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Validity of Substances of Abuse Detection in Hair by Biochips Array Technology

Ahmed R Ragab[1,2*], Maha K Al-Mazroua[2], Mona A El-Harouny[1], Moustaf M Afify[3], Mohamed Omran[2], Calrole Katbai[2], Mohamed A Al-Qurnay[2], Ismaiel Al Saeed[2] and Faisyl Al-Zweide[2]

[1]*Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Mansoura University, Mansoura, Egypt*

[2]*Dammam Poison Control Center, Dammam, Eastern Region, Ministry of Health, Saudi Arabia*

[3]*Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Beni Suef University, Beni Suef, Egypt*

[*]**Corresponding author:** Ahmed R Ragab, Dammam Poison Control Center, Dammam, Eastern Region, Ministry of Health, Saudi Arabia, Tel: 966540990033; E-mail: ahmedrefat1973@yahoo.com

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Abstract

Using hair as a biological specimen to analyze drug abuse has been receiving elevated attention during last years because of flexibility of collection procedure and because hair does not autolysis like other body fluids or tissues. Hair testing provides a broad-spectrum detection window after drug exposure than urine testing. Hair analysis has specific criteria that it provides a way of obtaining information that cannot be acquired by other commonly used forensic toxicology analytical procedures, such as blood or urine analysis. In the hair matrix, many xenobiotics are trapped permanently, in disparity to the situation with blood or urine where they are only identifiable for a few hours or days. Subsequently substance of abuse detection by hair analysis should be the target procedure in the clinical and forensic toxicology aspect when the evaluation of repeated or chronic exposure to a drug is indicated, e.g. in the case of pre-employment/workplace testing, criminal affairs or a driving license restoration. In the current study, seventy cases of the application of hair analyses using this technique for the determination of substances of abuse (opiates, cannabis, amphetamine, barbiturate, benzodiazepines, and cocaine) are investigated. Analytical toxicology procedure for detection of substance of abuse is critical important: Biochips array technology that confirmed with GC/MS has proved to be a highly sensitive and specific technique for the detection of substances of abuse at very low concentrations in hair except for cannabis.

Keywords: Biochips array technology; Substance of abuse; Toxicological hair analysis; Substances of abuse; Cannabis; Opiates; Cocaine; Amphetamine

Introduction

The accessibility of detecting substances of abuse and other toxic agents in hair has delivered considerable interest. Substances of abuse incorporated in hair matrix remain unchanged for a long time, thus creating a wider spectrum of substances of abuse detection than that obtained from the analyses of substances of abuse in blood or urine. Monitoring of abused substance of provides a valuable informative data for the accuracy of diagnosis and the progress of management of the patient. It has been and repeatedly shown the information for the diagnosis and personal history of substance of abuse is far from the accurate [1].

Many substances present in the blood will be passed rapidly to the hair follicle and, if they are capable of transport the cell membrane, will enter the hair matrix where they remain almost unchanged because they do not generally participate in metabolic reactions. The hair growth rate on average is 1.0-1.5 cm per month, so hair that is only a few centimetres long allows a retrospective study of substances of abuse consumption over duration of several months [2].

Usually most of substance abusers tend to deny or under-report illicit drug consumption list for fear of consequences. Urine sample is too problematic to handle as an only suitable sample for detection of substances of abuse because it cause infection, needs refrigeration or freezing for (long-term) storage, and must be collected under direct/indirect observation with special sample precautions for collections [3]. Considering the limitations of self-reports of substance abuse such laboratory testing in highly important either for monitoring the progress of individual cases. The most commonly used screening methods use immunology-based assays. Confirmation by chromatographic analysis is randomly required for clinical purposes and obligatory applied for medico-legal situations [4].

The aim of the current study was to investigate the validity method of drug hair analysis by Biochips Array Technology screening procedure. Furthermore, the usefulness degrees of different bioassays for drugs of abuse like urine and hair tests are compared with self-report results.

Subjects and Methods

Data collection

In the current study, known substance abusers (70 cases) from psychiatric clinic (Riyadh and Dammam) Al-Amal hospitals were enrolled. The research was approved by Dammam Poison Control Center- ministry of health ethics committee. An interview protocol was designed, which incorporated the DSM-IV and ICD-10 diagnostic criteria. The interview protocol also involved questions about the period of most recent/old abuse of various substances.

Toxicological investigations

Urine investigations: Urine samples were taken by clinical staff as part of the routine medical screening procedure at the day of admission intake and were analyzed by the poison control center for the presence of opiates, amphetamines, cannabinoids, barbiturates, benzodiazepines, and cocaine using standard immunoassay screening tests and cut-off levels proposed by the manufactures (Abbott TDs). There were GC/MS and LC/MS/MS confirmatory procedures to all positive results for more accurate results.

Hair investigations: At the initial assessment, each patient was asked to give a hair specimen for analysis after completion of the interview. Hair segments were cut at the base of the crown as close to the scalp as possible at a middle part of occipital region. The hair specimens were then wrapped in aluminium foil with the root ends marked enclosed in an envelope at room temperature. Specimen shorter than 3 cm were analyzed. Longer specimen were cut in three segments each 3 cm in length and analyzed.

Hair specimens were subsequently washed for 2 hours with 10 ml of Acetone. After decanting the acetone allowing hair drying overnight, the hair samples were grinded manually to transform into a powder condition. The hair sample was weighted to obtain 30-100 mg of hair powder and place in a glass test tube with a screw cap. Two ml of methanol was added to the glass test-tube containing the hair sample and its cap was tightly screwed. The mixture was heated at 80◦C for 1 hour. The tube was centrifuged after cooling to room temperature. 1.5 ml of the clear methanolic phase was separated and leaved to dryness. 0.8 ml of Tris buffer was added to 0.2 ml of methanol {Buffer: 12.1 g Tris (hydroxyl methyl) amino methane in 100 ml of water, hydrochloric acid is added until the pH is 8}. The mixture was diluted with deionized water (1:100). Then, the mixture was vortexed for 1 minute [5]. Finally, the supernatant is the effective sample that can be aspirated and applied to the biochip immuno-analyzer array technology analyzer (Randox/Drugs of Abuse BAT Array).

The prepared hair samples were analyzed by conventional gas chromatographic-mass spectrometric (GC/MS) procedures to confirm the positivity of the results. Substances of abuse analysis of the prepared hair samples were performed using validated GC/MS methods. The analysis of opiates morphine (MO), 6-monoacetylmorphine (6 MAM), codeine (COD) and its main metabolite benzylecogonine (BE), amphetamine (AP), methamphetamine (MAP), 3,4-methylendioxyamphetamine (MDMA), 3,4-methylendioxymethamphetamine (MDMA),) was performed after alkaline hydrolysis followed by liquid-phase extraction (6,7). The analysis of cannabinoids (⌂9-tetrhydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD) was performed after solid phase extraction SPE [6]. The cut-off values for the urine and hair tests used in the study are summarised in Table 1.

Results and Discussion

The studied group of substance abusers in psychiatric clinic (Riyadh and Dammam) Al-Amal hospitals consisted of 70 men 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 substance 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 12.9 cm. A proximal segment of up to 3 cm was analysed in 53 cases (seg. 1), an intermediate segment (seg. 2) in 14 cases, and a third (distal) segment (rest) in 3 cases (seg.3).

Cut-off Level (ng/ml)/(ng/mg)		
Drugs of Abuse Type	**Urine Sample (ng/ml)**	**Hair Sample (ng/mg)**
Opiates	300 ng/ml	Morphine (MO): 0.1, *6-MAM: 0.1 & Codeine 0.05 and: DHC:0.1
Amphetamines	300 ng/ml	Amphetamines (AP)designer drugs: 0.10
Cannabinoids	25 ng/ml	THC***: 0.14, Cannabinol (CBN), 0.12 Cannabidoil(CBD):0.1
Cocaine	300 ng/ml	Cocaine(COC): 0.1 & Benzyleconine (BE)0.10

Table 1: Cut-off levels for the urine and hair tests used in the study. *6-Mono-Acetyl Morphine; **Di-hydrocodiene, *** Tetra-Hydo, Cannabinol.

In Table 2, the samples prevalence rates of drug abuse of toxicological analyses and self-report are compared. A hair test was evaluated as positive if any segment showed a positive result by Biochips Array Technology followed by and GC/MS confirmed procedures. This was done because self-reports showed various periods concerning past substance abuse.

With regard to the study group cannabis abuse was admitted by the majority of self-report (51.4%), followed by amphetamine (28%), and opiates (27.1%). Benzodiazepine and Cocaine abuses were not significant in this cohort. Except for opiates, the comparison between self-reported substance abuse and urine analysis at admission showed only a low correlation. In contrast to urinalysis, hair analyses for substances of abuses revealed consumption in more cases. After comparison of self-reports and the results of hair analysis for opiates and amphetamines it was clearly that the use of opiates and amphetamines were under reported.

Furthermore, the relatively high number of cases 'all cases', which tested hair 'negative', and urine 'positive' or recent self-report 'positive' for cannabinoids is indicator for assays' failure to detect THC in the hair. This phenomenon was also described by other scientific research groups [7,8], and can be explained by the assays' limited sensitivity to detection of cannabis in hair samples. On the opposite side, other scientific researcher's states a scientific evidence of detection of cannabis abuse by means of hair analysis should involve the sensitive detection of the THC metabolite THC carboxylic acid (THC-COOH) in a very lower detection limit, (pg) detection unit [9-11].

The hair test results among the self-reported substance abuser are demonstrated in Tables 3-6. All positive results from Biochips Array Technology screening procedure were confirmed by GC/MS confirmatory procedure and they have been given 100% accuracy of results zero percentage of pseudo positivity/negativity results. By GC/MS confirmatory procedures 6 MAM was found in higher concentrations than MO in opiate positive samples. The concentration ratio 6 MAM/MO was always >1.4 in the proximal segment. COD was detected in 4 samples in concentrations above 0.7 ng/mg only when 6 AM and MO were found in significantly higher hair concentration, so that a consumption of a COD preparation was excluded. THC was not detected in any case. COC was detected only in one case.

As described above an interview protocol involved two questions about the period and dose of recent substance abuse. Dose amount and

length of duration/concentration relationships in head hair tests were calculated by linear regression. We found a significant finding, after heroin abuse (indicated by grams per day) significant dose concentration relationships were found for MO ($r=0.84$; $p<0.001$) and 6 MAM ($r=0.82$; $p<0.01$). For all other substance of abuse, no correlations between the dose of substance of abuse and hair level of the substance or its metabolites have been found. An explanation for this could be attributed to the self-reported dose patterns are poor approximation of the real amount of amphetamine dosage abused in addition to the degree of purity of the illicit amphetamines is markedly variable and completely unknown [12].

In most of the studied cases hair, segmentation data showed an increase in opiates and amphetamines concentrations from proximal-to-distal parts. Considering the study group, this phenomenon can be explained by diffusion and especially the abused substance incorporation via sweat and sebum secretions [13,14].

	Cannabinoids		Amphetamines		Opiates	
Self-report	36	51.40%	28	40%	19	27.10%
Urine test	25	35.70%	12	17.10%	8	11.40%
Hair test*	0	0%	19	27.10%	9	12.90%

Table 2: Comparative relationship of the prevalence/percentage substances of abuse figures between the toxicological laboratory detection and self-report documentation. (n=70). *Hair test by biochips array technology confirmed by GC/MS confirmatory procedures.

Amount of abuse	Amphetamines Total case (36)		Cannabis Total cases (28)		Heroin Total cases (19)	
Small	1 tablet/d	8	< 4 cig/d	21	< 0.5 gm/d	2
Medium	2-3 tablets/d	23	4-8 cig/d	4	0.5-1 gm/d	2
Large	> 3 tablets/d	5	>8 cig/d	3	>1.0 gm/d	15*

Table 3: Amount of substances of abuse by self-reported data. *P value <0.05.

Duration of Abuse		Number of cases
Minimum: 1.5 years	Short(<3 years)	16 Case (22.9%)
Maximum: 30 years	Intermediate (3-7 years)	18 Case (25.7%)
Means +SD): 9.6 + 7.6 years	Long (> 7 years)	36 Case (51.4%)

Table 4: Duration of substance of abuse by self-reported data.

Conclusion

Opiates, amphetamines and cannabis tests are typical examples of different bioassays of urine and hair that cannot comparable with each other's, because they represent different time frames of toxicological detection in biological samples. By means of hair toxicological analysis, opiates and amphetamines abuses are detectable up to 7 months after a single >1 gm of heroin and >20 mg of amphetamines respectively [15]. A positive urine test can only be expected within 2-3 days after consumption of the opiates or amphetamines. If one compares hair and urine assays for opiates or amphetamines, it is reasonable to expect a lot of hair positive and urine negative results, considering the windows of detection for each assays.

Amphetamines (Total No 19 cases)	Heroin (Total No 9 cases)
Positive Hair Test with positive self-report and urine samples	
13 (68.4%)	4 (44.4%)
Positive Hair Test with to negative self-report and/or urine samples	
6 (31.6%)	

Table 5: The relationship among positive findings in self-reports, urine and hair drugs testing. *P value < 0.05.

	Opiates			Amphetamines
	6 AM (ng/mg)	MO (ng/mg)	COD (ng/mg)	AP (ng/mg)
N	9	5	4	19
Minimum	0.1	0.1	0.1	0.3
Maximum	46.7	18.2	0.75	0.8
Means ± SD	4.24 ± 2.11	1.72 ± 0.4	0.28 ± 0.13	0.53 ± 0.16

Table 6: Hair test result from substance abuser in various hair segments (ng/mg).

In contrast, cannabinoids are slowly excreted via urine sample, and as a sequelae of toxicological urine analysis has a wide detection spectrum for cannabis than it does for opiates and amphetamines (metabolites). Additionally, it is a known fact that tetra-hydro-cannabinol THC and especially THC-COOH have a very low excretory pathway into hair, with 3600-folds differences between excretory rate of opiates and that of THC-COOH. In contrast to opiates and amphetamines, hair lacks the sensitivity to detect cannabinoids [16,17].

In conclusion, biological specimens (such as urine, blood, hair, saliva and breath) are very helpful as resources for different procedures of detection of substances abuse. Despite of their efficiency, all have variable degree of limitation in detection of abused substances, the parameter of selection a best sample for substance of abuse detection depends on multiple factors as the time of exposure, duration, degree and frequency of substance abuse.

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