and child development, may even be tailored by including intervention protocols and specific teaching methods that have been reported to be effective at improving skills like language and literacy, learning, math, communication, and behavior in children with FASD.[31,32] In the future, additional

studies focusing on the effectiveness of such interventions will be needed to investigate the full benefits of such programs.

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