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Comparison of urine and hair testing for drugs of abuse in the control of abstinence in driver's license re-granting

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The purpose of the study was to compare the detection rate of illicit drugs in urine and hair specimens. The samples were taken from subjects trying to regain their revoked driver's license after a drug- or alcohol-related traffic offence. In 2010, we screened 14 000 urine and 3900 hair samples for amphetamines, methamphetamines, cannabinoids, cocaine, opiates, methadone, and benzodiazepines as well as for ethylglucuronide. We used the low threshold values of the new German guidelines for Medical Psychological Assessment (MPA). Positive screening tests were confirmed with gas chromatography–mass spectrometry (GC-MS), gas chromatography-tandem mass spectrometry (GC-MS/MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The results show that positivity rates for methamphetamines, MDMA, cocaine, and monoacetylmorphine were 1.7-, 5.7-, 3.8- and 9.3-fold higher in hair than in urine. In contrast, the detection rate for benzodiazepines was higher in urine than in hair (oxazepam, 0.21% versus 0%, nordiazepam 0.10% versus 0.03%). The positivity rate in hair for ethylglucuronide was 6-fold (12.7%) that for urine testing (2.1%).

The study reveals that in the control of abstinence in the context of driving license re-granting there are in part large differences of positivity rates for some drugs or metabolites between hair and urine samples. These differences should be kept in mind by physicians and psychologists in traffic medicine who are ordering the drug testing. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: drug testing; ethylglucuronide; hair analysis; urine testing; control of abstinence; driving license

Introduction

For many years, urine has been the preferred specimen for drug of abuse testing in monitoring abstinence. The most common illicit drugs can be detected in urine for a few days after cessation of use. Other matrices such as oral fluid and hair are now rapidly gaining recognition and their use in forensic sciences is growing. Several papers recently addressed the question of whether urine or hair is more effective in disclosing drug of abuse consumption in pre-employment testing,[1] in driver's license re-granting,[2,3] in patients participating in methadone-substitution programme,[4] and prior to organ transplantation.[5] Generally these studies revealed that hair analysis identified illegal drugs more often than urine testing. However, the number of subjects tested was often small and the cut-off values in urine were much higher than those used in the present work. With respect to the amphetamine test, there was generally no differentiation between amphetamines and methamphetamines. Furthermore, the investigations did not include benzodiazepines with one exception[1] or ethylglucuronide which is a marker of alcohol consumption.

The purpose of the present study was to retrospectively compare the detection rate of drugs of abuse and ethylglucuronide in urine and in hair in subjects wanting to regain their driver's license. According to the present German regulations, abstinence from drugs or alcohol is generally required for a period of 12 months. This is monitored by either six urine tests and/or hair tests as recommended by the new guidelines for Medical Psychological Assessment (MPA)[6] which came into force 1 July 2009. We compared the results in urine and hair using the lowered cut-off values of the new guidelines.

Materials and methods

Analytical procedures

Drug screening in urine and hair samples was performed using CE-labelled enzyme-linked immunosorbent assay (ELISA) kits for amphetamines, methamphetamines, cannabinoids, cocaine/benzoylecgonine, opiates, methadone, and benzodiazepines that were obtained from NAL-vonMinden GmbH (Regensburg, Germany). For the hair testing, the ELISA calibrators were prepared in drug free hair matrix using 10 mg aliquots of hair at concentrations recommended by the new Guidelines for driving license re-granting (Table 1).

The validated ELISAs were run on a BEP 2000 Advance®System from Siemens Healthcare Diagnostics.

The urine samples were first tested for ethylglucuronide (EtG) with immunoassay (DRI-EtG EIA, ethyl glucuronide immunoassay; Microgenics Corp. (Passau, Germany). on an AU5400 analyser (Beckman Coulter).

The confirmation analyses for drugs of abuse in urine and hair were performed according to previously published GC-MS methods after sample preparation using solid-phase extraction (SPE) cartridges, deuterated internal standards, appropriate derivatizations, and selected ion monitoring (SIM) mode.[7] Positive samples for EtG

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Table 1. Comparison of old and new cut-off values in urine and hair after introduction of new German guidelines for Medical Psychological Assessment (MPA) for driving license re-granting (6)

	Old cut-offs in urine (ng/mL)	New MPA cut-offs in urine (ng/mL)	Old cut-offs in hair (ng/mg)	New MPA cut-offs in hair (ng/mg)
Amphetamines and designer-amphetamines	500	50	0.2	0.1
THCOOH / THC	50	10 (after hydrolysis)	0.1	0.02
Cocainics	300	30	0.5	0.1
Opiates	300	25	0.2	0.1
Methadon	200	50		0.1
Benzodiazepines	200	50		0.05

in urine were confirmed with GC-MS (Shimadzu GC-MS-QP-2010) in SIM mode at a cut-off of 100 ng/mL The determination of EtG in hair was carried out using the recently published headspace solid-phase microextraction-GC-MS/MS method.[8] The lower limit of detection (LLOD) of the method was 0.5 pg/mg hair and the lower limit of quantification (LLOQ) 2.8 pg/mg hair.

Table 2 presents the cut-off limits for EtG in hair used at our laboratory, which are in line with the German MPA guidelines.[6] An EtG concentration in hair above 30 pg/mg hair is evidence of chronic excessive alcohol consumption. This is conform with the consensus value of the Society of Hair Testing (SOHT).[9] Concentrations between 7 and 30 pg/mg indicate moderate consumption and values <7 pg/mg are suggestive of abstinence or very low alcohol intake.

The ELISA screening tests and the GC-MS confirmation methods in urine and hair were accredited according to DIN EN ISO/IEC 17025 for forensic purposes as required by the new MPA guidelines [6] using the validation guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh).[10] The details have been described separately.[11]

Samples

In accordance with the new MPA guidelines,[6] the traffic psychologists ordering the drug tests generally requested from the applicants, who had lost their driver's license, six urine tests and additional hair analyses to cover the 12-month period during which abstinence had to be proven. Urine collection had to take place within 24 h of short notice and occurred under supervision. Hair samples were tested in periods of time during which drugs and EtG were not tested in urine. There was no evidence of any pre-selection of subjects and the psychologists ordering the analyses confirmed that there was generally a random testing of urine and hair.

In 2010, 14 000 urine and 3900 hair samples were analyzed for drugs of abuse and approximately the same number of samples were analyzed for EtG. The exact figures are shown in Tables 2 and 3.

Statistics

The detection rates of amphetamines, cannabinoids, cocaine/benzoylecgonine, opiates, methadone, benzodiazepines, and ethylglucuronide in urine and hair were compared using the chi-square test. Significant differences are indicated with an asterisk ($* = p < 0.01$) (Tables 2 and 3).

Results

Amphetamines and methamphetamines

In 2010, we tested 14 000 urine and 3900 hair samples at the new MPA threshold values shown in Table 1. The percentage of positive methamphetamines and MDMA (methylenedioxymethamphetamine) results confirmed by GC-MS was significantly higher in hair (0.20% and 0.23%) than in urine (0.12% and 0.04% respectively) ($p < 0.01$). In contrast, the detection rate for amphetamines and MDA (methylenedioxyamphetamine) did not significantly differ between urine and hair. MDEA (methylenedioxyethylamphetamine) was found neither in urine nor in hair.

Cannabinoids

The percentage of positivity of cannabinoids in urine (3.21%) and hair (3.35%) was in the same range and did not differ significantly (Table 3).

Cocaine and benzoylecgonine

The high proportion of positive results for cocaine and benzoylecgonine in hair compared with urine is presented in Table 3. In 3.18% of the subjects tested for abstinence, benzoylecgonine was found in hair, compared with 0.83% in urine ($p < 0.01$). The detection of this metabolite in hair or urine can be taken as an indication of cocaine consumption. Since benzoylecgonine can be produced by the hydrolysis of cocaine, the concentration of this metabolite in hair should be at least 5% of the parent substance to be highly

Table 2. Detection rates of ethylglucuronide (EtG) in urine and hair. Number of urine and hair samples tested in 2010, cut-off values in both samples and detection rates of ethylglucuronide (percentages) in urine and hair as confirmed with GC-MS and GC-MS/MS respectively. Significant differences between detection rate in urine and hair are indicated with an asterisk ($* = p < 0.01$)

	Urine samples number	Cut-off ng/mL	Detection rate in urine %	Hair samples number	Cut-off pg/mg	Detection rate in hair %
Ethylglucuronide	13,441	≥100	2.1	3,952	>7	12.7 *
				3,952	7 – 29	7.8
				3,952	>30	4.9

Table 3. Detection rates of drugs of abuse in urine and hair. Number of urine and hair samples tested in 2010, cut-off values in both samples and detection rate of drugs of abuse (percentages) in urine and hair as confirmed with GC-MS or LC-MS/MS. Significant differences between detection rates in urine and hair are indicated with an asterisk (* = p <0.01)

	Urine samples number	Cut-off ng/ml	Detection rate in urine %	Hair samples number	Cut-off ng/mg	Detection rate in hair %
Amphetamines	14,177	≥50	1.46	3,925	≥0.1	1.32
Methamphetamines	14,177	≥50	0.12	3,925	≥0.1	0.20 *
MDA	14,177	≥50	0.02	3,925	≥0.1	0.02
MDMA	14,177	≥50	0.04	3,925	≥0.1	0.23 *
THCOOH	14,774	≥10	3.21			
THC				4,209	≥0.02	3.35
Cocaine	14,168	≥30	0.06	4,124	≥0.1	4.49 *
Benzoylecgonine	14,168	≥30	0.83	4,124	≥0.05	3.18 *
Morphine	14,172	≥25	0.52	3,941	≥0.1	0.23
Monoacetylmorphine	14,172	≥10	0.03	3,941	≥0.1	0.28 *
Codeine	14,172	≥25	0.15	3,941	≥0.1	0.43 *
Dihydrocodeine	14,172	≥25	0.02	3,941	≥0.1	0.13 *
Methadon	14,156	≥50	0.45	3,883	≥0.1	0.59
EDDP	14,156	≥50	0.44	3,883	≥0.1	0.54
Diazepam	14,163	≥50	0	3,854	≥0.05	0
Nordiazepam	14,163	≥50	0.10	3,854	≥0.05	0.03
Oxazepam	14,163	≥50	0.21 *	3,854	≥0.05	0
Aminoflunitrazepam/ flunitrazepam	14,163	≥50	0.02	3,854	≥0.05	0.03
Bromazepam	14,163	≥50	0.02	3,854	≥0.05	0.08
Alprazolam	14,163	≥50	0	3,854	≥0.05	0
Lorazepam	14,163	≥50	0	3,854	≥0.05	0

suggestive of endogenous incorporation.[12] This criterion was used in the classification of the hair results. All hair samples positive for benzoylecgonine (n: 131) were also positive for cocaine. Cocaethylene, which is a marker of simultaneous consumption of cocaine and alcohol, was positive in 0.75% of the hair samples tested.

Opiates

The metabolite monoacetylmorphine (MAM) is a marker of heroin consumption. With 0.28% it was found significantly more often in hair than in urine (0.03%)($p<0.01$)(Table 3). This was also the case for codeine and dihydrocodeine. In contrast, morphine was detected more often in urine with a positivity rate of 0.52% versus 0.23% in hair (Table 3). The level of significance was between $p<0.05$ and $p<0.01$.

Methadone

The detection rates of methadone or EDDP (2-ethylidene-1, 5-dimethyl-3.3-diphenyl-pyridolin), which is the main metabolite of methadone, did not differ between urine and hair samples (Table 3).

Benzodiazepines

The benzodiazepines analyzed are presented in Table 3. The detection rates of nordiazepam and oxazepam in urine were 0.10% and 0.21%, respectively. The difference with hair was statistically significant for oxazepam ($p<0.01$). All in all, the number of benzodiazepines detected in urine and hair was small in the collective studied. Diazepam, alprazolam, and lorazepam were found neither in urine nor in hair, and aminoflunitrazepam/flunitrazepam and bromazepam were measured in only a small number of urine and hair samples (Table 3).

Ethylglucuronide

With 12.7%, the percentage of positive EtG results in hair was much higher than the 2.1% in urine (Table 2). 7.8% of those positives in hair demonstrated EtG concentrations between 7 and 29 pg/mg which is considered evidence for moderate alcohol consumption and 4.9% had values above 30 pg/mg which is evidence for chronic excessive alcohol abuse.[9]

Discussion

The major findings of this study are the much higher percentage of positive cocaine and benzoylecgonine results and the significant higher detection rate of methamphetamine, MDMA, monoacetylmorphine, codeine, and dihydrocodeine as well as ethylglucuronide in hair compared with urine.

On the other hand, the percentage of positive benzodiazepines (oxazepam, nordiazepam) was higher in urine than in hair.

In several studies, the detection rate of drugs of abuse was compared between urine and hair. The number of subjects tested was often small. In a pre-employment study,[1] a large number of subjects (n: 9700) were tested using both hair and urine samples. The rate of drug positive hair assays was approximately five times that of urinalysis. A limitation of this study actually was that in pre-employment testing, the applicant to be monitored can anticipate the use of a drug test, and therefore can abstain from drug consumption for a few days with the result of a negative

urine test. Under these circumstances, a hair analysis is far more likely to reveal drug use than a urine test. This could in part account for the 0.83 % positive cannabis results in urine which is far less than in our study (3.21%). The differences can also in part be explained by the lower threshold values (50 ng/ml) we used.

In a pilot study, Kronstrand *et al.*[2] compared the detection rates of drugs in urine and hair in a similar group of subjects as ours in the context of driving license re-granting. Ninety-nine hair samples and 198 urine samples were analyzed with higher threshold values than in the present study. They found higher detection rates for amphetamines in hair but did not distinguish between amphetamines and methamphetamines. Cocaine was found in hair not in urine. The detection rates of benzodiazepines were higher in urine than in hair. These results agree with what we found.

The Drug Testing Index of 2009[13] reported that in the general US workforce hair testing showed higher levels of cocaine and methamphetamine use than urine testing; however, the thresholds used in urine were much higher than those which were applied in the present work.

The higher detection rate of several drugs of abuse in hair is consistent with what we know about accumulation in hair and excretion of those substances in urine. Nakahara *et al.*[14] investigated systematically the incorporation rate (ICR) of 20 frequently used drugs in hair in animal experiments (Table 4). The ICR was defined as the ratio of the drug concentration in rat hair to the area under the plasma concentration versus time curve (AUC). The ICR correlated with lipophilicity and basicity of the drugs which are essential characteristics for their incorporation into the hair. Cocaine, methamphetamines, and monoacetylmorphine are more lipophilic than their more polar metabolites benzoylecgonin, amphetamine, and morphine which enter the hair to a lesser extent.[12] As can be seen in Table 4, cocaine was found in animal experiments to have the highest incorporation rate in hair. This could in part also explain the high proportion of positive cocaine results in humans. Moreover, the detection time of cocaine in urine is very short (between 6 and 12 h)[15] which may have accentuated the difference. Benzoylecgonine can be detected in urine for 2 to 3 days [15] which may explain the higher detection rate in urine of 0.83% compared with 0.06% for cocaine. The factors responsible for the high percentage of benzoylecgonine positive hair samples (3.18%) are less clear, taking into consideration its low incorporation rate in experimental studies (Table 4).[14]

The percentage of positive amphetamine results in hair was about the same as in urine (1.32% versus 1.46%). Methamphetamines were detected more often in hair (0.20% in hair compared with 0.12% in urine), which could be explained by the higher ICR of methamphetamines (Table 4), the mean urinary excretion half-lives of methamphetamines and amphetamines being about the same with 23 and 22 h, respectively.[16]

Table 4. Incorporation rate (ICR) of frequently used drugs of abuse in hair in animal experiments. The ICR was defined as the ratio of the drug concentration in rat hair to the area under the plasma concentration versus time curve (AUC). The data originate from the work of Nakahara and coworkers (14)

	ICR (conc in hair/AUC)
Amphetamines	0.15
Methamphetamines	0.29
MDA	0.5
MDMA	0.6
Cocaine	3.6
Benzoylecgonine	0.003
Morphine	0.03
Monoacetylmorphine	0.21

Monoacetylmorphine has a short urinary excretion time between 5 and maximally 10 h, which could account for the low detection rate in urine. It accumulates well in hair, as can be expected from the relatively high ICR value (Table 4).

The percentage of positive cannabis results in hair and urine was in the same range. The long excretion time of THC-COOH in urine for up to several days in regular consumers and the high incorporation of THC in hair due to its lipophilicity are consistent with the high rate of positive results of over 3% in urine and hair. Probably most regular cannabis consumers among those subjects tested trying to regain their driver's license are detected either with a urine test or a hair test.

The most obvious argument for the higher positivity rate of drugs of abuse in hair compared to urine is the larger window of detection. Since benzodiazepines were found more often in urine than in hair, other explanations for this paradox have to be looked for. Taking into account the long urinary excretion time (nordiazepam, 7-aminoflunitrazepam, bromazepam), the high urinary concentrations of some benzodiazepines tested (nordiazepam, oxazepam, bromazepam) and the fact that they do not accumulate well in hair matches with the findings that these substances were found more often in urine than in hair. In hair only 5 of 3854 hair samples were positive for benzodiazepines (Table 3), and it seems legitimate to question whether a screening for seven benzodiazepines in hair as proposed by the new German guidelines is cost-effective.

In 2009, we compared the results of 800 hair samples with those of 4800 urine samples and we found a much higher detection rate of ethylglucuronide in hair than in urine.[17] With the present study, we confirmed these differences in 2010 with a larger number of subjects. The percentage of positive EtG results in hair (12.7%) was significantly higher than in urine (2.1%). So more than 10% of the candidates wanting to attest their alcohol abstinence had EtG values in hair documenting alcohol consumption (≥7 pg/mg) and 4.9% provided evidence for a chronic excessive alcohol intake (>30 pg/mg).

A strong argument for hair testing is its better acceptance by the clients. If urine tests have to be performed six times per year, as recommended by the German Guidelines for driver's license re-granting, it means that the client has to be at the disposal of the driving license issuing agency for urine collection over a period of twelve months. Kronstrand *et al.*[1] reported that clients preferred hair sampling, considering it a better means to prove their abstinence than urine testing.

Besides the large window of detection which allows a retrospective investigation of drug or alcohol abuse, hair testing has the additional advantages that sample collection is straightforward, the risk of adulteration is low, and the transport of a hair sample to the laboratory does not present problems.

In conclusion, hair testing is a highly efficient procedure to disclose drugs of abuse. It can disclose more cases of cocaine and heroin abuse as well as chronic excessive alcohol consumption. On the whole, the differences of detection rates observed for drugs of abuse in hair and urine have a comprehensible physiological and pharmacokinetic basis, even if some differences, such as the high positive rate of benzoylecgonine in hair, cannot be explained on the basis of its ICR. The study reveals that in the control of abstinence in the context of driving license re-granting, there are in part large differences of positivity rates for some drugs or metabolites

between hair and urine samples. These differences should be taken into account by traffic physicians and psychologists ordering the drug testing.

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