Hair Ethyl Glucuronide is Highly Sensitive and Specific for Detecting Moderate-to-Heavy Drinking in Patients with Liver Disease

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Abstract — **Aims:** Hair ethyl glucuronide (EtG) is a promising biomarker of moderate-to-heavy alcohol consumption and may have utility in detecting and monitoring alcohol use in clinical populations where alcohol use is of particular importance. This study evaluated the relationship between hair EtG and drinking in patients with liver disease. **Methods:** The subjects ($n = 200$) were patients with liver disease who presented for care at a university medical center. Alcohol use during the 3 months preceding participation in the study was assessed, and a sample of hair was obtained for EtG testing. Classification of drinking status (any drinking or averaging at least 28 g per day) by hair EtG was evaluated, as well as the effects of liver disease severity and demographic and hair care factors. **Results:** The area under the receiver operating characteristic curve for detecting an average of 28 g or more per day during the prior 90 days was 0.93. The corresponding sensitivity and specificity of hair EtG ≥ 8 pg/mg for averaging at least 28 g of ethanol per day were 92 and 87%, respectively. Cirrhosis and gender may have a modest influence on the relationship between drinking and hair EtG. **Conclusion:** Hair EtG was highly accurate in differentiating subjects with liver disease averaging at least 28 g of ethanol per day from abstainers and lighter drinkers.

INTRODUCTION

Unhealthy alcohol use can be either a primary or a secondary cause of liver disease, often coexisting with other primary liver diseases such as chronic Hepatitis B or Hepatitis C virus infection (Zakhari and Li, 2007; Szabo *et al.*, 2010). Reduction in alcohol use or abstinence from alcohol in these conditions is thus an important component of treatment. However, it is clear that a substantial minority of patients with alcohol-related liver disease under-report their drinking (Staufer *et al.*, 2011), and other errors can also occur in clinical assessments that are dependent on patient-reported drinking. Laboratory testing for alcohol use has traditionally suffered from poor sensitivity and specificity, but newer biomarkers are likely to have greater accuracy in patients with liver disease. One such marker is ethyl glucuronide (EtG), which is a direct product of ethanol metabolism by the UDP-glucuronyltransferase detoxification system in the liver (Wurst *et al.*, 2000). EtG in urine has been shown to identify recent drinking in liver disease patients with good sensitivity and specificity (Erim *et al.*, 2007; Staufer *et al.*, 2011; Stewart *et al.*, 2012). EtG also accumulates in hair through sweat and blood, and hair EtG has been validated as a biomarker of chronic heavy alcohol consumption in populations such as subjects with alcohol dependence or pregnancy, in clarifying potentially alcohol-related medicolegal issues, and for other purposes (Yegles *et al.*, 2004; Politi *et al.*, 2006; Wurst *et al.*, 2008; Bendroth *et al.*, 2008; Pragst and Yegles, 2008). These findings clearly demonstrate that hair EtG has a potentially important role in characterizing alcohol use in human studies of alcoholic liver disease or other alcohol-related diseases, and may have a role in clinical settings when objective determination of alcohol use is a critical component of medical decision-making, as has been suggested in organ transplant patients (Haller *et al.*, 2010). While hair EtG has mainly been used to detect chronic heavy drinking, it is important to note that moderate drinking

is also of interest for liver health, as lesser alcohol use (e.g. 30 g daily) can lead to or exacerbate liver disease in susceptible individuals (Zakhari and Li, 2007). We report here on the performance of hair EtG for detecting any alcohol use and drinking that averages two or more standard US drinks per day (i.e. ≥ 28 g ethanol/day) in liver disease patients.

METHODS

Subjects

This was a cross-sectional study that included the first 212 study subjects participating in an ongoing clinical epidemiologic study of alcohol use at a university medical center, 200 of whom had sufficient hair for EtG analysis. Subjects were recruited during their visits to the Hepatology clinics or during hospitalization for liver disease, and all the study data for a given subject were collected on the same day. The exclusion criteria were minimal and included encephalopathy sufficient to impair provision of informed consent or accurate recall, and prior liver transplantation. In order to obtain as accurate an estimate of specificity as possible, efforts were also made to exclude subjects who were clinically suspected by the hepatologists to be drinking but who were reporting abstinence. Subjects who consented to provide a confidential report on their alcohol use that would be accessible only to research personnel not involved in their health care were then interviewed and provided blood, urine and hair samples, and received \$40 as compensation for their time and effort. The study was approved by the Institutional Review Board of the Medical University of South Carolina (federal wide assurance number for human subjects protection 00001888).

Determination of alcohol consumption

Alcohol use was assessed by trained research staff using alcohol timeline followback methods (Sobell and Sobell,

1992). The type, brand and serving size of alcoholic beverages were obtained, and then converted into grams of ethanol on the basis of serving size, alcohol content and the density of ethanol. As a reference, one standard drink in the USA contains ~14 g of ethanol (e.g. the amount of ethanol in a 12-oz beer containing 5% ethanol, 5 oz of wine containing 12% ethanol or 1.5 oz of 80-proof liquor). Since EtG accumulates in hair over months, past 90-day drinking was the focus of this report. We also measured other biomarkers that were useful for estimating the veracity of self-report. These included the ethanol metabolite blood phosphatidylethanol (PEth) (Aradottir *et al.*, 2006; Stewart *et al.*, 2009) and urine EtG (Halter *et al.*, 2008).

Laboratory measures

Scalp hair was preferentially sampled. When hair length was greater than 3.8 cm, only the proximal 3.8 cm was utilized for EtG testing, while shorter lengths were processed in full. Regardless of length, a minimum of 100 mg of hair was required for analysis. Hair EtG was assayed by a contracted laboratory (US Drug Testing Laboratories, Des Plaines, IL, USA) using a liquid chromatography-tandem mass spectrometry assay, with a limit of detection of 2 pg EtG/mg hair and a limit of quantitation of 8 pg/mg (Morini *et al.*, 2006). The same laboratory performed liquid chromatography-tandem mass spectrometry assays for urine EtG (Dresen *et al.*, 2004) and blood PEth (Gunnarsson *et al.*, 1998).

Chart review

After confirming that signed informed consent had been provided, a physician reviewed each subject's electronic medical record to extract data on liver disease etiology and severity. The source documentation included Hepatology clinical notes, endoscopy results, laboratory values, reports of imaging studies and liver biopsy results.

Use of other biomarkers to detect under-reporting of alcohol use

Because underestimation of alcohol use occurs and this phenomenon is a substantial barrier in determining the true specificity of alcohol consumption biomarkers, we utilized a conservative strategy for detecting under-reported drinking and minimizing its influence on study results. Those who reported drinking histories during the prior days to weeks that were not consistent with urine EtG and blood PEth results were considered to have provided inaccurate drinking histories and were excluded from additional analyses. Specific rules for exclusion based on self-report and laboratory values were as follows:

- (1) Report no drinking during the prior 7 days but urine EtG >100 ng/ml (Halter *et al.*, 2008).
- (2) Report <14 g ethanol/day average over the last 28 days but PEth >125 ng/ml (Stewart *et al.*, 2010).
- (3) Report no drinking during the past 14 days and no heavy drinking days (exceeding 60 g in a single day) during the past 6 weeks but PEth >20 ng/ml [based on

elimination of PEth from blood (Varga *et al.*, 2000; Gnann *et al.*, 2012)].

Data analysis

Receiver operating characteristic analysis was conducted to estimate the area under the curve (AUC) for hair EtG in detecting any prior 90-day drinking and drinking that averaged 28 or more g/day over that time frame, a level at which hair EtG is more likely to become detectable (Kronstrand *et al.*, 2012). These analyses were repeated after excluding the 7% of subjects that provided non-scalp hair samples. The sensitivity and the specificity for positive hair EtG (i.e. at or above the 8 pg/mg limit of quantitation) in identifying these levels of drinking were estimated by contingency table analysis. We also evaluated a 30 pg/mg cutoff, which has been suggested for differentiating chronic heavy drinking from lighter drinking and abstinence (Pragst *et al.*, 2010). Finally, because 28 g or more/day does not represent chronic heavy drinking (often considered an average of at least 60 g of ethanol/day) and a lower cutoff may be optimal for more moderate drinking, we also estimated the sensitivity of hair EtG for drinking averaging 28 or more grams per day at cutoffs that were 90 and 95% specific for that level of drinking in the study sample. Logistic regression methods were utilized to estimate interactions of drinking with demographic factors (ethnicity, gender and age), hair care factors (hair coloring in the past 90 days and frequency of washing) and liver disease severity. Severity was estimated by the presence of cirrhosis vs. non-cirrhotic disease, with cirrhosis limited to subjects with biopsy-proven cirrhosis, current ascites from chronic liver disease or known esophageal varices.

RESULTS

Subject characteristics

The first 212 subjects participating in the ongoing study were enrolled in order to obtain 200 hair samples (12 subjects had insufficient hair for sampling). This included 107 men and 93 women, with a mean age of 51.4 years (standard deviation 11.2), of whom there were 158 non-Hispanic whites, 41 non-Hispanic blacks and 1 Hispanic white. Fifty-five percent of the subjects were classified as cirrhotic.

Subject selection and preliminary qualitative results

Seven out of 73 subjects with positive hair EtG results and 2 out of 127 subjects with negative hair EtG were excluded from analysis on the basis of the exclusion rules presented in the methods section. After this additional selection, there were two outlying subjects with positive hair EtG who reported a total of 0 and 2 standard drinks during the prior 90 days. Both reported heavy drinking within 6 months of recruitment, and these subjects were included in all analyses. Thus, 191 subjects were evaluated [53.4% men, 79.1% non-Hispanic whites, average age 51.2 (SD 11.3), 53.9% with cirrhosis, 57.1% reporting some drinking during the prior 3 months, 34.6% with detectable hair EtG and 27.2%

Table 1. Sample characteristics stratified by self-reported drinking status

	Past-90-day abstinence (<i>n</i> = 82)	Drinking averaging under 28 g per day (<i>n</i> = 57)	Drinking averaging 28 or more grams/day (<i>n</i> = 52)
Mean age (standard deviation)	53.0 (10.6)	49.2 (13.4)	50.7 (9.3)
Gender (% male)	51.2	49.1	61.5
Ethnicity (% non-Hispanic white)	81.7	77.2	76.9
Cirrhotic (%)	72	33.9	46.2
Positive hair EtG (%)	1.2	28.1	90.4
Hair EtG ≥ 30 pg/mg (%)	1.2	15.8	80.8
Median EtG (interquartile range)	(only 1 positive subject)	0 (0–10)	100 (31–187)

Table 2. Sensitivity and specificity of EtG^a

Hair EtG cutoff concentration	Sensitivity	Specificity
8 pg EtG/mg hair	—	—
Any past-90-day drinking (%)	58 (49–67)	99 (96–100)
Average ≥ 28 g/day (%)	90 (82–98)	88 (82–93)
30 pg EtG/mg hair	—	—
Average ≥ 28 g/day (%)	81 (70–91)	93 (89–97)

^a95% confidence intervals are enclosed in parentheses.

with hair EtG ≥ 30 pg/mg]. Subject characteristics stratified by drinking status are listed in Table 1.

Alcohol use in self-reported drinkers with and without detectable hair EtG

Forty-six subjects reported some alcohol use (median 3.1 g per day, interquartile range 0.7–13.6 g per day, range 0.1–68.5 g per day), but did not have hair EtG ≥ 8 pg/mg. Sixty-three subjects reported some alcohol use (median 56 g per day, interquartile range 27.7–144.1 g per day, range 0.3–310 g per day) and had hair EtG ≥ 8 pg/mg. The amount of alcohol consumed by these groups significantly differed (Wilcoxon $P < 0.001$). In the latter group, the median hair EtG concentration was 88 pg/mg (interquartile range 30–165 pg/mg, range 8–4860 pg/mg). The one reported abstainer during the 90 days preceding hair sampling who had a positive result had an EtG concentration of 124 pg/mg. There was only modest correlation between average daily alcohol consumption during the past 90 days and hair EtG concentration in subjects with detectable hair EtG (Spearman correlation = 0.38, $P = 0.002$).

Performance of hair EtG in detecting any drinking and drinking averaging ≥ 28 g per day

ROC analysis for hair EtG showed a modest AUC of 0.79 [95% confidence interval (CI) 0.74–0.84] for any prior 90-day drinking, and a high AUC of 0.93 (95% CI, 0.88–0.97) for averaging at least 28 g per day over the prior 3 months. These results were similar in the sample with scalp hair ($n = 179$), and there were an insufficient number of subjects with non-scalp hair to evaluate separately ($n = 12$). The overall sensitivity and specificity estimates are listed in Table 2. The results indicate that hair EtG ≥ 8 pg/mg performed particularly well in detecting subjects averaging at least 28 g per day, and illustrate the trade-off between sensitivity and specificity when using the alternative 30 pg/mg cutoff. In this study population, an EtG cutoff of 15 pg/mg

Table 3. Accuracy of hair ethyl glucuronide ≥ 8 pg/mg by gender and cirrhosis

Past-90-day drinking	Sensitivity*	Specificity*
Any drinking		
Gender effects		
Men	72 (60–83)	100
Women	41 (27–55)	98 (93–100)
Cirrhosis effects		
Cirrhosis	65 (51–79)	98 (95–100)
No cirrhosis	54 (42–66)	100
Average ≥ 28 g/day		
Gender effects		
Men	97 (91–100)	83 (74–92)
Women	80 (62–98)	93 (87–99)
Cirrhosis effects		
Cirrhosis	100	94 (88–99)
No cirrhosis	82 (68–96)	80 (70–90)

*95% confidence intervals are enclosed in parentheses.

was 90% specific for averaging at least 28 g daily, with a sensitivity of 87% (95% CI, 77–96). A cutoff of 40 pg/mg was 95% specific for that magnitude of drinking, with a sensitivity of 71% (95% CI, 59–83).

Logistic regression results demonstrated statistically significant interactions ($P < 0.05$) of drinking with gender and cirrhosis on the development of positive hair EtG results. Sensitivity and specificity estimates stratified by these factors are presented in Table 3, and suggest that detection of lighter drinking may be greater in men and detection of heavier drinking may be enhanced in cirrhotic subjects. Similarly, there was a marginally significant interaction ($P = 0.060$) between drinking and reporting hair coloring during the prior 90 days. We could not differentiate this effect from gender, as nearly all subjects reporting hair coloring were women. However, in an analysis limited to women, there was no significant interaction with hair coloring ($P = 0.269$).

DISCUSSION

This study evaluated the utility of hair EtG for detecting any drinking and moderate-to-heavy drinking during the prior months in patients with liver disease. Results generally reflected previous findings in other populations and confirmed the utility of hair EtG in patients with liver disease. Hair EtG had a modest correlation with the total amount of alcohol ingested during the prior 90 days, and will therefore have limited utility for estimating how much alcohol an individual has been consuming. However, hair EtG ≥ 8 pg/mg

was highly sensitive and specific for detecting drinking that averaged at least 28 g per day in that time period.

The measurement of hair EtG would provide a strong supplement to other clinical assessments of drinking in patients with liver disease. Use of hair EtG would also improve classification of drinking in studies of alcoholic liver disease, where inaccurate self-reported drinking represents a threat to study validity. A cutoff of 8 pg/mg may be most useful for detecting drinking in excess of 28 g per day, but some lighter drinkers will exceed this cutoff. This would usually be at a low concentration, given the statistically significant albeit modest correlation between the amount of alcohol consumed and EtG concentration, the reasons for which have been discussed (Pragst *et al.*, 2010). When false positive results for this degree of drinking are particularly problematic, the use of a higher cutoff such as 15 pg/mg will further increase specificity, whereas concentrations ≥ 8 pg/mg essentially rule out abstinence during the preceding months. In this current study, the only subject reporting 90-day abstinence whom we did not exclude on the basis of mismatches with other biomarkers had been a very heavy drinker within the past 6 months, and the positive results may have reflected the presence of non-growing hair and more remote drinking.

The results of this study suggest that lighter drinking is less likely to result in detectable hair EtG in women, and others have reported diminished accuracy of hair EtG in women. Additional research is needed to adequately characterize any gender effects and to distinguish such effects from hair care practices. Severity of liver disease may modestly influence the accuracy of hair EtG (i.e. somewhat better performance in cirrhotics vs. non-cirrhotics), but hair EtG performed well regardless of cirrhosis.

Strengths of this study include the evaluation of hair EtG in a population that is susceptible to alcohol-related liver damage and in whom self-reported drinking can be misleading. In situations where insufficient hair is available, nail matrix has potential as a substitute (Jones *et al.*, 2012), but it has not been thoroughly validated. Limitations of this study include reliance on self-reported drinking to validate hair EtG. This is clearly an imperfect standard, but our use of timeline followback methods, avoidance of subjects who were clinically suspected of under-reporting alcohol use and use of other biomarkers to detect under-reporting likely provided accurate summary measures on the relationship between drinking and hair EtG. Also, this was not a random sample of subjects with liver disease, which may bias sensitivity estimates (e.g., sensitivity for any drinking would depend on the distribution of drinking in the population). However, our sensitivity estimate for the more clinically relevant outcome of averaging at least 28 g of ethanol per day is likely accurate. Hair lengths varied in this study according to an individual's hair style, and shorter lengths would be expected to have diminished sensitivity for more remote drinking. In addition, we did not specifically inquire about harsh hair treatments such as bleaching, which can result in false negative hair EtG measurements (Morini *et al.*, 2010). Kidney disease may lead to impaired clearance of EtG from serum and possibly result in a higher hair EtG concentration, and our results should not be extended to individuals with substantially reduced kidney function. Finally, assays for hair EtG are complex and have not been standardized between all laboratories that may offer testing, although efforts are

underway to rectify this (Society of Hair Testing, 2012). For use in clinical settings, it would be important to ensure performance of a validated assay by an experienced laboratory.

In conclusion, hair EtG seems likely to improve classification of drinking status in research studies of alcohol-related liver disease, and may have a role in clinical settings such as liver transplant candidacy assessment, where accurate characterization of drinking during the prior months will have a strong influence on medical decision-making.

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