Assessing maternal alcohol consumption in pregnancy: comparison of confidential postnatal maternal interview and measurement of alcohol biomarkers in meconium

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Keywords
Neonatology
Child Health
Infant Development

Word count 2470

This is a peer reviewed, accepted author manuscript: Henderson, E. M., Tappin, D., Young, D., Favretto, D., & Mactier, H. (2023). Assessing maternal alcohol consumption in pregnancy: comparison of confidential postnatal maternal interview and measurement of alcohol biomarkers in meconium. *Archives of Disease in Childhood*. https://doi.org/10.1136/archdischild-2022-325028

Abstract

Objective: Knowledge of alcohol consumption in pregnancy is important for early identification of children with fetal alcohol spectrum disorder. We investigated whether alcohol biomarkers fatty acid ethyl esters (FAEEs) and ethyl glucuronide (EtG) in meconium are predicted by maternal or newborn demographics and/or correlate with early confidential postnatal self-report of alcohol consumption in pregnancy.

Design: Anonymised, observational population-based study.

Setting: Inner-city maternity unit, Glasgow, UK.

Patients: Singleton mother/infant dyads delivering every fourth day.

Interventions: Mother: confidential postnatal interview. Baby: meconium sample for FAEEs and EtG.

Results: 840/908 mothers consented. 370 (46.4%) reported alcohol consumption in pregnancy, generally of modest amount; for 114 (13.6%) this was after 20 weeks' gestation,. Alcohol consumption in later pregnancy was more commonly reported by older (31.3 *v* 29.5 years) women of white British ethnicity ($p<0.05$). Their babies were on average 118g heavier ($p=0.032$). FAEEs were identified in all meconium samples; concentration was ≥600 ng/g in 39.6%. EtG concentration was ≥30 ng/g in 14.5%. Neither biomarker was associated with maternal age, body mass index or socioeconomic status but when EtG was ≥30 ng/g, the mother was less likely to identify as white British (71.3% vs 81.8%, $p=0.028$). Sensitivity of

FAEEs ≥600 ng/g and EtG ≥30 ng/g were 43.1% and 11.6% respectively at identifying postnatal self-report of alcohol use in later pregnancy (specificities 60.6% and 84.8%). *Conclusions:* Higher rates of alcohol consumption are reported when newly delivered mothers are asked detailed, confidential questions in a systematic way. Meconium biomarkers do not reliably detect alcohol consumption in later pregnancy.

Key Messages

What is already known on this topic

- Knowledge of alcohol consumption in pregnancy is important in targeting public health messaging and to help early identification of children at risk of fetal alcohol spectrum disorder (FASD).
- Fatty acid ethyl esters (FAEEs) and ethyl glucuronide (EtG) can be measured from infant meconium but utility to document the pattern of alcohol consumption in pregnancy in an unselected UK population is not known.

What this study adds

- Higher rates of alcohol consumption in pregnancy are reported in the early postnatal period when mothers are asked detailed, confidential questions in a systematic way.
- FAEEs and EtG measured in meconium have low sensitivity for self-reported modest alcohol consumption after 20 weeks' gestation.

How this study might affect research, practice or policy

- Measurement of alcohol biomarkers in meconium cannot currently be recommended as a reliable indicator of alcohol exposure in later pregnancy in isolation.

Contributorship statement

EMAH participated in study design, recruited all subjects, undertook some of the statistical analyses, wrote the first draft of the manuscript and participated in all subsequent revisions. DT participated in study design and critically reviewed the draft manuscript. DY advised on data analysis and reviewed the draft manuscript. DF supervised laboratory analyses and reviewed the draft manuscript. HM conceived and supervised the study, contributed to the draft manuscript and critically reviewed all manuscript revisions. All authors approved the final manuscript.

Introduction

The aetiology of fetal alcohol spectrum disorder (FASD) is complex but necessarily includes prenatal alcohol exposure (PAE) (1-3) Earlier diagnosis of FASD with targeted interventions results in better outcomes and may provide opportunity to provide interventions to caregivers to support safer future pregnancies (4, 5) but can be hampered by difficulty in ascertaining PAE. Report of alcohol use during pregnancy may be unreliable for reasons including under-reporting, limited handover of information to child health services (6) and over-representation of children with FASD in the accommodated population (7).

The use of alcohol biomarkers is imperfect but may be complementary to self-report in managing adult patients with alcohol use disorder(8). Alcohol biomarkers may also have a role in assessing PAE (11-14) with biomarkers in meconium highlighted as worthy of further population-based research (9-13). Meconium forms in the fetus from 16-20 weeks' gestation and offers an ethically acceptable, non-invasive method of seeking to determine PAE in the latter half of pregnancy(9). Both fatty acid ethyl esters (FAEEs) and ethyl glucuronide (EtG) can be measured in meconium; (14) FAEEs result from fetal metabolism whereas EtG in meconium reflects both maternal and fetal alcohol metabolism and is considered less prone to variation from nutritional factors and/or endogenous production(15). Concentrations of FAEEs in meconium ≥600 ng/g have been related to regular alcohol consumption during pregnancy of >2 drinks daily (equivalent to 28 g ethanol or 3.5 UK units) (16). EtG >30 ng/g had moderate-substantial agreement with self-reported PAE after 19 weeks' gestation in a relatively heavy drinking population (10). While PAE does not necessarily result in PAE (cite McGuire), FAEE concentration in meconium correlates inversely with educational attainment and meconium EtG has been linked to childhood cognitive deficits and attention deficit hyperactivity disorder-related behaviour (17)(18).

In a previous anonymised study of an unselected population, concentrations of FAEEs and EtG in meconium were respectively ≥600 ng/g in 42% and ≥30 ng/g in 15% despite only 3% of mothers reporting alcohol consumption in pregnancy (9). No association was seen between either biomarker and any maternal or infant demographic although there was a trend towards more babies of affluent mothers having elevated meconium FAEEs. We sought to determine in a larger study if a structured, confidential early postnatal interview would be associated with more common reporting of alcohol in pregnancy, and if alcohol biomarkers in meconium would correlate with self-reported alcohol consumption and/or any maternal or infant demographics.

Methods

This was an observational population-based study within a large obstetric-led maternity unit in the relatively deprived city of Glasgow. All mothers delivering a live singleton infant within each fourth consecutive 24-hour period were eligible. Women admitted to labour suite were provided with written information and a plastic bag in which to collect the baby's first meconium nappy. Written informed consent was sought as soon as possible after delivery by a single researcher; if the mother declined, retained samples were discarded. Meconium samples were transferred directly from the nappy to a plain universal container, avoiding contact with baby wipes. Containers were labelled and frozen at -40°C and thermally protected during shipping to the University of Padova, Italy.

Assessment of alcohol consumption in pregnancy

Alcohol consumption in pregnancy was assessed by confidential health questionnaire administered by a single researcher immediately after consent for participation in the study. Mothers who reported alcohol consumption were asked more details including timing, type and amount of alcohol; this was recorded on an individualised calendar using a modified

time-line follow back (TLFB) method and related to gestation. Participants were encouraged to utilise diaries and/or social media to prompt recollection. Demographic information obtained from case notes included maternal age, gravidity, postcode of residence, ethnicity and body mass index (BMI), infant gestation, birth weight and occipitofrontal head circumference (OFC). The Scottish Index of Multiple Deprivation 2016 (SIMD16) based on mother's postcode at delivery was used as a measure of socioeconomic deprivation and divided into deciles (19) (1 least deprived).

Laboratory Analyses

Liquid chromatography/mass spectrum analysis was utilised for measurement of FAEEs (ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate) and EtG. 200 mg meconium was sonicated for 15 minutes with 20 ng EtG-d5 and 200 ng of FAEE d-5. The supernatant was added to an aminopropyl solid-phase extraction cartridge, preconditioned with 2 ml methanol, water and acetonitrile (ACN). FAEEs were eluted with 2 ml hexane and EtG elution with 2 ml water. The two mixtures were dried using nitrogen stream and recovered with 50 µl of ACN (FAEEs) and 50 µl methanol (EtG). FAEEs were detected following separation using a C8 reversed-phase column. A C18 reversed-phase column was used in isocratic mode for EtG detection. Acquisition was in multiple reactions monitoring for all the analytes in positive mode for FAEEs and negative mode for EtG. Lower limit of quantification (LOQ) values were 10-15 ng/g for summed FAEEs and 10 ng/g for EtG.

Statistics

Between group comparisons were done using Z-tests for two proportions for categorical data and t-tests for numerical data. Pearson correlations were used to determine relationships between numerical measurements. The performance of FAEEs and EtG to predict alcohol

consumption was quantified by computing sensitivity, specificity, positive and negative predictive values. All analyses were done using Minitab (version 18) at a 5% significance level.

The study was approved by West of Scotland Research Ethics Committee 3 (15/WS/0110); This work was supported by funding was from Yorkhill Children's Charity grant number (YRSS/CRF/2014/01).

Results

1021 singleton infants were born on 71 study days (Figure 1). 840 (92.5%) eligible mothers consented of whom 828 completed a health questionnaire.

Smoking and alcohol consumption

223/828 mothers (26.9%) declared smoking prior to pregnancy; 145 (17.5% of the entire cohort) continued to smoke during pregnancy. A further two mothers started smoking in the current pregnancy (Table 1). 384 (46.4%) mothers reported consuming alcohol at any point in pregnancy; for the majority (252) this was only prior to knowledge of pregnancy. 114 (13.8%) reported alcohol beyond 20 weeks' gestation; three reported one or more episodes of binge drinking ($\geq$ five UK units/40 g ethanol). Alcohol consumption was less common in mid-pregnancy (5.3% of all mothers).

Any alcohol consumption in pregnancy was more common in women who identified as white British ($p<0.05$) and/or smoked in pregnancy ($p<0.05$). Self-reported alcohol beyond 20 weeks' gestation was more common in mothers aged >35 years ($p=0.0004$) and who identified as white British ($p<0.05$) (Table 1).

Babies with declared PAE after 20 weeks' gestation had a higher mean birthweight (3470 *vs* 3352 g (p=0.032)); this was not explained either by gestation or maternal BMI and was not accompanied by change in OFC.

Fatty acid ethyl esters (FAEEs)

FAEEs were detected in all samples; total concentration ranged from 22.2 to 7549.8 ng/g. Using ≥600 ng/g to define a positive result, 282 (39.6%) were positive.

The likelihood of meconium positive for FAEEs was not related to maternal age, BMI, SIMD score, ethnicity or previously having had a baby (table 2). When meconium was positive, mothers tended to have been less likely to smoke during pregnancy (14% *vs* 20%, p=0.071).

Mean birthweight was greater for those infants whose meconium was positive for FAEEs (3425 *vs* 3331 g, p=0.032)). Mean OFC did not differ.

Self-reported alcohol consumption was linked with 702 meconium samples. When infant meconium was positive or negative for FAEEs, 41/280 (14.5%) or 54/422 (13%) mothers respectively reported alcohol consumption after 20 weeks' gestation (NS). Of the eight mothers that reported drinking at least three units of alcohol on any occasion after 20 weeks' gestation, only three infants' meconium samples were positive for FAEEs (707, 847 and 996 ng/g respectively). When FAEE concentration was > 2000 ng/g (n=21), none of the mothers reported consuming alcohol in pregnancy.

Ethyl glucuronide (EtG)

EtG was detectable in 293/712 samples but below LOQ in 96. When EtG was quantifiable (n=197), median concentration was 32.2 ng/g, IQR 17.9 to 55.8 ng/g; results were skewed with mean concentration 162.1 ng/g (SD 871). EtG was ≥30 ng/g (positive) in 103 (14.5%)

samples. Infants with meconium positive for EtG were less likely to have a mother identifying as white British (71.3% *vs* 81.8%, p=0.028). When meconium was positive for EtG, mothers tended to have been less likely to smoke during pregnancy (13.6% *vs* 18.5%) and mean birthweight was marginally greater (3418 *vs* 3365 g) but neither difference was significant (table 3).

When meconium was positive for EtG, 10.7% of mothers reported alcohol consumption after 20 weeks' gestation compared to 14% of mothers when meconium was negative. Eight mothers reported drinking at least three units of alcohol on any occasion after 20 weeks' gestation; only one meconium had detectable EtG (17.3 ng/g).

Correlation between FAEEs and EtG in meconium

Overall, there was no correlation between FAEEs and EtG. There was a weak positive correlation in those meconium samples positive for both FAEEs and EtG (n=50) (Figure 2) (Pearson's coefficient= 0.283, p value=0.044).

Sensitivity and specificity of alcohol biomarkers

Using maternal self-report of alcohol after 20 weeks' gestation as gold standard, the sensitivities of FAEEs $\geq$600 ng/g and EtG $\geq$30 ng/g in meconium were 43.1% and 11.6% respectively (Table 4). Combination of FAEEs $\geq$600 ng/g and EtG $\geq$30 ng/g had high specificity for alcohol consumption in later pregnancy (92.4%), but very low sensitivity (5.3%). The positive predictive value of two positive infant biomarkers for self-reported alcohol consumption in later pregnancy was 9.8%.

Discussion

In this unselected population, almost half of newly delivered mothers reported drinking alcohol in any point in pregnancy compared to 3% in a pilot study of a similar population(9). Most alcohol consumption was before awareness of pregnancy. Thus higher rates of alcohol consumption are reported when mothers are asked detailed, confidential questions in a systematic way by a single researcher with no influence on the mother's care.

The prevalence of alcohol consumption in pregnancy in the Scottish population was examined via national Infant Feeding Surveys (IFS) in 2010 and 2017 and in the 2010-11 birth cohort (20-22). In 2017 at six weeks postnatal, 12% mothers reported alcohol consumption during pregnancy(22), comparable with 13.8% reporting drinking after 20 weeks' gestation in the current study, conducted between 2015-16. Self-report therefore underestimates drinking alcohol in the earliest stages of pregnancy, when the embryo is potentially at greatest risk of damage(23). In 2010 40% of mothers reported alcohol consumption during pregnancy, albeit generally of modest amount (<3 UK units)(21); whether this change in reporting over a seven year period including much public health messaging around the dangers of PAE reflects a true reduction or more inhibition about reporting alcohol in pregnancy is not clear. A similar retrospective reporting system noted a reduction in alcohol consumption during pregnancy in Scotland between 2005/6 and 2010/11(20). The 2017 IFS and current study are consistent in that almost half of women of reproductive age in Scotland regularly consume alcohol prior to conception (25,26). Since up to 40% of pregnancies are unplanned(20), this has implications for public health messaging. In our study mothers who self-reported alcohol consumption beyond 20 weeks' gestation were more likely to be older, again similar to the IFS(21).

Although we are reasonably confident that self-reporting of frequency of alcohol consumption was accurate, those mothers who reported drinking in pregnancy may have

under-estimated the amount. It is also possible that mothers who declined to participate, or who provided consent but no meconium sample, were more likely to have consumed alcohol (selection bias). Inaccurate self-report may reflect lack of recollection, underestimation of poured volume and concern about clinician judgement(24). Any comparison with maternal self-report either through individualised or standardised risk questionnaires such as TWEAK, MAST, CAGE must be close to delivery (16, 25, 26) since accuracy of reporting of alcohol consumption reduces with time from delivery (27) and, as evidenced in the current study, patterns of alcohol consumption may change during pregnancy.

If clinicians are to make early diagnosis of FASD to allow early more effective intervention, a marker of PAE needs to be sensitive to allow as many affected infants as possible to receive intervention and specific to not subject unaffected infants to unnecessary intervention and the mother to the stigma of an alcohol-related disorder. Using maternal self-report of alcohol after 20 weeks' gestation as a gold standard, the sensitivities of meconium FAEEs >600 ng/g and EtG >30 ng/g in this study were 43.1% and 11.6% respectively. Specificities were 60.6 and 84.8% respectively with a combination of FAEEs $\geq$600 ng/g and EtG $\geq$30 ng/g achieving a specificity of 94.8% for alcohol consumption in later pregnancy. Self-report of alcohol after 20 weeks' gestation was more common in older mothers of white British ethnicity, but this association was not seen with either alcohol biomarker; indeed, meconium was more likely to be positive for EtG if the mother did not identify as white British.

Previous publications report higher sensitivity of FAEEs and/or EtG in meconium as a marker of PAE (10, 25, 26, 28, 29). Chan *et al.* concluded a summed value of > 600 ng/g for four FAEEs was indicative of PAE using a cohort of six "confirmed heavy alcohol consumers" compared to 207 non-drinkers (16, 28). Using maternal self-report of heavy drinking (3.5 units daily and/or thrice weekly binge drinking) as the gold standard, others

have reported sensitivity of FAEEs in meconium ≥600 ng/g at 100% with specificity varying from 13 - 98.4% (28, 30). Himes *et al.* determined that EtG >30 ng/g had the highest sensitivity (81.8%) and specificity (75%) of any other biomarker or combination of biomarkers, using a TLFB method of self-report as the gold standard (n=107) (10).
The strengths of this large population-based study are the high rates of recruitment, the consistency of confidential, early postnatal interviewing and the prompt freezing of samples designed to minimise *in vitro* production of FAEEs(31). In the current study the prevalence of FAEEs >600 ng/g and EtG >30 ng/g was very similar to a pilot study of a comparable unselected Scottish maternity population, suggesting reproducibility of laboratory analyses (11).

A weakness is the lack of inclusion of known heavy alcohol consuming mothers despite attempts to recruit high consumers of alcohol. It is unclear whether this was due to lack of confidence in identifying mothers or staff reluctance to refer to the research team.

Conclusions

Higher rates of alcohol consumption are reported when newly delivered mothers are asked detailed, confidential questions in a systematic way. FAEEs and EtG measured in meconium have low sensitivity and specificity for self-reported alcohol consumption after 20 weeks' gestation in an unselected Scottish population. Measurement of these alcohol biomarkers in meconium in isolation cannot currently be recommended for the identification of newborns at risk of FASD.

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	Entire recruited population (N=840)	Ever drank alcohol in pregnancy (N=384)	Drank alcohol after 20 weeks' gestation (N=114)	Did not drink alcohol after 20 weeks' gestation (N=713)
Maternal age (years) mean (SD)	N=840	384	114	713
	29.8 (5.7)	29.5 (5.8)	31.3 (5.7)	29.5 (5.6)
% aged > 35 years	19.5	20.6	31.6*	17.4*
Maternal BMI (kg/m^2) median (IQR)	N=822	380	112	697
	25.8 (22.9 – 30.3)	27.0 (16.0-53.0)	25.4 (23.0 – 29.8)	25.8 (22.8 – 30.3)
SMID decile median (IQR)	N=831	379	110	709
	3 (1 – 6)	4 (1-7)	5 (2- 8)	4 (1 – 6)
Ethnicity (% White British)	N=803	370	107	686
	80.3%	90.5%	91.6%*	79.7%*
Smoked during pregnancy	N=828	383	113	712
	17.8%	20.1	13.3	18.5
Previous child (%)	N=822	375	111	699
	56.3%	54.1%	54.1%	56.8%
Infant sex (% male)	N=840	384	114	713
	50.1%	50.8%	48.2%	50.6%
Gestation (weeks) mean (SD)	N=837	382	113	711
	38.9 (1.7)	38.9 (1.5)	39 (1.3)	38.9 (1.7)
Birthweight (g) mean (SD)	N=838	383	113	712
	3367 (526)	3369 (511)	3470 (474)*	3352 (532)*
OFC (cm) Mean (SD)	N=825	375	111	702
	34.6 (1.5)	34.7 (1.5)	34.7 (1.5)	34.6 (1.5)

Table 1 Maternal and infant demographics in relation to maternal consumption of alcohol in mid-late pregnancy * (P=<0.05)

	Meconium sample resulted (N=712)	FAEE ≥600 ng/g (N=282)	FAEE <600 ng/g (N=430)
Maternal age (years) mean (SD)	N=712	282	430
	29.8 (5.6)	30.1 (5.3)	29.7 (5.8)
Maternal BMI (kg/m^2) median (IQR)	N=696	274	422
	25.7 (22.8 – 30.1)	25.6 (23 – 30.6)	26.9 (22.5 – 30)
SIMD decile median (IQR)	N=704	280	424
	3 (1 – 6)	3 (1 – 7)	3 (1 – 6)
Ethnicity N= (% White British)	N=682	276	406
	547 (80.2%)	218 (79%)	329 (81%)
Smoked during pregnancy N= (%)	N=703	279	424
	126 (17.9%)	41 (14.7%)	85 (20%)
Previous child N= (%)	N=699	280	419
	390 (55.8%)	118 (57.9%)	191 (54.4%)
Infant sex N= (% male)	N=712	282	430
	365 (51.3%)	145 (51.4%)	220 (51.2%)
Gestation (weeks) mean (SD)	N=709	282	427
	38.9 (1.6)	39 (1.5)	38.8 (1.7)
Birthweight (g) mean (SD)	N=710	282	428
	3373 (526)	3425 (523)	3331 (525)
OFC (cm) Mean (SD)	N=700	276	424
	34.6 (1.4)	34.7 (1.4)	34.6 (1.5)

Table 2. Maternal and infant demographics in relation to meconium positive (≥600 ng/g) or negative for FAEEs

	Meconium sample resulted (N=712)	EtG ≥30 ng/g (N=103)	EtG <30 ng/g (N=609)
Maternal age (years) mean (SD)	N=712	103	609
	29.8 (5.6)	29.4 (6.5)	29.9 (5.6)
Maternal BMI (kg/m^2) median (IQR)	N=696	100	596
	25.7 (22.8 – 30.1)	26.9 (22.8 – 30)	27.1 (22.7 – 30.2)
SIMD decile median (IQR)	N=704	101	603
	3 (1 – 6)	2 (1 – 6)	3 (1 – 6.5)
Ethnicity (% White British)	N=682	101	581
	547 (80.2%)	72 (71.3%)	475 (81.8%)
Smoked during pregnancy	N=703	103	600
	126 (17.9%)	14 (13.6%)	112 (18.5%)
Previous child (%)	N=690	101	598
	390 (55.8%)	53 (52.5%)	337 (56.4%)
Infant sex (% male)	N=712	103	609
	365 (51.3%)	47 (45.6%)	318 (52.2%)
Gestation (weeks) mean (SD)	N=709	103	606
	38.9 (1.6)	38.8 (1.8)	38.9 (1.6)
Birthweight (g) mean (SD)	N=710	103	607
	3373 (526)	3418 (524)	3365 (526)
OFC (cm) Mean (SD)	N=700	101	599
	34.6 (1.4)	34.8 (1.5)	34.6 (1.4)

Table 3 Maternal and infant demographics in relation to meconium sample positive (≥ 30 ng/g) or negative for EtG

	Self-reported alcohol consumption in pregnancy at any time (n=328)				Self-reported alcohol consumption in pregnancy after 20 weeks' gestation (n=95)			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
FAEEs ≥600 ng/g	41.2	61.2	48.2	54.3	43.1	60.6	14.6	87.2
EtG ≥30 ng/g	13.1	84	41.7	52.4	11.6	84.8	10.7	86
FAEEs >600 ng/g and EtG >30 ng/g	6.7	92.2	43.1	53	5.3	92.4	9.8	86.2

Table 4. Sensitivity, specificity, positive predictive value, and negative predictive value (percentages) of infant biomarkers for FAEEs and EtG as ascertained by confidential postpartum maternal interview.