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Assessing maternal alcohol consumption in pregnancy: Does phosphatidylethanol measured from day five newborn blood spot cards have any value? An observational, population-based study.

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Abstract

Objective: Prenatal alcohol exposure (PAE) places children at risk of fetal alcohol spectrum disorder (FASD) but ascertainment of PAE is problematic. Early intervention for children at risk of FASD may help mitigate long term difficulties. Phosphatidyl ethanol (PEth), a metabolite of alcohol, is incorporated into red cell membranes and can be measured in dried blood spot cards (DBS). In the UK DBS samples are collected on day five for routine newborn screening. We sought to examine if PEth measured from DBS correlates with postnatal maternal self-report of alcohol consumption in pregnancy.

Design: Observational population-based study. Comparison of infant PEth concentration and self-report of maternal alcohol use during pregnancy.

Setting: Large maternity unit in Glasgow, Scotland.

Participants: All singleton mother-infant dyads delivered during each fourth consecutive 24-hour period.

Interventions: Mother: direct, confidential immediate postnatal interview by a single researcher examining alcohol use during pregnancy. Infant: one extra DBS collected coincident with routine newborn screening if bleeding continued.

Results: 92.5% of eligible mothers agreed to participate. 510 DBS were obtained of which 502 were successfully analysed. 216 (43%) samples contained PEth at a concentration of ≥ 8 ng/ml and 148 (29.5%) at ≥ 20 ng/ml. The sensitivity of PEth ≥ 8 ng/ml and ≥ 20 ng/ml in

identifying women who self-reported modest alcohol use after 36 weeks' gestation was 50% and 36.4% respectively.

Conclusion: PEth measured from DBS obtained on day five of life does not reliably identify modest PAE after 36 weeks' gestation from maternal self-report.

Key Messages

What is already known on this topic

- Knowledge of prenatal alcohol exposure (PAE) is critical to the diagnosis of fetal alcohol spectrum disorder but can be difficult to ascertain.
- Phosphatidylethanol (PEth), a metabolite of ethanol, can be measured from newborn dried bloodspot cards but the utility of this in identifying PAE is not known.

What this study adds

- PEth assayed from newborn blood spot cards obtained coincident with routine newborn screening at 96 - 120 hours of age has low sensitivity and specificity for self-reported modest alcohol consumption after 36 weeks' gestation.

How this study might affect research, practice or policy

- PEth measured retrospectively from stored UK newborn blood spot cards cannot be recommended as a reliable indicator of PAE in later pregnancy.

Contributorship statement:

EMAH participated in study design, recruited all participants, 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 statistical analysis and critically 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 subsequent manuscript revisions. All authors approved the final manuscript.

Introduction

Despite clear guidance(1), women continue to drink alcohol in pregnancy(2). Prenatal alcohol exposure (PAE) places children at risk of fetal alcohol spectrum disorder (FASD) with implications for behaviour and learning(2, 3). Children with FASD present a specific neurodevelopmental profile but despite diagnostic guidelines are commonly misdiagnosed(4). Diagnosis of FASD and stability in early childhood, including fewer placements, is associated with better outcomes(5). The increased likelihood of a sibling having FASD should prompt family screening and intervention when a positive diagnosis is made(6).

A history of alcohol consumption in pregnancy is key to the diagnosis of FASD but is often difficult to ascertain. Mothers commonly under-report drinking alcohol in pregnancy(7) and children with FASD are over-represented in the accommodated population(8) with limited opportunity to explore PAE.

Alcohol biomarkers present an objective tool for assessment of PAE. Fatty acid ethyl esters (FAEE) and ethylglucuronide (EtG) detectable in meconium are the best described infant alcohol biomarkers (9) and have been related to neurodevelopmental outcomes (10, 11). Phosphatidylethanol (PEth) formed from ethanol by phospholipase incorporates into cell

membranes with a half-life of 3.5-9.8 days and is detectable for up to three weeks in adults. PEth concentration is related to both amount and timing of alcohol exposure, with half-life shorter in chronic alcohol consumers (12, 13). PEth was detectable in 16.9% of a random selection of newborn dried blood spot cards (DBS); concentration was ≥ 20 ng/ml in 6.5 % (14). In 28 newborns of moderate chronic or binge-drinking mothers, PEth ≥ 8 ng/ml was 100% specific and 32.1% sensitive for PAE, more sensitive than any maternal alcohol biomarker(15). Baldwin *et al.* reported PEth ≥ 8 ng/ml in 4% of 250 anonymised stored DBS from a single midwestern state and another US study reported PEth ≥ 20 ng/ml in 8.4% of randomly selected newborns. Concentrations of PEth were higher in Hispanic and African American infants and in urban areas (16). In a rural, deprived US population with high prevalence of substance misuse, PEth was ≥ 8 ng/ml in 8.1% of newborn samples overall but with considerable variation (2.3 – 17.1%) between different regions (17). Factors predicting a positive sample included smoking, preterm birth, birth weight < 2000 g and absence of breast feeding. In a large, prospective study, 45.8% of mothers in Uruguay and 43.9% of mothers in Brazil had detectable PEth in a DBS although only 6% and 9.1% respectively reported third trimester alcohol consumption. PEth was almost twice as likely to be detected in the newborn compared to maternal DBS; the authors had no explanation for this (18).

In the UK, newborn screening DBS are routinely stored. If PEth measured from stored DBS was a valid method of ascertaining PAE, this could have potential for retrospective ascertainment of PAE in children presenting with signs consistent with FASD. To date no study has reported PEth concentrations in DBS obtained within 96 to 120 hours of birth in line with the UK national newborn screening programme.

We report PEth in DBS obtained in an unselected Scottish newborn population and related to confidential immediate postnatal maternal self-report of alcohol consumption in pregnancy.

Methods

This study was part of a larger observational population-based study of alcohol consumption in pregnancy including analysis of meconium. The eligible population comprised all mothers delivering a live singleton infant within each fourth consecutive 24-hour period. Site was a large obstetric-led maternity unit covering part of the relatively deprived population of the city of Glasgow and its suburbs. Women likely to deliver on a study day were provided with a plastic bag to collect the infant's first meconium soiled nappy; written informed consent sought as soon as possible after delivery. If the mother declined, any samples were discarded. Regardless of whether meconium collection was successful, if the mother was still in agreement, the community midwife tried to obtain an additional DBS following routine newborn blood spot screening (96 to 120 hours of age). No further heel stick was performed if the infant did not continue to bleed. The DBS was dried in room air, kept from direct sunlight and received at the study centre within 72 hours where it was immediately vacuum packed prior to freezing at -40°C .

Assessment of alcohol consumption in pregnancy

PAE was assessed by confidential early postnatal questionnaire administered by a single researcher soon after obtaining written informed consent. This included timing of confirmation of pregnancy and smoking and alcohol consumption during pregnancy.

Mothers who reported any alcohol in pregnancy were asked more details with a modified time-line method(19). Both type and quantity of alcohol were documented by the researcher

on a calendar individualised with date of delivery and gestation. Recollection was encouraged by use of telephones, diaries and/or social media. Alcohol consumption was calculated based on manufacturers' information and Drinkaware®(20) and recorded in UK units (8 g ethanol) for time periods: <12 weeks, 12-20 weeks, >20 weeks and > 36 weeks of pregnancy.

Demographic information from casenotes included maternal age, parity, ethnicity, body mass index (BMI), gestation, birth weight and occipitofrontal head circumference (OFC). The Scottish Index of Multiple Deprivation 2016 (SIMD16) was used as a measure of socioeconomic deprivation, calculated from postcode of residence and expressed in deciles(21).

Laboratory analysis

DBS were shipped on dry ice (-40°C) to the University of Padua. From each DBS a 30 µl sample was removed by a manual punch and extracted in 2 ml methanol before solvent evaporation in a nitrogen stream. The dried extract was dissolved with isopropanol (100µl) and analysed by liquid chromatography mass spectrometry. Calibration curves were prepared on DBS by spotting 30 µl of blank blood, obtained from an alcohol abstinent subject, fortified at a concentration of 20, 40,100, 400, 800 and 1600 ng/ml of each PEth homologue. The laboratory undergoes bi-annual proficiency testing for the biomarkers.

Statistics

Chi-squared tests were used to investigate associations between categorical variables and t-tests or paired t-tests for numerical variables. PEth ≥ 8 and ≥ 20 ng/ml were investigated as predictors of alcohol consumption by computing sensitivity, specificity, and positive and negative predictive values. 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).

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Results

1021 mothers delivered singleton infants during 71 study days. Seven mothers were ineligible; two infants born at home, one infant transferred for specialist neonatal care, one early neonatal death, one infant placed in care, one mother without capacity to consent, one mother too unwell to consent. 908 (89.5%) of eligible mothers were approached, of whom 840 (92.5%) provided written, informed consent. 668 DBS were returned to the study centre; 510 contained blood and were sent for PEth assay. 502 samples proved suitable for analysis (Figure 1). 827 (98.5%) recruited mothers completed the questionnaire.

46.4% of all mothers reported drinking alcohol at some point in pregnancy; 114 (13.8% of the recruited population) stated they had consumed alcohol after 20 weeks' gestation and 40 of these 114 mothers reported alcohol after 36 weeks' gestation (Supplemental Table). Only three mothers reported one or more episode of binge drinking (\geq five UK alcohol units).

65.6% of mothers who declared some alcohol consumption in pregnancy stated that this was prior to knowledge of being pregnant. Consuming alcohol in pregnancy was more commonly reported by women of white British ethnicity and those who had not previously had a baby (Table 1 & supplemental); women who reported alcohol after 20 weeks' gestation were more likely to be aged >35 years ($p<0.05$). Infants of this latter group of mothers had a higher mean birth weight ($p<0.05$), with a difference of approximately 100 g compared to no reported alcohol consumption. Difference in birth weight was not explained by maternal BMI.

PEth was detectable in 262 (52%) samples; concentrations ranged from 2.4 to 3991.6 ng/ml. Results were highly skewed; mean PEth concentration was 100.5 (SD 416.3) ng/ml and the median concentration 5 ng/ml.

Comparison with maternal and infant demographics was made utilising two cut off values for PEth, 8 ng/ml and 20 ng/ml (Table 2).

PEth cut off 8 ng/ml

216 (43%) DBSs contained ≥ 8 ng/ml PEth. For these “positive” results, median PEth concentration was 29.5 (IQR 16.3, 57.5) ng/ml (mean concentration 120.7 ng/ml). There was no relationship between maternal age, parity, BMI or SIMD16 decile and likelihood of ≥ 8 ng/ml PEth. When PEth was ≥ 8 ng/ml the mother was more likely to self-identify as white British (86.6 vs 79.1%, $p=0.028$) and to have smoked during pregnancy ($p=0.047$); infants tended to be heavier at birth ($p=0.052$). Reported alcohol consumption after 20 weeks’ gestation was not more common than when PEth concentration was < 8 ng/ml (14.8% versus 12.2%, $p=0.396$).

PEth cut off 20 ng/ml

PEth concentration was ≥ 20 ng/ml in 29.5% DBS. These mothers were younger ($p=0.023$), had a higher BMI ($p=0.038$) and were more likely to be socioeconomically deprived ($p<0.05$) and to have smoked in pregnancy ($p=0.022$). 12.8% mothers reported alcohol consumption after 20 weeks’ gestation, very similar to 13.6% mothers who declared alcohol consumption in later pregnancy when infant PEth was < 20 ng/ml. PEth ≥ 20 ng/ml did not predict birth weight or OFC.

Eight mothers reported ≥ 5 weekly units of alcohol after 36 weeks' gestation. DBS were analysed for five infants; PEth concentrations were 0, 0, 21.4, 21.7 and 22.9 ng/ml.

There was no correlation between PEth in DBS and either FAEEs (Pearson co-efficient-0.019, $p= 0.695$) or EtG measured in meconium (Pearson co-efficient -0.009, $P= 0.843$). Meconium analyses for FAEEs and EtG will be reported in a separate manuscript.

Sensitivity and specificity

Both ≥ 8 ng/ml and ≥ 20 ng/ml PEth in DBS had low sensitivity and specificity for self-reported alcohol consumption in later pregnancy, after either 20 or 36 weeks' gestation. The positive predictive values of PEth ≥ 8 ng/ml and ≥ 20 ng/ml for self-reported alcohol consumption after 36 weeks' gestation were 5.1% and 5.4% respectively (Table 3).

Discussion:

Since the half-life of PEth is less than 10 days, PEth concentrations in the newborn can be predicted at best to reflect alcohol consumption in the last month of pregnancy. In this unselected early postnatal population 13.8% of mothers self-reported alcohol consumption in the second half of pregnancy, but barely one third stated that this was after 36 weeks' gestation. Corresponding infant day five PEth ($n=22$) had a sensitivity of 50% (≥ 8 ng/ml) and 36.4% (≥ 20 ng/ml) to identify these mothers (Table 3).

It is important to underscore that when women self-report that they have drunk alcohol during pregnancy, they are very unlikely to have misrepresented *any* alcohol use although the timing and amount of alcohol intake reported may differ from reality. In the absence of a gold

standard for identifying prenatal alcohol consumption, declarations made by newly delivered mothers in a secure, confidential environment and obtained in a consistent way by an independent researcher were likely to be reasonably accurate particularly when a participant reported *any* alcohol use compared with *no* alcohol use.

Recruitment rate for this study (82.8% of the entire eligible population) was high despite requirement for written consent. 510 DBS represented 56.2% of eligible participants; almost all samples proved suitable for analysis. Collection of the DBS by the midwife coincident with routine newborn screening did not require further written consent by the mother, reducing the likelihood of selection bias. The study population was representative of the relatively deprived population of the west of Scotland; consistent with the high prevalence of alcohol consumption in this population, 80.5% of mothers declared pre-pregnancy alcohol consumption. A weakness of this study is the lack of recruitment of known heavy alcohol consuming mothers.

Understanding the pattern of alcohol consumption in pregnancy is important, both to identify children at risk of FASD, and to examine the effectiveness of public health messaging. Previous studies of newborn PEth suggest that this may be a useful tool in retrospective assessment of PAE, but the majority did not relate infant PEth to maternal alcohol history. Newborn PEth concentration of ≥ 8 ng/ml was 32% sensitive for PAE in a small study of known heavy alcohol-consuming mothers, more sensitive than any maternal alcohol biomarker and 100% specific(15). Within our study, infant PEth ≥ 8 ng/ml was more sensitive for modest PAE after 36 weeks' gestation as determined by early postnatal maternal self-report (50%) but specificity was only 56.9%. Mothers less frequently reported alcohol

consumption in the last month of pregnancy, making it unlikely that newborn infant PEth is a reliable biomarker of alcohol consumption earlier in the pregnancy.

30.3% of mothers in this study stated they had drunk while pregnant, but prior to knowledge of pregnancy. This is fewer than the 44% of pregnant mothers (mostly in later gestation) who declared alcohol consumption prior to knowledge of pregnancy in a contemporaneous, anonymised Scottish antenatal survey (22); the reasons for this are not clear. Unfortunately, alcohol consumption as declared at maternity booking was too poorly documented in the current study to be compared with postnatal self-report. 46.4% of mothers in the early postnatal period declaring some alcohol consumption in pregnancy is more than double the 20% stating this at 10 months postnatal in another similar Scottish population(23), consistent with recall of alcohol consumption diminishing after delivery(10). One third of mothers reporting alcohol in early pregnancy raises concerns about the effectiveness of pre-pregnancy public health messaging, particularly as approximately one half of pregnancies are unplanned(24).

To date studies of infant PEth have all used DBS obtained within the first 48 hours of life; this is the first study to report measurement of PEth on day five of life. We had predicted that infant PEth on day five would be lower than concentrations measured within the first 48 hours, but we found more positive results than most previous reports. *In vitro* production of PEth in whole blood samples is recognised, but this does not occur in filter paper cards stored at room temperature for 48 hours(23). In the current study relatively prompt storage of DBS at -40C should have protected PEth from late degradation(25, 26). Rates of alcohol consumption in different populations are influenced by multiple factors, but even in a targeted maternity population with suspected drug misuse, commonly associated with

significant alcohol consumption, only 26% of infant DBS (cord blood) had PEth \geq 8ng/ml compared to 43% in our study(27, 28). Higher than expected PEth might reflect polycythaemia secondary to deferred cord clamping(29) and relative polycythaemia may also explain the finding of higher PEth levels in newborns compared to their mothers(18). Early infant haemolysis could account, at least in part, for PEth increasing over the first few days; confirming this would require serial PEth measurements compared to haemocrit. Contamination in sampling from either alcohol hand gel or wipes is an unlikely explanation for elevated PEth concentrations as PEth is a metabolite of alcohol, dependent upon *in vivo* metabolism.

Conclusions: PEth assayed from newborn DBS cards obtained coincident with routine newborn screening at 96-120 hours of age has low sensitivity and specificity for self-reported modest alcohol consumption after 36 weeks' gestation. PEth measured retrospectively from stored UK newborn blood spot cards will not be a reliable indicator of PAE.

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	Dried blood spot card result (N=502)	Ever drank alcohol in pregnancy* (N=227)	Never drank alcohol in pregnancy* (N= 273)	Drank alcohol after 20 weeks' gestation* (N= 67)	Did not drink alcohol after 20 weeks' gestation* (N= 431)
Maternal age (years) mean (SD)	N=502 29.6 (5.7)	227 29.5 (5.9)	271 29.8 (5.6)	67 31.7 (5.7)	431 29.3 (5.7)
Maternal BMI (kg/m ²) median (IQR)	N=489 27.3 (22.9 – 30.1)	225 27.0 (6.3)	260 27.5 (23 – 30.2)	65 27.2 (23 – 30.4)	420 27.3 (22.9 – 30.1)
SMID decile median (IQR)	N=497 3 (1 – 6)	223 3 (1 – 6)	270 3 (1 – 7)	64 5 (2 – 8)	429 3 (1 – 6)
Ethnicity (% White British)	N=482 82.4%	221 90.5	257 75.5	62 90.3	416 81.3
Parous mothers (%)	N=491 59.5%	222 50.5	266 66.9	66 62.1	422 59
Smoked during pregnancy	N=497 18.3%	226 21.7	270 15.6	66 9.1	430 19.8
Mode of delivery (% vaginal)	N=502 60%	227 61.8	271 59	67 53.7	431 61.3
Infant sex (% male)	N=502 48.2%	227 48.5	271 48.3	67 47.8	431 48.5
Gestation (weeks) mean (SD)	N=500 38.9 (1.6)	225 38.9 (1.5)	271 38.9 (1.7)	66 39 (1.3)	430 38.9 (1.7)
Birthweight (g) mean (SD)	N=501 3389.1 (540)	226 3401.7 (529)	271 3386.4 (542)	66 3475.5 (504)	431 3378.9 (540)
OFC (cm) Mean (SD)	N=492 34.7 (1.6)	220 34.7 (1.6)	268 34.6 (1.6)	65 34.8 (1.7)	423 34.7 (1.6)
APGAR score mean (SD)	N=492 9 (1.3)	224 9 (1.3)	265 9 (1.3)	65 9 (1.5)	424 9 (1.2)

Table 1. Maternal and infant demographics in relation to postnatal self-report of alcohol consumption in pregnancy

*Postnatal questionnaire including self-report of alcohol in pregnancy available for 498 mothers

	Dried blood spot card result (N=502)	PEth \geq 8ng/ml (N=216)	PEth <8ng/ml (N=286)	PEth \geq 20ng/ml (N=148)	PEth <20ng/ml (N=354)
Maternal age (years) mean (SD)	N=502 29.6 (5.7)	216 29.3 (6)	286 30.0 (5.6)	148 28.7 (5.9)	354 30.0 (5.6)
Maternal BMI (kg/m ²) median (IQR)	N=489 27.3 (22.9 – 30.1)	211 26.4 (23 – 31)	278 25.7 (22.7 – 29.9)	144 27 (23.2 – 32)	345 25.5 (22.7 – 29.9)
SMID decile median (IQR)	N=497 3 (1 – 6)	214 3 (1 – 6)	283 3 (1 – 7)	147 2 (1 – 5)	350 3 (1 – 7)
Ethnicity (% White British)	N=482 82.4%	209 86.6%	273 79.1%	141 85.1%	341 77.4%
Previous pregnancy (%)	N=491 59.5%	212 60.4%	279 58.7%	145 60%	346 59.2%
Smoked during pregnancy	N=497 18.3%	215 22.3	282 15.2	147 24.5	350 15.7
Mode of delivery (% vaginal)	N=502 60%	216 61.5%	286 58.7%	148 64.1%	354 58.2%
Infant sex (% male)	N=502 48.2%	216 46.8%	286 49.3%	148 47.3%	354 48.6%
Gestation (weeks) mean (SD)	N=500 38.9 (1.6)	216 39 (1.4)	284 38 (1.8)	148 39.1 (1.3)	352 38.8 (1.8)
Birthweight (g) mean (SD)	N=501 3389.1 (540)	216 3428.2 (500)	285 3359.4 (567)	148 3457 (491)	353 3360 (557)
OFC (cm) Mean (SD)	N=492 34.7 (1.6)	213 34.8 (1.5)	279 34.5 (1.7)	146 34.9 (1.4)	346 34.6 (1.7)
APGAR score mean (SD)	N=492 8.6 (1.3)	215 8.6 (1.3)	277 8.6 (1.3)	147 8.6 (1.5)	345 8.6 (1.2)

Table 2 Maternal and infant demographics in relation to PEth concentration in DBS

	Self-reported alcohol consumption in pregnancy after 20 weeks' gestation (n=67)				Self-reported alcohol consumption after 36 weeks' gestation (n=22)			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PEth ≥ 8 ng/ml	47.8	57.4	14.8	87.6	50	56.9	5.1	96.1
PEth ≥ 20 ng/ml	28.4	70.1	12.8	86.3	36.4	70.6	5.4	96

Table 3 Sensitivity, specificity, positive predictive value, and negative predictive value of infant biomarkers for PAE as ascertained by confidential postpartum maternal interview.

