Forensic Science International 279 (2017) 187–191

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Ethyl glucuronide in keratinous matrices as biomarker of alcohol use: A correlation study between hair and nails

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ARTICLE INFO

Article history:
Received 14 April 2017
Received in revised form 29 July 2017
Accepted 21 August 2017
Available online 26 August 2017

Keywords:
Ethyl glucuronide
Alcohol
Keratinous matrices
Nails
Hair
Correlation

ABSTRACT

To quantify alcohol use, objective, specific and sensitive long-term alcohol markers are necessary. Ethyl glucuronide (EtG), a direct metabolite of alcohol, accumulates in keratinous matrices such as hair and nails, and is a specific and sensitive long-term biomarker for the detection of chronic alcohol consumption. So far, research has primarily focused on the detection of EtG in hair, and studies on its measurement in nails are scarce. In this article, we assessed EtG concentrations in hair, finger- and toenails from the same individuals in order to evaluate the direct correlation between the matrices. To this end, a total amount of 45 hair, 41 fingernail, and 13 toenail samples were collected from patients treated for alcohol use disorders at two psychiatric centers in Belgium. Samples were analyzed by gas chromatography-tandem mass spectrometry. Hair EtG concentrations ranged from <LLOQ to 1149 pg/mg (median = 164 pg/mg, IQR [42; 283]). Fingernail EtG concentrations ranged from <LLOQ to 4090 pg/mg (median = 250 pg/mg, IQR [74; 645]). Toenail EtG concentrations ranged from 127 to 3792 pg/mg (median = 687 pg/mg, IQR [379; 1370]). EtG levels in hair and nails were significantly and positively correlated (p-values < 0.001, r = 0.70 and r = 0.62, respectively). Higher concentrations were present in finger- and toenails compared to hair, which might be attributed to the slower growth rate of nails, resulting in increased accumulation of EtG. Hence, nail analysis may be interesting when low concentrations of EtG are expected, e.g. to discriminate between teetotalers and social drinkers. In addition, the current study proposes preliminary cut-off values for EtG concentrations in fingernails: >123 pg/mg for chronic excessive alcohol consumption, 59–123 pg/mg for moderate alcohol consumption, and <59 pg/mg for alcohol abstinence. In light of these results, nails may be a useful alternative to hair samples for monitoring of long-term alcohol consumption, e.g., in cases where hair is not available. Further studies are needed to establish cut-off values for EtG levels in nails.

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1. Introduction

The harmful use of alcohol is a worldwide problem and causes a large disease, social and economic burden in societies. Alcohol abuse is directly and indirectly responsible for more than 40 diseases; most notably alcohol use disorders, liver cirrhosis, cancers and injuries. Overall, 5.1% of global mortality and 4.1% of disability-adjusted life years (DALYs) lost are directly attributable to alcohol [1]. Moreover, both heavy drinking and alcohol use disorders are associated with a range of negative socio-economic effects, e.g. diminished work productivity, traffic accidents, and criminality [2].

There is a growing need for objective, specific and sensitive long-term alcohol markers for both detection and monitoring of harmful and/or chronic alcohol consumption. Clinical and forensic

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http://dx.doi.org/10.1016/j.forsciint.2017.08.022
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applications for such alcohol markers are multiple: from the assessment of excessive alcohol use (e.g. in treatment settings and in forensic cases) to the monitoring of alcohol abstinence, (e.g. during pregnancy, in child custody cases and in liver transplant procedures due to alcoholic liver failure).

Screening for alcohol abuse through questionnaires can be problematic due to participant's denial or recall bias. Indirect alcohol biomarkers, such as aspartate amino transferase (AST-GOT), gamma-glutamyl transferase (GGT) and mean corpuscular volume (MCV) are mainly correlated to liver damage, and are influenced by age, gender and somatic pathologies. As carbohydrate-deficient transferrine (CDT) has a short half-life (8–12 days), its use to detect chronic alcohol consumption is limited. Direct alcohol markers, such as ethyl glucuronide (EtG), a minor phase II metabolite of alcohol, have the advantage of reflecting direct alcohol presence without the biases that influence indirect measures [3].

EtG accumulates in keratinized matrices, such as hair and nails, where it remains detectable for several weeks to months depending on the length of the hair or nail [4]. In comparison to more 'traditional' matrices, such as blood and urine, hair and nails are particularly interesting owing to their long detection window and their ease of sample collection, transport and storage. Over the past years, the monitoring of alcohol consumption through the detection of EtG in hair has been widespread, since EtG in hair has proved to be a reliable specific and sensitive long-term biomarker for the detection of chronic and excessive alcohol consumption [5,6]. The Society of Hair Testing (SoHT) has stated its confidence in EtG as a marker for alcohol and has established guidelines and cut-off values for EtG concentrations in hair [7]. An EtG concentration in hair of >30 pg/mg suggests chronic excessive alcohol consumption, a concentration between 7 and 30 pg/mg corresponds to moderate alcohol consumption, and an EtG concentration of <7 pg/mg does not contradict self-reported abstinence [8].

Research has primarily focused on the detection of EtG in hair, whereas studies on its measurement in nails are sparse [9,10]. However, nail analysis offers multiple advantages over hair analysis [11,12]. First, nails grow slower than hair (3 mm/month for fingernails and 1 mm/month for toenails compared to 1 cm/month for head hair), and would thus allow a more significant accumulation of EtG [13]. This can be relevant in situations where low EtG concentrations need to be determined, e.g., for the differentiation between teetotalers and moderate alcohol consumers. Second, nails can provide an alternative option in cases where hair is not (sufficiently) available (e.g., alopecia, newborns). Third, cosmetic hair treatments have been proven to reduce EtG content in hair, which would be avoided through nail analysis [11,14]. Possible disadvantages of nails can be insufficient sample amount in cases of (finger) nail biting, presence of nail diseases (e.g. bacterial or fungal infections), and effects of nail polishing or cleaning.

To the best of our knowledge, only two studies compared the presence of EtG in hair and nails [15,16]. The studies included hair and fingernails collected from a group of college-students with a limited alcohol consumption. Only Berger et al. considered cosmetic treatment of hair and nails. In the current study, we evaluated EtG concentrations in paired hair and nail samples (both finger- and toenails) from real-life alcohol dependent patients, to investigate the differences and correlations between both matrices. In this way, we evaluated the applicability of nail samples as an alternative to hair for the monitoring of long-term alcohol consumption.

2. Materials and methods

2.1. Reagents

Ethyl glucuronide (EtG) and the internal standard ethyl glucuronide-D_5 (EtG-D_5) in methanol were purchased from Medichem (Stuttgart, Germany). Pentafluoropropionic anhydride (PFPA) was obtained from Sigma Aldrich (Bornem, Belgium). Methanol, ammonium hydroxide solution (25%), ethyl acetate, formic acid (98–100%), and acetone were supplied by Biosolve (Valkenswaard, the Netherlands). All chemical and reagents were of analytical purity grade. Stock solutions of EtG (10 ng/μL) and EtG-D_5 (10 ng/μL) were prepared in methanol. The working solutions were prepared in methanol by further dilution of the stock solutions. All solutions were stored at −20 °C.

2.2. Sample collection

Hair and nail samples were collected from patients engaged in treatment for alcohol use disorders at the psychiatric centers of ZNA Stuivenberg and Multiversum together with an informed consent. The Ethical Committee of the University Hospital of Antwerp (UZA) and the local Ethical Committees of ZNA Stuivenberg and Multiversum approved the study (Belgian registration number B30020169233). Hair samples were collected from the vertex posterior region of the head and cut as closely to the scalp as possible. The first 3 cm segment from the proximal end was used for further analysis. Nail samples were obtained by clipping of the distal edges of all ten finger- and toenails. Finger- and toenail samples were collected and analyzed separately. Samples were stored in aluminum foil at room temperature until analysis.

2.3. Data on alcohol consumption

For all patients, the diagnosis of alcohol use disorder was made by the treating psychiatrist. Together with the hair and nail samples, a detailed anamnesis of past alcohol consumption was taken. Patients were asked about their alcohol consumption in the past 12 weeks using either the Timeline Follow-back (TLFB) method or an in-house questionnaire. The total amount of alcohol (in grams) consumed in the past 12 weeks, and per week, was calculated for each subject.

2.4. Data on cosmetic treatment

Patients were asked whether or not they had bleached, dyed, permed or thermally straightened their hair in the past year, as cosmetic hair treatment may lead to a degradation or removal of EtG in hair [8,17]. Nail polishing, the use of acetone and other nail treatment were also recorded. Patients reporting any cosmetic treatment of the hair or nails were excluded from the study.

2.5. Sample preparation and materials

Nail and hair samples were processed according to a previously described method [18,19]. Briefly, samples were washed with water and acetone, dried, cut into 1–2 mm pieces, and pulverized for 5 min at 30 Hz in a ball mill of type MM400 (Retsch, Haan, Germany). Then, an accurately weighed portion between 15 and 35 mg of each sample was transferred to a tube. To this tube, 2 mL of water was added and the samples were ultrasonicated for 2 h with an ELMA TI-H-15 ultrasonication bath (Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany). Next, samples were spiked with 2.4 ng of EtG-D_5 as internal standard, vortexed, and centrifuged at 5000 rpm for 10 min using a Sigma centrifuge (Osterode am Harz, Germany). Solid-phase extraction was then performed by transferring the supernatant to Oasis® MAX (60 mg, 3 mL) extraction cartridges which were acquired from Waters (New Bedford, MA, USA). The cartridges were placed on a Supelco VisiprepTM SPE Vacuum Manifold (Bellefonte, CA, USA) with 24 ports, and conditioned with 2 mL of methanol and 2 mL of water.

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