

## Evaluation of the Addiction History of a Dead Woman After Exhumation and Sectional Hair Testing

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In Greece, sectional hair analysis, in addition to clinical examination, has been used as a valuable tool for the confirmation of a person's history of drug use. The present report concerns the toxicologic analysis of the exhumed remains and hair samples of an 18-year-old woman. Postmortem toxicologic analysis of blood and urine confirmed recent opiate and cannabis use and indicated that death was associated with heroin abuse. Several months later, the woman's family asked for exhumation and reexamination of the body, insisting that the cause of death was homicide. The investigating judge ordered exhumation and new medicolegal examination of the body. The investigation of the drug profile along the hair shaft was undertaken by analyzing hair sections 1 cm from the hair root for morphine, 6-monoacetylmorphine, heroin, and cannabinoids. The total lengths of the hair samples ranged from 8 to 11 cm. The total morphine levels in the hair sections corresponding to the 3-month period before death were significantly lower (1.5–2.85 ng/mg) than those of the 4- to 10-month period before death (7.4–14.8 ng/mg). An interpretation of these results may be occasional drug use (with considerable attenuation of use during the last 3 months before death). Decrease of tolerance to heroin caused by abstinence and relapse in use could have been the cause of death.

**Key Words:** Morphine hair profile—Exhumed hair—Addiction history.

A substantial amount of research regarding the application of hair testing in the service of law and other forensic purposes has been performed in the recent years (1,2). Scientists from the United States, Europe, and Japan have validated these methods, showing the strengths and limitations of hair testing (3–5). Although many questions concerning scientific issues of hair testing are still to be answered, hair testing for drugs is generally considered to be a valuable analytic tool to demonstrate long-term use of drugs (2). The application of this new tool in the investigation and documentation of human drug use is increasing, as is the submission of hair testing results in legal proceedings (6). Several studies have provided contradictory evidence about the presence of a dose–response relationship between drug use and measured drug in hair (6). The most recent data confirm that the concentrations of several drugs (e.g., cocaine, phenytoin, carbamazepine, morphine) in hair reflect the quantity of the drugs consumed (7–11).

Hair analysis is a powerful tool for the diagnosis of poisoning and death associated with opiate addiction (8,12). A good correlation of drug dose administration and drug hair level has also been documented for phenytoin (9) and carbamazepine (2). The simultaneous quantification of opiates, cocaine, and cannabinoids in hair, as well as the effects of environmental contamination and washing, has been studied (13,14). Actually, hair tests provide unique laboratory evidence and may considerably assist the evaluation of systematic past drug abuse (2,15).

The results of hair sectional analysis of heroin abusers suggest that there is a good correlation between the distribution of morphine along the hair shafts and the history of drug use. Exceptions can be explained by the accumulation of heroin metabolites in different body compartments as a result of

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chronic heroin abuse (11). The link between the declared quantity of consumption and the concentration measured in hair for cocaine and 6-monoacetylmorphine has also been demonstrated (7).

### CASE HISTORY

The body of a young woman was found in a state of early decomposition outside a vacant building in a park in Thessaloniki. A syringe was found next to the body. Analysis of biologic samples from the body revealed the presence of opiates and cannabinoids. Death was attributed to heroin abuse (death certificate from the Department of Forensic Pathology and Toxicology, University of Thessaloniki). Several months later, the woman's family asked the prosecutor for exhumation and a new autopsy of the body, alleging evidence of blunt force injuries, strangulation, and rape and stating that the woman had been free of drugs for the last months before her death. Because of the family's claims of possible organized crime involvement and official cover-up, and the resulting media attention, the investigating judge ordered exhumation and new medicolegal examination of the body. The examination was performed by a group of six forensic pathologists (three examiners from the Forensic Pathology Service of the Ministry of Justice in Athens and three independent examiners from different university departments) 7 months postmortem. Sampling of exhumed remains of the woman's body and hair was performed, and the Toxicology Service Ministry of Justice and the Unit of Toxicology and Criminal Chemistry, Department of Medicine, University of Crete performed the toxicologic analysis.

No further autopsy findings on the exhumed body were revealed because of the advanced decomposition and the partial skeletonization. No fractures were found, and no evidence of application of blunt force was identified.

A basic question of the investigating judge, apart from the existence of fatal blunt injuries, strangulation, and rape, concerned the clarification of a recent history of drug abuse. The Toxicology Laboratory of the Ministry of Justice in Athens confirmed only the presence of cannabinoids in the exhumed remains. We were asked to elucidate the history of the woman's heroin abuse from the hair samples.

### METHODS

#### Chemicals and Reagents

Reagents and standards, all of analytic grade, were obtained from Sigma (Sigma Chemical Co, St. Louis, MO, U.S.A.). The solvents used were

high-performance liquid chromatography grade and were obtained from Lab-Scan (Stillorgan Ind. Park Co., Dublin, Ireland).

#### Hair Samples

Black scalp hair from volunteer non-drug users was collected and stored at room temperature in glass vials until use. The hair was determined to be drug free before the preparation of the spiked standards. Head hair was also sampled from addicts and detainees with self-reported heroin use of an average of six doses daily for >1 year. Similar sampling was performed in different periods during their preliminary imprisonment for detainees who admitted to chronic heroin abuse. Hair was analyzed by gas chromatography-mass spectrometric (GC-MS) methods according to slightly modified procedures already described (2,11,13,15,16).

#### Preparation of Standard Curves

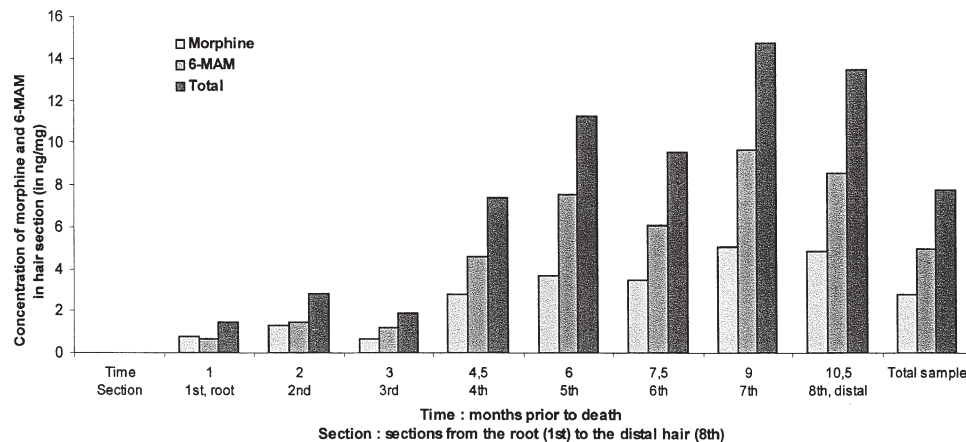
Six separate stock solutions containing morphine, 6-monoacetylmorphine, heroin,  $\Delta$ -9 tetrahydrocannabinol (THC), cannabidiol, and cannabinol were prepared in methanol at a concentration of 100  $\mu$ g/mL and stored at 0°C. From each of these initial stocks, five new diluted solutions containing all three compounds were prepared and used for the fortified drug-free human hair standards. The final fortified hair standard concentrations of the mentioned opiates were 0, 2.5, 5, 7.5, 10, and 15 ng/mg, and of cannabinoids were 0, 0.1, 0.25, 0.4, 0.5, and 1.0 ng/ml.

#### Hair Preparation and Gas Chromatography-Mass Spectrometry Analysis

Hair preparation (washing, dissolution, hydrolysis) and extraction procedures were performed by use of various methods according to well-documented analytic works. For opiates, alkaline, acidic, and solvent extraction were applied (2,13,13), whereas the extraction of cannabinoids followed the basic hydrolysis of the samples (13). Extraction residues for opiate analysis were derivatized with 50  $\mu$ L bis-trimethylsilyltrifluoroacetamide at 80°C for 30 minutes. Extraction residues for analysis of cannabinoids were dissolved in 25  $\mu$ L methanol before injection.

#### Instrumental Method

Electron ionization mass spectrometry was performed on a Finnigan Mat (GCQ ThermoQuest, Austin, TX, U.S.A.) mass spectrometer coupled to a Finnigan Mat gas chromatography and a DB-5MSITD, 30 m  $\times$  0.25 mm, 0.25-mm film thickness capillary column. Helium (99.999%) was used as



**FIG. 1.** Morphine hair profile. Mean concentrations of morphine, 6-monoacetylmorphine (6-MAM) and total morphine (ng/mg) in the sections (from the root to the distal hair) for the two different total hair samples analyzed.

carrier gas at a flow rate of 20 cm/minute. A 1- $\mu$ L aliquot of each sample was injected in the column at split mode. The column temperature was initially 180°C for 1 minute, then was increased to 310°C at 10°C/minute and held at 310°C for 10 minutes. Under these conditions, the retention time of morphine was 13.12 minutes, of 6-monoacetylmorphine was 13.87 minutes, and of heroin was 14.55 minutes. The ions for morphine were m/z (mass-to-charge ratio) 234, 401, and 429; for 6-monoacetylmorphine were m/z 204, 287, 340, and 399; and for heroin were m/z 204, 327, and 369. The retention time of cannabidiol was 8.46 minutes, of  $\Delta^9$ -THC was 8.83 minutes, and of cannabiniol was 9.15 minutes. The ions for cannabidiol were m/z 231; for  $\Delta^9$ -THC were m/z 231, 299, and 314; and for cannabiniol were m/z 238, 295, and 310.

#### Quantitation of Hair Extracts

A 6-point curve was prepared daily by analyzing 40 mg of drug-free hair samples fortified with morphine, 6-monoacetylmorphine, and heroin at concentrations of 0, 2.5, 5, 7.5, 10, and 15 ng/mg hair and  $\Delta^9$ -THC, cannabidiol, and cannabiniol, and concentrations of 0, 0.1, 0.25, 0.4, 0.5, and 1.0. The recovery for opiates was 76% ( $y = 567.48x + 230.8$ ,  $R^2 = 0.9902$ ) and for cannabinoids was 72%. The detection limit for morphine, 6-monoacetylmorphine, and heroin was 0.1 ng/mg; for cannabinoids it was 0.05 ng/mg.

#### RESULTS

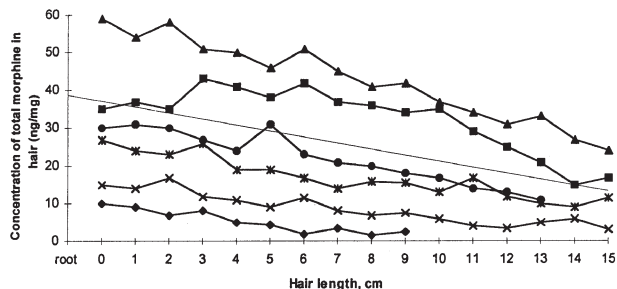
Figure 1 depicts data of mean morphine, 6-monoacetylmorphine, and total morphine concentrations in hair sections of two different total hair samples from the exhumed woman obtained by GC-MS analysis. The lengths of the first three sections from the root were 1 cm, whereas the five dis-

tal sections were 1.5 cm long. The concentration of mean total morphine obtained by GC-MS from two total hair samples was 7.8 ng/mg. Figure 1 demonstrates the morphine profiles in head hair sections from the exhumed woman. The concentrations of morphine and 6-monoacetylmorphine in the two different total hair samples did not differ significantly. The time intervals shown in Figure 1 illustrate the history of the use of heroin for 10.5 months, assuming a hair growth rate of 1 cm/month. The identification of 6-monoacetylmorphine is an indicator of heroin use (16). Heroin was identified in segments 4, 5, 7, and 8 at levels close to the limit of detection by use of the solvent extraction procedure and GC-MS, Singlet ion monitoring mode. The concentrations of  $\Delta^9$ -THC and cannabiniol found in hair sections ranged from 0.1 to 0.5 ng/mg.

#### DISCUSSION

Accepting no significant influences in hair matrix while the body was buried (14,17), hair growth rate of 1 cm/month, and results revealing a close relationship of 6-monoacetylmorphine hair levels in segments and actual heroin abuse (11), we concluded that hair samples provided us information about the last 10 months of the woman's life and confirmed her heroin abuse. Morphine was detected in blood and other autopsy specimens at the time of death (blood 0.4  $\mu$ g/mL, urine 1.1  $\mu$ g/mL).

The findings of toxicologic testing at autopsy may not be helpful in opiate-related deaths. Other than the fact that the drug was taken, not much else can be inferred from a single blood level. Quantitative measurement of drug at autopsy is of value only when the results are combined with evidence obtained by thorough examination of the death scene, a review of the deceased's history, and examination of the body. Moreover, the extent of tol-



**FIG. 2.** Total morphine (morphine and 6-monoacetylmorphine) hair profile from six representative heavy users of heroin.

erance cannot be determined from the autopsy. The final diagnosis depends on appropriately weighing all these factors (18).

Morphine levels in hair sections corresponding to the 3 months before death were significantly lower (1.5–2.85 ng/mg) than those of the 4 to 10 months before death (7.4–14.8 ng/mg). A 7-month hair profile indicated (mean 11.33 ng/mg) that the subject fell into the category of a heavy heroin user (2,7,8). Morphine levels in the hair of drug users varies significantly as reported in the literature. This is because there is usually no grouping of these data into categories of occasional drug users, systematic drug users, and heavy users in addition to many other already mentioned factors (e.g., ethnicity, melanin content, lipophilicity of hair matrix). We randomly sampled hair from common heavy heroin abusers and detainees who admitted to heavy chronic heroin abuse, and conducted sectional hair testing for these samples. The above subjects reported intake of 4 to 10 heroin doses daily. The morphine hair profiles found for these addicts are illustrated in Figure 2. Morphine levels in hair sections ranged from 10 to 60 ng/mg (19). Generally, a reduction of drug concentrations in hair from the first to the subsequent segments was observed. This is caused by drug degradation over time or by drug extraction from hair by washing and hair cosmetics. The possibility of migration of the drug along the hair shaft cannot be excluded, thus making difficult the decision about when actually drug intake was stopped, based on the drug hair profile.

The 3-month period before death was associated with occasional drug use (considerable attenuation of abuse) and efforts to stop heroin (incompletely abstinent individual). High susceptibility to opioid overdose after periods of abstinence, owing to loss of tolerance, has been reported (12). In addition, occasional heroin use not characterized by dependence and tolerance is an increasing heroin addiction pattern that could lead to more cases of heroin

overdose. Medical staff running detoxification programs should be aware of the risk inherent in relapse to heroin use after a period of abstinence (12).

Taking into account several scientific issues concerning the drug fate into the hair shaft (e.g., whether drugs migrate within the hair shaft; how much bleaching, perming, and straightening reduce the amount of measured drug), we cannot neglect the possibility of incomplete abstinence from heroin use during the last months before death. This fact was supported by the police investigation, which documented a visit of the woman to a consultative station for addicts 4 months before her death.

Because no strangulation or blunt injury was revealed, the cause of death was determined to be heroin overdose related to the decrease of tolerance resulting from intentional or unintentional abstinence. The conclusion that a death is caused by narcotism can be based on examination of the scene where the body is found, investigation of the circumstances, history obtained from relatives and friends, autopsy examination (that demonstrates drug use and excludes other causes of death), and toxicologic analysis. Hair tests considerably assist the evaluation of the systematic present and past abuse of heroin and other drugs. Consequently, it may be used as valuable expert evidence during questioning and in court. The data given here strongly support the possibility of applying hair testing to the evaluation of systematic drug use of heroin, even when time has passed since the event.

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