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Can ethyl glucuronide in hair be determined only in 3 cm hair strands?☆

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ABSTRACT

This paper addresses the suitability of ethyl glucuronide in hair (EtGH) strands other than 3 cm for alcohol consumption. This issue will be addressed (a) by statistically comparing the distribution of EtGH results for 3 cm hair strands to other hair strands analysed from 4126 cases and (b) by examining the stability of EtGH in an 8 cm hair strand and two 12 cm hair samples of two volunteers and a post-mortem case using 1 cm segmental analysis. For 3464 driving license re-granting Medical and Psychological Assessment (MPA) cases, the detection of alcohol consumption using hair lengths longer than 3 cm was never significantly less than for 3 cm hair lengths, even up to 12 cm hair lengths analysed non-segmented. For 662 non-MPA cases, where, in contrast to MPA cases, generally no abstinence was required, an increase in the EtGH positivity rate was observed with increasing hair length analysed up to 9 cm, indicating that EtG-washout effects seem to play a minor role if any. For both MPA and non-MPA hair samples less than 3 cm, a drastic, significant increase in the number of positive EtGH samples were observed, compared to 3 cm hair lengths, strongly supportive of EtGH incorporation from sweat after a recent alcohol consumption. Segmental studies indicated that EtG is stable in the hair matrix up to 12 cm long, hence supporting the above results. Even though both the statistical and the stability studies are preliminary results which need to be confirmed by other studies, they both provide evidence for the determination of alcohol consumption using EtGH in hair lengths longer than 3 cm. Amendments to the Consensus of the Society of Hair Testing, the German driving license re-granting guidelines and EWDTS hair guidelines with respect to testing for abstinence and/or alcoholism are proposed for the benefit of the donors.

1. Introduction

Ethyl glucuronide in hair (EtGH) is a well-established direct alcohol biomarker, which can differentiate between chronic alcohol abuse, social drinking and abstinence [1–6]. Furthermore, EtGH provides new opportunities for a reliable retrospective evidence of alcohol consumption in otherwise unresolved cases in which false negatives or false positive results with respect to other less sensitive alcohol markers. Due to this and other advantages, EtGH is increasingly routinely being demanded in many contexts including pre-employment drug testing, child-custody cases and driving license re-granting [7,8]. A recent publication analysing a very heterogenous group of samples for whom the alcohol use and the hair washing frequency was not available suggested that "normal" hair hygiene might wash out EtGH [9]. The authors suggested that "*only the most recent month must reliably monitor abstinence or chronic alcohol abuse*." For somewhat other reasons, possibly an easier interpretation of combined EtGH and fatty acids ethyl esters (FAEE) analysis, the first Consensus of the Society of Hair Testing [10] suggested the use of 3 cm hair length for both alcohol markers. There are several studies which document the use of EtGH in hair lengths longer than 3 cm [5,8,11–15]. Unlike FAEE, many studies as the ones mentioned above have shown that EtG concentrations in hair are not dependent on hair length. Due to their peculiar incorporation mechanism in hair, FAEE present major disadvantages when compared to EtGH. Firstly, despite constant drinking behaviour, different FAEE concentrations were found to increase distally [5]. Consequently, investigation of shorter hair samples resulted in lower concentrations at the same drinking behaviour [5]. Secondly, during a drinking period, newly formed FAEE are deposited over the full hair lengths such that a previous change in the drinking pattern, such as a previous period of abstinence, cannot be demonstrated by lower FAEE concentrations in the corresponding hair segments, hence making segmental analysis for FAEE analysis impossible [5,16]. Thirdly, FAEE were found on hair even after strict abstinence, thus resulting in false positive results [5,16]. Thus FAEE cannot differentiate between social

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drinkers and teetotalers [5]. Hence, EtGH seems to be a better marker than FAEE, especially for alcohol abstinence assessment, as clearly stated recently by two separate publications [7,8]. Nonetheless, the stability of EtGH should be addressed in order to assess the suitability of hair lengths longer than 3 cm. Few studies have been published about the stability of EtGH. Reproducible results were obtained for a pool of hair collected from autopsies of alcoholics amounting to 250 pg/mg hair were stable over a 5-week period [1]. In another study, hair from two autopsy cases with EtG values of 110 pg/mg and 300 pg/mg hair varied less than 15% of the initial EtGH concentration over a 4 months' period when kept in the dark at room temperature [18]. Furthermore, segmental hair analysis performed for alcohol withdrawal treatment patients on hair strands up to 8 cm length successfully showed the different drinking behaviour including the beginning of alcohol abstinence or relapse and hence proofing the stability of EtG for several months in old hair segments [11]. The fact that EtGH seems to be stable over a period longer than 3 months justifies the use of hair lengths longer than 3 cm.

Another important factor to be considered when interpreting EtGH results is the incorporation mechanisms of EtGH. Even though, the precise mechanisms involved in the incorporation of EtGH, is not fully understood, various mechanisms have been proposed for the incorporation of drugs in hair including passive diffusion from blood capillaries into growing cells, diffusion from sweat or sebum secretions and from the external environment, such as through sweaty hands and alcohol containing cosmetic products [17]. EtG, being very hydrophilic and acidic, is more likely to be incorporated from sweat than from sebum. Moreover, since the intracellular pH of keratinocytes and melanocytes is more acidic than plasma [17], incorporation of deprotonated EtG from blood to the keratinocytes is thermodynamically unfavourable. This explains the very low concentrations of EtG found in hair.

Even though the first Consensus of the Society of Hair Testing [10] was strictly meant "for chronic excessive alcohol consumption", the suggestion of using 3 cm hair strand was very quickly adopted as regulative by other guidelines, such as the German driving license re-granting Guidelines [19] and Guidelines for European workplace drug and alcohol testing in hair [20], also for abstinence purposes. This has in some cases badly affected hair donors requesting an EtGH test in that their driving license or child custody case was unnecessarily delayed by months due to the scientifically unfounded statement 8 [10]: "The cut-off for EtG in hair to strongly suggest chronic excessive alcohol consumption is proposed at 30 pg/mg scalp hair measured *in the 0–3 cm proximal segment*." For this reason, the present study is intended to support existing publications documenting the utility of EtGH determination in hair lengths other than 3 cm and hence clarify this situation based on our recent findings.

2. Materials and methods

2.1. Statistical study comparing EtGH in 3 cm to other hair lengths

The hair samples used for the statistical investigation were prepared and measured by a previously published method [21] validated fully according to forensic guidelines [22,23]. For the statistical investigation, 4126 hair samples were tested for EtG: 3464 samples were tested for abstinence in order to regain their revoked driving license, while 662 were medico-legal cases. The former needed to undergo the Medical and Psychological Assessment (MPA), in which abstinence from alcohol is required for at least 1 year as part of the driving license re-granting process and hence are referred to throughout this paper as "MPA cases". The other cases are referred to as "non-MPA cases" and represent other medico-legal cases such as child custody. Information regarding alcohol consumption was either not given or was not reliable and hence could not be used in the investigation. However, the division of the samples in MPA and non-MPA reduces the heterogeneity of this large. The EtGH results were classified in three EtG concentrations: (1) less than 7 pg/mg as recommended by the new German guidelines for driving ability diagnostics [19] and other publications [5,7,8,12,20] indicating abstinence or very seldom alcohol consumption, (2) between 7 and less than 30 pg/mg, indicating social drinking habits less than 60 g pure ethanol per day [6,20] and (3) greater than 30 pg/mg hair indicating chronic excessive drinking greater than 60 g pure ethanol per day [10,20]. The EtGH results for the 3 cm hair lengths were compared to shorter and longer hair lengths analysed. The differences observed were tested for significance at the 99% significance level using a self-made Chi squared test using Microsoft Excel.

2.2. Stability study

The method used for extraction of the hair samples for the stability studies has been described previously [12]. The hair specimens were obtained from two volunteers whose alcohol daily intake (EDI) was between 30 and 40 g and a post-mortem case of a well-known female heavy drinker with regular alcohol consumption above 60 g ethanol per day and were cut in 1 cm segments and analysed for EtG.

3. Results and discussion

3.1. EtG in hair method validation

The validation results were presented in detail in previously published HS-SPME–GC–MS/MS method [21] and GC–MS/NCI method [12]. The limits of quantification (LOQ) were 2.8 and 2.3 pg/mg respectively and the limits of detection (LOD) were 0.6 and 0.7 pg/mg respectively using linear regression at 99% significance level and S/N ratio respectively; hence well below the "abstinence" cut-off at 7 pg/mg hair.

3.2. Statistical study comparing EtGH in 3 cm to other hair lengths

In Germany, before the publication of the Consensus of the Society of Hair Testing on hair testing for chronic excessive alcohol consumption 2009 [10], hair lengths other than 3 cm were routinely analysed for EtG for the context of driving license re-granting Medical and Psychological Assessment (MPA). The results for 962 different hair lengths analysed in 2009 are shown in Fig. 1.

3.2.1. EtGH in hair lengths longer than 3 cm

The percentage positive samples, i.e. samples with [EtGH] > 7 pg/mg hair, hence excluding alcohol abstinence or rare consumption found in hair lengths between 3 cm and 6 cm, between 6 cm and 9 cm and between 9 cm and 12 cm were 30%, 26% and 26% respectively compared to 21% for 3 cm hair lengths, see Fig. 1. To our surprise, the percentage positive samples, were not less for hair strands longer than 3 cm as was expected due to the washout effect attributed to the hydrophilic nature of EtG as reported previously [9]. These results were statistically tested for significance using the Chi squared test at the 99% confidence levels.

As shown in Table 1, there was no significant difference between the results obtained for hair samples 3 cm long and the results obtained for other hair lengths greater than 3 cm up to 12 cm. Assuming the same alcohol consumption over the investigated periods, this indicates that possibly hair strands longer than 3 cm are also suitable to be analysed for EtG.

Integrating the more recent MPA EtGH results for 2010 to those above, resulted in a similar distribution to Fig. 1 as seen in Fig. 2. The percentage positive samples, i.e. samples with [EtGH] > 7 pg/mg hair, hence excluding alcohol abstinence or rare consumption, found in hair lengths between 3 cm and 6 cm, between 6 cm and 9 cm and between 9 cm and 12 cm were 28%, 21% and 17% respectively compared to 13% for 3 cm hair lengths, see Fig. 2. As previously mentioned due to the fact that the suggestion of using 3 cm hair strand was adopted as regulative by the German driving license re-granting MPA guidelines [19], a significant increase in the number of 3 cm hair lengths analysed can be observed in this