Occupational Exposure to Alcohol-Based Hand Sanitizers: The Diagnostic Role of Alcohol Biomarkers in Hair

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Occupational exposure to alcohol-based hand sanitizers: the diriment role of alcohol biomarkers in hair

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Abstract

Ethyl glucuronide (EtG) and Fatty Acid Ethyl Esters (FAEEs) in hair are effective direct biomarkers of ethanol ingestion, whose analytical determination can be used to discriminate between chronic and occasional ethanol intake. Ethanol is a compound widely spread in the workplaces (clinics, hospitals, etc.) and is present in considerable amounts in mouthwash for oral cleaning, medications, cosmetic products, hydro-alcoholic disinfectants and antiseptics for hands.

This study examined the ethyl alcohol exposure derived from hand-disinfectants (in gel form), simulating the typical occupational situation of medical-health workers (healthcare workers, nurses, surgeons, etc.) who daily and frequently wash hands with antiseptic sanitizer. Two types of hand disinfectants with 62% w/w of ethanol content were daily applied on the hands of a teetotaler for 20 times a day, for 4 consecutive weeks, thus simulating a typical workplace situation and a cumulative dermal exposure to ethanol of approximately 1100 grams. Different matrices (head, chest and beard hair, urine) were regularly sampled and analyzed using a UHPLC-MS/MS validated method for ethyl glucuronide and a (HS)SPME-GC-MS validated technique for FAEEs.

The data obtained showed that a significant dermal absorption and/or inhalation of ethanol occurred, and that the use of detergents produce urinary EtG concentrations both higher than the ~~clinical and forensic~~ cut-offs normally used for clinical and forensic analyses (either 100 and 500 ng/mL, depending on the context) ~~respectively)~~. The concentrations of the ethanol metabolites in the keratin matrices were respectively below the cut-off of 7 pg/mg for EtG and below 0.5 ng/mg for FAAEs (0.35 ng/mg for ethyl palmitate). In conclusion, the ~~excessive~~ regular use of alcohol-

based hand sanitizers can affect the concentration of urinary EtG and lead to positive analytical results, particularly when specimens are obtained shortly after sustained use of ethanol containing hand sanitizer. On the other hand, direct biomarkers of alcohol abuse in the keratin matrix are capable of distinguishing between ethanol consumption and incidental exposures.

Keywords

EtG; FAEE; ethylglucuronide; alcohol biomarkers; hair; hand sanitizers; workplace testing

Introduction

The direct determinations of alcohol in blood and exhaled breath represent the most common analyses used to ascertain recent ethanol consumption in forensic cases, but they do not provide any clue about the possible incidence of chronic alcohol ~~ab~~use. In recent years, the detection of direct ethanol biomarkers in unconventional (keratin) matrices has become very popular to assist~~in~~ clinical and toxicological diagnostics, in order to ascertain either chronic alcohol ~~ab~~use or abstinence (1-4). In particular, ethylglucuronide (EtG) is a direct biomarker with high diagnostic sensitivity and ~~diagnostic~~ specificity for heavy or chronic alcohol use, and represents an indicator of alcohol intake in the short (serum), medium (urine), and long term (keratin matrix). Together with EtG, fatty acids ethyl esters (FAEEs) determined in hair samples have also become important diagnostic markers for alcohol consumption for both forensic and clinical purposes (2). These metabolites are possibly used to support the results of EtG analysis in case of doubtful positive outcome (2,5). Considering the extensive use of ethanol in many commercial products other than alcoholic beverages, many circumstances of unintentional ethanol exposure may occur. In particular, the use of ethanol-containing hygiene products and disinfectants, that are marketed as gel or soaps for the cleaning and the disinfection of the hands skin, may produce false positive results (6-16). Hand washing and disinfection is aimed to physically remove dirt and most of the transient flora from the skin. Currently, in hospitals and healthcare facilities the hand hygiene is considered the most important prevention and control measure to reduce the spread of pathogens and thereby the transmission of infectious diseases (17,18). ~~The a~~Alcohol-based formulations are among the most effective for hands hygiene and disinfection, and they are recommended by the World Health Organization (WHO) because they are characterized by rapid and effective bactericidal activity, waterless use, easy application, time and cost saving (19). In healthcare professions, hand disinfectants are ~~constantly~~ frequently used during the work shift. Many of these disinfectants contain high ~~quantities~~ concentrations of ethanol as an active ingredient, so surgeons and healthcare assistants are

potentially exposed to ethanol absorption several times per day, through both the skin and the respiratory tract.

Positive findings of blood ethanol (6,8,11) and urinary EtG (7, 12-16) have been reported after sustained exposure to ethanol-based hand sanitizers. In many workplace testing programs, including healthcare professions, employees are tested for alcohol ~~ab~~use. Aim of the present study was to verify if the daily exposure to ethanol-containing hygiene products may induce significant concentrations of alcohol markers (namely EtG and FAEE above the cut-offs recommended by the Society of Hair Testing ~~recommended cut offs~~), especially in conditions of total abstinence from ethanol-containing beverages.

Materials and Methods o Experimental

Chemical, reagents and standard solutions

Ethyl glucuronide and ethyl glucuronide-d5 (EtG-d5), used as internal standard (IS), were acquired from Medichem® (Germany). Standard solutions of EtG and EtG-d5 were prepared in methanol at 10 µg/mL concentration and stored at -20°C. Ethyl myristate (E14:0), ethyl palmitate (E16:0), ethyl oleate (E18:1) and ethyl stearate (E18:0), n-heptane, dimethyl sulfoxide (DMSO), acetonitrile, methylene chloride, methanol, sodium phosphate dibasic dihydrate (Na_2HPO_4• 2 H_2O), potassium phosphate monobasic (KH_2PO_4) and formic acid were obtained from Sigma-Aldrich (Milan, Italy). The deuterated standards ~~D5~~ ethyl myristate-d5, ~~D5~~ ethyl palmitate-d5, ~~D5~~ ethyl oleate-d5, ~~D5~~ ethyl stearate-d5 were provided by Toronto Research Chemicals (TRC).Stock solutions of FAEEs, as well as the deuterated analogues, were prepared in n-heptane (1 mg/mL). A working solution containing all four D5-FAEE at 1 µg/mL concentration was prepared by dilution and used as the internal standard (ISTD). A solution containing the non-deuterated FAEEs at 1 µg/mL concentration for E14:0 and E18:1 and 4 µg/mL for E16:0 and E18:0 was also prepared. All solutions were stored in a ~~refrigerator~~ freezer at -20°C. Phosphate buffer (50 mM, pH=7.5) was prepared by dissolving 4.63 g of KH_2PO_4 and 11.75 g of Na_2HPO_4• H_2O in 1 L of deionized water.

All chemicals and reagents were of analytical purity grade. Ultra-pure water was obtained using a Milli-Q UF-Plus apparatus (Millipore, Bedford, MA, USA).

Study protocol

Two hand sanitizers, namely LH GEL(Lombarda H srl, Albairate, Milan, Italy) and ESOSAN GEL (Microtek Italy srl, Rovigo, Italy), were used in this study during a four weeks period. Both products ~~(LH GEL and ESOSAN GEL)~~ were hydroalcoholic gels containing 62% w/w ethanol, with different formulations of excipients. The exclusion criteria for participation in the study were: visible lesions on the skin of the hands and arms; ethanol use disorder or ethanol intake in any form before the start of the experiment; diabetes mellitus; pregnancy or breastfeeding; obsessive-compulsive disorder involving hand washing; skin sensitivity to ethanol; xerodermia; hepatic or renal dysfunction and symptoms of urinary tract infection.

After considering the exclusion criteria, a teetotaler, male, 25 years old, body mass of 80 kg, voluntary subject was involved in the study. The instructions provided by the WHO (20), the Centers for Disease Control and Prevention (CDC) (21) and the Société Française d'Hygiène Hospitalière (22) have been followed and respected throughout the month of study.

The application phase was organized in the following steps: (i) initial hands washing with soap and water and drying; (ii) application of 5 mL of gel (with graduated syringe) on the hand palm; (iii) hands rubbing to cover the whole skin surface (right above the wrists) in about 30 seconds and prosecution of the rubbing until the evaporation of all gel (about 1.5 min). These steps were repeated four consecutive times (2-3 min pause) at every hour, for a total of 20 applications per day. The first application started at 10.30 am while the last application was made at 3.00 pm.

The two types of gels were alternatively applied every week for 4 consecutive weeks, i.e. ESOSAN GEL during the 1st and 3rd week, LH GEL in the 2nd and 4th week. The volunteer was dermally exposed to about 2.8 g of ethanol for single application (5 mL of gel), up to a total of 55.2 g per day

(100 mL), with a cumulative dermal exposure to ethanol of approximately 1103.6 g (2000 mL) during the course of the study.

During the month preceding the study and the entire duration of it, the volunteer did not ingest ethanol from any sources, namely alcoholic beverages, food containing liquors (eg. soaked cakes, ice cream with rum, etc.), or fruit juices (which in some cases contain small amounts of alcohol). Furthermore, no mouthwashes, perfumes, aftershave, bug sprays (for dermal and environment use), counter medications such as syrups for cough, and any other types of hydro-alcoholic disinfectants were used.

Ethical approval for our research activity is granted by the Ethical Committee of the Azienda Ospedaliero-Universitaria San Luigi Gonzaga of Orbassano.

Samples collection

Samples were collected after obtaining informed consent. Urine specimens were stored in plastic aseptic vessels at –20°C until analysis for a maximum of four weeks. Keratin specimens were collected by a trained operator (23) and then stored in sterile plastic containers at room temperature until analysis.

Urine was collected every day (from Monday to Friday) at 9.30 am and 5.30 pm, for a total of 40 urine samples. Beard hair was collected every Monday for five consecutive weeks, for a total of five samples, while chest hair was collected at day 1 and 30 of the study, for a total of two samples. Finally, head hair was sampled at day 1 (measured length 3.5 cm), 15 (measured length 4.0 cm) and 30 (measured length 4.5 cm) of the study, for a total of three samples.

Samples treatment

Urine samples

One 200 µL aliquot from each urine sample was added with 20 µL EtG-d5 solution (1000 ng/mL final concentration); then was stirred for about 1 min and micro-centrifuged at 12000 rpm for 5 min

if turbid. A 50 µL aliquot was transferred and added with 950 µL of deionized water. Homogenization of the sample was produced by vortex for 30 seconds; a 100 µL aliquot of the supernatant was micro-centrifuged at 14000 rpm for 5 min if turbid. 2 µL were lastly injected in the Ultra High Performance Liquid Chromatography tandem mass spectrometry ~~MS/MS~~ (UHPLC-MS/MS) instrument. To minimize the physiological variations in urinary excretion, creatinine was measured by Jaffe method –using an Abbott Architect instrument (Abbott Laboratories, Abbott Park, IL, USA) and every EtG level was also expressed with respect to 100 mg/dL of excreted creatinine.

The concentration of urinary creatinine depends on two influencing factors of large intra-individual variability, namely meat intake with nutrition and exercise. These variations have been minimized, by adopting a regular diet and exercise over time during the month of treatment.

Keratin samples

For head hair, the proximal segment 0-3 cm was analyzed for each sample while chest and beard hair were analyzed in their total length. The pretreatment of keratin samples (hair, chest and beard hair) for EtG analysis was performed according to a routine standard method previously developed and validated (24). It consists of: two consecutive decontamination washes with methyl chloride and methanol (3 mL, 3 min); drying; grinding with a ball mill (two grinding cycles of 50 s each, with 30 s of pause, at 6000 rpm, using 6 steel balls in a sample holder of 2 mL); weighting of approximately 50 mg decontaminated sample; addition of EtG-d5 (100 pg/mg final concentration) and 720 µL of a deionized water/methanol mixture 35:5 (v/v); centrifugation at 4000 rpm for 5 min; incubation over night; ultra-sonication with an UCI-150 Ultrasonic Cleaning Bath (Raypa1, Ankara, Turkey) for 1.5 h; transfer of 100 µL extracting solution in a vial for UHPLC-MS/MS analysis.

The preparation of hair samples for FAEE determination was performed as in previously published methods (25-27). It involves: decontamination with two consecutive washes with heptane (3 mL

and 5 min of stirring each); removal of the solvent and drying over night; grinding with a ball mill (same conditions previously described); weighting of approximately 50 mg decontaminated sample; addition of FAEE-d5 (600 pg/mg final concentration); addition of 2 mL of heptane and 0.5 mL of DMSO; mild stirring overnight (16-17 h); centrifugation at 3000 rpm for 3 min; refrigeration at –20 °C for 30 min (DMSO solidifies); transfer of the supernatant liquid phase into a vial for headspace analysis; drying under heating at +70°C with a nitrogen stream; addition of 1 mL phosphate buffer 50 mM and capping of the vial with magnetic plug for the next SPME extraction.

Analytical methods

UHPLC-MS/MS method

Analyses of EtG in urine and keratin samples were performed using a Shimadzu Nexera 30 UHPLC-system (Shimadzu, ~~Duisburg, Germany~~Kyoto, Japan) interfaced to an AB Sciex ~~API~~ 5500 triple quadrupole mass spectrometer (~~AB~~ Sciex, Framingham, MA, USA~~Darmstadt, Germany~~) with an electrospray source (ESI) in the negative-ion mode. For chromatographic separation, an Acquity UPLC 1 BEH C18 column (100 mm × 2.1 mm i.d. × 1.7 µm, Agilent Technologies, Italy), ~~,~~ protected by a Acquity UPLC BEH C18 VanGuard Pre-column~~C18 guard column~~, was used. The column oven was maintained at +40°C. Elution solvents were water/formic acid 5 mM (solvent A) and acetonitrile/formic acid 5 mM (solvent B). The linear gradient concentration of elution solvents was (A:B; v/v): 97:3 for 0.2 minutes, then to 10:90 at 1.5 min for 0.30 minutes, and finally back to 97:3 at 2.5 minutes, followed by isocratic elution at for 1.8 min. The flow rate was 0.5 mL/min and total run time was 4.3 min. EtG and EtG-d5 were eluted in about 1 min. Data were recorded at unit mass resolution in the selected reaction monitoring (SRM) mode, using nitrogen as the collision gas. LOQ of the methods was 7 pg/mg and 10 ng/mL for the keratin and urine matrices, respectively.

HS-SPME-GC-MS method

Analyses of FAEEs in the keratin matrix were performed with a Flex A05-FLX-0001 autosampler (Est Analytical, West Chester Township~~Fairfield~~, OH, USA) for HS-SPME system, combined to a gas chromatograph Agilent Technologies 6890N Network GC System, interfaced to a single quadrupole 5975 Inert Mass Selective Detector with electron impact ion source. For HS-SPME, a Stableflex PDMS/DVB fiber (65 µm and i.d. 23 Ga, Supelco), provided by Sigma-Aldrich (Milan, Italy) was used operating with the following conditions: fiber preheating 10 min at 250°C; agitation and rotation for 5 min at 250 rpm and 90°C; headspace adsorption for 30 min at 90°C; desorption for 1 min at 250°C. For chromatographic separation, a DB-5 capillary column (30 m × i.d. 0.25 mm × 0.25 µm) was used. The injection mode was splitless and the following temperature program was applied: 1 min at 140°C, 25°C/min up to 265°C, 15°C/min up to 300°C, 2 min at 300°C. The total run time was about 10 min, with helium as carrier gas (1.6 mL/min). The temperatures of the injector, the interface, the ion source and the quadrupole were 250°C, 280°C, 230°C, and 150°C, respectively. LOQ values were between 0.01 and 0.09 ng/mg for ethyl myristate and ethyl oleate, respectively.

Results and discussion

Determination of EtG in urine samples (EtG-u)

Forty urine samples were collected in one month, corresponding to the period of gels application. The ~~twenty~~ samples taken every day in the morning (9.30 am) for a total of twenty samples were negative to EtG (<LOQ) while the samples collected at the end of the working day (i.e. 5.30 pm, after 20 applications) contained measurable amounts of EtG. The EtG in urine ~~EtG-u~~ concentrations (EtG-u) and the relative values normalized with respect to the creatinine concentration are reported in Table 1. From the data relative to the EtG-u determination, it is evident that the observed concentrations are comparable with those obtained from similar studies previously published (Table 2). In fact, the EtG-u concentrations measured in the present study are in the range from 10 to 1150

ng/mL (Table 1), as in the studies employing comparable treatment conditions. In accordance with the conclusion established in the studies of Reisfield et al (12) and Rosano and Lin (14), the results below the LOQ, observed in the morning samples, indicate that EtG is rapidly excreted and does not accumulate in urine following multiple exposures to ethanol. It can be concluded that ~~EtG was~~ the urinary EtG concentrations were below the 10 ng/mL LOQ on every testing occasion, meaning that EtG was ~~completely~~largely eliminated from the urinary flow during the 16 h elapsed between the last sampling at the end of the day (5:30 pm) and the beginning of the following day (9:30 am). Conversely, the intensive use of ethanol-containing hand disinfectants exposes the operator to a significant transdermal absorption and/or inhalation, as the data reported in Table 1 clearly show. This exposure may produce urinary concentrations of EtG greater than 100 and 500 ~~clinical and forensic~~ng/ml cut-off values ~~(28)~~, simulating conditions just like after the intake of alcoholic beverages. The EtG-u absolute values ranged from 10 to 306 ng/mL, except for an unusual value of 1150 ng/mL during the third week (Table 1). Taking into account the normalized EtG concentrations, more homogeneous values are observed, with several cases exceeding the clinical cut-off (100 ng/mL). In particular, most values of the EtG-normalized concentrations are grouped within the range between 100 ng/ml and 500 ng/ml. One hypothesis that may account for the anomalous value of 1150 ng/ml (506 ng/ml after creatinine correction) is that the persistent rubbing of the hands with alcohol detergents caused a desquamation of the outermost part of the skin, resulting in the outer horny layer to become thinner and allowing ethanol to be more effectively adsorbed through the skin. ~~On the other side~~Conversely, the determination for EtG in the keratin matrix produced a negative outcome relative to the intake of alcohol. The EtG results in beard, chest and head hair were consistently below the LOQ of the method (7 pg/mg), namely lower than the cut-off values normally chosen for abstinence assessment (7 pg/mg) and much lower than that used to ~~ascertain~~ assist the diagnosis of chronic alcohol abuse (30 pg/mg) (2~~9~~8). Coherently with hair EtG results, also the concentrations of FAAEs in head and beard hairs were all far below 0.5 ng/mg

(0.35 ng/mg for ethyl palmitate), namely the cut-off values used to verify the occurrence of chronic alcohol abuse (2~~9~~8).

Conclusions

The outcome of this study disclosed that the continuous use of alcohol-based hand disinfectants can lead to a positive alcohol intake result in some types of tests, such as the EtG determination in urine. This type of result is particularly significant for the professions (i.e. typically, ~~–~~surgeons and healthcare assistants) that make use of hand sanitizers frequently, (i.e., several times per day) and eventually undergo through regular workplace alcohol testing~~, a typical condition occurring for surgeons and healthcare assistants~~. Such a risk of false positive result should always be taken into consideration when urinary EtG is used as a marker of ethanol consumption.

Unlike the high concentrations found in urine, the EtG results in the investigated keratin matrices were consistently below the LOQ value and therefore were not affected by use of disinfectants containing ethanol. The negative results provided by the determination of FAEEs in head hair and beard hair correlate with EtG in that their concentration was close the LOQ and much lower than the cut-off value. Therefore, the exposure to alcohol by means of hand disinfectants ~~is not likely~~did not ~~to~~ produce false positive classification of the exposed subject as chronic alcohol abuser.

In conclusion, our study demonstrated (albeit based on a single case) that the use and daily application of hydro-alcoholic gel for hand hygiene can lead to positive results in the analysis of urinary EtG, but the same exposure is not likely ~~cannot~~ to increase ~~the~~ EtG and FAEEs levels in the keratin matrix. The analysis of urinary EtG should ~~always~~ preferably be combined with hair EtG (and hair FAEEs) in order to ~~carry out an accurate~~assist the medical staff in making a correct diagnosis of alcohol ~~abuse~~ use disorders or abstinence, in particular in those workplace situations where an unwanted exposure to alcohol may occur.

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Table 1. Summary of urinary EtG concentrations and creatinine during the 20 days period of dermal exposure to hand sanitizer (urine collected at 5.30 pm).

Week	Day	EtG (ng/mL)	Creatinine (mg/dl)	EtG normalized (ng/mg of creatinine)
1	**1**	115	26	442
	2	103	53	194
	3	85	62	137
	4	129	37	349
	5	61	74	82
	Average	***99***	***50***	***241***
2	**1**	10	53	19
	2	86	77	112
	3	77	131	59
	4	16	61	26
	5	10	31	32
	Average	***40***	***71***	***50***
3	**1**	290	82	354
	2	306	113	271
	3	1150	227	507
	4	157	78	201
	5	10	50	20
	Average	***383***	***110***	***271***
4	**1**	170	52	327
	2	20	16	125
	3	283	194	146
	4	11	35	31
	5	24	22	109
	Average	***102***	***64***	***148***
	Min value	**10**	**16**	**19**
	Max value	**1150**	**227**	**507**
	Average (20 days)	**156**	**74**	**177**

Table 2. Comparison of literature results reporting urinary EtG concentrations after dermal exposure to ethanol-based hand sanitizers.

Study [reference]	Partecipants	Sanitizer (ethanol % w/w)	Site of application	Ethanol amount per application	Days of treatment (*n. applications/day*)	Max EtG ng/mL	Max EtG ng/mg creatinine
Presented	1	LH Gel (62%) Eosan Gel (62%)	Hand/wrist	5 mL (2.8 g)	20 *(20)*	1150	506
Reisfield et al. [12]	11	Purrel (62%)	Hand/wrist	1 mL (0.55 g)	3 *(120)*	2001	1528
Rohrig et al. [13]	4	Germ-x (62%)	Hand/wrist	n/a	2 *(8-32)*	62	n/a
Jones et al. [7]	2	Purrell (62%)	Hand/wrist	0.91 mL (0.5 g)	1 *(8)*	103	58
	2	Purrell (62%)	Hand/forearm	3.64 mL (2.0 g)	1 *(8)*	713	799
Rosano et Lin [14]	9	Avagard D (61%)	Hand/wrist	1 mL (0.5 g)	5 *(20)*	114	n/a
Arndt et al. [15]	5	Desderman (75%)	Hand/wrist	3 mL (1.9 g)	1 *(32)*	2100	1700
Skipper et al. [16]	24	n/a (62%)	Hand/forearm	2 squirts	1 (*15*)	n/a	348
	1	n/a (62%)	Hand/forearm	2 squirts	2 (*8-16*)	n/a	770

n/a: not available