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Prenatal Alcohol Exposure Prevalence as Measured by Direct Ethanol Metabolites in Meconium in a Native American Tribe of the Southwest

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Abstract

Background: While Fetal Alcohol Spectrum Disorders (FASD) represent a significant public health problem, Native Americans are underrepresented in population and targeted screening programs. Prior reports suggest that Native American tribal communities may have higher prevalence of alcohol use during pregnancy; however, systematic examination using ethanol biomarkers is lacking.

Methods: This study utilized data collected through the Navajo Birth Cohort Study (NBCS) – a birth cohort study of a Southwestern tribal community. Prevalence of prenatal alcohol exposure (PAE) was assessed by a battery of meconium biomarkers among 333 NBCS participants. Meconium samples were analyzed for nine individual fatty acid ethyl ester (FAEE) species, ethyl glucuronide (EtG), and ethyl sulfate (EtS) by LC-MS/MS.

Results: Participants were recruited from 5 hospitals at the Navajo Nation located in Arizona (Chinle, Tséhootsooí, Tuba City) and New Mexico (Gallup, Shiprock). All participants identified as Native American; most reported personal income of <$20,000 per year (71.3%), and ≤high

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school education (55.3%). The most prevalent biomarker was EtS (7.8%) followed by ethyl oleate (6.9%); 5.4% of the sample were positive for at least 2 biomarkers.

Conclusions: Results of this study on the prevalence of PAE in the Navajo Nation, obtained for the first time with an objective comprehensive panel of meconium biomarkers, indicate that the rates in the NBCS may be comparable to the general U.S. population and are in accord with recent U.S. national survey estimates. Our findings emphasize that drinking behaviors among Native American communities in the United States can vary, and generalization across all Native American populations is not warranted.

Keywords

Prenatal alcohol; meconium; Native American; pregnancy; prevalence

INTRODUCTION

Alcohol consumption during pregnancy places a fetus at risk for fetal alcohol spectrum disorders (FASD), which entail lifelong physical and neurological impairments. Recent studies in the U.S. general population indicate that prenatal alcohol exposure (PAE) and FASD may be more prevalent than previously thought, with 10% of pregnant women self-reporting alcohol use in the past 30 days (Tan et al., 2015) and as many as 1.1–5% of school-aged children meeting criteria for FASD (May, Chambers, et al., 2018). Estimated prevalence of PAE varies widely according to surveillance method (May et al., 2009), and among different populations as affected by socioeconomic factors that influence drinking behaviors and access to preventive care (May et al., 2009; Roozen et al., 2016). Identifying high-risk populations is critical for providing appropriate interventions, and obtaining reliable prevalence estimates for specific sub-populations is a key step in this process.

Direct ethanol biomarkers in maternal blood, hair, urine, placenta, umbilical cord tissue and blood, meconium, and blood collected via newborn heel lancing have been previously examined for their utility in detecting PAE (Bakhireva & Savage, 2011; Joya et al., 2012; Montag, 2016). Meconium, an infant's first stool, has been heralded for having the longest window of detection, providing ability to detect PAE incurred as far back as 20 weeks' gestation or more. Additional advantages include non-invasiveness and the ability to identify moderate and episodic PAE. Over 20 compounds produced in response to PAE are detectable in meconium (Joya et al., 2012). Fatty acid ethyl esters (FAEE), which do not cross the placental barrier, accumulate in meconium as a result of ethanol metabolism by the fetus (Burd & Hofer, 2008). FAEE in meconium have demonstrated substantial sensitivity and specificity (Bearer et al., 2003) for heavy PAE, and are widely used in prevalence studies (Hastedt et al., 2013; Himes et al., 2015; Pichini et al., 2012). Bearer and co-authors reported that a positive test for meconium FAEE correctly identifies 72% of pregnant women who consume ≥1 drink per week during the third trimester (Bearer et al., 1999). The presence of FAEE in meconium is predictive of subsequent mental and psychomotor delays in children at 6.5 months, 1 year, and 2 years of age (Peterson et al., 2008), and poorer cognitive development at ages 9, 11, and 15 years (Min et al., 2015).

In addition to FAEE, ethyl sulfate (EtS) and ethyl glucuronide (EtG) are considered highly reliable indicators of PAE (Himes et al., 2015). Some evidence suggests that EtS and EtG in meconium may be more stable than FAEE (Himes et al., 2014). Given that the risk of having an infant with cardinal features of fetal alcohol syndrome (FAS) is increased with a greater number of positive alcohol biomarkers (Stoler et al., 1998), meconium offers a non-invasive medium for testing multiple ethanol metabolites simultaneously. To our knowledge, only 4 prior studies (Table I) have assessed meconium EtS, EtG, and FAEEs within the same study population.

We are not aware of any previous studies utilizing biomarkers to estimate prevalence of PAE specifically among Native Americans. Existing research has relied primarily on maternal self-report, records review, and FASD active case ascertainment (Duimstra et al., 1993; Fox et al., 2015; May et al., 2009). Findings from these studies have suggested that Native American tribal communities may represent a high-risk group. However, past studies concentrated in a few geographic regions (Alaska and the U.S. Northern Plains) (Iyasu et al., 2002; Khan et al., 2013) may fail to represent heterogeneity of prenatal alcohol consumption behaviors among diverse people who identify as Native American. Moreover, analyses based on the National Survey on Drug Use and Health (NSDUH, 2005–2009, 2009–2013) have indicated that Native American adults, in general, are in fact more likely to abstain from alcohol than Whites or African Americans (Cunningham et al., 2016), and Native American pregnant women, in particular, are less likely to drink than pregnant women of other US racial/ethnic groups (Watt, 2012). The purpose of this study is to estimate PAE prevalence among newborns in the Navajo Nation, a region not studied in previous reports, using a comprehensive battery of ethanol biomarkers measured in meconium.

METHODS

Participants

This study utilized data collected through the ongoing Navajo Birth Cohort study (NBCS), which focuses on uranium exposure, birth outcomes, and development on the Navajo Nation (Hunter et al., 2015). The NBCS is a collaborative effort between the University of New Mexico (UNM), Centers for Disease Control and Prevention Agency for Toxic Substances and Disease Registry (CDC/ATSDR), Indian Health Services (IHS), Navajo Area IHS, the Southwest Research and Information Center, and the Navajo Nation Department of Health. For the parent study, pregnant women were recruited from all 110 chapters (political units equivalent to counties) of the Navajo Nation and followed-up through labor/delivery and postpartum. All study activities were reviewed and approved by the UNM, CDC, and Navajo Nation IRBs, as well as the U.S. Office of Management and Budget. The study design was informed and approved by community partners and leaders. All patients signed informed consent to participate. Minors were included, as 25% of births on Navajo Nation are in women <18 years of age. Minor consents were co-signed by a parent or guardian, with the minor re-consented if she reached maturity during the study. The consent had participants specifically check if they were willing to provide meconium for analysis of alcohol metabolites, since meconium has a cultural use; therefore, providing the sample was not mandatory for participation in the NBCS.

The following inclusion criteria were used for pregnant women: a) age: 14–45, b) lived on Navajo Nation for at least five years at any time in their life, c) agreed to receive prenatal care and deliver at one of five participating IHS or PL638 hospitals (Chinle, AZ; Tséhootsooí, AZ; Gallup, NM; Shiprock, NM; Tuba City, AZ); and d) willing to have their child followed-up for biological sample collection and developmental assessment through the first year of life. The study was explained to women upon their initial presentation at a participating hospital for pregnancy confirmation. Those interested were told they could enroll at the hospital any time after pregnancy confirmation and before delivery. Average enrollment was at 23.3±10 gestational weeks.

Data collection

Meconium sampling and analysis occurred over a 3.5-year period between April 2013 and November 2016. During that timeframe, 638 cohort infants were born. Maternal consent for meconium collection was obtained from 570 (89.3%) participants. Among this 570, 361 (63.3%) specimens were collected, and 333 (92.2% of collected samples) were analyzed for ethanol biomarkers. Nursery staff, trained by the study team, collected two quarter-sized aliquots of meconium which were placed in a sealed plastic container inserted within a brown paper bag to protect from light. Samples were placed in −80° C study-specific freezers at each participating collection site within 1–1.5 hours from collection (samples were stored at 4° C temperature before transfer to ultralow temperature freezers), batched, and shipped on dry ice to the University of Maryland for analyses.

Measures

At the University of Maryland, EtG, EtS, and FAEE samples were prepared and analyzed via LC-MS/MS using the previously described methodology (Himes et al., 2014). Briefly, EtG, EtS, and FAEEs were extracted from the same aliquot using a methanol-based liquid extraction followed by solid phase extraction using SLE+ cartridges for FAEE and Evolute-AX cartridges for EtS/EtG (Biotage). EtG-d5 and EtS-d5 were used as internal standards in the EtG/EtS analyses, and E17:0 (non-natural FAEE) was used as the internal standard in the FAEE analyses to assess recovery. Average recoveries were as follows: EtS, 94%; EtG, 71%; FAEE, 57%. These recoveries are consistent with previous published work (Himes et al., 2014).

EtG/EtS analysis: EtG and EtS were quantified by LC-MS/MS using the methodology described by Himes et al. (Himes et al., 2014), performed on a Dionex U3000 UPLC coupled to a Thermo TSQ triple quadrupole mass spectrometer using ESI operated in negative ion mode. Limits of detection (LOD, as defined by signal:noise >3) for EtS and EtG were both 1.0 ng/g. Limits of quantitation (LOQ, as defined by signal:noise > 10) for EtS and EtG were 3.0 ng/g and 5.0 ng/g, respectively.

FAEE analysis: FAEEs were quantified by LC-MS/MS using the methodology described by Himes et al. (Himes et al., 2014), performed on a Dionex U3000 UPLC coupled to a Thermo TSQ triple quadrupole mass spectrometer using ESI operated in positive ion mode. The LOD for FAEEs ranged from 10–25 ng/g and LOQ ranged from 15–50 ng/g. FAEE species that were quantified included: ethyl laurate (12:0) (LOD: 25 ng/g; LOQ 50 ng/g),

ethyl myristate (14:0) (LOD: 15 ng/g; LOQ 250 ng/g), ethyl palmitate (16:0) (LOD: 20 ng/g; LOQ 50 ng/g), ethyl palmitoleate (16:1) (LOD: 10 ng/g; LOQ 15 ng/g), ethyl stearate (18:0) (LOD: 20 ng/g; LOQ 50 ng/g), ethyl oleate (18:1) (LOD: 10 ng/g; LOQ 15 ng/g), ethyl linoleate (18:2) (LOD: 10 ng/g; LOQ 15 ng/g), ethyl linolenate (18:3) (LOD: 15 ng/g; LOQ 25 ng/g), and ethyl arachidonate (20:4) (LOD: 10 ng/g; LOQ 15 ng/g).

Self-reported alcohol consumption 12 months before enrollment: The Alcohol Use Disorders Identification Test-Consumption (AUDIT-C) screening was administered at enrollment as a screening tool to capture 'at risk' drinking in the preceding 12 months (standard reporting timeframe); thus, capturing both pre-pregnancy and early pregnancy time periods. AUDIT-C data were available on 289 out of 333 subjects (86.8%) included in the meconium analyses. AUDIT-C was chosen based on feedback provided by tribal leaders, clinicians, and community advisors, who emphasized the need for a screening measure that minimizes concerns about possible stigma. AUDIT-C has been demonstrated to perform with approximately equal accuracy to the full 10-item AUDIT and with superior accuracy compared to other alcohol self-assessment tools (Burns et al., 2010). It has been used in research to screen pregnant women for risky alcohol use and demonstrated high accuracy (Lopez et al., 2017; May, Hasken, et al., 2018). More in-depth measures (e.g., Timeline Follow-Back interview) were deemed inappropriate for this population due to high sensitivity related to alcohol use in the community, and a reluctance of participants to discuss alcohol use observed in previous studies.

Analysis

Descriptive statistics were performed to estimate the prevalence of PAE based on each meconium biomarker and their combination. Given that the biomarker data were not normally distributed, range and median concentrations were reported (values below LOQ were not used in these calculations). Due to lack of agreement in the field on cutoff concentrations for meconium biomarkers (Himes et al., 2015; Joya et al., 2012), values above the LOQ were considered 'positive'. For self-reported drinking, AUDIT-C score ≥3 was used as a cutoff for 'risky drinking' (Reinert & Allen, 2007). Analyses were performed in SAS version 9.3 (Gary, NC).

RESULTS

Patient characteristics are summarized in Table II. Mean maternal age at recruitment was 27.4± 6.0 years (range: 16.4–45.5 years). All participants identified as Native American. Most participants reported a personal (71.3%) and household (55.2%; data not shown) income of <$20,000 per year. The majority had ≤high school education (55.3%). While ceremonial tobacco use is prevalent in the tribal community (35.9% in this study), only 1 participant (1.8%) reported regular tobacco use. This is consistent with the low rate of cigarette smoking among Navajo relative to Northern Plains tribes (Nez Henderson et al., 2005).

Self-reported prevalence of 'risky' alcohol use (AUDIT-C ≥3) in the 12 months before enrollment was 12.5%. As shown in Table III, among meconium biomarkers, the highest concentration (median) was observed for ethyl palmitate (129.4 ng/g), laurate (93.3 ng/g),

and palmitoleate (47.6 ng/g). The most prevalent FAEE (% of participants with a concentration >LOQ) was ethyl oleate 23 (6.9%), followed by ethyl linoleate 17 (5.1%), ethyl palmitoleate 4 (1.2%); other ethyl esters were detectable in less than 1% of the sample (Table III). EtS and EtG were detected in 7.8% and 5.1% of the population, respectively.

The greatest overlap among individual biomarkers was observed between ethyl oleate and ethyl linoleate (11 subjects). Ethyl oleate also demonstrated some overlap with EtS (6 subjects), ethyl palmitoleate (2 subjects), and ethyl arachidonate (2 subjects). Ethyl linoleate demonstrated overlap with EtS (4 subjects). As shown in Figure 1, there were no subjects positive for all 3 of the most prevalent ethanol biomarkers (ethyl oleate, EtG, and EtS). There was minimal overlap between positive AUDIT-C and biomarkers, possibly due to the different time frames they captured (data not shown). In total, 5.4% of the sample were positive for ≥2 biomarkers.

DISCUSSION

In this population-based study, 5.4 % of specimens were positive for ≥2 meconium biomarkers, highly indicative of regular PAE. By contrast, nearly 17% of a population-based cohort from the U.S. Northern Plains and Cape Town, South Africa tested positive for two meconium biomarkers (EtS and EtG) (Himes et al., 2015). Native American communities have traditionally been regarded as ‘high-risk’ for PAE and FASD. However, in a meta-analysis of 8 population studies (not focused on Native Americans) from Canada, Germany, Italy, USA, Spain, and Uruguay, pooled prevalence of PAE as detected by FAEE in meconium was 18.9% (Lange et al., 2014) – much higher than observed in our study. In a cohort study which included a large proportion of Native American participants from the U.S. Northern Plains, PAE prevalence determined by a battery of meconium biomarker analyses ranged from 10.3% to 65.4% (Himes et al., 2015). In a German prospective cohort, 7.1% and 16.3% of subjects were positive for FAEE (summed concentration of four individual ethyl esters) and EtG, respectively (Bakdash et al., 2010). Similar to our findings, combined positivity for two meconium biomarkers (EtG and FAEE) was 5.5% (Bakdash et al., 2010). Finally, in a cross-sectional study using another direct ethanol metabolite, phosphatidylethanol (PEth), in newborn dry blood spots collected at UNM hospital, 6.5% were positive, indicative of late-pregnancy PAE (Bakhireva et al., 2013). Thus, PAE prevalence observed in the general population of New Mexico per PEth analysis (Bakhireva et al., 2013) is comparable to the prevalence we observe in the Navajo Nation; it is additionally consistent with NSDUH (2005–2009) data indicating lower self-reported past 30 day PAE rates among Native American women (8.7%) compared to White (12%) and African American (16.7%) women (Watt, 2012). Of note, the Navajo Area IHS has implemented a strong outreach program to increase awareness of the dangers of PAE, which may have additionally contributed to lower prevalence rates.

Our findings regarding ethyl oleate being the most prevalent FAEE and the one with the highest overlap with EtG/EtS are consistent with findings in the Cleveland cohort, where ethyl oleate was the best indicator of PAE (Bearer et al., 2003). The greatest overlap in our study was observed between ethyl oleate and ethyl linoleate, similar to the Himes et al. study (Himes et al., 2015). Somewhat surprising was the minimal overlap among EtG/EtS and

FAEE biomarkers; however, this is consistent with population-based studies in Spain and Italy (Morini et al., 2010; Pichini et al., 2012). While specific timeframe and magnitude of ethanol exposure cannot be determined from meconium biomarkers, Himes et al. found that EtG ≥ 30 ng/g had a moderate to substantial agreement with self-reported PAE at ≥19 weeks with a dose-response relationship for drinks per drinking day (DPDD), but not timing of consumption. In these studies, the odds of observing a positive EtG result (≥30 ng/g) was 9.1 times higher for women self-reporting DPDD between >0 and ≤3 DPDD than women reporting no drinking. The odds of observing a positive EtG of ≥30 ng/g increased to 22.6 for DPDD between >3 and ≤10 and increased to 29.4 when DPDD was ≤10 (Himes et al., 2015). Other studies suggested that a relationship between PAE and meconium FAEE is not linear with a potential threshhold at 3 drinks/week, corresponding to light/moderate alcohol consumption (Yang et al., 2015).

One limitation of this study is its lack of the Timeline Follow-back (TLFB) in-depth interview, or other similar methods, for obtaining more detailed self-reported PAE information spanning the full gestational period. For the parent study, implementation of the TLFB was deemed inappropriate by community advisors due to concerns about alcohol-related stigmas in the tribal community and a related reluctance to discuss alcohol use observed in previous studies. Thus, AUDIT-C was selected as this method covers the 12-months before enrollment, including pre-pregnancy. Pre-pregnancy drinking is likely perceived as less stigmatizing than gestational drinking, and has a demonstrated ability to predict risky drinking continuing into pregnancy (Anderson et al., 2014; Eichler et al., 2016). However, with the average enrollment at 23.3 weeks gestation, AUDIT-C captured an average window of 5 months before pregnancy in our study, thus direct comparison with ethanol biomarkers is not warranted. Many women with risky drinking behaviors pre-pregnancy may stop drinking upon pregnancy recognition (Handmaker et al., 2006; Pryor et al., 2017; Schmidt et al., 2017).

Another possible limitation is that meconium analyses were conducted on 52.2% of cohort births. Notably, 89.3% of participants gave consent for meconium analysis (participation rate), which was higher than in other studies (Zelner et al., 2012). Less than 100% sample collection (361 out of 570 consented or 63.3%) is expected given the recognized challenges associated with strained resources within medically underserved and rural hospitals (Genovesi, Hastings, Edgerton & Olson, 2014) which are further strained in neonatal units managing birthing complications and competing medical test needs, as noted by others (Vaught & Henderson, 2011). Of note, 28 meconium samples were collected but not analyzed due to limited funding. To ascertain the effect of potential selection bias on results, we compared prevalence of self-reported risky drinking (AUDIT-C ≥3) among 453 out of 570 meconium-consenting subjects who delivered during the meconium collection period and had complete AUDIT-C scores. This N included the subset reported in this paper, but is fewer than the 570 consenting mothers due to missing AUDIT-C scores. Among those 453 subjects, 312 had meconium samples collected and 141 did not. Prevalence of risky drinking was similar between these two subgroups (15.7% vs 17.0%; $p = 0.72$). Additionally, AUDIT-C ≥3 rates were similar among subjects who consented for meconium vs. those who did not (16.1% vs. 10.9%, respectively; p=0.31). These analyses demonstrate that selection biases are unlikely to have affected our prevalence estimates obtained from meconium testing.

Finally, generalizability of our findings might be limited, since this study included only Native American individuals. While a question with respect to difference in alcohol metabolic pathways between Native American and Caucasian populations has been raised (Ehlers, 2007), it appears we can expect to observe an effect size comparable to that observed in prior studies in Caucasian populations for FAEE, EtG, and EtS. These biomarkers are produced through nonoxidative metabolism of alcohol, distinctly different from the oxidative pathway involving alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). To our knowledge, observed genetic variations in alcohol metabolism have all been linked to the latter oxidative pathways, specifically to variants of ADH, ALDH, and CYP2E1 (Zakhari, 2006). In some Native American populations, roughly 6% have been shown to have an ADH1B*3 allele that leads to more rapid metabolism than the more common ADH1B polymorphisms (Wall et al., 2003). However, ADH and ALDH phenotypes among Native Americans in New Mexico were actually found to be very similar to Caucasian populations (Rex et al., 1985). As all of these known polymorphisms are in the oxidative pathway, and even if present in a small proportion of the study population, they would not affect the target biomarkers.

Our findings suggest that PAE prevalence in the Navajo Nation is at least comparable to rates in the general population and consistent with national self-reported PAE rates among Native Americans (Watt, 2012). The highest prevalence for a single biomarker (EtS) in the current study was 7.8%, and 5.4 % of specimens were positive for ≥2 biomarkers. This finding contrasts with widely-held perceptions of Native Americans as ‘high-risk’ for alcohol-related problems. Our research in the Navajo Nation, which is the first that we know of to present alcohol biomarker prevalence data in an all-Native American pregnancy cohort, highlights the heterogeneity of Native American communities. Although prior studies among other Native American groups found higher rates of self-reported drinking and FASD (Duimstra et al., 1993; Fox et al., 2015; Khan et al., 2013; May et al., 2009), our findings emphasize that drinking behaviors among Native American populations in the country can vary dramatically. Emerging research on urban and rural Native American veterans (Westermeyer et al., 2009) found lower rates of alcohol-related illness among women compared to men, while a study of high school students found widely similar drinking levels and behaviors between White and Cherokee Nation women (Komro et al., 2016). Generalization across all Native American populations is not warranted, and emerging data, expanded upon by our own findings, refutes long-held stereotypes about increased alcohol use among Native Americans (Cunningham et al., 2016).

The lower prevalence might also demonstrate success from the Navajo Nation’s efforts to reduce alcohol-related risks in the community. Navajo Nation IHS clinicians in many service units where participants received care reported initiation of a strong campaign in recent years to educate patients on the risks of alcohol consumption during pregnancy, which may be contributing to the lower rates of consumption observed in this study, and further highlights the importance of such efforts. While the effectiveness of this campaign could not be directly evaluated, and we have insufficient data to assess change in consumption over time, evaluations of targeted intervention programs in tribal communities are emerging (Hanson et al., 2017; Montag et al., 2015) and should be the focus of future efforts. It is worth noting that the effectiveness of these interventions has not always proven to be an

improvement over standard care (Montag et al., 2015). Working with clinicians adopting educational and other interventions to track resulting changes would help in evaluating effectiveness and identifying how factors such as prenatal care compliance influence outcomes. Such data could inform future preventive efforts incorporating holistic community-based participatory approaches to address multiple reproductive health challenges in Native communities.

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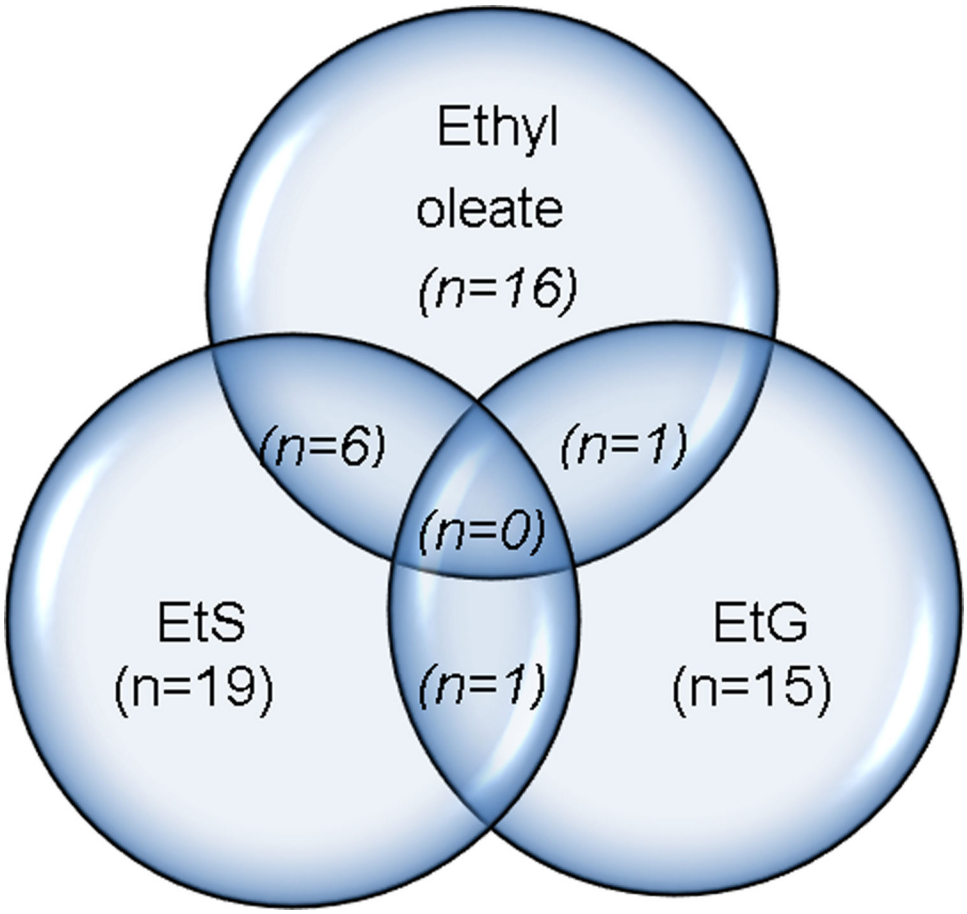

Figure 1.

Overlap between the Most Prevalent Biomarkers

*Total prevalence for each biomarker shown is the sum of all numbers within each of the corresponding circles of the diagram (e.g., total number of subjects positive for EtS is 26: 19+6+0+1)

Table I.

Literature Review: Prevalence of Prenatal Alcohol Exposure by Meconium Biomarkers

Author/year	Study design	Study population	Meconium biomarkers	Self-reported alcohol use	Major findings/ comments
Himes SK. et al, 2015 [10]	Prospective multi-site PASS cohort	US Northern Plains, and Cape Town, South Africa 108 meconium samples: 33 with no PAE, others stratified by timing and amount of PAE.	9 FAEE (ethyl linolenate, palmitoleate, arachidonate, linoleate, palmitate, oleate, and stearate), EtG, and EtS Analysis: LC-MS/MS	Prospective repeated TLFB interviews	Meconium biomarkers with the highest agreement with self-reported PAE in the second part of pregnancy was EtG ≥ 30 ng/g (kappa=0.57). No association between EtG and EtS; EtG was negligibly associated with individual and summed FAEEs. Associations among individual FAEEs were moderate-to-strong (ρ=0.50–0.89). Correlations between ethyl oleate/EtG and ethyl oleate/EtS were modest (Spearman correlation coefficients 0.38 and 0.27, respectively).
Morini L. et al, 2010 [30]	Prospective cohort study	99 meconium samples: 49 from Italy 50 from Spain	7 FAEE (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and arachidonic acid ethyl esters), EtG, and EtS Analysis: LC-MS/MS	Information on alcohol use obtained from medical records	PAE by EtG (>LOD): 82.8% in both cohorts PAE by EtS (>LOD): 19.2% in both cohorts PAE by FAEE (≥2 nmol/g): 10.4% in Italy and 34% in Spain (22.2% combined) Significant correlations between EtG and ethyl laurate, linolenate, linoleate, oleate and total FAEEs. Significant correlations between EtS and ethyl arachinodate, linoleate, palmitate, oleate and total FAEEs. Correlation coefficients were not reported.
Morini L. et al, 2010 [40]	Cross-sectional study in public hospitals	185 meconium samples: 80 from Italy 105 from Spain	7 FAEE (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidonic acid ethyl ester), EtG, EtS. Analysis: LC-MS/MS	A structured questionnaire administered at each trimester (type of the questionnaire not specified)	Median, min, max, and 97.5 percentile concentrations for each biomarker were presented by abstaining women and those with uncertain exposure. Meconium EtG (≥2 nmol/g) was determined to be more sensitive and specific than EtS and FAEE
Pichini S. et al, 2009 [41]	Cross-sectional study in public hospitals	177 meconium samples: 96 from Italy 81 from Spain	7 FAEE (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and arachidonic acid ethyl esters), EtG, EtS. Analysis: LC-MS/MS	Assessment by questionnaire (type of the questionnaire or timing of administration not specified)	PAE by EtG (>5 ng/g): 81.5% in Italy and 95.5% in Spain. PAE by EtS (>1 ng/g): 46.9% in Italy and 51.9% in Spain. PAE by FAEE (≥2 nmol/g): 8% in Italy and 42% in Spain. No correlation was found between EtG or EtS concentrations and the total amount or each of the 7 FAEEs in the meconium samples from either cohort.

PAE: Prenatal alcohol exposure; FAEE: Fatty acid ethyl esters; EtG: Ethyl glucuronide; EtS: Ethyl sulfate; LC-MS/MS: Liquid chromatography–tandem mass spectrometric

Table II:

Description of the study population (n=333*)

Patient Characteristics	Mean + SD
Maternal age (years)	27.4 ± 6.0
Gestational age at recruitment (weeks)	23.3 ± 10.0
Gestational age at delivery (weeks)	38.7 + 1.8
Birth weight (g)	3,356 + 541
Birth length (cm)	50.2 + 2.8
APGAR score – 1 min	8.3 + 1.0
APGAR score – 5 min	9.1 + 0.6
Marital status:	N (%)
Married/cohabitating	253 (83.5)
Separated/divorced	23 (7.6)
Single	27 (8.9)
Maternal education:	
Less than high school grad	68 (22.4)
High school grad/GED	100 (32.9)
Some college/vocational or higher	136 (40.8)
Annual income:	
Less than $19,999	214 (71.3)
$20,000-$39,999	30 (10.0)
≥ $40,000	10 (3.3)
Do not know/Refused to answer	46 (13.8)
Gravidity (primigravida)	78 (25.6)
Parity (nulliparous)	90 (29.7)
Tobacco use:	
Regular use	1 (1.8)
Ceremonial purposes only	120 (35.9)
Placement of an infant in NICU	3 (0.92)

*Sample size may vary due to pairwise deletion of missing data

Table III:

Distribution and Prevalence of Positive Meconium Biomarkers in the Study Population (n=333)

Measures of PAE	Median (ng/g)	Range (ng/g)	Prevalence (>LOQ)N (%)
Fatty acid ethyl esters (FAEEs):			
Ethyl laurate E12:0	93.9	0.0–93.9	1 (0.3)
Ethyl myristate E14:0	0.0	0.0–0.0	0 (0.0)
Ethyl palmitate E16:0	129.4	78.8–180	2 (0.6)
Ethyl palmitoleate E16:1	47.6	20.2–197	4 (1.2)
Ethyl stearate E18:0	0.0	0.0–0.0	0 (0.0)
Ethyl oleate E18:1	30.4	16.2–325	23 (6.9)
Ethyl linoleate E18:2	47.1	17.9–168	17 (5.1)
Ethyl linolenate E18:3	49	30.9–67.1	2 (0.6)
Ethyl arachidonate E20:4	15.8	15.4–35.2	3 (0.9)
Ethyl glucuronide (EtG)	30.8	9.4–3440.2	17 (5.1)
Ethyl sulfate (EtS)	7.8	3.1–37.7	26 (7.8)
Positive for ≥ 2 biomarkers	--	--	18 (5.4)