Ethyl glucuronide in nails : method validation, influence of decontamination and pulverization, and particle size evaluation

Ethyl glucuronide in hair of non-excessive alcohol consumers: correlations and gender influence

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ABSTRACT

Hair ethyl glucuronide (hEtG) is a marker for the detection of retrospective alcohol consumption. Correlations between the amounts of alcohol consumed and hEtG concentrations have been reported earlier, but little has been published regarding the consumption of relatively low amounts of alcohol and the associated hEtG concentrations close to the limit of quantification (LOQ). Here, we investigate hEtG concentrations in non-excessive <60 g/day alcohol consumers and address the role of gender differences on hEtG concentrations within this population. Daily alcohol consumption over a period of the past 3 months before hair collection was assessed using the Timeline Follow Back interview in 130 non-excessive <60 g/day alcohol consumers. Participants showed large variation in consumption: between 10 g (±1 consumption) and 3300 g (±330 consumptions) within the prior 3 months. Eight individuals abstained from alcohol and served as a control group. HEtG was measured using gas chromatography-mass spectrometry in the 0-3 cm hair segment and varied between <LOQ and 29.8 pg/mg, showing a linear correlation with the amounts of alcohol consumed (r=0.81; p<0.001). Gender had no influence on this correlation (gender effect: p<0.001), nor on the nominally detected EtG concentrations (p<0.001). In conclusion, a correlation between hEtG and the amount of alcohol consumed and a lack of gender difference could be shown for non-excessive alcohol consumption, confirming the results for excessive alcohol drinkers. We propose to lower the current hEtG cut-off for abstinent assessment to 5 pg/mg hair.

Keywords: ethyl glucuronide; hair; alcohol dependence; alcohol biomarker; gender

Introduction

Ethyl glucuronide (EtG) is a minor phase II alcohol metabolite that accumulates in hair and can be used to assess retrospective alcohol consumption over a time window of several months[1]. According to Society of Hair Testing (SoHT) guidelines, hEtG concentrations ≥7 pg/mg strongly suggest repeated alcohol consumption, whereas concentrations ≥30 pg/mg indicate excessive chronic alcohol consumption of >60 gram alcohol per day during several months[2]. A previous study reported correlations between hEtG concentrations and the amount of alcohol consumed in alcohol-dependent individuals, whereas no effect of gender on the correlation was observed[3]. The correlation between hEtG and amounts of alcohol consumed has previously been noted in other studies as well[4-6]. In 32 alcohol consumers, hEtG concentrations were <7 pg/mg in 12 participants consuming low daily amounts of alcohol (0-20 g alcohol/day)[5]. In 11 volunteers consuming <20 g alcohol/day, hEtG concentrations could not be detected with a limit of detection (LOD) of 2 pg/mg[6]. Another study detected daily consumption of two glasses of wine in only 4 of the 7 male participants (hEtG concentration: 5-11 pg/mg), while the daily consumption of one glass of wine was detected in only one of 12 female drinkers (hEtG concentration: 3 pg/mg)[7]. In individuals with higher (up to 60 g/day) alcohol consumption, hEtG concentrations correlated well with the estimated daily alcohol intake[6]. In general, few studies (often also with small sample size) report on the correlation between hEtG and amounts of non-excessive <60 g/day alcohol consumption. Also, no studies report on the effect of gender on hEtG concentrations in non-excessive <60g/day alcohol consumers. One study paid attention to the aspect of gender by presenting findings separately for males and females[6], but, because of a lack of female participants consuming low amounts of alcohol and a relatively low amount of study participants (N = 21), did not present results on gender differences[6]. Here, we report on the correlation between non-excessive amounts of alcohol consumed <60 g/day and hEtG concentrations in alcohol

consumers and we investigate the influence of gender hereon. For the first time, these are reported in a large sample of 130 well-characterized non-excessive alcohol consumers that provided detailed information on their daily alcohol consumption of the prior three months.

Materials and methods

Hair samples were collected from alcohol consumers recruited at the University Campus of the University of Antwerp, Belgium and the wider metropolitan area. Participants were included when aged 18-60 years old, having hair length $\geq$ 3 cm, and being able to report on their consumption over the prior months.

Gender, age, body mass index (BMI), hair color, shampoo and conditioner use, and possible additional cosmetic treatments (including hair bleaching, permanent coloring, perming or straightening) were assessed. Alcohol consumption was recorded using the Timeline Follow Back interview (TLFB)[8].

For hEtG analysis, about 50 mg of scalp hair was collected from the vertex posterior. Hair samples were mechanically pulverized, and 30 mg powdered sample was accurately weighted for analysis. Using the protocol described earlier[9], EtG concentrations in the samples were analyzed using gas chromatography-mass spectrometry (GC-MS) in negative chemical ionization mode with 2 ng of EtG-D_5 as internal standard and pentafluoropropionic anhydride as derivatization agent. LOD was 0.70 pg/mg hair and the lower limit of quantification (LLOQ) was 2.10 pg/mg hair. Quality control (QC) results are depicted in Figure 1. The CVs (%) of the two QC samples were 13.1 and 11.7 %, respectively.

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Data were analyzed on normality using Shapiro-Wilk tests. Differences in participant characteristics between males and females were analyzed using parametric student T-tests or with nonparametric Mann-Whitney U tests where appropriate. Data are presented as mean ± standard deviation (SD) or as median ± interquartile range (IQR), with a p-value <0.05 considered statistically significant. The correlation between hEtG concentrations and amount of alcohol consumed was assessed using linear regression. To address the role of gender on this correlation, regression analysis was performed with hEtG as the dependent variable, and with gender, alcohol consumption, and the interaction between gender and alcohol consumption as the independent variables. Separate regression analyses per gender were performed to assess whether gender influenced the nominally detected hEtG concentrations.

Results

130 individuals were included in the study, of which the majority (77%) was female. Participants had a mean age of 28.8 ± 12.3 years and a mean BMI of 22.5 ± 3.7. There were no statistical differences in age or BMI between males and females (all p>0.290). Three participants had blond, 86 brown, 4 black, 4 white/grey, and 4 red hair. Of these, 13% (all female) had colored hair, and 3% (all female) had bleached hair. None reported hair perming or straightening. Eight individuals (3 males and 5 females) reported abstaining from alcohol in the prior 3 months (all had hEtG concentrations <LOD) and are further described as a control group. In alcohol consumers, the total alcohol consumption was 705 ± 858 gram pure alcohol over the prior 3 months (median ± IQR; range 10-3300 g/3 months). Males consumed statistically more alcohol (median ± IQR: 1180 ± 1083 g/3 months) than females (median ± IQR: 620 ± 776 g/3 months; p=0.001). As a rough approximation, this corresponds with an approximate daily alcohol intake of 13 g pure alcohol for males and 7 g pure alcohol for females. HEtG concentrations were 4.0 ± 7.3 (median ± IQR; range <LOD–29.8 pg/mg).

Including all participants, a statistically significant positive correlation was observed between hEtG concentrations and the total amounts of alcohol consumed over the prior 3 months (Pearson r = 0.81; p<0.001). When removing the 8 abstainers from analysis, the correlation remained significant (Pearson r=0.80; p<0.001).

In individuals reporting alcohol consumption, 28 had hEtG results below LOD (23%; 2 males, 26 females). Their alcohol consumption ranged between 40 and 1220 g pure alcohol for the males and between 30 and 880 g pure alcohol for females.

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Separate analysis of males and females showed that the correlation between hEtG and amounts of alcohol consumed remained significant independent of gender (males: Pearson r=0.79; p<0.001; females Pearson r=0.79; p<0.001; Figure 2). Additionally, gender had no effect on the correlation between hEtG and the amount of alcohol consumed (p=0.113).

It has been shown that hair coloring and bleaching reduces hEtG concentrations, especially with repeated hair treatments[10]. When removing the female participants with colored and bleached hairs from analysis, we observed no differences in the correlation between hEtG concentration and amounts of alcohol consumed (Pearson r=0.80; p<0.001).

Discussion

This study provides evidence for a correlation between hEtG concentrations and amounts of alcohol consumption in non-excessive <60 g/day alcohol-consuming individuals. This is the first time that these data are presented in such a large group of individuals who provided detailed daily estimates of alcohol consumption over the prior months. This study thereby confirms and extends previous findings that gender has no influence on the detected hEtG

concentrations in hair when assessing non-excessive <60 g/day alcohol consumption, and addresses correlations between low amounts of ingested alcohol and hEtG concentrations close to LOD values.

Previous studies showed that permanent hair coloring and bleaching decreases the EtG concentrations in hair[9-10]. In this study, when removing the participants with hair coloring and bleaching, no significant difference was noted on the correlation between hEtG and amounts of alcohol consumed. Since coloring was kept to a minimal in the prior three months or, more probably, because only 4 participants had bleached hairs, this may have had little influence on the total correlation. To be noted, 28 participants reported consuming alcohol, but had hEtG concentrations < LOD. Of these, two were male and 26 were females, including the 4 females having had hair coloring. In light of this study, this would imply that female participants who reported hair coloring would have higher hEtG incorporation than measured by hEtG analysis. Therefore, one should be careful when interpreting hEtG results in these individuals. Because no information was recorded on the amounts of coloring/bleaching sessions in the prior three months, this is speculative.

When comparing the results of this study with the 7 pg/mg cut-off proposed by the SoHT[2] to reflect alcohol consumption, we noticed that up to 28 individuals who reported drinking alcohol daily and in moderate amounts (between 1 and 3 glasses per day) have hEtG concentrations below the 7 pg/mg cut-off. This proposes that, for abstinence assessment, the 7 pg/mg cut-off should perhaps be lowered. From the results of the present study, we propose a lower cut-off for abstinence assessment of 5 pg/mg hair. However, in order to measure low hEtG amounts with adequate certainty, it is important that the analytical technique has a LLOQ well below the 7 pg/mg cut-off. This is the case with the used GC-MS method, as well as with more sensitive GC tandem MS (GC-MSMS) methods[11]. Reducing the cut-off should

thus only be considered if the techniques used are adequate to measure this. This aspect warrants further research.

Conclusion

Hair EtG shows a linear positive correlation with the amounts of alcohol consumed in the prior months. Also with non-excessive <60 g/day alcohol consumption, gender does not influence hEtG levels, such that the interpretation of hEtG results can occur independent of gender.

Acknowledgements

None.

Conflict of Interest

No conflict declared.

Ethical Approval

The study was approved by the Ethical Committee of the Antwerp University Hospital and has been performed in accordance with the ethical standards in the 1964 Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

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Figure Legends

Figure 1: Quality control (QC) results for two quality control samples (QC1 and QC2) with the minimal and maximal analyzed concentrations, and the respective reference values (QC1 ref and QC2 ref) with their interval ranges. Error bars represent standard deviations.

Figure 2: Plot of the correlation between ethyl glucuronide concentrations in hair (hEtG) in the 0-3 hair segment and the total amount of alcohol consumed in the prior three months in males (left) and females (right).