Detection of the Direct Alcohol Biomarker Ethyl Glucuronide (EtG) in Hair
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ABSTRACT

Ethyl glucuronide (EtG) is considered to be a promising candidate marker of alcohol consumption, but exhibits a short window of detection in blood or urine. Keratinized tissues are known to retain foreign substances and to provide a greater retrospective window of detection than body fluids. Therefore, post-mortem hair, skin swabs, and stratum corneum samples were collected from four subjects with a reported history of alcohol misuse and from seven subjects with a report of regular, socially accepted drinking behavior, and were investigated for EtG. Additionally, certain specimens were collected from three children, who had not yet consumed any alcoholic beverages. EtG was detectable in most of the hair and stratum corneum samples as well as in perspiration stains from alcohol-consuming subjects. The results indicated that EtG might be formed locally in very small and highly variable amounts. The most important finding was that EtG cannot be expected to be generally detectable in keratinized tissues or perspiration stains from alcohol-drinking subjects, whereas a positive result is always associated with recent alcohol consumption.

Implication of this study

This German paper was the first to publish EtG hair data. There were 11 post-mortem cases to supplement a previous unpublished presentation at a conference. This GC/MS EI method used methyl glucuronide as the internal standard with a LOD of 5000 pg/mg using 50 mg of specimen.
**Determination of Ethyl Glucuronide in Human Hair by SPE And LC–MS-MS**


**ABSTRACT**

A method for the sensitive and selective determination of ethyl glucuronide (EtG) in hair has been developed using solid phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). Washed and cut hair segments were extracted by ultrasonication (3 h, 50 °C) and the extracts were cleaned-up with aminopropyl SPE columns. LC–MS/MS analysis was performed using a polar-end capped phenyl-hexyl-RP-phase with negative mode electrospray ionization (ESI) using a triple quadrupole mass spectrometer (Sciex API 365) with a turboionspray source and post-column addition of acetonitrile for enhanced sensitivity. The MS/MS transitions monitored were m/z 221 => 75 for EtG and 226 => 75 for D5-EtG as an internal standard. The method was selective and sensitive, with a detection limit of 51 pg/mg hair at a signal-to-noise ratio of 3:1. The mean recovery was 96%, with an intra- and inter-day precision of less than 11.7% at a concentration of 200 pg/mg. The linearity was assessed in the range of 25–2000 pg/mg hair, with a correlation coefficient of 0.997. The method was successfully applied to 97 human hair samples which were taken at autopsies from persons with known alcoholism or were obtained from alcoholics who were hospitalized for ethanol withdrawal, from social drinkers and from children having not consumed any alcohol. Although, approximately two-third of the alcoholics showed EtG concentrations in hair of higher than 51 pg/mg (up to >4000 pg/mg), in one-third the EtG concentration was below the detection limit. However, only in one of five hair samples of “social drinkers”, the EtG concentration was above the detection limit (51 pg/mg). No EtG has been detected in the hair of children. These investigations demonstrate that heavy alcohol consumption may be but not necessarily has to be detectable by EtG analysis in hair.

**Implication of this study**

Using a total of 97 cases (27 alcoholic post-mortem, 60 alcoholics undergoing treatment, five social drinkers, and five children), the study demonstrated that all of the children were negative, one social drinker was detected with 55 pg/mg, 49 of 87 heavy drinkers were positive with concentrations ranging from 51 to 13000 pg/mg. The method achieved adequate sensitivity using SPE clean-up, an API 365 LC-MS/MS with the post-column addition of acetonitrile and an isotopically labeled internal standard (EtG-d5). The method achieved a LOD of 51 pg/mg using 100 mg of hair. The study was unable to demonstrate a correlation between the amount of drinking reported and the measured concentration of EtG in the hair samples.
Diagnosis of Chronic Alcohol Consumption Hair Analysis For Ethyl-Glucuronide

ABSTRACT

This paper describes a procedure for the detection and quantification of ethyl-glucuronide (EtG) in hair samples. During method development the efficacy of extraction of EtG from hair was compared in four extraction methods: (a) methanol; (b) methanol:water (1:1); (c) water; and (d) water:trifluoroacetic acid (9:1). In addition, three derivatizing agents were compared as well: N,O-bistrimethylsilyl-trifluoroacetamide (BSTFA): trimethylchlorosilane (TMCS) (99:1), pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA). Water was found to be the best extracting solvent and PFPA the best derivatizing agent. Both provided the highest recoveries, with cleaner extracts and more stable derivatives. The final method is as follows: about 100 mg of hair are sequentially washed with water and acetone. The decontaminated sample is finely cut with scissors, then the deuterated internal standard (EtG-d5) and 2 mL of water are added. After sonication for 2 h, the sample is maintained at room temperature overnight. Derivatization is performed with PFPA. Derivatives are injected into a GC–MS system in the electronic impact mode. The method shows linearity over the range of concentrations from 0.050 to 5 ng/mg. Detection and quantification limits are 0.025 and 0.050 ng/mg, respectively. Mean recoveries for the three studied concentrations (low, medium and high) are higher than 87%. The coefficients of variation in intra- and inter-assay precision are always lower than 7%. The method is being routinely applied in our lab for the diagnosis of chronic alcohol consumption.

Implication of this study

This Spanish paper reported seven cases from divorce court. The results ranged from 50 to 750 pg/mg. This GC/MS EI method achieved an LOD of 25 pg/mg using 100 mg hair. This paper was the first to propose EtG in hair for the diagnosis of chronic alcohol consumption. The authors indicated that this method was used routinely in their laboratory in Sevilla, Spain.
Comparison of Ethyl Glucuronide And Fatty Acid Ethyl Ester Concentrations in Hair of Alcoholics, Social Drinkers And Teetotalers

ABSTRACT

In previous investigations hair analysis for ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEE) proved to be suitable for the detection of excessive alcohol consumption. The aim of this study was to compare EtG and FAEE concentrations in hair of alcoholics, social drinkers and teetotalers. Hair samples from 10 alcoholics in withdrawal treatment, 11 fatalities with documented excessive alcohol consumption, four moderate social drinkers who consumed up to 20 g ethanol per day, and three strict teetotalers were analyzed. After external degreasing with n-heptane, extraction with a dimethyl sulfoxide/n-heptane mixture and headspace solid-phase microextraction of the extracts, four fatty acid ethyl esters (FAEES) (ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate) were analysed by gas chromatography–mass spectrometry (GC–MS) with deuterated internal standards. EtG was determined by GC–MS/NCI after ultrasonication of the samples with H2O, cleanup by SPE with aminopropyl columns and PFP derivatisation. The following concentrations were measured for the four groups: teetotallers EtG < 0.002 ng/mg, FAEE 0.05–0.37 ng/mg, moderate social drinkers EtG < 0.002 ng/mg, FAEE 0.26–0.50 ng/mg, alcoholic patients EtG 0.030–0.415 ng/mg, FAEE 0.65–20.50 ng/mg and the fatalities with alcohol history EtG 0.072–3.380 ng/mg, FAEE 1.30–30.60 ng/mg. The results confirm that by using a cut-off value of the sum of FAEE > 1 ng/mg and/or a positive EtG result in hair, excessive alcohol consumption can be identified using hair analysis. However, no significant correlation between the EtG and FAEE concentrations in the positive cases could be shown. Segmental analysis of some of the specimens did not reveal the same distribution for EtG compared to FAEE in hair, and no chronological accordance compared to the self-reported alcohol consumption could be observed for both parameters. These different results of both methods are discussed in terms of differences between EtG and FAEE in mechanism of formation and incorporation into hair and elimination from hair.

Implication of this study

The study from Germany reported three teetotalers and four social drinkers as negative, all 10 alcohol treatment patients between 42 to 415 pg/mg and 11 post-mortems from 72 to 3,380 pg/mg. This GC/MS-NCI method achieved an LOD of 2 pg/mg using 30 mg of hair. The authors suggest using FAEE and EtG in hair in tandem as a means to detect excessive alcohol consumption. However, there was no correlation between EtG and FAEE in hair.
A method for the determination of ethyl glucuronide (EtG) in hair samples, using liquid chromatography/electro-spray tandem mass spectrometry (LC/ESI-MS/MS), was developed and validated. The treatment of hair samples was as follows: to 100 mg of washed (dichloromethane followed by methanol, 1 ml each) and cut (1–2 mm) material, 700 μl of water, 20 μl of internal standard solution (penta-deuterated EtG, D5-EtG, 500 μg/l) and 20 μl of methanol were added. Samples were incubated at 25 °C overnight and then ultra-sonicated for 2 h. Finally, 8 μl of the centrifuged solution (13 000 rpm) were analyzed by LC/ESI-MS/MS in negative ion mode. The surviving ions of EtG and D5-EtG were monitored together with the following MRM transitions: m/z 221 → 75, m/z 221 → 85 (EtG) and m/z 226 → 75, m/z 226 → 85 (D5-EtG). The method exhibited a mean correlation coefficient better than 0.9998 over the dynamic range (3–2000 pg/mg). The lower limit of quantification (LLOQ) and the limit of detection (LOD) were 3 and 2 pg/mg respectively. The intra- and inter-day precision and accuracy were studied at four different concentration levels (3, 5, 56 and 160 pg/mg) and were always better than 7% (n = 5). Matrix effects did not exceed 20%. The method was applied to several hair samples taken from autopsies of known alcoholics, from patients in withdrawal treatment, from social drinkers, from adult teetotalers and from children not exposed to ethanol, with EtG concentrations globally ranging from 2 to 4180 pg/mg.

Implication of this study

This Italian study, which used an assay with an LOD of 2 pg/mg, differentiated between alcohol involved post-mortem cases (LOD to 4180), alcohol withdrawal treatment patients (LOD to 434 pg/mg) and social drinkers (LOD to 34 pg/mg). This study was instrumental to establish the Society of Hair Testing cutoff of 30 pg/mg.
**Ethyl Glucuronide in Hair: Is it a Reliable Marker of Chronic High Levels of Alcohol Consumption?**

**ABSTRACT**

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.

**Implication of this study**

This Italian study used a detailed questionnaire to determine the ethanol daily intake from a variety of risk groups. This study reported that they were able to discriminate seven teetotalers as negative, 21 alcoholics (4-435 pg/mg) and 21 social drinkers (LOD to 35.4 pg/mg). The authors reported that a correlation exists between EtG in hair and reported ethanol daily intake.
ABSTRACT

Hair differs from other materials used for toxicological analysis because of its unique ability to serve as a long-term storage of foreign substances with respect to the temporal appearance in blood. Over the last 20 years, hair testing has gained increasing attention and recognition for the retrospective investigation of chronic drug abuse as well as intentional or unintentional poisoning. In this paper, we review the physiological basics of hair growth, mechanisms of substance incorporation, analytical methods, result interpretation and practical applications of hair analysis for drugs and other organic substances. Improved chromatographic–mass spectrometric techniques with increased selectivity and sensitivity and new methods of sample preparation have improved detection limits from the ng/mg range to below pg/mg. These technical advances have substantially enhanced the ability to detect numerous drugs and other poisons in hair. For example, it was possible to detect previous administration of a single very low dose in drug-facilitated crimes. In addition to its potential application in large scale workplace drug testing and driving ability examination, hair analysis is also used for detection of gestational drug exposure, cases of criminal liability of drug addicts, diagnosis of chronic intoxication and in postmortem toxicology. Hair has only limited relevance in therapy compliance control. Fatty acid ethyl esters and ethyl glucuronide in hair have proven to be suitable markers for alcohol abuse. Hair analysis for drugs is, however, not a simple routine procedure and needs substantial guidelines throughout the testing process, i.e., from sample collection to results interpretation.

Implication of this paper

This paper was the first invited review article that included the detection of EtG detection in hair.
Segmental Determination of Ethyl Glucuronide in Hair: A Pilot Study

ABSTRACT

Ethyl glucuronide (EtG) is a minor metabolite of ethanol. Its detection in hair is more and more studied in both clinical and forensic context for the purpose of alcohol abuse monitoring. In this pilot study, hair specimens from 15 patients included in a treatment program after alcohol abuse cessation, were segmented and analyzed for EtG. The results were then compared to their self-reported past alcohol consumption and to their blood biomarkers values (GGT, MCV, ASAT, ALAT). EtG concentrations measured in hair varied from 8 to 261 pg/mg. The pattern of EtG concentration detected in the different hair segments matched with the drinking history of patients, displaying variations (increase and decrease) in alcohol consumption and also time of cessation. Results also demonstrated the existence of a significant correlation ($r_p = 0.5357; p = 0.0390$) between EtG concentration in hair and the amount of alcohol intake. Variations in the EtG concentrations with respect to hair segments may provide an overview of the drinking history of patients. Moreover, EtG concentration in hair may help to estimate the daily alcohol intake.

Implication of this study

This study out of Luxembourg analyzed the self-report, hair and blood from 15 alcohol treatment patients with results ranging from 24-261 pg/mg and demonstrated further evidence of the existence of the correlation between EtG in hair and the reported amount of ethanol consumption.
Comparison of Ethyl Glucuronide in Hair With Phosphatidylethanol in Whole Blood as Post-Mortem Markers of Alcohol Abuse


ABSTRACT

Ethyl glucuronide (EtG) is a direct metabolite of ethanol and has been used as a marker of alcohol abuse in both urine and hair. This study investigated the value of EtG testing in post-mortem hair for diagnostic improvement of alcohol abuse in forensic medicine. Material from 70 consecutive medico-legal autopsies was collected in accordance with the recommendations on ethics by the Swedish National Board of Forensic Medicine. A method for determination of EtG in hair samples was developed using ultra performance liquid chromatography/electrospray tandem mass spectrometry (UPLC/ESI-MS/MS; LOQ, 2.5 pg/mg). The result of the EtG analysis was compared with the findings of phosphatidylethanol (PEth) in femoral whole blood, as measured by high performance liquid chromatography with an evaporative light-scattering detector (HPLC–ELSD; LOQ, 0.22 mmol/l). Evaluation of liver histology and anamnestic evidence of alcohol abuse of the deceased were taken in consideration for the interpretation. Measurable levels of EtG were present in 49 of the 70 autopsy cases whereas PEth was present in 36. Thirty-nine cases had EtG levels above the cutoff limit (~30 pg/mg) compared with 29 for PEth (0.7 mmol/l). Fifteen cases had EtG as exclusive indicator for alcohol abuse compared with four cases for PEth. These findings suggest that measurements of EtG in hair may provide improved diagnostic information on alcohol abuse, due to a long retrospective time-window for detection and stability of EtG in hair in the decaying cadaver. However, an EtG level below the cutoff does not completely exclude previous alcohol abuse.

Implication of this study

A Swedish group analyzed the hair of 70 consecutive post-mortem cases that were at high risk of alcohol involvement. There were 49 positives with the EtG in hair concentrations ranging from 7.5 to 10,400 pg/mg. The authors indicate that a negative does not prove abstinence but demonstrates the utility of EtG hair determinations in post-mortem analysis.
Markers of Chronic Alcohol Use in Hair: Comparison of Ethyl Glucuronide And Cocaethylene in Cocaine Users
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ABSTRACT

Two direct ethanol metabolites, namely ethyl glucuronide (EtG) and cocaethylene (CE), in the hair of cocaine (COC) users were compared in this study. Hair samples (n = 68) were submitted to the determination of EtG (by liquid chromatography-electrospray-tandem mass spectrometry) and of COC and metabolites, including CE (by gas chromatography-mass spectrometry). Quantitative and qualitative results were compared. No quantitative correlation was found between EtG and CE, as well as between EtG and the cocaethylene concentration divided by the concentration of COC and its metabolites (benzoylecggonine and egonine methylester, as COC equivalents). Nevertheless, many factors are supposed to affect the amount of the two substances incorporated in the hair matrix, such as the subject’s habits in ethanol and COC use, genetic variability in the metabolism of both substances, and the different chemical and physical properties of EtG and CE. When establishing a cut-off of 4 pg/mg for EtG and of 200 pg/mg for CE, 47 samples tested positive for EtG and 41 samples tested positive for CE; 12 samples out of the 47 EtG-positives tested negative for CE (25%), whereas 6 samples out of the 41 CE-positives tested negative for EtG (15%). According to these data, EtG appears to be a more sensitive and specific marker of non-moderate alcohol users than CE.

Implication of this study

The study evaluated 68 cases positive for cocaine in hair. From this high-risk population, there were 47 EtG hair positives ranging from LOD to 184 pg/mg. However, the hair samples positive for EtG did not correlate to cocaethylene, a cocaine plus ethanol metabolite.
Ethyl glucuronide (EtG) is a minor metabolite of ethanol that can be detected in hair. In some specific situations, head hair can be missing, and therefore, alternative anatomical locations of hair are of interest. In this study, paired hair specimens (head hair and pubic hair) from eight social drinkers were analyzed for EtG. Each sample was decontaminated by two dichloromethane bashes (5 ml) for 2 min. After cutting into small pieces, about 50 mg of hair was incubated in 2 ml water in the presence of 10 ng of EtG-d5, used as internal standard and submitted to ultra-sonication for 2 h. The aqueous phase was extracted by SPE using Oasis MAX columns. The hair extract was separated on an ACQUITY BEH HILIC column using a gradient of acetonitrile and formate buffer. Detection was based on two daughter ions: transitions m/z 221–85 and 75 and m/z 226–75 for EtG and the IS, respectively. This laboratory is using a positive cut-off at 50 pg/mg. All eight head hair specimens were negative for EtG at a limit of quantitation fixed at 10 pg/mg. Surprisingly, EtG was identified at high concentrations in pubic hair, in the range 12–1370 pg/mg. It appears, therefore, that it is not possible to document the drinking status of a subject by simply switching from head hair to pubic hair.

Implication of this study

This French study evaluated eight cases that were identified as social drinkers. The head hair specimens were negative and the pubic hair specimens were positive, which ranged from 12 to 1370 pg/mg. This observation is not surprising because pubic hair is older, coarser and is routinely contaminated with EtG tainted urine. Similar observations have been reported with other drugs of abuse.
Ethylglucuronide Determination in Urine And Hair From Alcohol Withdrawal Patients

**ABSTRACT**

Two methods for the determination of ethyl glucuronide (EtG) in urine and in hair have been developed by liquid chromatography–tandem mass spectrometry. These two methods were fully validated, including linearity (0.25–100 μg/mL in urine; 0.05–5 ng/mg in hair; r² > 0.99, n = 5), limits of detection (0.1 μg/mL in urine, 0.025 ng/mg in hair) and quantitation (lowest level of the calibration curve), extraction efficiency (> 55%), within-day and between-day imprecision and bias (CV and mean relative error < 15%), matrix effect, and relative ion intensity. These methods have been applied to 541 urine samples and 17 hair specimens collected from 156 alcohol withdrawal patients. The determination of ethanol versus EtG in urine was compared, and also the convenience of EtG determination in hair. EtG in urine and in hair proved to be a powerful tool for monitoring abstinence in these patients.

**Implications of this study**

Originating out of Spain, the study analyzed 17 withdrawal patients with EtG hair concentrations between 90 and 640 pg/mg. The method did not use a SPE clean-up and proved to have inadequate sensitivity.
The Diagnosis of Alcoholism Through The Identification of Biochemical Markers in Hair

ABSTRACT
Alcoholic beverages and the heavy problems linked to their abuse have been familiar in human societies since the beginning of recorded history. Alcoholism is a social, economic and medical question, that involve a wide population in almost all ethnic groups, and the evidence of alcohol abuse is often very difficult mainly for the capability of abusers to keep secret their trouble. Hence the clinician needs various information to make the exact diagnosis, including the acquisition of the complete history of the patient and the investigation of clinical signs. Today, the study of such a topic could be markedly improved by the systematic use of laboratory tests, such as blood ethanol, serum gamma-glutamyl transferase (γ-GT), the mean corpuscular volume of erythrocytes (MCV) and the carbohydrate-deficient transferrin (CDT), currently the most specific marker of alcohol abuse. In the last years some minor ethanol metabolites in hair matrix (in particular ethyl glucuronide and fatty acid ethyl esters) have been studied, for the unique ability of hair to serve as a long-term storage of xenobiotics with respect to the temporal appearance in blood. Over the last 20 years in fact, hair testing has gained increasing attention for the retrospective investigation of chronic drug abuse because of the window of drug detection is dramatically extended to weeks and months. The chance to detect minor ethanol metabolites in hair have been proposed in the early 2000 and ethyl glucuronide and fatty acid ethyl esters seem to satisfy the prerequisites requested by the alcoholism diagnosis. Ethyl glucuronide (EtG) is a non-volatile, water soluble, direct metabolite of ethanol. It has received much recent attention as a sensitive and specific biological marker of alcoholism. Formed in the liver via conjugation of ethanol with activated glucuronate, EtG remains detectable in serum, plasma, and hair for days after ethanol abuse. The use of this marker detected in hair alone and complementary with other biological state markers is expected to lead to significant improvement in treatment outcome, therapy efficacy and cost reduction. Fatty acid ethyl esters (FAEE) are products of the non-oxidative ethanol metabolism, which are known to be detectable in blood about 24 h after the last alcohol intake. After deposition in hair they should be suitable long-term markers of chronically elevated alcohol consumption. It was shown by some investigations that FAEE are also present in sebum, that there is no strong difference in their concentrations between pubic, chest and scalp hair, and that they are detectable in hair segments after a 2 months period of abstinence. From these experiences it follows that the measurement of FAEE concentrations in hair is a useful way for a retrospective detection of alcohol abuse, able to discriminate between heavy drinkers and teetotallers. Moreover the use of FAEE into neonatal hair to objectively identify children exposed to alcohol in utero may be a helpful approach to diagnose fetal alcohol spectrum disorder.

Implications of this article
This article was written as a review of numerous alcohol biomarkers in hair for the textbook "The Handbook of Neuropsychiatric Biomarkers, Endophenotypes and Genes".
ABSTRACT

Ethyl glucuronide (EtG) is a minor and direct metabolite of ethanol. EtG is incorporated into the growing hair allowing retrospective investigation of chronic alcohol abuse. In this study, we report the development and the validation of a method using gas chromatography–negative chemical ionization tandem mass spectrometry (GC–NCI-MS/MS) for the quantification of EtG in hair. EtG was extracted from about 30mg of hair by aqueous incubation and purified by solid-phase extraction (SPE) using mixed mode extraction cartridges followed by derivation with perfluoropentanoic anhydride (PFPA). The analysis was performed in the selected reaction monitoring (SRM) mode using the transitions m/z 347→163 (for the quantification) and m/z 347→119 (for the identification) for EtG, and m/z 352→163 for EtG-d5 used as internal standard. For validation, we prepared quality controls (QC) using hair samples taken post mortem from 2 subjects with a known history of alcoholism. These samples were confirmed by a proficiency test with 7 participating laboratories. The assay linearity of EtG was confirmed over the range from 8.4 to 259.4 pg/mg hair, with a coefficient of determination ($r^2$) above 0.999. The limit of detection (LOD) was estimated with 3.0 pg/mg. The lower limit of quantification (LLOQ) of the method was fixed at 8.4 pg/mg. Repeatability and intermediate precision (relative standard deviation, RSD%), tested at 4 QC levels, were less than 13.2%. The analytical method was applied to several hair samples obtained from autopsy cases with a history of alcoholism and/or lesions caused by alcohol. EtG concentrations in hair ranged from 60 to 820 pg/mg hair.

Implication of this study

Originating out of Switzerland, the study analyzed seven alcohol-related post-mortem 61 to 819 pg/mg using GCMSMS with an LOD of 3 pg/mg.
Comparison of Ethyl Glucuronide in Hair With Carbohydrate-Deficient Transferrin in Serum as Markers of Chronic High Levels of Alcohol Consumption

ABSTRACT
This study was designed with the aim to compare sensitivity and specificity of ethyl glucuronide in hair (HEtG) and carbohydrate-deficient transferrin (CDT) in serum as markers of heavy drinking. Eighty-six volunteers, including teetotalers, social, and heavy drinkers, were interviewed to evaluate their ethanol daily intake (EDI) during the last 2-week and 3-month periods. HEtG determination was performed by a fully validated LC–MS–MS procedure and ranged from <LOD (2 pg/mg) to 890.5 pg/mg. CDT was measured by immunonephelometry or by HPLC. Sensitivity and specificity of the two markers as indicators of an EDI higher than 60 g/day were calculated, with cut-off at 27 pg/mg (HEtG) and 2.5% (CDT). Considering the EDI of the last 2 weeks, HEtG showed equal selectivity (0.93 for both HEtG and CDT-immunonephelometry; 0.70 for both HEtG and CDT-HPLC) and 2 times the sensitivity of either of the two CDT methods (1.00 vs. 0.44 for CDT-immunonephelometry; 0.96 vs. 0.50 for CDT-HPLC). The same difference in performances but with higher absolute sensitivity and selectivity values for HEtG were observed considering the EDI of the last 3-months (selectivity: 1.00 for both HEtG and CDT-immunonephelometry, 0.89 and 0.78 for HEtG and CDT-HPLC, respectively; sensitivity: 1.00 vs. 0.47 for CDT-immunonephelometry; 0.98 vs.0.51 for CDT-HPLC). Our results indicate that HEtG, as compared to CDT measured using different methods, is a selective marker of ethanol heavy chronic use providing considerably higher sensitivity.

Implications of the study
This Italian paper demonstrated improved sensitivity and specificity for EtG hair over the indirect biomarker CDT and self-report.
Hair Analysis Versus Conventional Methods of Drug Testing in Substance Abusers Seeking Organ Transplantation

ABSTRACT

As substance abusers need to demonstrate abstinence prior to transplant, valid/reliable drug tests are needed. Patients may deny use, fearing surgery will be delayed. Breath, blood and urine tests have brief detection windows that allow patients to evade detection. Routine laboratory tests do not include all substances of abuse. Hair analysis overcomes these barriers, increasing the likelihood that active users will be identified. This study compared results for alcohol, opioids and cocaine based on 445 self-report, breath, urine and hair samples from 42 patients who had been denied a transplant due to recent substance abuse. Compared to hair toxicology, sensitivity for conventional drug tests was moderate for cocaine and opioids, but poor for alcohol. Of positive hair tests, only half were corroborated through other tests. In contrast, specificity was high across tests and substances, with positive findings from conventional tests confirmed through hair toxicology. Based on a 90-day detection window for hair analysis, two negative tests suggest 6 months of continuous abstinence. Hair testing should be considered as an alternative approach for monitoring substance use in the transplant population, either as a routine procedure or when the veracity of findings from conventional tests is in doubt.

Implication of this study

The study tested 42 liver transplant patients at a New York City hospital at four different times. The assay produced 26 positives and demonstrated the utility of using EtG hair to monitor clandestine drinking among this demographic.
**Estimating Driver Risk Using Alcohol Biomarkers, Interlock Blood Alcohol Concentration Tests And Psychometric Assessments: Initial Descriptives**


**ABSTRACT**

**Aim**
To identify alcohol biomarker and psychometric measures that relate to drivers’ blood alcohol concentration (BAC) patterns from ignition interlock devices (IIDs).

**Design, setting, participants, measurements**
In Alberta, Canada, 534 drivers, convicted of driving under the influence of alcohol (DUI), installed IIDs and agreed to participate in a research study. IID BAC tests are an established proxy for predicting future DUI convictions. Three risk groups were defined by rates of failed BAC tests. Program entry and follow-up blood samples (n = 302, 171) were used to measure phosphatidyl ethanol (PETH), carbohydrate deficient transferrin (%CDT), gamma glutamyltransferase (GGT) and other biomarkers. Program entry urine (n = 130) was analyzed for ethyl glucuronide (ETG) and ethyl sulphate (ETS). Entry hair samples were tested for fatty acid ethyl esters (FAEE) (n = 92) and ETG (n = 146). Psychometric measures included the DSM-4 Diagnostic Interview Schedule Alcohol Module, Alcohol Use Disorders Identification Test (AUDIT), the time-line follow-back (TLFB), the Drinker Inventory of Consequences (DRINC) and the Temptation and Restraint Inventory (TRI).

**Findings**
Except for FAEE, all alcohol biomarkers were related significantly to the interlock BAC test profiles; higher marker levels predicted higher rates of interlock BAC test failures. PETH, the strongest with an overall analysis of variance F ratio of 35.5, had significant correlations with all nine of the other alcohol biomarkers and with 16 of 19 psychometric variables. Urine ETG and ETS were correlated strongly with the IID BAC tests.

**Conclusions**
The findings suggest that several alcohol biomarkers and assessments could play an important role in the prediction and control of driver alcohol risk when re-licensing.

**Implication of this study**
This study followed 137 driver interlock DUI offenders from Canada. The levels found ranged from 0.7 to 487 pg/mg. The authors reported that EtG hair results were well-correlated with self-report in this study and that EtG levels were predictive of ignition interlock failed attempts.
Effect of Bleaching on Ethyl Glucuronide in Hair: An in vitro Experiment
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ABSTRACT

Introduction: Ethyl glucuronide in hair (HEtG) has recently gained great attention, because of its high sensitivity and specificity in the diagnosis of chronic alcohol abuse. Due to its high polarity hydrophilicity, a strong hair treatment followed by a shampooing may lead to removal/degradation of this molecule from hair matrix.

Aim: To set up an in vitro study in order to evaluate the ability of bleaching of modifying HEtG test results. Methods: Thirty hair samples from teetotalers (n = 5), social drinkers (n = 4) and heavy drinkers (n = 21), after an informed written consent, were collected and divided longitudinally into four aliquots. The first aliquot was kept untreated and was processed following the method routinely used in our lab for the determination of HEtG (double washing with methanol/dichloromethane, overnight incubation in water, and LC–MS/MS analysis, LLOQ: 3 pg/mg). To the other three aliquots a commercially available bleaching solution was applied, according to the manufacturer’s instructions. One out of the three aliquots was submitted to the analysis by following the same procedure used for the untreated sample. The other two were submitted to a purification step before LC–MS/MS analysis, by using two different SPE cartridges (aminopropyl and dimethyl butylamine).

Results: HEtG levels in the untreated samples from social drinkers and heavy drinkers ranged from 7.7 to 149.0 pg/mg. All the samples from teetotalers tested negative. The treated samples processed without any SPE extraction and with aminopropyl cartridges showed a relevant ion suppression for both EtG and D5-EtG (IS) signals. Samples treated with the bleaching solution and extracted with dimethyl butylamine cartridge allowed to sensitively reduce ion suppression (less than 35%) and to verify that EtG, after a strong treatment like bleaching, completely disappears.

Conclusions: This in vitro study showed that HEtG disappears from hair matrix after a strong hair treatment. It is not clear whether the mechanism involved is chemical degradation or physical removal from the damaged keratinic matrix. However, owing to the highly hydrophilic character of the compound, the second mechanism seems more likely to occur. Finally, bleaching solutions could lead to a heavy ion suppression of this metabolite that may be avoided by using an SPE purification before instrumental analysis.

Implication of this study

This Italian study evaluated 30 cases: five negative teetotalers, four positive social drinkers with 7-15 pg/mg and 21 heavy drinkers 35.8 to 149 pg/mg. The authors demonstrated that bleaching affects the EtG hair test.
** Combined Use of Fatty Acid Ethyl Esters And Ethyl Glucuronide in Hair For Diagnosis of Alcohol Abuse: Interpretation And Advantages **

** ABSTRACT **

In this study the combined use of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) for diagnoses of chronically excessive alcohol abuse is investigated at 174 hair samples from driving ability examination, workplace testing and child custody cases for family courts and evaluated with respect to the basics of interpretation. Using the cut-off values of 0.50 ng/mg for FAEE and 25 pg/mg for EtG, both markers were in agreement in 75% of the cases with 103 negative and 28 positive results and there were 30 cases with FAEE positive and EtG negative and 13 cases with FAEE negative and EtG positive. As the theoretical basis of interpretation, the pharmacokinetics of FAEE and EtG is reviewed for all steps between drinking of ethanol to incorporation in hair with particular attention to relationships between alcohol dose and concentrations in hair. It is shown that the concentrations of both markers are essentially determined by the area under the ethanol concentration in blood vs. time curve AUCEtOH, despite large inter-individual variations. It is demonstrated by calculation of AUCEtOH on monthly basis for moderate, risky and heavy drinking that AUCEtOH increases very strongly in the range between 60 and 120 g ethanol per day. This specific feature which is caused by the zero-order elimination of ethanol is a favorable prerequisite for a high discrimination power of the hair testing for alcohol abuse. From the consideration of the different profiles of FAEE and EtG along the hair and in agreement with the literature survey, a standardized hair segment 0–3 cm is proposed with cut-off values of 0.5 ng/mg for FAEE and 30 pg/mg for EtG. This improves also the agreement between FAEE and EtG results in the cases of the present study. A scheme for combined interpretation of FAEE and EtG is proposed which uses the levels of abstinence and the double of the cut-off values as criteria in addition to the cut-offs. Considering the large variations in the relationship between ethanol dose and FAEE and EtG concentrations in hair, the combined use of both parameters strongly increases the accuracy of the diagnosis by mutual confirmation and identification of false positive or false negative results due to biological variations or analytical errors.

** Implication of this study **

This paper reported 174 cases from Germany. There were 99 cases that were less than 7 pg/mg and 75 cases that ranged 7-5270 pg/mg. The authors recommended that the long-term markers, such as EtG hair, were proportional to the Area Under the Blood Alcohol Curve as opposed to the raw number of drinks consumed. For example, an individual that has a couple of binge episodes will more likely be positive than someone who routinely sips a couple a drinks daily (never achieved an appreciable blood alcohol content).
Impact of Hair-Care Products on FAEE Hair Concentrations in Substance Abuse Monitoring
doi: 10.1007/s00216-011-4685-0

ABSTRACT

Previous studies have indicated that the use of high-ethanol-content (>65%) hair-care products may elevate fatty acid ethyl ester (FAEE) concentrations in hair. In this case series, nine individuals were identified by FAEE analysis to be chronic alcohol abusers in the context of child-welfare substance abuse monitoring. Based on patient claims of moderate or no alcohol consumption, the presence of ethanol in the patients’ hair-care regimens was investigated. Samples were additionally tested for the presence of ethyl glucuronide (EtG). From a total of nine patients, 12 hair samples were submitted for analysis. Patient histories were obtained as well as Material Safety Data Sheets (MSDS) listing hair-care product ethanol content. Hair samples were pre-washed to remove external contamination and analyzed for FAEE and EtG by GC-MS. According to the Society of Hair Testing consensus guidelines, FAEE levels exceeding 0.50 ng/mg and/or EtG levels exceeding 30 pg/mg indicate chronic excessive alcohol consumption. Upon initial analysis, the nine samples exhibited positive FAEE findings ranging from 0.496 to 4.984 ng/mg. MSDS review revealed the presence of ethanol from 10% to 95% by volume in at least one hair care product used by each individual. Results of the EtG analysis ranged from 1.9 to 23.5 pg/mg. These findings indicate that regular use of products with ethanol content as low as 10% can impact FAEE results. EtG analysis should be used to confirm FAEE findings and appears to be unaffected by hair-care products, likely due to alternative mechanisms of incorporation.

Implication of this study

This study analyzed the hair from nine patients that were contesting positive FAEE hair results that were claiming moderate to no drinking. The determined EtG concentrations ranged from 1.9 to 23.5 pg/mg. The authors demonstrated that the presence of ethanol in numerous hair care products generated false positive FAEE results, whereas, the EtG results were unaffected. This study clearly demonstrated that EtG detection in hair was the preferred analyte for long-term drinking detection.
Ethyl Glucuronide in Human Hair After Daily Consumption of 16 or 32g of Ethanol For 3 Months

ABSTRACT

The overall objectives of the study were to develop a sensitive method for ethyl glucuronide (EtG) determination in hair and then investigate if a low or moderate intake of ethanol could be differentiated from total abstinence. Forty-four subjects were included in the study, 12 males (7 drinkers and 5 abstinent) and 32 females (14 drinkers and 18 abstinent). The study lasted 3 months and the female drinkers consumed one glass (16 g of ethanol) and the males consumed two glasses (32 g of ethanol) of wine (13.5–14%) daily. Hair samples were collected as close as possible above the skin and the proximal 2 cm were analyzed for EtG. Hair was cut into pieces of about 0.5 cm length and washed before incubation overnight in water and then extracted on Clean Screen EtG Carbon columns. The LC/MS/MS system consisted of a Waters ACQUITY UPLC connected to an API4000 triple quadrupole instrument. Two transitions for EtG and one for the internal standard EtG-D5 were measured. The method was linear from 60 to 10,000 pg/sample. Imprecision studies were performed at three levels as well as with an authentic sample. Total imprecision was 16% at 200 pg/sample, 8% at 1000 pg/sample, 6% at 8000 pg/sample and 13% at 29 pg/mg in the authentic sample. Of those who drank two glasses of wine every day, four had measurable amounts of EtG in their hair (5–11 pg/mg), and in only one of the females drinking one glass of wine EtG was quantified (3 pg/mg). Among the 23 abstinent subjects two had traces of EtG in the hair. We conclude that persons who ingested 16 or 32 g of ethanol daily for 3 months presented with low concentrations of EtG in hair, well below the proposed threshold for over consumption set at 30 pg/mg. In addition, none of those who ingested 16 g/day had concentrations over the proposed abstinence threshold of 7 pg/mg.

Implication of this study

A Swedish group evaluated 22 controls and 21 participants consuming one to two drinks per day for 90 days. The controls and 16 participants were negative. Five of the participants had low levels of EtG detected in their hair ranging from 3-11 pg/mg. This study confirms that the detection of EtG in hair is dependent on the drinking behavior as opposed to simply the number of drinks. The males in this study drank 180 drinks over a 90-day period, but only consuming two drinks per day and therefore not obtaining appreciable blood alcohol content. None was above the arbitrary 30 pg/mg cutoff.
Correlation of the Alcohol Biomarker Ethyl Glucuronide in Fingernails and Hair to Reported Alcohol Consumed

M. Jones, J. Jones, D. Lewis, C. Plate, M. Fendrich, L. Berger, & D. Fuhrmann. (2011, June). Poster session at the annual conference for the Research Society on Alcoholism, Atlanta, GA.

ABSTRACT

The goal of this study was to determine if a relationship existed between the reported number of drinks consumed over a 90 day period and the measured concentration of the direct alcohol biomarker ethyl glucuronide (EtG) in the fingernails and hair of a college-aged population. This IRB-approved study was conducted by enrolling 606 consented college students from the University of Wisconsin-Milwaukee, determining the number of drinks reported over a 90 day period using the time line follow back (TLFB) interview instrument, and collecting fingernail clippings and head hair from each study participant. Biological specimens were sent to USDTL to determine EtG levels. EtG was analyzed by liquid chromatography combined with tandem mass spectrometry. One method of data analysis utilized was determining the Pearson correlation coefficient between each individual’s fingernails and hair EtG levels and the number of drinks that the individual reported to have consumed in the previous 90 days to providing the nail and hair samples. For 271 females and 180 males enrolled in this study the Pearson correlation coefficient for EtG in fingernails and number of drinks reported was 0.5153 (p < .0001) and 0.6376 (p < .0001), respectively. This indicates a large positive correlation between the amount of EtG present in the fingernails and the reported number of drinks consumed. For 178 males the Pearson correlation coefficient for EtG in hair and number of drinks reported was 0.5548 (p < .0001), again indicating a large positive correlation between the number of drinks reported consumed and the amount of EtG present in the hair. For 276 females the Pearson correlation coefficient for EtG in hair and number of drinks reported was 0.2752 (p < .0001), indicating only a small positive correlation between the number of drinks reported consumed and the EtG present in the hair. Thus, for EtG in hair, there is a gender bias. These findings demonstrate that the levels of the direct alcohol biomarker EtG in fingernails and hair have significant correlations with the number of drinks reported consumed. These findings further demonstrate that fingernails are the preferred specimen for EtG analysis, as they lack the gender bias seen with hair.

Implications of this study

This study demonstrated a significant correlation between EtG hair, EtG nail and self-reported ethanol intake. The study showed that nail was a suitable alternative to hair for EtG testing. The study further demonstrated a gender bias in testing hair for the direct alcohol biomarker EtG. Lastly, there is further evidence that there appears to be at least two factors that affect the uptake of EtG by nails and hair. First, the actual amount of alcohol consumed over time and second, the drinking style by which the alcohol is consumed (non-hazardous versus hazardous drinking).
Chemometric Evaluation of Nine Alcohol Biomarkers in a Large Population of Clinically Classified Subjects: Pre-eminence of Ethyl Glucuronide Concentration in Hair For Confirmatory Classification.

doi: 10.1007/s00216-011-5314-7

ABSTRACT

An important goal of forensic and clinical toxicology is to identify biological markers of ethanol consumption that allow an objective diagnosis of chronic alcohol misuse. Blood and head hair samples were collected from 175 subjects-objectively classified as non-drinkers (N=65), social drinkers (N=51) and active heavy drinkers (N=59)-and analyzed to determine eight traditional indirect biomarkers of ethanol consumption [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (γ-GT), alkaline phosphatase (ALP), mean corpuscular volume (MCV), carbohydrate-deficient transferrin (CDT), and cholesterol and triglycerides in blood] and one direct biomarker [ethyl glucuronide (EtG) in head hair]. The experimental values obtained from these determinations were submitted to statistical evaluations. In particular, Kruskal-Wallis, Mann-Whitney and ROC curve analyses, together with principal component analysis (PCA), allowed the diagnostic performances of the various biomarkers to be evaluated and compared consistently. From these evaluations, it was possible to deduce that EtG measured in head hair is the only biomarker that can conclusively discriminate active heavy drinkers from social and non-drinkers, using a cut-off value of 30 pg/mg. In contrast, a few indirect biomarkers such as ALP, cholesterol, and triglycerides showed extremely low diagnostic abilities and may convey misleading information. AST and ALT proved to be highly correlated and exhibited quite low sensitivity and specificity. Consequently, either of these parameters can be discarded without compromising the classification efficiency. Among the indirect biomarkers, γ-GT provided the highest diagnostic accuracy, while CDT and MCV yielded high specificity but low sensitivity. It was therefore concluded that EtG in head hair is the only biomarker capable of supporting a confirmatory diagnosis of chronic alcohol abuse in both forensic and clinical practice, while it was found that γ-GT, CDT, MCV, and AST--whether used alone or in combination--do not allow the conclusive classification of subjects according to ethanol consumption. However, a diagnostic strategy combining these four parameters could be formulated in order to create a multivariate model capable of screening suspected active heavy drinkers.

Implications of this study

This study compared the diagnostic performance of EtG in hair with eight indirect alcohol biomarkers in blood (AST, ALT, ALP, GGT, MCV, CDT, cholesterol, and triglycerides) to identify heavy drinkers in a clinically diagnosed population (n = 175). EtG showed very high sensitivity and specificity and was the only alcohol biomarker able to “conclusively discriminate” between heavy and non-heavy drinkers (98% correctly diagnosed). The study also demonstrated that EtG seems to be unaffected by previous occurrences of liver disease in patients.
Determination of Ethyl Glucuronide Levels in Hair For The Assessment of Alcohol Abstinence.

ABSTRACT

This study examined the potential of a highly sensitive LC-MS/MS method for the determination of EtG in head hair (i) to ascertain alcohol abstinence, (ii) to estimate the basal level of EtG (sub-ppb concentrations) in head hair in a population of alcohol abstainers and (iii) to suggest a revision of cut-off values for assessing alcohol abstinence. An UHPLC-MS/MS protocol previously developed was modified and validated again to detect low EtG levels in head hair samples from a population of 44 certain abstainers and teetotalers. Basal level of EtG in hair was determined by a standard addition quantification method. The validated UHPLC-MS/MS method allowed detecting and quantifying 0.5 and 1.0 pg/mg of EtG in hair, respectively. EtG concentrations lower than 1.0 pg/mg were determined for 95% of abstainers; 30% of them had non-detectable (<0.5 pg/mg) EtG values. Two samples evidenced EtG concentrations higher than 1.0 pg/mg that were subsequently explained by unintentional ethanol exposure. The method's feature of high analytical sensitivity makes it particularly suitable for alcohol abstinence ascertainment and, in the same time, allows to tentatively estimate basal EtG concentrations in hair around 0.8±0.4 pg/mg. This finding opens a discussion on the possible origin of basal EtG concentration and potential sources of bias in the evaluation of alcohol abstinence. Cut-off value in the range of 1.0-2.0 pg/mg can be reliably proposed to support alcohol abstinence.
**Ethyl Glucuronide Identified in Commercial Hair Tonics.**
doi: 10.1016/j.forsciint.2013.05.010

**ABSTRACT**

**Background:** Ethyl glucuronide (EtG) in hair is considered as a specific marker of ethanol consumption. Prompted by a report of positive EtG hair testings due to hair treatment with an EtG containing hair lotion, commercially available herbal hair tonics from supermarkets, drug-stores, and health food stores were analyzed for the presence of EtG and ethyl sulfate (EtS).

**Methods:** LC-MS/MS (QTRAP 5500 mass spectrometer) was done in multiple reaction monitoring (MRM), enhanced product ion (EPI) and MS(3) mode. The lower limit of quantitation was 0.05 mg/L for EtG and the cut-off for the detection of EtS 0.01 mg/L.

**Results:** Altogether 11 hair tonics from 8 manufacturers were tested, with 1 product in 3 different lots. EtG ranged between 0.07 and 1.06 mg/L (7 products from 4 manufacturers) and was almost identical in the 3 lots of 1 product (1.01-1.06 mg/L). EtS was found in 3 out of the 11 hair tonics.

**Conclusions:** EtG is quite frequently present in commercially available herbal hair tonics. Using EtG in hair as a marker of alcohol (ab)use, one has to consider external sources of EtG and has to assess the use of hair care products, esp. if the patient denies any ethanol intake. Whether EtS is a more reliable alcohol (ab)use marker, as sometimes discussed, should be critically assessed against the background of its broad use in large amounts in industrial chemistry.
A SPME-GC/MS Procedure For The Determination of Fatty Acid Ethyl Esters in Hair For Confirmation of Abstinence Test Results

ABSTRACT

Fatty acid ethyl esters (FAEE), direct metabolites of ethanol, are suitable alcohol markers that can be detected in different tissues. The determination of FAEE in hair can help to evaluate social and excessive alcohol consumption. Due to the presence of FAEE in the hair of teetotalers, proving alcohol abstinence seems to be impossible. To verify these results, an solid phase micro extraction-gas chromatography/mass spectrometry procedure for the determination of the four FAEE: ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate in hair was validated with special focus on low concentration levels. Besides very high sensitivity (limits of detection between 0.005 and 0.009 ng/mg), good results for linearity, precision and accuracy, recovery and stability were achieved. In addition, 73 hair samples with measured ethyl glucuronide (EtG) concentrations between 4 and 10 pg/mg were analyzed for FAEE. By using the following cut-offs: EtG: 7 pg/mg, FAEE: 0.2 ng/mg a satisfying matching rate of 72.6% was found. This shows that FAEE can be determined to verify borderline EtG concentrations even in the context of abstinence tests. However, the diversified influencing factors on analyte concentrations in hair, which may explain the large deviations between EtG and FAEE results observed in some cases, have to be mentioned when interpret ambiguous results.
Detecting Alcohol Abuse: Traditional Blood Alcohol Markers Compared to Ethyl Glucuronide (EtG) And Fatty Acid Ethyl Esters (FAEEs) Measurement in Hair.

ABSTRACT

Alcohol abuse is a common problem in society; however, the technical capabilities of evaluating individual alcohol consumption using objective biomarkers are rather limited at present. In recent years research has focused on alcohol markers using hair analysis but data on performance and reliable cut-off values are still lacking. In this study 169 candidates were tested to compare traditional biomarkers, such as carbohydrate-deficient-transferrin (CDT), gamma glutamyl transferase (GGT), aspartate amino transferase, alanine amino transferase and the mean corpuscular volume of the erythrocytes, with alcohol markers detectable in hair such as ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs). This study revealed that EtG, GGT and CDT showed the best results, demonstrating areas under the curve calculated from receiver operating characteristics of 0.941, 0.943 and 0.899 respectively. The lowest false-negative and false-positive rates were obtained by using a combined interpretation system for hair EtG and FAEEs. All markers demonstrated only low to moderate correlations. Optimum cut-off values for differentiation between social and chronic excessive drinking calculated for hair EtG and FAEEs were 28 pg/mg and 0.675 ng/mg, respectively. The critical values published in the "Consensus on Alcohol Markers 2012" by the Society of Hair Testing were confirmed.
An Evaluation of Washing And Extraction Techniques in The Analysis of Ethyl Glucuronide And Fatty Acid Ethyl Esters From Hair Samples.
doi: 10.1016/j.jchromb.2014.01.049

ABSTRACT

Ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs) are alcohol metabolites measured in hair and are after a decade of research thought to be the best markers in hair to indicate alcoholism and abstinence Forensic Sci. Int. 218 (2012) 2. A great body of work concerning EtG and FAEEs detection in hair has been performed. However, no recent extensive comparison has been made concerning washing and extraction procedures. This work shows that the washing procedure of dichloromethane followed by a methanol rinse of the hair sample removes more than 16% of the FAEEs and 50% of the total EtG that is present in and on the hair. A review of ten washing protocols (where the removal is categorised: high, medium or low) showed that a relatively high percentage of FAEEs was removed and "medium" amount of EtG compared to the other washing protocols. This work shows promising results for the extraction of the FAEEs and the combined extraction of FAEEs and EtG by using 30min of sonication with methanol. More FAEEs were recovered from hair with methanol than with any other extraction solvent including the commonly used dimethyl sulfoxide/heptane mixture. When the sonication time was increased a higher percentage of transesterification of the FAEEs was observed, the extraction was "dirtier" as solids and a colour change was observed whereas the extraction efficiency did not increase. Therefore, washing the hair sample with dichloromethane and methanol followed by an addition of 1ml of methanol and sonication for 30min to extract the FAEEs and EtG from hair is recommended for FAEEs as well as for the combined analysis of EtG and FAEEs. A linear calibration curve ($r^2>0.99$) was obtained for all analytes.

ABSTRACT

Background: To assess the debated diagnostic performance of ethyl glucuronide in the 3-cm proximal scalp hair fraction (HEtG) as a marker of chronic excessive drinking.

Methods: In July 2012/May 2013, after a systematic search through the Medline, Ovid/Embase, Web of Science and Scopus databases, 8 studies were included in the pooled analysis, that report raw single data on HEtG concentration and Self-reported Daily Alcohol Intake (SDAI). A Receiver Operating Characteristic curve analysis and a Spearman rank order correlation test were used. A meta-analysis was performed following the PRISMA and Cochrane recommendations, comprising quality and bias assessments.

Results: The pooled analysis showed that 30 pg/mg could be a useful cut-off value for HEtG to detect a SDAI > 60 g/day, and demonstrated a parabolic direct correlation between HEtG and SDAI data (rho 0.79; 95% CI 0.69-0.87; p<0.001). The meta-analysis found an overall HEtG sensitivity of 0.96 (95% CI 0.72-1.00) and specificity of 0.99 (95% 0.92-1.00); a nomogram to predict the post-test probability of exhibiting the targeted condition in the general population was built. Significant variability among the included studies was detected, which is mainly explained by true heterogeneity in the presence of publication bias.

Conclusions: With the available data we conclude that HEtG is a promising marker for identifying chronic excessive drinking. Nonetheless, larger and well-designed population studies are required to draw any definitive conclusions on the significance and appropriateness of its application in the forensic setting.
ABSTRACT

Background: Ethyl glucuronide (EtG) is a minor alcohol metabolite that has been proposed as a stable marker in hair to detect and quantify alcohol consumption over long time periods.

Methods: We provide an outline of currently available techniques for EtG hair sample analysis and highlight the pitfalls related to data interpretation. The literature of EtG analysis has been reviewed from January 1980 up to August 2013. In addition, we present an overview of the clinical and forensic studies which have used EtG quantification in hair as a marker for alcohol consumption/abstinence and we provide suggestions for future research.

Results: EtG is a stable marker in hair that can be used to detect and quantify alcohol consumption over long time periods. This alcohol metabolite remains in hair after complete elimination of alcohol. Currently, there are three main analytical techniques used to quantify EtG in hair: gas chromatography-mass spectrometry (GC-MS), gas chromatography-tandem mass spectrometry (GC-MS/MS), and liquid chromatography-tandem mass spectrometry (LC-MS/MS). No standardized protocols are yet available for the analysis of EtG levels in hair samples, and the current protocols vary in sample preparation and extraction procedures. Variables such as hair length, cosmetic treatment, gender, and pathophysiological conditions influence the final results and should be taken into account.

Conclusions: EtG quantification in hair is a useful tool for the objective detection of alcohol consumption over extended time periods, but care should be taken when interpreting the results.