Results of hair analyses for drugs of abuse and comparison with self-reports and urine tests

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Abstract

Urine as well as head and pubic hair samples from drug abusers were analysed for opiates, cocaine and its metabolites, amphetamines, methadone and cannabinoids. Urine immunoassay results and the results of hair tests by means of gas chromatography–mass spectrometry were compared to the self-reported data of the patients in an interview protocol. With regard to the study group, opiate abuse was claimed from the majority in self-reports (89%), followed by cannabinoids (55%), cocaine (38%), and methadone (32%). Except for opiates the comparison between self-reported drug use and urinalysis at admission showed a low correlation. In contrast to urinalysis, hair tests revealed consumption in more cases. There was also a good agreement between self-reports of patients taking part in an official methadone maintenance program and urine test results concerning methadone. However, hair test results demonstrated that methadone abuse in general was under-reported by people who did not participate in a substitution program. Comparing self-reports and the results of hair analyses drug use was dramatically under-reported, especially cocaine. Cocaine hair tests appeared to be highly sensitive and specific in identifying past cocaine use even in settings of negative urine tests. In contrast to cocaine, hair lacks sensitivity as a detection agent for cannabinoids and a proof of cannabis use by means of hair analysis should include the sensitive detection of the metabolite THC carboxylic acid in the lower picogram range.

Keywords: Hair analysis; Urine test; Drugs of abuse; Self-report; Maintenance

1. Introduction

Monitoring prescribed and non-prescribed drug use during treatment programs provides valuable information for the diagnosis and management of patients. It has been repeatedly shown that the information on personal history of drug use is far from accurate [1–3]. Fearing consequences, most users tend to deny or under-report illicit drug consumption. Urine testing is a feature of most official methadone maintenance programs. Unfortunately, urine is too problematic to handle because it cause infections, requires refrigeration or freezing for (long-term) storage, and must
often be collected under observation. Considering the limitations of self-reports of drug use such testing is highly important either for monitoring the progress of individual patients or for assessing the effectiveness of particular interventions in controlled clinical trials. The most commonly used screening methods use immunology-based assays. Confirmation by chromatographic analysis is often not required.

However, hair tests offer some advantages over urine assays [4,5]; the collection of hair specimens is less embarrassing and less intrusive for study subjects. Hair analysis allows a cumulative reflection of long-term abuse. Another advantage of hair samples is that they can be stored at room temperature and do not need to be quickly analysed after collection. Moreover, the window of detection of drug abuse in hair tests is considerably wider than that of urine assays and is only limited by the length of the hair. It typically ranges from a week to several months compared with 2–3 days with urinalysis.

The aim of the present study was the collection and assessment of positive hair test results to demonstrate the robustness and applicability of our analytical methods. Segmentation data (head hair) and pubic hair concentration results are compared and, additionally, dose concentration relationships are calculated. Furthermore, the usefulness of different bioassays for drugs of abuse like urine and hair tests are compared with self-reports.

2. Materials and methods

2.1. Collective

For the study, known drug users (51 persons) from a psychiatric clinic were enrolled. The project was approved by the local ethics committee. An interview protocol was chosen, which incorporated the DSM-IV and ICD-10 diagnostic criteria. The interview protocol also included questions about the period of most recent use of various drugs.

2.2. Toxicological tests

2.2.1. Urine tests

Urine samples were taken by clinical staff as part of the routine medical screening at the day of admission intake and were analysed by the clinical laboratory for the presence of opiates, cocaine metabolites, cannabinoids, amphetamines and methadone using standard immunoassay screening tests and cut-off levels proposed by the manufacturer (Abbott TDx). There were no attempts to confirm positive results.

2.2.2. Hair tests

At the initial assessment, each patient was asked to provide a hair sample for analysis after completion of the interview. Hair segments were cut at the base of the crown as close to the scalp as possible. The samples were then wrapped in aluminium foil with the root ends marked, enclosed in an envelope, and coded. Additionally, patients were asked to provide a pubic hair sample.

Samples shorter than 3 cm as well as pubic hair samples were analyzed. Longer samples were cut in two segments each 3 cm in length and analyzed.

Hair samples were subsequently washed for 5 min in 5 ml of deionised water, dichloromethane and petroleum ether, respectively. After drying, the hair samples were cut into small pieces of about 1 mm. The washing solutions were analysed by conventional gas chromatographic–mass spectrometric (GC/MS) procedures to exclude contamination. Drug analysis of the washed hair samples was performed using validated GC/MS methods. The analysis of opiates (morphine (MO), 6-acetylmorphine (6AM), codeine (COD), dihydrocodeine (DHC) and cocaine (COC) and its main metabolite benzoylcegonine (BE) was performed after solid-phase extraction (SPE), according to the method of Cone and coworkers [6,7]. Fully automated procedures for the analysis of cannabinoids (Δ⁹-tetrahydrocannabinol (THC), cannabionol (CBN), cannabidiol (CBD)), methadone (METH), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and amphetamine as well as designer drugs (amphetamine (AP), methamphetamine (MAP), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), 3,4-methylenedioxyphenyl-2-butanamine (BDB) and N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) after alkaline hydrolysis followed by headspace solid-phase microextraction (HS-SPME) were described previously [8,9].

The cut-off values for the urine and hair tests used in the study are summarized in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Opiates</th>
<th>Cocaine</th>
<th>Methadone</th>
<th>Cannabinoids</th>
<th>Amphetamines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine test (ng/ml)</td>
<td>200</td>
<td>300</td>
<td>250</td>
<td>25</td>
<td>300</td>
</tr>
<tr>
<td>MO: 0.1</td>
<td></td>
<td></td>
<td>METH: 0.10</td>
<td>THC: 0.14</td>
<td>AP designer drugs: 0.10</td>
</tr>
<tr>
<td>6AM: 0.1</td>
<td></td>
<td>BE: 0.2</td>
<td>EDDP: 0.10</td>
<td>CBN: 0.12</td>
<td></td>
</tr>
<tr>
<td>COD: 0.05</td>
<td></td>
<td></td>
<td></td>
<td>CBD: 0.10</td>
<td></td>
</tr>
<tr>
<td>DHC: 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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3. Results and discussion

The cohort of drug users in the psychiatric clinic consisted of 39 men and 12 women aged 20–53 years (mean 31.7 years). From all patients a urine sample for immunological drug screening was taken on admission, additionally self-reports of recent drug abuse had been given. Hair samples of the head were obtained from all patients and specimen length varied from 1 to 42 cm with a mean length of 12.9 cm. A proximal segment of up to 3 cm length was analysed in 47 cases (seg. 1), an intermediate segment (seg. 2) in 31 cases and a third (distal) segment (rest) in 24 cases (seg. 3). A pubic hair sample was obtained from 36 persons.

3.1. Prevalence rates

In Table 2 the sample prevalence rates of drug abuse of the toxicological analyses and self-reports are compared. A hair test was evaluated as positive if any segment showed a positive result. This was done because self-reports showed various periods of time concerning past drug abuse.

With regard to the study group opiate abuse was admitted by the majority of self-reports (89%), followed by cannabinoids (55%), cocaine (38%), and methadone (32%). Amphetamine abuse was not significant in this cohort. Except for opiates the comparison between self-reported drug use and urinalysis at admission showed only a low correlation. In contrast to urinalysis, hair analyses revealed consumption in more cases. Even the good agreement between self-reports and urine test results concerning methadone can be explained by the fact that these patients took part in an official methadone maintenance program. Comparing self-reports and hair test results for methadone, methadone abuse in general was under-reported, especially by people who did not participate in a substitution program. After a relaxation of regulations concerning so-called take-home prescription rules, an increase of methadone availability on the black market has been observed [10]. After comparison of self-reports and the results of hair analyses for cocaine it was obvious that the use of cocaine was dramatically under-reported. Cocaine hair tests appear to be highly sensitive and specific in identifying past cocaine use even in settings of negative urine tests. These findings are in concordance with similar studies [1,3,11–15]. According to Magura and Kang [13] the under-reporting of cocaine abuse might be attributable to several factors: patients might have been suspicious or fearful of the research interviewers; cocaine use is stigmatized; cocaine use is rather infrequent and, consequently, patients do not define themselves as using the substance at all. Yet, self-report and urine test alone miss most drugs, and especially cocaine users in different risk populations [11,16–21]. Hair tests improve detection significantly and thus can enhance various evaluations; for example the effects of prenatal cocaine use on fetal and child development [22–24].

Otherwise the relatively high number of cases, which tested hair “negative”, and urine “positive” for cannabinoids is indicative for the assays’ failure to detect THC in the hair. This phenomenon was also described by other groups [3,25], and can be explained by the assays’ limited sensitivity. In our opinion a proof of cannabis use by means of hair analysis should include the sensitive detection of the THC metabolite THC carboxylic acid (THC-COOH) in the lower pg range [26–28].

3.2. Head hair results

The head hair test results among the self-reported drug users are demonstrated in Table 3. 6AM was found in higher concentrations than MO in opiate positive samples. The

<table>
<thead>
<tr>
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<th>Cocaine</th>
<th>Cannabinoids</th>
<th>Amphetamines</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>54</td>
<td>40</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Maximum</td>
<td>41.2</td>
<td>16.8</td>
<td>0.8</td>
<td>211.6</td>
</tr>
<tr>
<td>Mean (ng/mg)</td>
<td>3.43</td>
<td>1.34</td>
<td>0.3</td>
<td>13.75</td>
</tr>
<tr>
<td>Median (ng/mg)</td>
<td>1.6</td>
<td>0.83</td>
<td>2.85</td>
<td>0.81</td>
</tr>
</tbody>
</table>
concentration ratio 6AM/MO was always >1.3 in the proximal segment, but decreased to the intermediate and distal segments. COD was detected in 16 samples in concentrations above 0.8 ng/mg only when 6AM and MO were found in significantly higher concentrations, so that a consumption of a COD preparation was excluded. DHC was not detected in any case. COC was the characteristic substance in order to detect a cocaine abuse and the concentration ratio BE (if present) to COC was always >0.05. THC and METH were found more frequently than CBD or CBN or EDDP, respectively.

3.3. Dose concentration relationship in hair tests

As described above an interview protocol included questions about the period and dose of recent drug abuse. Dose concentration relationships in head hair tests were calculated by linear regression. Surprisingly, after heroin abuse (indicated by grams per day) significant dose concentration relationships were found for MO ($r = 0.82; p < 0.001$) and 6AM ($r = 0.80; p < 0.001$). For all other drugs no correlation between ingested dose and hair concentration of the drug or metabolites was found. An explanation for this could be that the self-reported dose patterns are poor approximations of the real amount of drug used and of course the purity of the illicit drugs is variable and unknown.

3.4. Pubic hair concentrations

The pubic hair concentrations are given in Table 4. Drug concentrations in the pubic hair samples showed higher or equal concentrations when compared to the proximal or intermediate segment from the head hair. This known fact [29–34] can be used for plausibility control in forensic cases. In contrast to the head hair results MO and BE were found in higher concentrations than 6AM or COC in pubic hair. Because of differences in the growth cycle a larger amount of pubic hair is known to be in catagen and telogen phases. Therefore, higher concentrations of drugs can be expected. On the contrary, high levels of analytes present in urine (MO, BE) may contaminate pubic hair. Also, additional hydrolytic processes in pubic hair have to be taken into account, when the results are compared to head hair. However, in some cases higher concentrations were found in the distal segment of the head hair. In these cases the window of detection in the pubic hair sample was considered to be wider than in a pubic hair sample. Considering all this, pubic hair analysis seems to be an alternative if head hair is not available, although the ability to reconstruct a longitudinal history of drug use is not possible using this sample material. In principle, body hair should not be used in aptitude tests (e.g. in driving ability cases), because no comparison to head hair results is feasible [27,28].

3.5. Hair segmentation data

In most cases hair segmentation data showed an increase in drug concentration from proximal-to-distal orientation. Considering the study group, this phenomenon can be explained by a more frequent drug abuse in the past. Yet diffusion and especially drug incorporation via sweat and sebum has to be taken into consideration. In principle, this diffusion into the hair does not interfere with the ability of the test to identify a drug user, however, this fact complicates the segmental regression of the level of drug expected in hair per unit dose and may explain drug concentrations in the distal segments that deviated from self-reports. Drug concentrations in the pubic hair samples were higher or similar concentrations than in the proximal or second segment from the scalp. In spite of the fact that pubic hair is shorter, due to its different three-stage growth cycle it probably represents the same time period as head hair. However, in some cases higher concentrations were found in the third segment of the head hair. In these cases the window of detection in the head hair sample was considered to be wider than in a pubic hair sample.

4. Conclusions

Cocaine and cannabis tests are typical examples of different bioassays of urine and hair that are not comparable, because they represent different time frames. By means of hair analysis, cocaine abuse is detectable for 2–6 months after a single 25–35 mg dose of drug administered intravenously [35]. A positive urine test can only be expected within 2–3 days after consumption of the drug. If one compares hair and urine assays for cocaine, it is reasonable to expect a lot of hair positive and urine negative results, considering the windows of detection for each assay. In contrast cannabi-
noids are slowly excreted via urine, and as a consequence urine analysis has a broader detection window for cannabinoids than it does for cocaine (metabolites). Additionally, it is a known fact that THC and especially THC-COOH have a very low incorporation rate (ICR) into hair, with a 3600-folds difference between ICR of cocaine and that of THC-COOH, the drug with the lowest ICR investigated [36]. In contrast to cocaine, hair lacks the sensitivity to detect cannabinoids [3,25].

In summary biological specimens (such as urine and hair and for other purposes saliva, breath, and blood) are very useful as objective indicators of substance abuse. However, as all have inherent and different limitations in measuring the timing, duration, frequency, and intensity of drug use, the specimen of choice depends on the circumstances and special analytical problems.

References