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Ethyl glucuronide in hair: is it a reliable marker of chronic high levels of alcohol consumption?

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ABSTRACT

Aims This study aims to investigate the relationship between ethanol daily intake (EDI) and the levels of ethyl glucuronide in hair. **Design** Ethyl glucuronide concentration was determined in hair samples from different classes of ethanol drinkers and results were compared with the reported information about drinking habits. **Setting** Pavia, Italy. **Participants** Twenty-two known alcoholics, 21 volunteers self-reporting an EDI from 2 to 60 g, and seven teetotallers were involved in this study. **Measurements** Ethyl glucuronide determination in hair samples was performed by liquid chromatography-tandem mass spectrometry (limit of detection: 2 pg/mg, lower limit of quantification: 3 pg/mg). **Findings** Current known alcoholics ($n = 21$) had ethyl glucuronide hair concentration in the range 4.0–434.7 pg/mg (average: 62.8, median 37.4 pg/mg); ethyl glucuronide was not detected in hair samples from teetotallers ($n = 7$); all volunteers reporting an EDI of at least 30 g ('non-moderate drinkers' according to the US Department of Health and Human Services) tested positive for ethyl glucuronide (cut-off: 4 pg/mg). All volunteers declaring an ethanol daily intake higher than 40 g ('heavy drinkers' according to the World Health Organization, Regional Committee for Europe) tested positive for this compound (cut-off: 5 pg/mg). The application of a cut-off of either 4 pg/mg or 5 pg/mg resulted in one false positive, coming from a volunteer asserting an ethanol daily intake of 30 g. No false negatives were found. **Conclusions** The concentration of ethyl glucuronide in hair appears to correlate with EDI.

Keywords Alcohol abuse, ethanol daily intake, ethyl glucuronide, hair.

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INTRODUCTION

In 2004 the World Health Organization (WHO) estimated that more than 76 million people world-wide have a diagnosable alcohol use disorder, resulting in a considerable global burden both in terms of morbidity and mortality. Overall there is a causal relationship between alcohol consumption and more than 60 types of disease and injury and, specifically, alcohol is reportedly responsible for about 20–30% of oesophageal cancer, liver cancer, cirrhosis of the liver, homicide, epileptic seizures and motor vehicle accidents world-wide [1]. WHO estimated that alcohol causes 1.8 million deaths and a loss of 58.3 million of the so-called disability-adjusted life years. Unintentional injuries alone account for about one-third of the 1.8 million deaths [1].

From the diagnostic viewpoint, one of the major issues lies with the difficulty in identifying specific and sensitive markers of mid-term and long-term ethanol abuse. In the forensic and clinical fields, such markers would provide an important diagnostic tool in all cases where monitoring ethanol use/abstinence is an issue, such as withdrawal treatment, work-place testing, driving licence reissue/renewal, minors' adoption, divorce proceedings, etc.

Among the suggested markers of mid/long-term alcohol intake, ethyl glucuronide (EtG), a phase II metabolite of ethanol, has been proposed recently [2]. Despite the high hydrophilicity of this compound, probably accounting for only a moderate incorporation into hair [3], EtG has been detected in hair samples of chronic alcohol abusers [4–9].

In comparison with other clinical parameters used typically in the diagnosis of high levels of alcohol consumption (e.g. carbohydrate-deficient transferrin, gamma-glutamyltranspeptidase and mean corpuscular volume), EtG has the advantage of being a direct metab-

olite and therefore a significantly more specific marker. EtG formation has been demonstrated recently to be catalysed by the UDP-glucuronosyltransferase (UGT) superfamily of enzymes. As any functional difference in one UGT would probably be masked by a combination of other UGT isoforms, EtG levels in the human body are not liable to be influenced by genetic polymorphism [10]. Unfortunately, at present a strict correlation between the pattern of alcohol use and hair EtG concentration has not been demonstrated.

In order to study the possibility to correlate EtG hair concentration with ethanol use, hair samples from different users (known alcoholics, 'social drinkers' and teetotallers) were collected together with all the information available about the donors' drinking habits. The EtG concentration in hair samples was determined by liquid chromatography–tandem mass spectrometry and results were compared with the reported ethanol daily intake (EDI).

MATERIALS AND METHODS

Hair samples

Known alcoholics at the beginning of an in-patient withdrawal treatment at the alcohol-dependency unit of the Fondazione S. Maugeri (Pavia, Italy) in the period October 2004–June 2005 were considered in this study and identified as group A ($n = 22$). This group comprised 13 females and nine males, in the age ranges 29–62 (mean: 44.5, median 44) and 36–59 (mean: 46.7, median 42) years, respectively. Group B comprised 21 volunteers who were asked to complete a written questionnaire about their drinking habits. The questionnaire included the following information: age, sex, body weight, amount (drinks/day) and type of alcoholic beverages consumed, frequency of drinking (days/week) during the 6-month period preceding the collection of hair. This group included nine females, ranging in age from 28 to 53 years (mean: 34.3, median: 30.0), body weight from 47 to 70 kg (mean: 55.7, median: 56.0); and 12 males in the age range from 25 to 54 years (mean: 38.1, median: 41.5), body weight from 66 to 85 kg (mean: 75.7, median: 75.5). The average EDI of each subject of this group was estimated on the basis of the answers provided to the questionnaire, considering that a standard drink contains approximately 15 g of ethanol [11]. The estimated EDI ranged from 2 to 60 g (mean: 23.6, median: 15.0) and from 11 to 64 g (mean: 27.0, median: 19.3) for females and males, respectively. Seven teetotallers, including two children not exposed to ethanol, were classified as group C (age range from 7 to 37, mean: 23.9, median: 26.0).

Hair samples were collected with scissors from the posterior vertex as closely as possible to the skin and the proximal segment (3–5 cm long) was submitted to analysis. A written informed consent to participate in the study was obtained from the all the individuals.

Sample preparation and analysis

A minimum of 50 mg of hair was analysed as a single measurement using the method described elsewhere [12] by liquid chromatography–electrospray tandem mass spectrometry (LC-ESI-MS-MS) after organic solvent decontamination (dichloromethane and methanol), overnight water incubation and 2-hour ultrasonication. Pentadeuterated EtG was used as internal standard. The analytical method was fully validated and reached a lower limit of quantification (LLOQ) of 3 pg/mg and a limit of detection (LOD) of 2 pg/mg. EtG identification was accomplished by monitoring two precursor-to-product transitions (m/z 221 → 75 and 221 → 85) beside the surviving ion (m/z 221 → 221). Pentadeuterated EtG was used as internal standard for quantification (transitions monitored: m/z 226 → 75, 226 → 85, and surviving ion, 226 → 226). The transitions m/z 221 → 75 and m/z 226 → 75 were used for quantification.

RESULTS AND DISCUSSION

EtG concentration in hair samples from group A are summarized in Table 1. All patients tested positive, with the

Table 1 EtG hair concentrations in withdrawal treatment patients (group A).

Patient, sex, age (years)	*EtG hair concentration (pg/mg)*
A01, M, 53	43.6
A02, M, 40	76.6
A03, F, 38	186.5
A04, F, 55	45.6
A05, F, 52	434.7
A06, F, 56	105.5
A07, M, 57	24.7
A08, M, 41	96.2
A09, M, 36	9.3
A10, F, 45	39.3
A11, M, 59	12.1
A12, M, 42	86.9
A13, F, 42	13.6
A14, F, 29	46.2
A15, F, 49	49.5
A16, F, 39	4.7
A17, F, 62	< LOD
A18, M, 52	35.4
A19, M, 40	29.2
A20, F, 44	20.9
A21, F, 30	17.3
A22, F, 37	4.0

LOD: Limit of Detection

Table 2 EtG hair concentrations in withdrawal patients found by various authors.

	Yegles et al. 2004 [8]	*Janda et al. 2002 [6]*	*Alt et al. 2000 [4]*	*Our data*
Sample number	10	42	4	22
LOD (pg/mg)	2	51	Not reported	2
Minimum EtG hair concentration (pg/mg)	30	< LOD	119	< LOD
Maximum EtG hair concentration (pg/mg)	415	984	388	434.7
Average EtG hair concentration (pg/mg)	159.5	163.4	260.3	62.8
Relative standard deviation (%)	86.8	127.4	47.8	149.3
Median EtG hair concentration (pg/mg)	108	93	267	37.4

LOD: Limit of Detection

exception of patient A17. According to this subject's clinical records, she had been abstinent from ethanol for 6 weeks before entering the alcohol dependency unit. The range of levels measured in this group (excluding A17) was 4.0–434.7, and the average and median EtG concentrations were 62.8 and 37.4 pg/mg, respectively. The EtG range for females was 4.0–434.7 pg/mg, mean 74.5, median 39.3 pg/mg; the EtG range for males was 9.3–96.2 pg/mg, mean 46.0, median 35.4 pg/mg.

EtG hair concentrations of patients entering a withdrawal treatment reported by other authors are listed in Table 2 [4,6,8]. The data reported by Skopp *et al.* [5] have not been included in this table due to the relatively high limit of quantification of the analytical method used, making it difficult to compare the results from that study with those obtained by more sensitive procedures. None of these authors found a correlation between EtG hair levels and self-asserted alcohol intake, or between EtG hair levels and other markers of alcohol abuse (such as fatty acid ethyl esters, as described by Yegles *et al.* [8]). Some of the authors [5,6] report cases (two of four patients by Skopp [5], 11 of 42 patients by Janda *et al.* [6]) of known current alcoholics who tested negative for EtG in hair. However, these false negatives might be attributed to the relatively high LOD of their analytical method (i.e. 51 pg/mg [6], 2200 pg/mg [5]). No false negatives were observed by Yegles (10 patients, LOD = 2 pg/mg [8]) and, with the exception of case A17, in our study (22 patients, LOD = 2 pg/mg). False negatives might also depend on a low efficiency of extraction of EtG from the hair matrix. EtG extraction from hair is usually performed by incubation and sonication of the samples in water and/or organic solvent (i.e. methanol and acetonitrile) [4–8,12]. The accuracy of the process is influenced by a number of factors, such as the efficiency of the previous washing procedure in removing the lipid component of hair, the pulverization mode (when included) and, above all, the incubation/sonication solvent, temperature and time. In fact, if the keratinic matrix is not completely digested (as in all published methods on EtG, including the one adopted in this study) bringing the analytes into solution, the efficiency of the extraction procedures is hardly tested and compared [13].

Table 3 EtG hair concentrations and self-reported EDI in volunteers (group B).

Volunteer, sex, age (years)	*EtG hair concentration (pg/mg)*	*Self-reported EtOH intake (g/day)*
B01, F, 30	11.5	60
B02, M, 40	4.2	32
B03, M, 25	< LOD	28
B04, F, 28	3.1	11
B05, F, 33	< LOD	9
B06, M, 26	< LOD	11
B07, M, 26	< LOD	13
B08, M, 43	5.1	30
B09, F, 31	< LOD	4
B10, F, 28	< LOD	19
B11, M, 43	< LOD	15
B12, M, 54	< LOD	17
B13, F, 29	< LOD	15
B14, F, 53	6.7	47
B15, M, 52	35.4	60
B16, F, 49	26.2	45
B17, F, 28	< LOD	2
B18, M, 44	< LOD	15
B19, M, 25	3.0	21
B20, M, 47	11.5	64
B21, M, 32	< LOD	17

LOD: Limit of Detection

EtG hair concentrations of group B are reported in Table 3. The overall average and median hair concentrations were found to be 5.1 and 0 pg/mg, respectively.

In the processing of data from group B, good correlation ($R^2 = 0.8591$ using a second-grade polynomial function) was found by plotting EtG hair concentration versus EDI normalized by the individual body weight in kilograms (Fig. 1). Good linear correlation ($R^2 = 0.8525$) was also found by considering the two highest EtG hair

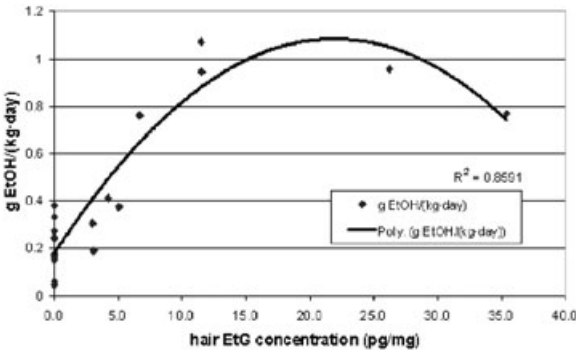

Figure 1 EtG hair concentration (pg/mg) of group B versus self-asserted EDI (g/day) normalized by the individual body weight in kilograms

Table 4 Correlation between EtG hair result and ethanol daily intake using different discriminants between moderate and non-moderate use and different EtG cut-offs in subjects of group B.

	Females		*Males*		*All subjects*	
Discriminant EDI (g/day)	15	20	30	40	30	40
EtG cut-off (pg/mg)	4	5	4	5	4	5
False positives	0	0	1	1	1	1
True positives	3	3	3	2	6	5
False negatives	1	0	0	0	0	0
True negatives	5	6	8	9	14	15
Total	9	9	12	12	21	21

values as outliers and excluding them arbitrarily from the elaboration.

A further attempt to correlate EDI and EtG hair concentration was made by dividing this group into two subgroups on the basis of the self-reported EDI. According to the 2005 *Dietary Guidelines for Americans* [11], an EDI of up to one standard drink (roughly corresponding to 15 g) for women and up to two standard drinks (30 g) for men indicates moderate drinking. By using a cut-off of 4 pg/mg, all women declaring an EDI up to 15 g were found negative and all those reporting an EDI higher than 15 g/day tested positive, with the exception of one individual (B10), asserting an intake of 19 g/day. All men reporting a standard intake of more than 30 g/day tested positive and of all the 12 individuals drinking less than 30 g/day, only one (B08) was positive for EtG (Table 4). In this case the volunteer reported an EDI at the upper limit of the 'moderate' drinker definition (30 g/day) and earlier total monolateral nephrectomy (that might reasonably impact EtG excretion and, consequently, disposition). By setting a common discriminant at 30 g/day for both sexes, all volunteers stating an EDI greater than 30 g (i.e. non-moderate drinkers) were found positive (Table 4) and in all but one (B08) hair samples from moderate drinkers EtG was lower than 4 pg/mg.

The report on alcohol in the WHO European Region [14] defines 'heavy drinkers' as males drinking at least 40 g/day and females drinking at least 20 g/day. By using these discriminants and raising the cut-off to 5 pg/mg, only women falling under the heavy drinkers' category tested positive for EtG (Table 4). All heavy drinking men tested positive, and among moderate drinkers the only false positive was, again, volunteer B08 (Table 3). Because no women declared an EDI between 20 and 40 g, no differences were observed when considering a common discriminant at 40 g/day for both sexes (Table 4).

These results are similar to those presented by other authors regarding social drinkers. Janda [6] analysed hair from 'social' drinkers (i.e. reporting a maximum EDI of 30 g), and found one sample of five above the LOD (51 pg/mg): 55 pg/mg. Yegles [8] and Alt [4], who both defined as 'social' drinkers individuals stating a maximum EDI of 20 g, found no EtG-positive hair in this group ($n = 4$ and $n = 6$, respectively).

CONCLUSIONS

According to the data of the present study it seems possible, when the analytical method fulfils sensitivity and specificity requirements, to correlate EtG hair concentration to EDI as:

- all teetotallers tested negative ($n = 7$);
- a good correlation was found between EtG in hair and normalized (by body weight) EDI ($n = 21$);
- it was possible to define a cut-off level discriminating between moderate and non-moderate drinkers. This cut-off was set at 4 or 5 pg/mg depending on the discriminant EDI between moderate and non-moderate drinkers adopted (15–30 g or 20–40 g); and
- all current alcoholics at the beginning of an in-patient withdrawal treatment tested above the 4.0 pg/mg cut-off ($n = 21$).

These results, if confirmed on a wider number of cases, indicate that the determination of EtG in hair is a viable tool for assessing non-moderate alcohol intake.

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References

1. World Health Organization (WHO). *Global status report on alcohol*. Available at: http://www.who.International/substance_abuse/publications/global_status_report_2004_overview.pdf (accessed 7 October 2005).

2. Wurst F. M., Skipper G. E., Weinmann W. Ethyl glucuronide—the direct ethanol metabolite on the threshold from science to routine use. *Addiction* 2003; **98**:51–61.
3. Pragst F., Spiegel K., Sporkert F., Bohnenkamp M. Are there possibilities for the detection of chronically elevated alcohol consumption by hair analysis? A report about the state of investigation. *Forensic Sci Int* 2000; **107**:201–23.
4. Alt A., Janda I., Seidl S., Wurst F. M. Determination of ethyl glucuronide in hair samples. *Alcohol Alcohol* 2000; **35**:313–14.
5. Skopp G., Schmitt G., Potsch L., Dronner P., Aderjan R., Mattern R. Ethyl glucuronide in human hair. *Alcohol Alcohol* 2000; **35**:283–5.
6. Janda I., Weinmann W., Kuehnle T., Lahode M., Alt A. Determination of ethyl glucuronide in human hair by SPE and LC-MS/MS. *Forensic Sci Int* 2002; **128**:59–65.
7. Jurado C., Soriano T., Gimenez M. P., Menendez M. Diagnosis of chronic alcohol consumption. Hair analysis for ethylglucuronide. *Forensic Sci Int* 2004; **145**:161–6.
8. Yegles M., Labarthe A., Auwarter V., Hartwig S., Vater H., Wennig R. *et al.* Comparison of ethyl glucuronide and fatty acid ethyl ester concentrations in hair of alcoholics, social drinkers and teetotallers. *Forensic Sci Int* 2004; **145**:167–73.
9. Klys M., Scislowski M., Rojek S., Kolodziej J. A fatal clomipramine intoxication case of a chronic alcoholic patient: application of postmortem hair analysis method of clomipramine and ethyl glucuronide using LC/APCI/MS. *Legal Med* 2005; **7**:319–25.
10. Foti R. S., Fisher M. B. Assessment of UDP-glucuronosyltransferase catalyzed formation of ethyl glucuronide in human liver microsomes and recombinant UGTs. *Forensic Sci Int* 2005; **153**:109–16.
11. US Department of Health and Human Services. *Dietary guidelines for Americans 2005*. Washington, DC: US Department of Health and Human Services, US Department of Agriculture; 2005. Available at: http://www.healthierus.gov/dietaryguidelines (accessed 7 October 2005).
12. Morini L., Politi L., Groppi A., Stramesi C., Polettini A. Determination of ethyl glucuronide in hair samples by liquid chromatography/electrospray tandem mass spectrometry. *J Mass Spectrom* 2006; **41**:34–42.
13. Polettini A., Stramesi C., Vignali C., Montagna M. Determination of opiates in hair. Effects of extraction methods on recovery and on stability of analytes. *Forensic Sci Int* 1997; **84**:259–69.
14. World Health Organization (WHO). *Report on Alcohol in the WHO European Region.* Available at: http://www.euro.who.int/Document/RC55/ebd01.pdf (accessed 7 October 2005).

