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Short communication

Hair ethyl glucuronide as a biomarker of alcohol consumption in alcohol-dependent patients: Role of gender differences

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ABSTRACT

Background: Ethyl glucuronide (EtG) is a minor alcohol metabolite that accumulates in hair and is proposed as a stable marker for the detection of chronic and excessive alcohol consumption above a cut-off level of 30 pg/mg hair. A correlation between drinking behavior and EtG hair concentrations is observed, but large variability exists.
Aims: To investigate the correlation between alcohol consumption and hair EtG concentrations in alcohol dependent patients, and the effect of gender differences as a factor for the variability on this correlation.
Methods: EtG was measured by gas chromatography coupled to mass spectrometry in the hairs (first 3 cm) of 36 alcohol dependent patients (25 males/11 females) starting and alcohol detoxification program. Factors that possibly influence EtG content in hair (except age and gender) were excluded. Detailed retrospective alcohol consumption was obtained over the last 3 months using the Timeline Follow Back interview.
Results: Median total alcohol consumption over 3 months was 13,050 g pure alcohol (range 60–650 g/day). Hair EtG concentrations varied between 32 and 662 pg/mg. There was a statistically significant linear and positive correlation between hair EtG and amounts of alcohol consumed (Pearson $r = 0.83$; $p < 0.001$), in both males (Pearson $r = 0.83$; $p < 0.001$) and females (Pearson $r = 0.76$; $p = 0.007$).
Conclusions: There is a linear correlation, with no significant effect of gender, between hair EtG concentrations and amounts of alcohol consumed in alcohol-dependent individuals. Analysis of EtG in hair can be applied to estimate retrospective alcohol consumption in both male and female alcohol dependent subjects using the same cut-off.

1. Introduction

Ethyl glucuronide (EtG) is a minor metabolite of alcohol that has gained attention as a biomarker for the detection of excessive/chronic alcohol consumption (reviewed in Crunelle et al., 2014). EtG accumulates in hair (Aderjan et al., 1994) and can be used to determine chronic alcohol consumption retrospectively over a large period of time (months to years depending on hair length). The Society of Hair Testing (SoHT; www.soht.org) proposes an EtG cut-off of 30 pg/mg hair to establish excessive chronic alcohol consumption (>60 g pure ethanol per day over several months). EtG quantification in hair has high sensitivity and specificity compared to blood alcohol biomarkers (Høiseth et al., 2009; Kharbouche et al., 2012), a non-invasive sample collection and easy storage conditions, making hair EtG a useful tool for the objective detection of alcohol use.

Considerable variation is observed in hair EtG levels of alcohol consumers (Alt et al., 2000; Høiseth et al., 2009; Kharbouche et al.,

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2012; Kronstrand et al., 2012; Politi et al., 2006; Stewart et al., 2013; Yegles et al., 2004). A few studies showed a correlation between hair EtG levels and amounts of alcohol consumed (Appenzeller et al., 2007; Kerekes et al., 2009; Politi et al., 2006), while other studies found no or modest correlations (Stewart et al., 2013; Yegles et al., 2004). The lack of correlation between hair EtG levels and amounts of alcohol consumed could be explained by several factors, such as by the presence of variables that may influence EtG incorporation in hair (i.e., hair treatments, pathophysiological conditions, or factors underlying alcohol metabolism such as age and gender). Of these, gender can influence EtG incorporation in hair, but, in contrast to other variables, the influence of gender on measured hair EtG levels has not been investigated. Upon similar alcohol consumption, females have higher blood alcohol concentrations (BAC) than males (Sutker et al., 1983), and presumably eliminate alcohol faster than males (Jones, 2010), resulting in shorter but higher BAC peaks. As EtG is incorporated into hair primarily through the blood supply, the question arises whether, as a result of differences in BACs, males and females differ in their EtG incorporation in hair.

Here, we investigated the correlation between alcohol consumption and hair EtG in alcohol-dependent patients, as these are participants that consume substantial amounts of alcohol over considerable time periods of months to years. Additionally, we investigated if there was an effect of gender on this correlation.

2. Methods

2.1. Samples

Hair samples were collected from alcohol-dependent patients recruited from a Belgian addiction treatment center (Psychiatric Center Broeders Alexianen, Boechout). Patients were included when aged 18–60 years, having a diagnosis of alcohol dependence, and entering in-patient treatment for alcohol dependence. Exclusion criteria were: hair shorter than 3 cm; gastrointestinal-, liver- or kidney-pathologies; and/or bleached, permed or cosmetically straightened hair, factors that influence EtG content in hair (Crunelle et al., 2014; Ettlinger et al., 2014; Kerekes and Yegles, 2013). Hair coloring was allowed.

This study was approved by the Ethical Committee of the Antwerp University Hospital. Participants gave written informed consent.

2.2. Measures

2.2.1. Hair EtG. Two hair locks were collected from the vertex posterior of the head, and combined into one hair strand used for hair EtG analyses. For analysis, a mean amount of 31.2 ± 1.0 mg hair was used.

Hair samples were analyzed using gas chromatography mass spectrometry (GC–MS) described previously, using 2 ng of EtG-d5 as internal standard and heptafluorobutyric anhydride as derivatization agent (Kerekes et al., 2009; Kerekes and Yegles, 2013). EtG analyses were performed in negative chemical ionization with a limit of detection (LOD) of 0.02 pg/mg hair and a limit of quantification (LOQ) of 0.08 pg/mg hair.

2.2.2. Alcohol consumption. The Timeline Follow Back method (TLFB; Sobell and Sobell, 1992) was used to retrospectively estimate daily alcohol consumption (including amounts of alcohol) of the past 3 months. The Alcohol Use Disorders Identification Test (AUDIT; Saunders et al., 1993) was used to assess the degree of harmful consumption of alcohol.

2.2.3. Patient' characteristics and hair treatment. Gender, age, body mass index (BMI) and hair treatment (shampoo and conditioner use, hair color) were assessed using an in-house developed questionnaire.

2.3. Statistical analyses

Differences in age, BMI and alcohol consumption between males and females were analyzed using parametric student *T*-tests or with nonparametric Mann–Whitney *U* tests where appropriate. All data are presented as mean ± standard deviation (SD) or as median ± interquartile range (IQR) where appropriate. A *p*-value <0.05 was considered statistically significant.

Linear regression was used to assess correlations between hair EtG and alcohol (amount) consumption. To assess gender differences, regression analysis was performed with hair EtG as the dependent variable, and with gender, alcohol consumption, and the interaction between gender and alcohol consumption as the independent variables. The significance of the latter term then indicates whether gender influences the effect of alcohol consumption on the measured hair EtG values. A separate linear regression analysis addressed whether there was an influence of gender on the nominal EtG hair levels.

Table 1
Patients' characteristics and characteristics of the proximal (0–3 cm) hair segment of 36 alcohol-dependent patients.

	Male (n = 25)	Female (n = 11)	p-value
Age (years)	46 ± 8	41 ± 7	0.069 NS
BMI	25 ± 4	26 ± 9	0.513 NS
AUDIT score	29 ± 6	28 ± 8	0.574 NS
Total alcohol consumption/3 months (g)	14,400 ± 14,210*	8820 ± 6750*	0.036
Hair color			
Black (%)	16%	18%	
Brown (%)	56%	45%	
Red (%)	0%	9%	
Blond (%)	28%	28%	
Hair coloring			
Patients with color treatments (%)	0%	55%	<0.001
Number of treatments in past 3 months	–	1 ± 1	–
Shampoo use (times/week)	4 ± 2	3 ± 2	0.201 NS
Conditioner use (times/week)	20%	64%	0.011

Data are presented as mean ± standard deviation (SD) or as median ± interquartile range (*)
Abbreviations: BMI: body mass index; AUDIT: alcohol use disorder identification test; NS: non significant.

3. Results

3.1. Patient characteristics

Thirty-nine Caucasian alcohol-dependent individuals were included. Of these, 36 had alcohol consumptions >60 g/day and are further described (25 males and 11 females). Participants were abstinent from alcohol for 2 ± 1 day when hair samples were collected. Patient characteristics (age, BMI) did not differ between males and females (Table 1). Table 1 provides an overview of the patients/hair characteristics. Mean AUDIT scores were 28 ± 6, confirming severe harmful alcohol consumption (cut-off score 20; Babor et al., 1989). Mean age was 45 ± 8 years.

3.2. Correlations between hair EtG and alcohol (amount) consumption

Total median consumption over the last 3 months (90 days) was 13,050 ± 12,020 g pure alcohol (range 60–650 g/day). Hair EtG concentrations varied between 32 and 662 pg/mg hair, with a linear and positive correlation with amounts of alcohol consumed (Pearson r = 0.83; B = 0.116; t = 8.798; p < 0.001). Sensitivity and positive predictive value of hair EtG analyses were both 100%.

3.3. Effects of gender differences on the correlation between hair EtG and alcohol consumption

Females drank fewer alcohol over the total 3 months (8820 ± 6750 g) compared to males (14,400 ± 14,210 g; p = 0.036). In both males and females, there was a linear and positive correlation between hair EtG and the amounts of alcohol consumed (males: Pearson r = 0.83, B = 0.115, t = 7.020, p < 0.001; females: Pearson r = 0.76, B = 0.119, t = 3.511, p = 0.007) (Fig. 1). There was no effect of gender on the correlation between hair EtG and alcohol (amount) consumption (p = 0.947) and there was no effect of gender on the nominally detected levels of hair EtG (p = 0.122). Age