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Hair ethyl glucuronide and serum carbohydrate deficient transferrin for the assessment of relapse in alcohol-dependent patients

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ABSTRACT

Objectives: Ethyl glucuronide in hair (hEtG) and serum carbohydrate deficient transferrin (%CDT) are valuable markers for alcohol abuse, but their diagnostic accuracy to monitor abstinence and relapse is unclear. Here, we investigate to what extent repeated measurements of hEtG and %CDT can be used to monitor relapse in alcohol-dependent patients during abstinence treatment.

Design and methods: HEtG and %CDT were measured in individuals starting treatment for alcohol dependence both at treatment entry and 3 months later. Alcohol consumption and relapse episodes were recorded using the Time Line Follow Back and by alcohol breath and urine tests, and correlated with hEtG and %CDT measurements.

Results: Fifteen patients completed the study, of which nine had one or more relapses. Hair EtG and serum %CDT identified whether a relapse occurred in 78% and 57% of cases, respectively. Only hEtG correlated with the amount of alcohol consumed before treatment entry (Pearson $r = 0.92$; $p < 0.001$). The specificity of %CDT to assess abstinence during treatment was 100%. HEtG had a specificity of only 17%; however, in all patients who remained abstinent, hEtG decreased with >85% from initial values. Mean hEtG, but not %CDT, differed significantly between patients who relapsed and patients who remained abstinent ($p = 0.034$).

Conclusions: HEtG was more sensitive than serum %CDT to assess relapse in alcohol-dependent patients and was positively correlated with the amounts of alcohol consumed. In contrast, serum %CDT was more specific for assessing abstinence. We highlight the benefit of repeated measurements of hEtG and serum %CDT for monitoring abstinence during treatment.

1. Introduction

Monitoring abstinence in alcohol-dependent patients is highly relevant in both legal and medical settings. The detection of alcohol abstinence and relapse can have a major impact on legal decisions for individuals convicted for alcohol related offenses or individuals in high risk work settings. Also, it influences healthcare decisions such as the eligibility for liver transplantation in case of end stage alcoholic cirrhosis or the intensification of psychiatric support [1–2].

Direct measurement of ethanol in blood or breath remains the golden standard to detect recent alcohol use. However, the short half-life of ethanol of several hours decreases the sensitivity and thus utility of these methods to detect alcohol relapses in the prior weeks. The limited diagnostic value of indirect markers such as liver tests, reflecting tissue damage, fueled the search for additional biomarkers. Carbohydrate deficient transferrin (%CDT) is an iron transporter protein that can be detected in serum to assess excessive alcohol use [3–4]. Improvements in the analytical techniques to quantify serum %CDT levels have increased its sensitivity and specificity [5–6]. Serum %CDT has a half-life of circa 15 days and thus gives an indication of the alcohol use in the last 2 to 4 weeks [7]. Ethyl glucuronide (EtG) is a phase II metabolite of alcohol that accumulates in hair [8]. Studies have shown that EtG in scalp hair (hEtG) is a highly sensitive and specific marker for the detection of chronic moderate and excessive alcohol consumption [8–9]. HEtG concentrations in the proximal 3 cm hair length correlate well with the consumed amount of alcohol in the last 3 months in alcohol-dependent individuals [10]. HEtG thus has a broader detection window compared

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with ethanol blood levels and serum %CDT. On the other hand, hEtG would have limited diagnostic value when assessing alcohol use of the past week compared to %CDT, because EtG-containing hair needs to first grow out of the hair follicle to the visible hair part to be detectable upon analysis. To address both time windows (recent use and chronic use), it has been suggested that hEtG and %CDT biomarkers may be combined for a more complete picture of both previous and current alcohol use [11].

The kinetic behavior of serum %CDT and hEtG in alcohol-dependent patients after alcohol withdrawal has been underreported. A large multicenter study showed that %CDT decreased significantly from initial levels between 1 and 4 weeks following abstinence, and that relapse during the course of the study correlated with a 30% increase in %CDT levels in the first week following the relapse [12]. For hEtG, a study in 15 alcohol-dependent patients showed that hEtG declined only moderately after the start of abstinence, probably due to the presence of non-growing hair [8]. Earlier studies comparing %CDT and hEtG investigated abstinence assessment after chronic alcohol consumption [13–14], thereby lacking repeated measurements (i.e., within one individual). These studies were performed in the context of drunk driving where alcohol use in the prior months could be underreported [13–14], whereas the latter study [13] reports hEtG results with a cut-off of 30 pg/mg hair which is not representative for abstinence assessment (Society of Hair Testing (SoHT) guideline on hEtG [15]). Information on the duration of abstinence required for a negative test outcome is of major importance for the interpretation of a single test result in order to detect alcohol relapse or confirm sustained abstinence [16]. So far, few studies focused on the assessment of alcohol relapse based on multiple measurements [16]. We hypothesize that the combined measurement of serum %CDT and hEtG will be an improved decision tool especially for the assessment of alcohol relapse over a broad time window. Therefore, we investigated whether hEtG and/or %CDT can be used as an objective tool for the detection of relapse in alcohol-dependent patients.

2. Material and methods

2.1. Participants

Alcohol-dependent individuals starting an in-patient treatment for alcohol dependence were recruited from a Belgian addiction treatment center (Broeders Alexianen, Boechout, Belgium). Patients were required to have hair length > 3 cm and be >18 years old. Patients were excluded from study participation if they entered an alcohol detoxification program in the prior 3 months or if they had an alcohol-free period of more than 7 consecutive days in the 3 months prior to study inclusion. Patients with liver and kidney diseases were also excluded (assessed by serum transaminases, bilirubin and creatinine laboratory test values >3× reference interval). The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The study was approved by the Ethics Committee of the Antwerp University Hospital (registration number: B300201215330) and all included patients signed informed consent.

2.2. Questionnaires and sample collection

Alcohol consumption was recorded using the Alcohol Timeline Follow Back Interview (TLFB; [17]), a widely used and validated tool to assess daily retrospective alcohol consumption and relapse [18]. Alcohol consumption was recorded at baseline (i.e., at treatment entry) to assess retrospective alcohol consumption in the three months prior to entry, and was re-assessed following three months treatment to assess relapse. Urine alcohol and alcohol breath tests were performed by the treatment center sporadically as part of the clinical routine and the information was used to verify the relapses documented by patients on the TLFB at the end of the study. Age, gender and body mass index (BMI) were recorded at baseline. Hair characteristics (length, color, daily washing and cosmetic treatments) were assessed by interview at baseline and three months later.

At baseline and 3 months later, a strand of hair (approximately 50 mg) was collected from the vertex posterior as close as possible to the scalp for hEtG measurements, in accordance with the Consensus on Hair Testing guidelines provided by the SoHT (see further). In parallel, blood from the arm vein was collected in a serum tube (4 cm^3) for %CDT measurements (see further).

2.3. Hair EtG measurements

Collected hair strands were stored in aluminum foil at room-temperature until analysis. The proximal 3 cm hair segment was used for hEtG measurement, in order to obtain data on alcohol consumption of the prior three months. Hairs were washed with water and acetone to remove external contamination, and pulverized to powder using a ball mill (Retsch MM400). Of the pulverized sample, 30 mg was carefully weighted and used for further analysis using gas chromatography–mass spectrometry (GC/MS) in negative chemical ionization mode following solid-phase extraction on Oasis MAX cartridges and derivatization with pentafluoropropionic anhydride (PFPA), as reported earlier [19]. Our protocol for the measurement of EtG in hair is presented in Table 1. The limit of detection (LOD) was 0.7 pg/mg and the lower limit of quantification (LLOQ) was 2.1 pg/mg. The method validation was reported earlier [20]. According to the SoHT guidelines, a hEtG result ≥7 pg/mg is considered a positive result, i.e. indicates regular alcohol consumption. A hEtG result ≥30 pg/mg proposes chronic excessive alcohol consumption of >60 g alcohol per day over several months [15]. External quality control was assessed through participation to two international inter-laboratory comparison schemes organized by the SoHT (www.soht.org) and by Arvecon (www.arvecon.de).

2.4. Serum CDT measurements

Collected serum tubes were centrifuged at 5000 rpm for 10 min and the serum was stored at −20 °C until analysis. %CDT was analyzed using capillary electrophoresis on the Capillarys 2 (Sebia). A cut-off of 1.6% (% of di-sialo-transferrin compared to total transferrin) was used as a positive test result [6].

2.5. Statistics

Statistical analyses were performed using SPSS IBM software version 20, and all data were checked for normality using Shapiro–Wilk tests. Patient characteristics, alcohol consumption, hEtG concentrations and %CDT measurements between relapsing and abstinent patients were analyzed using independent sample T-tests (for parametric data), Mann–Whitney U tests (for non-parametric data) or Chi-square tests

Table 1
Analytical protocol for the extraction and measurement of ethyl glucuronide in hair.

Place 30 mg of powdered hair sample in a vial and add
20 μL EtG-d5 (0.2 μg/mL)
2 mL water
Close the vial, vortex for 15 s, and ultrasonicate the sample for 2 h
Centrifuge for 10 min at 2500 *g*
Condition OASIS MAX cartridge with 2 mL methanol and 2 mL water
Place the aqueous phase of the sample on the cartridge and add
1 mL 5% NH_3 in water
2 mL MeOH
Dry the cartridge under vacuum
Add 2 mL 2% formic acid in methanol and dry the effluent using N_2
For derivatization, add 100 μL PFPA and vortex
Close immediately and heat to 60 °C for 30 min
Dry the sample with N_2
Add 50 μL ethyl acetate, vortex, and place in screw cap vial
Inject 1 μL into the GC–MS system