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Ethylglucuronide in maternal hair as a biomarker of prenatal alcohol exposure

Hilda L. Gutierrez [a], Lauren Hund [b], Shikhar Shrestha [a], William F. Rayburn [c], Lawrence Leeman [b,c], Daniel D. Savage [d], Ludmila N. Bakhireva [a,b,*]

[a] Department of Pharmacy Practice and Administrative Sciences, University of New Mexico, Albuquerque, NM, USA
[b] Department of Family and Community Medicine, University of New Mexico, Albuquerque, NM, USA
[c] Department of Obstetrics and Gynecology, University of New Mexico, Albuquerque, NM, USA
[d] Department of Neurosciences, University of New Mexico, Albuquerque, NM, USA

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ABSTRACT

While direct ethanol metabolites, including ethylglucuronide (EtG), play an important role for the confirmation of prenatal alcohol exposure (PAE), their utility is often limited by their short half-lives in blood and urine. Maternal hair allows for a retrospective measure of PAE for up to several months. This study examined the validity of hair EtG (hEtG) relative to self-reporting and five other biomarkers in 85 pregnant women. Patients were recruited from a UNM prenatal clinic, which provides care to women with substance abuse and addiction disorders. The composite index, which was based on self-reported measures of alcohol use and allowed us to classify subjects into PAE ($n = 42$) and control ($n = 43$) groups, was the criterion measure used to estimate the sensitivity and specificity of hEtG. Proximal segments of hair were collected at enrollment (average 22.0 gestational weeks) and analyzed by LC-MS/MS. At the same visit, maternal blood and urine specimens were collected for analysis of GGT, %dCDT, PEth, uEtG, and uEtS. The study population included mostly opioid-dependent (80%) patients, a large proportion of ethnic minorities (75.3% Hispanic/Latina, 8.2% American Indian, 4.7% African-American), and patients with low education (48.2% < high school). The mean maternal age at enrollment was 26.7 ± 4.8 years. Hair EtG demonstrated 19% sensitivity and 86% specificity. The sensitivities of other biomarkers were comparable (5–20%) to hEtG but specificities were higher (98–100%). Hair EtG sensitivity improved when combined with other biomarkers, especially with GGT (32.5%) and PEth (27.5%). In addition, validity of hEtG improved in patients with less frequent shampooing and those who did not use hair dyes/chemical treatments. These data suggest that hEtG alone is not a sufficiently sensitive or specific biomarker to be used separately for the identification of PAE, but might be useful in a battery along with other maternal biomarkers.

Introduction

Alcohol has been recognized as a human teratogen since the late 1960s to early 1970s. The term Fetal Alcohol Syndrome (FAS) was introduced (Jones, Smith, Streissguth, & Myrianthopoulos, 1974; Jones, Smith, Ulleland, & Streissguth, 1973; Lemoine, Harousseau, Borteyru, & Menuet, 2003) when a specific pattern of structural and neurobehavioral abnormalities was identified. Despite extensive research in this field during the past 40 years, concerted efforts continue in the primary and secondary prevention of Fetal Alcohol Spectrum Disorder (FASD). Recent findings from an active case ascertainment study demonstrated that as many as 2.4–4.8% of a representative middle-class community of young schoolchildren in the Midwestern United States were affected by FASD (May et al., 2014). Early and accurate recognition of alcohol consumption during pregnancy followed by targeted harm reduction strategies, such as brief intervention which can be administered by health professionals, are recognized tools to reduce the number of children affected by prenatal alcohol exposure (PAE) (Jones, Bailey, & Sokol, 2013). However, social stigma associated with alcohol use in pregnancy often leads to substantial maternal under-reporting.

The utility of supplementing a potentially unreliable maternal self-report with ethanol biomarkers to more comprehensively

* Corresponding author. University of New Mexico College of Pharmacy, 1 University of New Mexico, MSC 09 5360, Albuquerque, NM 87131, USA. Tel.: +1 505 272 2545; fax: +1 505 272 6749.
E-mail address: lbakhireva@salud.unm.edu (L.N. Bakhireva).

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capture PAE has long been recognized. Hair ethylglucuronide (hEtG) is a promising biomarker due to its high sensitivity and specificity for the identification of heavy chronic alcohol use. A meta-analysis conducted by Boscolo-Berto et al. (2014) found an overall sensitivity of 96% and specificity of 99% for hEtG in chronic alcohol users from eight studies. Furthermore, hEtG has a broader detection window (up to several months) compared to biomarkers measured in more traditional biological matrices such as blood and urine (Bakhireva & Savage, 2011). That is, urine or blood screening for alcohol biomarkers at the first prenatal visit will be negative in many pregnant women who discontinue drinking upon pregnancy recognition, while hair analysis can identify risky drinking in the periconceptional period – the most crucial period of organogenesis. In addition, hEtG, as a direct ethanol metabolite, has a higher specificity than traditional alcohol biomarkers, such as gamma glutamyltranspeptidase (GGT) and disialo carbohydrate-deficient transferrin (%dCDT), which are known to be affected by a number of maternal conditions and physiological changes in pregnancy. Specifically, advanced gestational age, iron deficiency, hypertension, and liver conditions have been reported to affect %dCDT in addition to excessive alcohol use (Bakhireva et al., 2012; Bianchi, Ivaldi, Raspagni, Arfini, & Vidali, 2011; De Feo et al., 1999; Kenan, Larsson, Axelsson, & Helander, 2011). One biological matrix with a comparable detection window to hair is meconium – a newborn's first stool. While ethanol biomarkers measured in meconium can capture PAE from approximately 20 gestational weeks onward, a number of challenges have been described with meconium analyses, including logistical difficulties with collection and storage, variations on laboratory analysis methods (Lange, Shield, Koren, Rehm, & Popova, 2014), and the possibility of increased false positive rates (Zelner, Hutson, Kapur, Feig, & Koren, 2012). While analysis of alcohol biomarkers in neonatal hair might present some cultural and esthetic challenges, obtaining a sample of maternal hair appears more feasible and acceptable.

Prior studies have examined the validity of hEtG in pregnant (Morini et al., 2013; Morini, Marchei, et al., 2010; Wurst et al., 2008) and non-pregnant women (Kronstrand, Brinkhagen, & Nyström, 2012; Politi, Morini, Leone, & Polettini, 2006) with mixed results. In two studies by Morini et al. (Morini, Marchei, et al., 2010; Morini et al., 2013), maternal alcohol consumption information was obtained from medical records rather than an in-depth face-to-face interview, which may be limiting in validating hEtG as a reliable biomarker. Differences in alcohol consumption patterns, the methods used to ascertain alcohol exposure, and timing of assessment might contribute to heterogeneity of findings. To our knowledge, none of the previous studies has compared the validity of hEtG to a battery of direct and indirect alcohol biomarkers. In this report, we examine the sensitivity and specificity of hEtG among 85 pregnant women and compare it with the performance of maternal biomarkers measured in more traditional media – maternal urine and blood.

Materials and methods

Study design and participant recruitment

Study participants were enrolled in the Biomarkers in Pregnancy Study (BIPS) (P.I.: Bakhireva) approved by the University of New Mexico (UNM) Human Research Review Committee. This study utilized a prospective cohort design with a baseline visit during one of the patient's first prenatal care visits and a follow-up visit during the hospital stay after delivery. The detailed study methodology has been described elsewhere (Bakhireva et al., 2012). Patients were recruited from a UNM prenatal clinic providing medical care to women with substance abuse and addiction disorders and had to meet the following initial eligibility criteria: (1) a singleton pregnancy; (2) plan to deliver at UNM Hospital; (3) be less than 32 weeks gestation; and (4) be able to give written consent. After enrollment, a semi-structured maternal interview was conducted which captured detailed information on alcohol and substance use in the periconceptional period and during pregnancy. Demographic (i.e., age, marital status, race/ethnicity, education, current employment, and health insurance status), medical, and reproductive health characteristics (i.e., any chronic conditions, complications during pregnancy, use of medications and vitamins, gravidity, parity, pregnancy dates) were also ascertained. Participants were also queried about the use of hair products (i.e., use of hair dyes and chemical treatment during the past 6 months and frequency of shampooing per week). Within 24 hours after delivery, a follow-up interview was administered to capture changes in alcohol and substance use since the enrollment interview as well as alcohol use in the 2 weeks before delivery. Information was abstracted from patients' electronic medical records regarding pregnancy and newborn outcomes, including any maternal and newborn complications and any diagnosis of major structural anomalies for the newborn.

Self-reported alcohol use and group allocation

The timeline follow-back (TLFB) interviewing technique (Sobell & Sobell, 1992) captured alcohol consumption in the periconceptional period (2 weeks before and 2 weeks after the first day of their last menstrual period [LMP]) and the 2 weeks before enrollment. Participants were asked to recall and report the specific types and number of drinks containing alcohol they consumed during those time periods. The reported quantities were converted into standard drink units (SDUs) based on the percentage of alcohol and quantity consumed. Reported quantity and frequency of alcohol use were then converted into absolute ounces of alcohol per day and per drinking day in periconceptional period (AAD0 and AADD0, respectively) and the 2-week period before enrollment (AAD1 and AADD1, respectively) as previously described (Bakhireva et al., 2012; Jacobson, Chiodo, Sokol, & Jacobson, 2002). Study participants were also administered a standard 10-question Alcohol Use Disorders Identification Test (AUDIT) questionnaire and were asked to report the maximum number of drinks consumed during 24 hours any time after LMP.

A total of 102 patients were enrolled into the study and were preliminarily classified into PAE ($n = 46$) or control ($n = 56$) groups based on the self-reported measures. Initial allocation in the control group required that participants meet all of the following criteria (1) no binge drinking episodes ($\geq$4 drinks/occasion) in the periconceptional period, (2) no more than two drinks per week in the periconceptional period (AAD0 $\leq$ 0.14), and (3) abstinence during the 2 weeks prior to enrollment per TLFB calendar (AAD1 and AADD1 = 0). The control group was further restricted to 44 participants after disqualifying patients who either admitted some alcohol use after the LMP but prior to the 2-week window immediately before enrollment ($n = 7$), had an AUDIT score $\geq$ 8 ($n = 3$), or both ($n = 2$). Allocation in the PAE group required that participants report either $\geq$3 drinks per week on average at enrollment (average AAD1 $\geq$ 0.21) or report at least one binge drinking episode at enrollment (AADD1 $\geq$ 2.0). Given the broad detectability window of hair biomarkers, patients recruited before 10 weeks of gestation (4 PAE and 1 control) were excluded from the analysis to avoid a potential confounding by alcohol use prior to the periconceptional period. Thus, the final sample size for this study was limited to 85 patients (43 controls and 42 PAE). Moreover, a subgroup analysis was conducted after further restricting the sample size to 52 patients (29 controls and