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Influence of repeated permanent coloring and bleaching on ethyl glucuronide concentrations in hair from alcohol-dependent patients

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ABSTRACT

Background: Ethyl glucuronide (EtG), a minor metabolite of alcohol, is used as a sensitive marker in hair to detect the retrospective consumption of alcohol. The proximal 0–3 cm hair segment is often used for analysis, providing information on alcohol consumption over the past 3 months. Using more distal segments would allow the detection of alcohol consumption over longer time periods, thereby addressing the chronicity of the consumption. In view of this, permanent coloring and bleaching were shown in vitro to alter EtG concentrations in hair, but no in vivo studies are available to prove or disprove this.

Aims: To investigate the influence of repeated bleaching and permanent coloring on EtG concentrations in vivo and to assess the stability of EtG concentrations in distal compared to proximal hair segments.

Methods: Hair samples from alcohol-dependent patients with uncolored/unbleached ($N = 4$), permanent coloration ($N = 5$) and bleached hair ($N = 5$) were analyzed in two to six 3 cm long segments for EtG concentrations, and alcohol consumption and hair cosmetic treatments were assessed.

Results: We observed that hair bleaching and permanent coloring reduces EtG concentrations by $82 \pm 11\%$ and $65 \pm 24\%$, respectively, with correlations between the number of cosmetic treatments and the decrease in EtG concentrations. EtG remained stable in untreated hair samples up to 18 cm.

Conclusions: EtG is a sensitive marker to assess chronic alcohol consumption up to 18 months in alcohol-dependent patients with no cosmetic hair treatments. However, in alcohol-dependent patients who color or bleach their hair, care should be taken when interpreting EtG measurements.

1. Introduction

Alcohol dependence is a chronic disorder with major impact on health and society. As a result, the detection of chronic alcohol consumption is of key importance in clinical and forensic settings. Biomarkers that detect the *chronicity* of the disorder, i.e., markers of alcohol consumption that remain detectable for long time periods, are therefore highly warranted. Ethyl glucuronide (EtG), a minor alcohol metabolite that incorporates into the hair matrix (for a review see [3]), has been proposed as a stable marker to detect and quantify the chronicity of alcohol consumption [9]. Dependent on hair length, alcohol use over time periods of several months can be detected using hair EtG measurements.

With a hair growth of about 1 cm per month, segmental analysis of hair strands allows the retrospective detection of alcohol consumption. While some studies propose that EtG concentrations remain stable in hair segments up to 12 cm [2], other studies report a washout of EtG over time, reported as 74% lower EtG concentrations in 3–6 cm hair segments compared to the proximal 3 cm in individuals who washed their hair daily [13]. Additionally, the stability of EtG in hair, and thus the interpretation of the results, can be questioned with the use of cosmetic treatments such as hair coloring or bleaching [14,4]. Bleaching discolors melanin pigments and is responsible for an oxidation of

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the S–S bonds and free amino groups in keratin, which destructs the hair and renders hair porous. One in vitro bleaching session reduced EtG levels with a mean of 74% [5]. Another study reported that EtG concentrations in hair were reduced to undetectable levels after an in vitro bleaching session [8]. Finally, Suesse et al. noted a 63% decrease in mean EtG concentration between samples with or without cosmetic treatment [12]. Hair coloring has also been observed to reduce hair EtG concentrations [12], while other studies propose that the use of coloring agents does not influence the EtG concentrations in hair [1]. Using a non-permanent hair-coloring product in vitro, no differences in EtG concentrations were observed [5]. Finally, EtG concentration in hair is not influenced by the use of alcohol-containing hair products [7], or by the use of hair spray, wax, gel, oil or grease [12].

For the purpose of detecting *chronic* alcohol consumption, hair segments longer than the proposed 0–3 cm should be analyzed. However, in segments >3 cm in length, repeated cosmetic treatments are expected in individuals with frequent hair treatments, and their influence on EtG concentrations may thus be more pronounced. Currently, to the best of our knowledge, no studies are available that report on the influence of repeated cosmetic treatments on EtG concentrations. Here, we investigate the in vivo stability of EtG in never-colored or -bleached hair segments. Additionally, we report on the influence of repeated cosmetic treatment (bleaching and permanent coloring) on EtG's stability by using hair segments of individuals who, in the past 18 months, repeatedly colored or bleached their hair. To detect changes in EtG levels upon repeated cosmetic treatments, we compare the proximal hair segments (supposed to reflect fewer cosmetic treatments) to the more distal segments up to 18 cm in samples with high EtG concentration, i.e., in hair samples from alcohol-dependent patients. As such, we evaluated whether hair segments further away from the scalp are useful to address the chronicity of the alcohol consumption, and how this is influenced by repeated cosmetic treatment.

2. Materials and methods

2.1. Participants and hair samples

Participants were alcohol-dependent patients entering an alcohol-detoxification program in Belgium. For the study purpose, males and females with hair >3 cm length and who provided full information on cosmetic hair treatments for the past 2 years were included between September 2013 and May 2014. Patients who both colored and bleached their hair in the past 18 months were exd, leaving patients to be included only when they had no cosmetic treatment in the prior 18 months, or when they exclusively colored or exclusively bleached their hair in the past 18 months. The number of cosmetic treatments per month was recorded. Hair straightening was excluded. Accurate estimation of retrospective alcohol use in the prior 3 months was assessed using the Time Line Follow Back interview [11]. Years of regular alcohol use and changes in alcohol consumption in the prior 18 months were also recorded, and were used to extrapolate the current consumption to the past 18 months.

Hair samples were cut as close as possible to the scalp from the vertex posterior. The hair strand was segmented in 3 cm-segments over the entire hair's length. These segments were analyzed, with a minimum of 2 segments and a maximum of 6 per participant (up to 18 cm).

This study has been approved by the ethics committee of the Antwerp University Hospital (Antwerp, Belgium). All participants gave written informed consent.

2.2. EtG measurements

Hair segments were washed with water and acetone, pulverized to powder using a ball mill, and analyzed using gas chromatography–mass spectrometry in negative chemical ionization following solid-phase extraction and derivatization with pentafluoropropionic acid (PFPA), as reported earlier [6]. The method had a limit of detection (LOD) of 0.70 pg/mg and a lower limit of quantification (LLOQ) of 2.10 pg/mg.

2.3. Statistical analyses

Statistical analyses were performed using SPSS IBM (version 20). Group differences in patient and hair characteristics were analyzed using parametric Student *t*-tests, non-parametric Mann–Whitney *U*-tests, or Chi-square tests where appropriate. Variables were checked for normality of their distribution using Shapiro–Wilk tests.

Between-group differences in changes in EtG concentrations were analyzed using a linear mixed model (LMM) to account for the dependence of the (repeated) measurements within subjects. Hair EtG, expressed as percentage from baseline (i.e., the initial measurement), was set as the dependent variable. This percentage was calculated assuming the first measurement as 100%. Because the alcohol consumption over time was not stable for all individuals, we introduced a correction factor to account for the alcohol consumption within that participant. The correction factor was the relative difference between the alcohol consumption between two segments (t_1/t_0) and was corrected at the obtained EtG hair segmental results. E.g., for a participant that drank 100 g of alcohol daily at t_0 and drank 200 g alcohol daily on t_2, the EtG result of the segment at t_2 was divided by a factor 2. Hair treatment (no treatment, coloring, or bleaching), the segment analyzed, and the interaction term "treatment × segment" were set as fixed factors. A random intercept for subject was included as random factors. The interaction effect treatment × segment was the primary outcome measure. To assess which groups differed significantly from one another, analyses were performed two-by-two. Correlations between changes in EtG concentrations and number of cosmetic hair treatments were analyzed using Pearson correlation analysis. A significance level of $p < 0.05$ was used as statistically significant for all statistical tests. Data are presented as means ± standard deviation.

3. Results

3.1. Patient and hair characteristics

We present data of 14 patients who had either unbleached/uncolored hair ($N = 4$), colored but unbleached hair ($N = 5$) and bleached but uncolored hair ($N = 5$). Hair lengths varied between 6 and 18 cm of length. Most included patients (13 of 14) were females, and ages did not differ between the groups (no treatment 49 ± 6 years; colored 41 ± 8 years; bleached 47 ± 9 years; ANOVA $p = 0.305$). Mean daily amounts of alcohol consumption were 134 ± 77 g alcohol per day (range: 60–293 g/day) and mean years of regular alcohol consumption were 9 ± 6 years (range: 1–19 years). These variables also did not differ across groups (ANOVA $p = 0.133$ and 0.774 respectively). Participants reported permanently coloring their hair every four to eight weeks. Participants reported bleaching their hair every six to eight weeks.

In all patients, EtG concentrations in hair at baseline (the proximal 0–3 cm, representing the alcohol consumption over the prior 3 months) were all above the current cut-off of 30 pg/mg hair (Society of Hair Testing, http://www.soht.org), providing 100% sensitivity for detecting chronic alcohol consumption above 60 g per day.