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Hair Testing for Drugs of Abuse

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A B S T R A C T

Hair testing for drugs of abuse is a developing technology, which offers the possibility of longer detection times than is commonly obtained with urine analysis. It is the main method for evaluation of an individual's drugs of abuse history. In many countries hair analysis is routinely used to detect drug abuse in forensic cases, occupational and traffic medicine and clinical toxicology. Hair analysis in pregnant women, neonates and infants is a useful tool for the detection of drug exposure in utero. In Croatia hair testing for drugs of abuse is performed at the Institute for Medical Research and Occupational Health. Three-year experience in drugs of abuse analysis in hair is described. In 331 hair samples (270 from adolescents and 61 from adults) opiates and metabolites, cocaine, methadone, and amphetamines were analyzed by gas chromatography/mass spectrometry. Most prevalent drugs of abuse in adolescents were amphetamines, and in adults heroin. From the examples cited and samples analyzed it is evident that hair testing is emerging as a reliable biological marker for cumulative account of individual exposure to drugs of abuse.

Key words: *hair testing, drug abuse*

Introduction

The prevalence of drug consumption in Europe

Consumption of drugs of abuse is growing and threatens to be a problem to the whole of humanity. An estimation of the proportion of the population that uses drugs, or that experimented with them, is basic information needed to assess the drug situation, to develop policies and to evaluate them. It is necessary to know in which group's drug use is concentrated and the patterns of use. According to the annual report on the European Union drugs problem 1 drug use is highest in Italy, Luxembourg, Portugal and the UK, and lowest in Belgium, Germany and the Netherlands, respectively. Cannabis remains the illegal drug most commonly used in all EU countries. About 15% of the population in Finland and Sweden, and 28–40% in Denmark, Spain, France, Ireland, the Netherlands, and the UK

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took cannabis. Up to 4% of EU adults, and 8% of 15–16 year old young people used amphetamines including »Ecstasy«. Cocaine has been experienced by 0.5 to 3%. Although less than one in 100 adults reports heroin use – it causes most problems (infectious disease, overdose).

For most drugs young adults (aged 15 to 34) present rates up to double the adult population. Perceived access to drugs by 15 to 16 year-old students increased in the period from 1995 to 1999 in all participating EU countries, except Ireland and the UK, where it decreased. In central and east European countries[1] (Croatia excluded) an increase in the experience of illicit drug use among school children (15 to 16 years old) was reported in 2000. This increase reflects mostly the use of cannabis, the commonest drug used among teenagers.

In the last 10 years the number of illicit drug addicts in Croatia[2] has almost trebled. The situation is most serious in the coastal area: data for 1999 indicate around 1,500 new drug addicts per year, of whom 85% are heroin addicts.

Identification of drug use

Recognizing drug abuse as a major problem world-wide, a number of rapid commercial immunoassay techniques for drugs of abuse testing in urine have been developed. Their results are satisfactory only for the first step in differentiating negative and positive urine samples. Recommended analytical protocol demands confirmation of the positive samples by a more specific technique, usually chromatographic[3]. However, sampling time of urine is critical because drugs, with the exception of marihuana, can be detected in urine 2 to 3 days after consumption[4]. Urine testing provides a reliable answer for short- term recent exposure. However, there is a need for a biological marker, which may yield a cumulative reflection of long-term exposure to illicit drugs. Hair analysis is a reliable tool for proving or excluding chronic drug abuse[5–8]. Hair is particularly advantageous as a biological matrix: it can be easily obtained without violating individual privacy, it is not easily adulterated, and it can be stored and transported without special precautions, due to its stability. Studies on Egyptian mummies[9] have provided evidence of the long-term stability of drugs in hair samples.

In 1979 Baumgartner et al.[10] first detected morphine in hair. Since then a great number of papers on hair testing for drugs of abuse has been published[11–16]. Normal hair grows in stages: the anagen (growing) stage; the catagen (transition), and telogen (resting) phase. The growth speed varies from 0.8 to 1.3 cm/month[17] and thus the history of drug abuse can be traced months back, depending on the length of the hair. There are many uncertainties concerning how drugs enter hair and factors that affect drug deposition and residence in hair. Possible routes of drug entry include diffusion from blood, sweat, sebum and skin and entry from the environment (contamination, passive exposure). Very little is known about the potential for environmental contamination of hair by drugs of abuse. It has been indicated that cocaine is adsorbed by hair when the cocaine base is vaporized in the presence of drug-free hair[18,19].

Washing techniques for the removal of cocaine from contaminated hair vary widely in reported efficiency[20]. Generally, appropriate decontamination procedures and the necessity of hair dissolution for adequate drug recovery are very important.

Several authors recently published results about a decreasing tendency of the hair drug content because of cosmetic treatment[21–24]. Bleaching, coloring, or permanent waving were found to affect the stability of incorporated drugs and to cause alterations of the fibers at an ultra-struc-

tural level. This may result in partial or complete loss of drug substances, depending on the particular drug molecule and on its concentration prior to cosmetic treatment.

The results of a few studies[25,26] have indicated the influence of hair pigmentation on the drug content in the hair fibers, i.e. there are differences between white, black, and Asian hair types.

As most hair in the posterior vertex area (85%) is in an active growing phase, and thus more likely to incorporate a drug, this region of the scalp is generally chosen as the sampling site. When head hair is not available or too short pubic or axillary hair is collected for drugs of abuse analysis[27].

Sectional analysis i.e. the analysis of hair segments is occasionally used, but shows little correlation between the time of drug administration and the position of the drug along the hair shaft[28]. Also, correlation between the drug dose and the amount of drug and metabolites found in hair is unclear at the present time and remains controversial[14–16]. Therefore, hair analysis for drugs of abuse reveals chronic drug consumption, but does not reflect the drug dose and time of drug administration.

Applicability of drug hair analysis

In some European countries hair analysis is routinely used as a tool of detection for drug abuse in forensic sciences[29], traffic medicine[30], occupational medicine and clinical toxicology[4,31]. In some of them testing of human hair is accepted by the Courts of Justice[32]. In forensic medicine hair can be used as a »calendar« of past exposure. Information concerning the abuse pattern of a subject in the weeks or months before death can be of the highest relevance for the forensic pathologist when the autopsy findings are unremarkable and the concentrations of drugs in blood are low[29]. Today hair testing is also applied in several areas of criminal investigation[33]. A person taken into custody for drug trafficking with no drugs detected in the hair will be sentenced as a dealer; with drugs detected in the hair such a person could be treated as an addict and be guaranteed extenuating circumstances[34].

Hair analysis from pregnant woman, neonates and infants has been shown to be a useful tool for the detection of drug exposure in utero[35–38]. Because the neonatal hair grows during the last 3 to 4 months of gestation, the presence of drugs may be an indicator of cumulative levels over the last trimester.

In university student's hair analysis was performed to evaluate the incidence of drug abuse[39].

Hair analysis can also help clinicians to decide whether the use of illicit substances (cannabinoids, amphetamines) has contributed to the etiology of psychosis or not[40].

Experience of hair drug analysis in Croatia

In Croatia hair testing for drugs of abuse is at the moment not referred to any law or decision of the government.

Hair testing for drugs of abuse has been performed only in the Clinical-Toxicological Chemistry Unit at the Institute for Medical Research and Occupational Health. Gas chromatographic/mass spectrometric (GC/MS) methods have been developed for the determination of opiates (morphine, codeine, heroin, and 6-acetylmorphine), cocaine, methadone, and amphetamines (amphetamine; methamphetamine; 3,4-methylene-dioxyamphetamine, MDA; 3,4-methylenedioxymethamphetamine, MDMA, »Ecstasy«, and 3,4-methylenedioxyethylamphetamine, MDEA) in hair[41]. For analysis hair samples, approximately 0.5 cm in diameter, were cut as close as possible to the scalp of the vertex posterior area, folded in alu-

minum foil, and the proximal and distal ends marked. Samples 2 to 4 cm long were analyzed, which represent approximately 2 to 4 months growth. It was not possible to analyze all drugs and metabolites simultaneously. They were divided into two groups; I. (morphine, codeine, heroin, 6-acetylmorphine, methadone, and cocaine), and II. (amphetamines). The methods included hair washing, cutting, extraction, derivatization, and GC/MS determination. It was decided to wash hair samples in dichlormethane twice, because tests showed that the third wash was always negative, although the two previous washes were positive. After washing, the hair was dried and cut into very small pieces of less than 1 mm, and 50 mg of each sample was analyzed. Methanol was chosen for extracting opiates and cocaine. The best recoveries for amphetamines were obtained after alkaline hydrolysis of hair. Hair samples for morphine, codeine, heroin, 6-acetylmorphine, methadone, and cocaine determination were cleaned up by solid-phase extraction, and for amphetamines by liquid extraction (ethyl acetate). A variety of derivatization reagents are used in the analysis of drugs of abuse. For the analysis it was found that a mixture of propionic acid anhydride and pyridine was very convenient and superior to N, O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane for the derivatization of codeine, 6-acetyl -morphine, and morphine. Heptafluorobutyric anhydride is recommended for the derivatization of amphetamines.

Methods were analytically validated for sensitivity, reproducibility, and accuracy. External quality assessment was verified through participation in the program Proficiency Test on Drugs of Abuse in Hair Testing, Munich, Germany.

The developed methods were applied in the hair analysis of 331 subjects (107 females; 224 males) who were suspected of drug abuse. A large majority of subjects (n=270; 14 to 19 years) were adolescents and the other subjects (n=61; 19 to 27 years) were adults. Their parents, older sister, brother, or other relative who wished to remain anonymous mostly accompanied them. For that reason the hair samples were identified only by first or a fictitious name, or by a number. Often hair samples were taken according to our instruction and either brought to the Unit or sent by the regional drug abuse prevention centers.

Only 101 samples (24 females, 77 males) were positive for drugs of abuse, and 230 were found negative. Although the low percent of positive results is rather unexpected, there is a plausible explanation. Namely, most hair samples were taken from young people whose parents were upset by their change in behavior and poor results in school or university, which lead them to suspect that their children were taking drugs. This suspicion was not altogether unjustified as most of them smoked marihuana[3]. However, a method has not yet been developed for the analysis of marihuana's psychoactive constituent tetrahydrocannabinol in hair.

Table 1 summarizes positive results of drugs of abuse (n=101) in which one or more than one kind of drug were found, and Table 2 shows the concentrations (range and median) of analyses found in drug consumers' hair.

In most subjects amphetamines were found alone (n=48) or with other drugs (n=6). The results of the hair analysis indicate that the leading amphetamine was MDMA (n=46), which confirmed »Ecstasy« consumption. »Ecstasy« is believed to be popular as a »dance drug« among the young; particularly those who attend »rave parties«. Opiates alone were measured in 20 hair samples, and with other drugs in 17 hair samples. The only specific heroin metabolite 6-acetylmorphine was found in all these samples confirming heroin

TABLE 1
POSITIVE HAIR SAMPLES (N=101) FOR PARTICULAR DRUGS AND COMBINATION OF DRUGS

Groups	Number of samples	%
Amphetamines	48	47.4
Amphetamines/opiates	2	2.0
Amphetamines/cocaine	3	3.0
Opiates	20	19.7
Opiates/cocaine	3	3.0
Opiates/methadone	8	7.9
Opiates/cocaine/methadone	3	3.0
Opiates/amphetamines/cocaine/methadone	1	1.0
Cocaine	4	4.0
Cocaine/methadone	2	2.0
Methadone	7	7.0

TABLE 2
THE CONCENTRATIONS OF PARTICULAR ANALYTES FOUND IN DRUG CONSUMERS' HAIR SAMPLES

Analyte	Number of positive samples	Concentration (ng/mg)	
		Range	Median
Amphetamine	16	0.37–16.35	2.43
Methamphetamine	7	0.30–2.01	0.60
MDA	7	0.18–1.81	0.74
MDMA	46	0.56–64.42	2.04
MDEA	–		
Heroin	21	0.27–63.57	2.20
6-Acetylmorphine	7	0.57–32.21	2.11
Morphine	28	0.56–5.05	1.10
Codeine	15	0.25–3.30	1.29
Cocaine	16	1.15–7.91	2.84
Methadone	21	1.12–55.70	5.20

consumption. Cocaine was present in 16 hair samples, in four alone; and methadone in 21 hair samples, in seven alone.

Apart from the living, hair samples (from the head and pubic regions) were also analyzed from dead persons suspected of drug overdose. In heroin consumers the 6-acetylmorphine concentration was always higher in pubic than in head hair and ranged from 1.10 to 23.08 ng/mg and 0.72 to 9.05 ng/mg, respectively.

In some cases hair was tested for drug abuse under special circumstances, when urine tests were not informative. Only by hair analysis for opiates is it possible to distinguish administration of pharmaceutical products (codeine, pholcodine), ingestion of poppy seed food (morphine, codeine), and true drug abuse (heroin, morphine). Namely, the only specific heroin metabolite 6-acetylmorphine is always present in the hair of heroin consumers,

but not in people who take analgesics with codeine or eat poppy seed cake[42].

From both the literature examples and results in the Unit it is evident that hair testing is emerging as a reliable biological marker for cumulative account of exposure to drugs of abuse. However, there are some pitfalls that must be taken into account in interpretation of the hair testing for drugs of abuse.

REFERENCES

1. EUROPEAN MONITORING CENTER FOR DRUGS AND DRUGS ADDICTION: Annual report on the state of the drugs problem in the European Union 2001. (Office for Official Publications of the European Communities, Luxembourg, 2001). — 2. SAKOMAN, S., Croat. Med. J., 41 (2000) 270. — 3. KARAČIĆ, V., LJ. SKENDER, Arh. Hig. Rada Toksikol., 51 (2000) 361. — 4. JAMES, W. H., D. D. MOORE, J. Child. Adolesc. Subst. Abuse, 7 (1997) 19. — 5. KINTZ, P., P. MANGIN, Forensic Sci. Int., 73 (1995) 93. — 6. GAILLARD, Y., G. S. PEPIN, Forensic Sci. Int., 86 (1997) 93. — 7. HOLD, K. M., D. G. WILKINS, D. E. ROLLINS, R. E. JOSEPH, E. J. CONE, J. Chromatogr. Sci., 36 (1998) 125. — 8. SEGURA, J., C. STRAMESI, A. REDON, M. VENTURA, C. J. SANCHEZ, G. GONZALES, L. SAN, M. MONTAGNA, J. Chromatogr. B., 724 (1999) 9. — 9. BALABANOVA, S., F. PARSCHE, W. PIRSING, Naturwissenschaften, 79 (1992) 358. — 10. BAUMGARTNER, A. M., P. F. JONES, W. A. BAUMGARTNER, C. T. BLACK, J. Nuclear Med., 20 (1979) 748. — 11. NAKAHARA, Y., K. TAKAHASKI, M. SHIMANINE, Y. TAKEDA, J. Forensic Sci., 36 (1991) 70. — 12. GOLDBERGER, B. A., Y. H. CAPLAN, T. MAGUIRE, E. CONE, J. Analyt. Toxicol., 15 (1991) 226. — 13. JURADO, C., M. P. GIMENEZ, M. MENENDEZ, M. REPETTO, Forensic Sci. Int., 70 (1995) 165. — 14. KAUERT, G., J. RÖHRICH, Int. J. Legal Med., 108 (1996) 294. — 15. PEPIN, G., Y. GAILLARD, Forensic Sci. Int., 84 (1997) 37. — 16. POLETTINI, A., C. STRAMESI, C. VIGNALI, M. MONTAGNA, Forensic Sci. Int., 84 (1997) 259. — 17. SACHS, H., Forensic Sci. Int., 70 (1995) 53. — 18. ROMANO, G., N. BARBERA, I. LOMBARDO, Forensic Sci. Int., 123 (2001) 119. — 19. KOREN, G., J. KLIN, R. FORMAN, K. GRAHAM, J. Clin. Pharmacol., 32 (1992) 671. — 20. CONE, E. J., D. YOUSEFNEJAD, W. D. DARWIN, T. MAGUIRE, J. Anal. Toxicol.,15 (1991) 250. – 21. KINTZ, P., A. TRACQUI, P. MANGIN, J. Forensic Sci., 38 (1993) 657. — 21. CIRIMELE, V., P. KINTZ, P. MANGIN, J. Anal. Toxicol., 19 (1995) 331. — 22. PÖTSCH, L., G. SKOPP, Forensic Sci. Int., 81 (1996) 95. — 23. JURADO, J., P. KINTZ, M. MENENDEZ, M. REPETTO, Int. J. Legal Med., 110 (1997) 159. — 24. SKOPP, G., L. PÖTSCH, M. R. MOELLER, Forensic Sci. Int., 84 (1997) 43. — 25. PÖTSCH, L., G. SKOPP, M. R. MOELLER, Forensic Sci. Int., 84 (1997) 25. — 26. KELLY, R. C., T. MIECZKOWSKI, S. A. SWEENEY, J. A. BOURLAND, Forensic Sci. Int., 107 (2000) 63. — 27. KINTZ, P., A. TRACQUI, P. MANGIN, J. Forensic Sci., 38 (1993) 657. — 28. TSATSAKIS, M. A., M. TZATZARAKIS, Pure Appl. Chem., 72 (2000) 1057. — 29. KRONSTRAND, R., G. GRUNDIN, J. JONSON, Forensic Sci. Int., 92 (1998) 29. — 30. MOELLER, M. R., Ther. Drug Monit., 18 (1996) 444. — 31. GOLDBERGER, B. A., A. G. DARRAY, Y. H. CAPLAN, E. J. CONE, J. Anal. Toxicol., 22 (1998) 526. — 32. DEVEAUX, A., P. KINTZ, J. P. GOULLE, B. BESSARD, G. PEPIN, D. GOSSET, Forensic Sci. Int., 107 (2000) 389. — 33. GAILLARD, Y., G. PEPIN, Forensic Sci. Int., 86 (1997) 49. — 34. UHL, M., Forensic Sci. Int., 107 (2000) 169. — 35. KLEIN, J., R. FORMAN, C. ELIOPOULUS, G. KOREN, Ther. Drug Monit., 16 (1994) 67. — 36. KOREN, G., Forensic Sci. Int., 70 (1995) 77. — 37. KLEIN, J., T. KARASAKOV, G. KOREN, Forensic Sci. Int., 107 (2000) 281. — 38. LAKSHMI, D., F. R. KATIKANENI, T. C. HULSEY, Biol. Neonate, 81 (2002) 29. — 39. QUINTELLA, O., A. M. BERMEJO, M. J. TABARENO, S. STRANO-ROSSI, M. CHIAROTTI, A. C. S. LUCAS, Forensic Sci. Int., 107 (2000) 273. — 40. SELTEN, J. P., I. J. BOSMAN, D. DE BOER, N. D. VEEN, Y. VAN DER GRAAF, R. A. A. MAES, R. S. KAHN, European Neuropsychopharmacology, 12 (2002) 27. — 41. SKENDER, LJ., V. KARAČIĆ, I. BRČIĆ, A. BAGARIĆ, Forensic Sci. Int., 125 (2002) 120. — 42. SKENDER, LJ., V. KARAČIĆ, I. BRČIĆ, Biochemia Med., 11 (2001) 79.

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PROCJENA ZLOUPORABE DROGA ANALIZOM KOSE

S A Ž E T A K

U novije vrijeme kosa kao biološki uzorak za analizu droga dobiva sve veći značaj. Za razliku od urina analiza kose proširuje mogućnost detekcije droga na mjesece, ovisno o duljini kose. Analiza droga u kosi u razvijenim zemljama koristi se u sudskoj medicini, medicini rada, pri prometnim prekršajima, kriminalnim slučajevima te u kliničkoj toksikologiji. U novorođenčadi se analizom kose utvrđuje je li majka za vrijeme trudnoće uzimala drogu. U Hrvatskoj se uzimanje droga analizom kose određuje jedino u Institutu za medicinska istraživanja i medicinu rada. Razvijene su metode za analizu opijata i metabolita, kokaina, metadona i amfetamina tehnikom plinske kromatografije sa spektrometrijom masa. Tijekom tri godine analiziran je 331 uzorak kose; 270 od adolescenata i 61 od odraslih osoba. U adolescenata je najčešće uzimana droga iz skupine amfetamina, dok odrasle osobe najčešće uzimaju heroin. Na temelju opisanih primjera iz literature i vlastitog iskustva može se zaključiti da je kosa dobar biološki pokazatelj za procjenu ponavljanog/kroničnog uzimanja droga.