Agreement between Maternal Self-reported Ethanol Intake and Tobacco Use During Pregnancy and Meconium Assays for Fatty Acid Ethyl Esters and Cotinine

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Reliance on self-reported use of tobacco and intake of ethanol during pregnancy is associated with a high probability of error. Use of biological markers, or biomarkers, potentially offers a more valid method to assess exposure. Although cotinine is an established biomarker for tobacco use, there is no established biomarker for in utero ethanol exposure. Recent reports suggest that fatty acid ethyl esters (FAEE) could serve this purpose. To assess agreement between maternal self-reported tobacco use and ethanol intake during pregnancy and detection of metabolites associated with tobacco use (cotinine) and ethanol intake (FAEE), the authors studied maternal histories and meconium samples obtained in November–December 1999 from 436 consecutive mother-infant pairs at a large urban regional perinatal center in Honolulu, Hawaii. Cohen's kappa coefficient and 95% confidence intervals were calculated. Moderate agreement was found between reported tobacco use during the third trimester and detected cotinine level (kappa = 0.53, 95% confidence interval: 0.39, 0.68); however, there was no agreement between reported ethanol intake during the third trimester and detected FAEE (kappa = –0.02, 95% confidence interval: –0.04, 0.00). No mother reporting ethanol intake during the third trimester had detectable FAEE. Findings support the need for additional refinement and validation of the use of FAEE as a biomarker for maternal ethanol intake.

Abbreviation: FAEE, fatty acid ethyl esters.

Reliance on self-reported use of tobacco and intake of ethanol during pregnancy is associated with a high probability of error. Numerous studies have documented substantial underreporting (1–4). Early identification of gestational exposure to illicit drugs, alcohol, and tobacco is important so that appropriate diagnostic and therapeutic interventions may be offered. In addition, mothers who continue to use these substances put their children at high risk for adverse health outcomes, including abusive and neglectful parenting, and should be targeted for postnatal support services (5–7).

The use of biological markers, or biomarkers, to identify drug exposure during pregnancy has been demonstrated for illicit and licit drugs such as cocaine, amphetamines, marijuana, opiates, and tobacco (5, 8–11). Toxicologic assay of neonatal urine at the time of birth has been the most commonly used methodology. However, because of the relatively short window of opportunity for detection, urine testing frequently gives false-negative results and underestimates drug exposure (6, 12). Recent reports have shown the superiority of meconium assays for detecting in utero drug exposure (10, 13, 14).

Cotinine, a nicotine metabolite, has been measured in both neonatal urine (15) and meconium (16). It is relatively stable, with a urinary half-life of approximately 24 hours (15, 17), and is an established biomarker for both active and passive tobacco exposure. Unlike the situation for nicotine, there is presently no conventionally accepted biomarker for diagnosing long-term in utero ethanol exposure (18).
Because the half-life of ingested ethanol is less than 1 hour (19), even mothers who drink daily during pregnancy may have negative ethanol toxicology screens if a drink has not been consumed within a few hours prior to presentation. Recent reports have identified fatty acid ethyl esters (FAEE), nonoxidative metabolites of ethanol that result from the transesterification of ethanol with fatty acids, as sensitive and stable biomarkers for ethanol exposure (18, 20–23). FAEE have a prolonged half-life compared with ethanol and its oxidative metabolites, and serum levels in adults have correlated well with ethanol ingestion (20, 22). Several authors have reported that both the placenta and meconium itself appear to have the necessary enzymes to produce these ethanol metabolites (24–26). In addition, a number of recent studies have reported increased levels of FAEE in the meconium of neonates exposed to ethanol in utero (25, 27, 28).

The purpose of this study was to assess the concordance between maternal self-reported tobacco use and ethanol intake during pregnancy and detection in neonatal meconium of the tobacco and ethanol metabolites, cotinine and FAEE, in a cohort of women giving birth at a large urban regional perinatal center.

MATERIALS AND METHODS

Consecutive newborns delivered at Kapi‘olani Medical Center for Women and Children in Honolulu, Hawaii, in November–December 1999 were enrolled as part of a pilot project to determine the prevalence of in utero drug exposure. The study protocol was reviewed and approved by the institutional review board at the medical center. The mother and nurse of each enrolled newborn were instructed to place all soiled diapers or other items containing meconium into a crib-side bag during the first 2 or 3 days after birth. These items were collected twice daily from each subject for the duration of the newborn infant’s hospitalization. For infants requiring toxicology screening for forensic purposes or clinical indications, meconium was collected first for that reason and then for study purposes. An attempt was made to collect as much meconium as possible during the hospitalization. Collected meconium samples for each subject were coded anonymously by using a numeric identifier. Samples were then placed into a single foil-wrapped container, weighed, frozen at –20°C, and sent in batches (100 samples per batch) to the US Drug Testing Laboratories in Des Plaines, Illinois. Samples weighing a minimum of 1 g were uniformly mixed and analyzed for ethanol metabolites (both total FAEE and individual FAEE subtypes, by gas chromatography/mass spectrometry under the positive chemical ionization mode) and cotinine (by enzyme-linked immunosorbent assay (STC Diagnostics Inc., Bethlehem, Pennsylvania)). Cutoff values for FAEE and cotinine were 50 ng/g and 25 ng/g, respectively.

Data on reported tobacco use and alcohol ingestion were extracted from the medical record. When expectant mothers were admitted to Kapi‘olani Medical Center for Women and Children, a triage nurse routinely asked them about their tobacco use and alcohol ingestion by using a standardized format. Tobacco use was documented by trimester of last use and packs smoked per day; alcohol intake was documented by trimester of last use and drinks per week. The medical record information was coded with the same unique numeric identifier used for the meconium specimens, and personal identifying information was then destroyed to ensure the anonymity of the study.

To assess agreement between maternal self-reported intake of ethanol and use of tobacco with detected biologic markers (FAEE and cotinine, respectively), Cohen’s kappa coefficient and 95 percent confidence intervals were calculated by using StatXact version 4.0.1 software (Cytel Software Corp., Cambridge, Massachusetts). By defining the presence or absence of FAEE and cotinine as the standards to which maternal self-reported ethanol intake and tobacco use are compared, we calculated the sensitivity, specificity, and positive predictive values for maternal self-reported ethanol intake and tobacco use (29).

RESULTS

During the study period from November 8, 1999, through December 17, 1999, 546 infants were delivered at Kapi‘olani Medical Center for Women and Children. Of these 546 infants, 17 (3.1 percent) were missed by the collection procedure, 72 (13.2 percent) had no meconium collected before hospital discharge, and 21 (3.8 percent) provided less than the minimum 1 g necessary for analysis. Of the specimens provided by 436 infants, 14 (3.2 percent) and 10 (2.3 percent) were of insufficient quantity to be analyzed for FAEE and cotinine, respectively. Therefore, for our final analysis, 422 and 426 infants provided samples that were analyzed for FAEE and cotinine, respectively.

Seventy-two (17.1 percent) of 422 meconium specimens were positive for FAEE, whereas 33 (7.7 percent) of 426 specimens were positive for cotinine. Information extracted from the medical record documented ethanol intake and tobacco use during the current pregnancy by, respectively, 23 (5.3 percent) and 58 (13.3 percent) of the 436 women. There was moderate agreement between reported tobacco use during the third trimester and detected cotinine level (kappa = 0.53, 95 percent confidence interval: 0.39, 0.68) (table 1); however, no agreement was found between reported ethanol intake during the third trimester and detected FAEE (kappa = –0.02, 95 percent confidence interval: –0.04, 0.00). Four mothers reported ethanol intake

| Table 1. Agreement* between maternal self-reported tobacco use during the third trimester of pregnancy and detected cotinine in meconium of 426 newborn infants delivered at Kapi‘olani Medical Center for Women and Children, Honolulu, Hawaii, November–December 1999 |
|---------------------------------|------------------|---|
| Maternal self-report | Cotinine in meconium | No. |
| Positive | Detected | 20 |
| Positive | Undetected | 17 |
| Negative | Detected | 13 |
| Negative | Undetected | 376 |

* Kappa = 0.53, 95% confidence interval: 0.39, 0.68; positive predictive value = 0.54.
TABLE 2. Agreement* between maternal self-reported ethanol intake during the third trimester of pregnancy and detected FAEE‡ in meconium of 411† newborn infants delivered at Kapi'olani Medical Center for Women and Children, Honolulu, Hawaii, November–December 1999

<table>
<thead>
<tr>
<th>Maternal self-report</th>
<th>FAEE in meconium</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Detected</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>Undetected</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>Detected</td>
<td>71</td>
</tr>
<tr>
<td>Negative</td>
<td>Undetected</td>
<td>336</td>
</tr>
</tbody>
</table>

* Kappa = –0.02, 95% confidence interval: –0.04, 0.00; positive predictive value = 0.
† FAEE, fatty acid ethyl esters.
‡ Eleven women reporting ethanol intake but for whom trimester was “unspecified” were excluded (one delivered an infant with detected FAEE in the meconium).

During the third trimester: two consumed one drink per day; one had two drinks per week; and, for one, frequency was unspecified. No FAEE were detectable in the meconium of infants born to these mothers (table 2). When detected cotinine was used as an indicator for tobacco exposure, the sensitivity and specificity of reported tobacco use during the third trimester were 61 percent and 96 percent, respectively. The positive predictive value of reported tobacco use during the third trimester was 54 percent (table 1).

DISCUSSION

When meconium is used as a screening medium, FAEE have been detected in mothers with a history of ethanol ingestion, although the distribution and relative concentrations of individual fatty acids have differed between published studies (25, 27, 28). It is difficult to assess the validity of FAEE as a biomarker for in utero ethanol ingestion because no “gold standard” has been identified for which to compare its performance characteristics. Previous studies have noted the presence of FAEE in adults given fixed amounts of ethanol (20), in the meconium of small numbers of known alcohol-exposed newborns (28), in meconium specimens “spiked” with ethanol (25), and in meconium spiked with FAEE (30). However, we know of only one previously published study that has attempted to assess the validity of FAEE as a biomarker for in utero ethanol exposure in a large, population-based sample of newborns (27).

Bearer et al. (27) reported on the use of ethyl linoleate (the predominant form of FAEE in their study) in meconium as a biomarker for prenatal ethanol exposure. Their published screening test parameters, sensitivity and specificity, were calculated by using the presence of ethyl linoleate as the positive screening test result; maternal self-reported ethanol intake or abstinence served as the gold standard. However, because maternal self-reports of ethanol ingestion are known to be unreliable (1–4), this is an unconventional method for calculating sensitivity, specificity, and positive predictive values for FAEE. Bearer et al. acknowledged in their study that histories of alcohol intake “may not be a gold standard for identifying abstainers” (27, p. 492). Additionally, their findings revealed disagreements not only for women reporting no alcohol consumption but also for some women who admitted consuming alcohol during the third trimester but whose infants’ meconium contained undetectable FAEE.

When there is no gold standard for which to compare, a validity assessment involving sensitivity and specificity calculations may not be possible. In such cases, some sense of the quality of one’s measurements may be more appropriately obtained by assessing the reliability of one’s measurements. The kappa coefficient provides an appropriate measurement of this reliability, because it reflects the degree of agreement between two imperfect measurement techniques (31). When Bearer et al.’s (27) data were reanalyzed by using the kappa coefficient, a rather weak agreement between maternal self-reports of ethanol intake and detected FAEE in meconium was demonstrated. In the situation in which women reported consuming at least three drinks per week during the third trimester, Bearer et al. (27) noted the sensitivity and specificity of ethyl linoleate to be 67 percent and 51 percent, respectively (p = 0.12, two-sided). However, the calculated kappa coefficient in this case was quite low: 0.12 (95 percent confidence interval: –0.03, 0.26).

In a second example, Bearer et al. (27) noted an increase in the reported “sensitivity” of the ethyl linoleate biomarker when applied to mothers reporting less ethanol ingestion (at least one drink per week in the third trimester). In this scenario, the sensitivity and specificity were 72 percent and 51 percent, respectively (p = 0.02, two-sided), although the kappa coefficient remained low: 0.19 (95 percent confidence interval: 0.04, 0.33) (table 3). When Bearer et al.’s (27) definition of maternal self-reported ingestion or noningestion of ethanol was considered the gold standard, the positive value of detected FAEE to correctly identify mothers who ingested ethanol during pregnancy remained suboptimal in both test scenarios (27 percent and 39 percent, respectively). When we used the kappa coefficient to assess the correlation

TABLE 3. Reanalysis of previously published data* on the concordance† between maternal self-reported ethanol intake (at least one drink per week) during the third trimester of pregnancy and detected FAEE‡ (ethyl linoleate) in meconium

<table>
<thead>
<tr>
<th>Maternal self-report</th>
<th>FAEE in meconium</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Detected</td>
<td>28</td>
</tr>
<tr>
<td>Positive</td>
<td>Undetected</td>
<td>11</td>
</tr>
<tr>
<td>Negative§</td>
<td>Detected</td>
<td>43</td>
</tr>
<tr>
<td>Negative§</td>
<td>Undetected</td>
<td>45</td>
</tr>
</tbody>
</table>

† Kappa = 0.19, 95% confidence interval: 0.04, 0.33.
‡ FAEE, fatty acid ethyl esters.
§ Women reporting alcohol abstinence in the month before pregnancy and throughout pregnancy.

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between maternal self-reported ethanol ingestion and FAEE detection, our findings were remarkably consistent with those reported by Bearer et al. (27).

Although our study included only a small number of women who acknowledged drinking in the third trimester, our purpose was to show how well the reported behavior (drinking or not drinking) agreed with the presence or absence of FAEE in meconium. We believe that our finding of FAEE in the meconium of infants whose mothers denied alcohol intake is as important as the absence of FAEE in the meconium of infants whose mothers admitted drinking in the third trimester.

Several authors have reported on the cytotoxic effects of FAEE (22, 32, 33) and on the possible association of FAEE with adverse infant and child health outcomes (34, 35). Indeed, we found associations between total FAEE and lower 1-minute Apgar scores (p < 0.01, two-sided) and between ethyl oleate and decreased birth weight (p < 0.01, two-sided) (author C. D., unpublished observations). It may well be that FAEE are related to adverse pregnancy outcomes; however, it does not appear that their presence specifically reflects maternal ingestion of alcoholic beverages. Ethanol is present in small amounts in a variety of food additives and flavorings as well as in both over-the-counter and prescription cold and cough syrups. Dietary fats or short-chain fatty acids produced in the colon by fermentation of dietary fiber may result in or effect the production of FAEE. In addition, it is also possible that medicines or food products will cause false-positive or negative test results (23).

Note that even nicotine, the major toxic compound associated with tobacco exposure, has been found as a naturally occurring entity in vegetables and teas (36).

Early identification of gestational exposure to alcohol through the development of a reliable and valid biological marker for such exposure is an important goal for the field of developmental toxicology. A recent publication measuring the prevalence of FAEE in meconium infers that they are an established biomarker for fetal alcohol exposure (37). Our findings, however, support the need for additional refinement and validation, and they suggest caution in prematurely applying FAEE in clinical care.

ACKNOWLEDGMENTS

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REFERENCES