

Sectional hair testing. Judicial and clinical applications*

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Abstract: Modern sophisticated analytical tools have enabled toxicologists to investigate hair specimens for the presence of drugs. Although great sensitivity and specificity in hair analysis have been already achieved, some concerns about bias due to hair color, lack of reference materials, and dose versus concentration relationship make the quantitative data sometimes debatable. The most commonly held opinion in this field nowadays indicates that hair analysis has sufficient scientific validity. Experts should always be aware and take into account limitations of hair analysis results and its evaluation. Unresolved scientific issues, however, should not obstruct the admissibility of strongly positive test results. Recent applications of hair testing include forensic investigations, epidemiological studies, gestational drug exposure, legal issues, clinical drug monitoring, and historical research. In this paper, the use of sectional hair tests to assess exposure to drugs of abuse (evaluation of toxicomania) and to assess compliance with carbamazepine, phenytoin and valproic acid therapy regime is presented. We conclude that hair drug versus time profiles give strong evidence that confirm chronic abuse, the diagnosis of drugs of abuse poisoning, and the state of addiction (toxicomania). Additionally, they may be used as a marker of the dosage history and the compliance of patients under long-term treatment with carbamazepine and phenytoin.

INTRODUCTION

Toxicological analysis of unusual biological samples (hair, saliva, exhalant, sweat, sperm, amniotic fluid) has many applications in several areas of medical, forensic, and environmental science [1,2]. These samples may provide additional information and thus have some advantages over the usual biological samples. The ease in the collection of a saliva sample is one of this method's advantages when compared to that of blood or urine sample collection.

Among the biological samples, hair can give the most information and thus sectional hair analysis is most widely used [3,4]. One of the basic advantages of using hair is that it keeps the information trapped into it for long periods. This is due to the absorption and trapping mechanisms that exist in the hair and take place mainly via the blood-circulating system in the hair follicle. Substances are trapped during keratinization of the newly formed cells. Sweat and sebaceous glands also play a basic role in the process of drug deposition in hair. Water-soluble drugs are excreted into sweat and sebum and may be incorporated after the hair emerges from the skin. Few or no regions in the hair are inaccessible to the external environments [5]. The removal of drugs from hair will depend upon several variables, not the least of which are the gels or solutions used to wash or treat the hair [5].

Environmental exposure of the hair should be taken into account, especially during result evaluation. All the factors that influence these processes additionally depend on the physicochemical properties of the substance under investigation, the anatomical, structural and morphological properties of the hair, and environmental conditions such as temperature, humidity, pollution, and competitive bonding

*Lecture presented at the 4th Congress of Toxicology in Developing Countries (4th CTOX-DC), Antalya, Turkey, 6–10 November, 1999. Other presentations are published in this issue, pp. 973–1066.

[5,6]. Since hair grows continuously at an average rate of 1 cm/month the time dependency of the accumulation of drugs and their metabolites in the shaft can be determined. The window of detection is limited only by the length of the hair and, typically, ranges from a week to months. The amount of drug incorporated into the hair depends on the extent and duration of exposure, the presence of competing cations of drugs, the chemical structure of the drug and prior treatment history of the hair [7,8]. Several studies have shown a dose–response correlation for drugs of abuse in hair while others suggest that the drugs in hair are influenced by other factors and not simply related to the quantity of drugs ingested [7–11].

Renewed efforts will be made to resolve the dilemmas if the exogenously applied drug can mimic drug usage, if there are inaccessible regions in hair, and if the drugs migrate along the hair shaft [5]. The role of melanin, the lipophilicity, and the pKa of analyte in drug binding have also been studied. There are many difficulties concerning the analysis of hair for drugs of abuse and the interpretation of the analytical results [4,5]. The main controversies have focused on the mechanisms of the appearance and binding to hair of the drugs and removal of external contamination. An understanding of the data and their interpretation is critical to the proper applications of hair testing [5,12].

So far, hair testing is applied in forensic investigations, epidemiological studies, gestational drug exposure, historical research, child autopsy and adoption cases, unemployment compensation cases, pre-employment testing, exclusion of evidence, military court-martials, and other legal cases [11–12].

The constitutionality of hair testing has been challenged and will continue to be debated in peculiar aspects. Court decisions indicate that hair testing might not be judged to be an unlawful invasion of privacy. The major technical area of disagreement in forensic cases will relate to the potential for false positives due to external contamination.

The present report summarizes our data with regard to certain judicial and clinical applications of sectional hair testing. We applied hair tests to obtain expert evidence for the evaluation of a person's toxicomania. We also apply sectional hair testing to evaluate compliance with the therapy regime in patients receiving antiepileptic drugs systematically.

MATERIALS AND METHODS

Hair sampling from addicts and detainees

Head, axillary, and pubic hair were sampled from subjects with self-reported heroin use of an average of six doses daily, for more than one year. Similar sampling was performed in different periods during their preliminary imprisonment for detainees who admitted chronic heroin abuse. Hair analysis was ordered by the Examining Judge during interrogation [13].

Hair treatment and analysis for morphine, 6-monoacetylmorphine, and heroin by gas chromatography-mass spectrometry

Hair samples were washed twice with methanol. The hair was cut into small segments (about 1 cm) and 40 mg of each hair section was homogenized in a minibeadbeater-8 (Biospec Products, Inc.) for 15 min. Two milliliters of methanol (HPLC grade) were added, and the samples were incubated at 42 °C for 18 h, centrifuged for 5 min, the methanol was separated and evaporated at 40 °C under a stream of nitrogen. The residue was dissolved in 2.5 mL of buffer, pH = 8.4. Extraction by mechanical shaking for 15 min with 3 mL of toluene/isoamylalcohol/n-heptane (70:10:20) followed. The organic phase was transferred to glass tubes and evaporated to dryness at 40 °C under a stream of nitrogen. The residue was derivatized with 50 mL of BSTFA at 80 °C for 30 min.

Instrumental method

Electron ionization (EI) MS analysis was performed on a Finnigan Mat (GCQ_{TM}) mass spectrometer coupled to a Finnigan Mat gas chromatography system and a Optima-5, 30 m × 0.32 mm, 0.25 mm film thickness capillary column. Helium (99.999%) was used as carrier gas at a flow rate of 20 cm/min.

A 1-mL portion of each sample was injected in the column at split mode. The column initial temperature was 180 °C for 1 min, then increased to 290 °C at 10 °C/min and was held at 290 °C for 10 min. The injector temperature was 270 °C and the ion source oven temperature was set at 200 °C. The electron multiplier voltage was 1500 V. Under these conditions the retention time of morphine, 6-monoacetylmorphine and heroin was 10.52, 10.61, and 12.08 min respectively. The ions for heroin were m/z 204, 327, and 369, and for morphine and 6-monoacetylmorphine were m/z 234, 401, and 429.

Patients and hair sampling

Hair samples from male and female patients, aged from five to seventy four years old and suffering mainly from epilepsy were collected for the study. The patients were receiving either carbamazepine (CBZ) (40 patients), phenytoin (PHT) (60 patients), or valproic acid (VPA) (40 patients) for periods ranging from two months to several years. Some of them were being treated in combination with phenobarbital but none of them was being treated with amitriptyline, chlorpromazine, imipramine, and nortriptyline. Hair samples (200 mg) were cut from the head area, as close as possible to the skin of the posterior vertex. The hair samples were cut into two or five segments along the hair shaft, each of 2 cm length with the first starting from the hair root. Blood samples from these patients were collected at the same time as the hair samples. Healthy individuals who were not taking the drugs were used as controls [14–16].

Hair treatment and high-pressure liquid chromatography analysis for CBZ and PHT

The samples of hair from the patients and controls (2 cm length, 30 to 50 mg), were added to 3 mL of a 1N NaOH solution, heated for 1 h at 100 °C, vortexed into a tube, and cooled at room temperature. Five milliliters of diethylether were added, vortexed for 3 min, centrifuged for 5 min, and the organic phase (supernatant) was transferred into new test tubes. The solvent was evaporated, and the residue was redissolved in 200 mL of saline.

Analysis was performed using HPLC. A series of CBZ and PHT standards in hair and samples from patients were treated in one sequence as described, and 50 mL of hair extract in saline was injected into the Spectra-Physics instrument. The effluent was monitored at 220 nm at a flow rate of 2.0 mL/min (ambient temperature, 25–28 °C). An S5 ODS2 column (10 cm × 4.6 cm) from SPHERISORB was used. The mobile phase was a mixture of acetonitrile (20%) and water (80%). The internal standard used for the procedure was a solution of flunitrazepam in acetonitrile (10 mg/mL). Good separation of PHT (R_f = 7.3 min), CBZ (R_f = 9.3 min), and flunitrazepam (R_f = 17.1 min) was achieved [14].

The standards used to obtain the calibration curve were created as follows: Blank hair was fortified with standard CBZ or PHT amounts (1.0, 2.0, 3.0, 4.0, 5.0, 10.0 mg) and were treated in exactly the same way as the unknown samples.

RESULTS

The results of sectional hair analysis are presented in Table 1 and in Figs. 1–6. Table 1 depicts data of total hair morphine concentration [heroin + 6-monoacetylmorphine (6-MAM) + morphine] in hair samples from head, axillary, and pubic regions and maximum levels of total morphine from segments of these samples, all obtained from imprisoned subjects.

Table 1 Morphine concentrations (ng/mg) in total samples and segments* found in hairs of detainees.

Sampling time	1 month	2–3 months	3–7 months
Sample	Total (segment)	Total (segment)	Total (segment)
Head	12–40 (60)	3–18 (36)	0.5–2 (16)
Axillary	14–55 (84)	2–28 (28)	1– 8 (14)
Pubic	15–68 (99)	1–30 (42)	0.5–13 (18)

* Only segments with maximum levels are presented.

Sampling was performed up to seven months after preliminary imprisonment. Figure 1 represents total morphine hair profiles from heavy heroin users abusing the drug chronically. Total hair samples are presented in 1-cm segments, each corresponding to approximately one month.

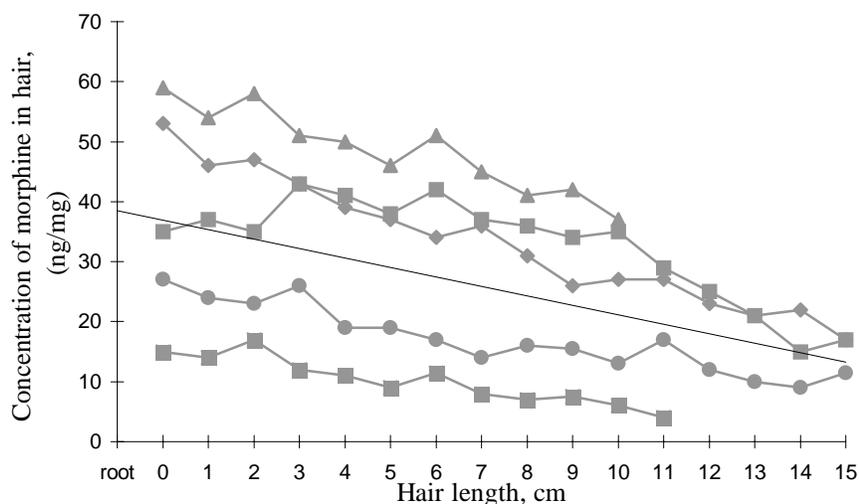


Fig. 1 Morphine hair profile from heavy heroin users. Addicts abusing chronically and systematically the drug.

Figure 2 shows CBZ concentration hair profiles for 7 patients receiving this drug systematically. Also, the mean and SD from the data of 40 patients (bank data). Figure 3 shows PHT concentration hair profiles of 6 patients receiving this drug systematically, as well as the mean and SD from the data of 40 patients (bank data).

DISCUSSION

Judicial applications

Evaluation of toxicomania is very significant for the giving of justice [8,9,13]. In the past, laboratory testing assisting evaluation of toxicomania included blood and urine analysis, both associated with short-term indication of use (several days). Segmental hair analysis offers the advantages of long-term confirmation of drug use, the pattern, and the magnitude of use.

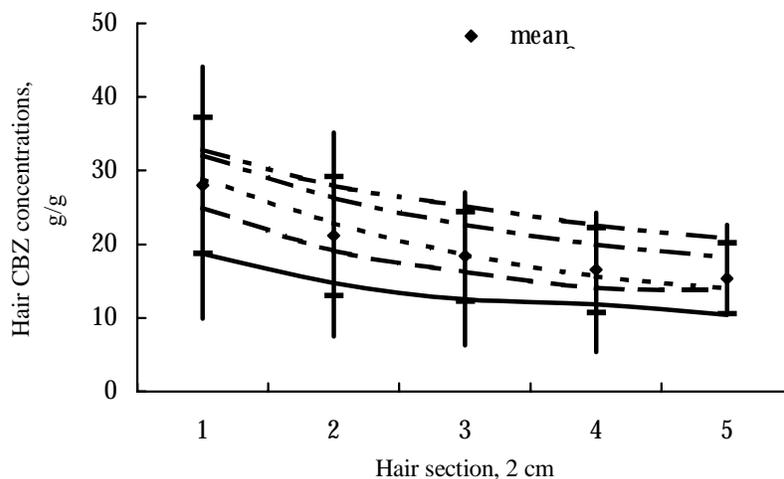


Fig. 2 CBZ hair profiles from seven patients in comparison with the mean \pm SD values obtained from the bank data.

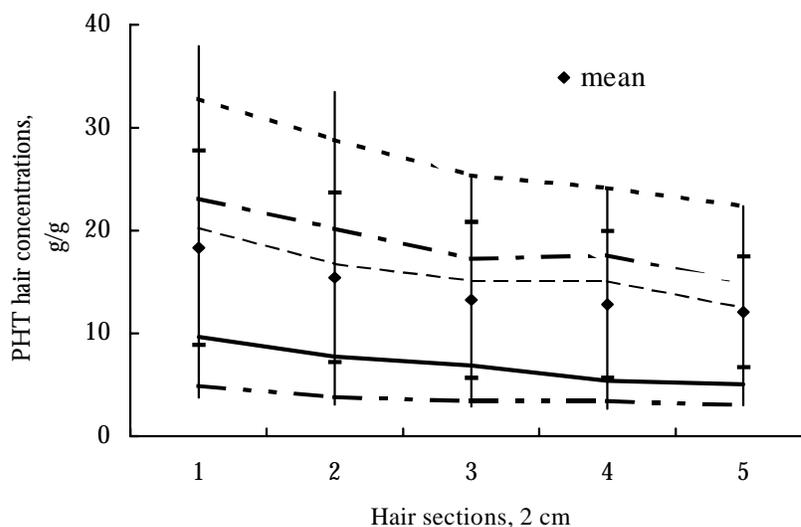


Fig. 3 PHT hair profiles from six patients in comparison with the mean \pm SD values obtained from the bank data.

Drug addicts, drug users, and traffickers arrested for possession and for use or trade of drugs are routinely examined in forensic medicine departments while under custody. Besides the expert report from the forensic pathologist, expert reports from a forensic psychiatrist and a toxicologist are mandatory to determine the state of drug dependence of an arrested person. These help determine whether the addict is able to terminate the drug abuse on their own. Laboratory evaluation in the majority of cases includes urine analysis, while hair testing may be ordered only under special circumstances when blood or urine and external examination is not informative.

Drug addicts must have special treatment according to the law (currently acting legislation, Law 1729/87, Articles 12, 13, 14 and Law 2161/93, Article 13). The acting legislation for the users of narcotics refers that possession for personal use is dependent upon the kind, the quantity, and the purity of the substance, and refers to the quantity limits that cover the use of a toxicomania for a certain period. In punishment, the degree of harm and grouping of substances is considered. An order of psychiatric,

forensic, and laboratory examination is obligatory to evaluate the existence, the kind, and the extent of dependence. This can be performed in psychiatric, medicolegal, and toxicological institutions or ordered from a list of experts in the field. After the examination a report is submitted to the Examining Judge or District Attorney. The report must contain data concerning the physical and psychological dependence of the person on a certain substance, the daily dose required to avoid withdrawal syndrome, and the influence of the drug on the behavior and conscience of the addict. Finally it should suggest a suitable treatment.

The Ministerial Enactment 3982/87 contains the common scientific criteria for the diagnosis of dependence. In Article 1 of the Enactment the assessment of narcotics use include: a) laboratory examination (sample collection no later than 48–72 h after last drug administration under controlled conditions and toxicological analysis of body fluids) and b) clinical examination in a public hospital or correctional institution for 5–7 days. During clinical examination scars due to vein puncturing and other evidence that indicate drug abuse are noted. Symptoms of withdrawal syndrome are sufficient evidence for drug addiction, and the syndrome is as recommended by the Ministry of Health. A full history must be obtained especially when no withdrawal syndrome is apparent.

Under Article 2, a patient is classed as an addict when at least three of the following criteria are fulfilled. The person:

- consumes substances in larger quantities or for longer periods than originally anticipated;
- has tried unsuccessfully to stop or reduce the drug use;
- spends a lot of time trying to obtain the drug, to use it, or under the influence of the drug;
- exhibits intoxication or withdrawal symptoms while expected to fulfil important obligations at work, school, or home;
- undertakes dangerous activities (e.g., driving of a car) while under the influence of the drugs;
- abandons important social, professional or entertaining activities due to drug abuse
- continues the drug abuse even though aware of a continuous or periodic social, psychological, or health problem caused by the drug;
- exhibits increasing tolerance to the substance, therefore, needs larger amounts to reach the desired effect;
- exhibits withdrawal symptoms and uses the substance to avoid them.

The expert report is constructed according to Articles 148 and 198 of the Criminal Procedure Law. It contains an exhaustive report of all the evidence and criteria of the previous articles, and indicates the most appropriate supportive (Article 12 of N1729/87) or therapeutic measures (Article 4 of N1729/87).

The general consideration for the criteria of dependence is that they are subjective, mostly based on the report from personal interview. Some may not be evident if a long time has elapsed from the arrest to the examination.

Cases in which hair testing might be mandatory involve drug users without any evidence of injections or nasal inflammation (smokers), users with some evidence of use, but no evidence of severity of use, detainees who confessed the systematic use of drugs when time has elapsed from arrest, or detainees examined by forensic psychiatrists after a period of obligatory abstinence (preliminary imprisonment).

In Crete, hair testing for drugs of abuse, which is not specially referred to in any law or decision of Ministry, has been ordered by District Attorneys (Prosecutor) in order to help elucidate legal issues, such as confirmation of drug use, extent of past drug use, systematic drug use, and severity of abuse. The Prosecutor is empowered to decide on sending the case to the Minor Crime Court or the Examining Judge, who may order preliminary imprisonment of the offender until the time of trial. In our experience, hair testing has been mainly ordered by the Examining Judge during interrogation in severe cases. Medical examiners and forensic psychiatrists who are ordered to examine the arrested person may also ask for a toxicological examination which includes hair testing.

It must be said that considerable time may have elapsed from the time of arrest until the receipt of the order for hair testing and the visit to the imprisoned. Pubic hair and axillary hair was the sample of

choice in cases when prisoners had no or short head hair. The final Crime Court will usually take place in less than a year from the time of preliminary imprisonment. During trial all interrogation data will be presented.

Our data on morphine levels (Table 1) in total hair samples from imprisoned abusers showed that even up to seven months and more after preliminary imprisonment, detection is possible. Morphine concentration in hair of detainees ranged from 0.2 to 130.5 ng/mg (mean 12.3 ng/mg). Based on a high number of case data (morphine levels in hair and self-reported drug use) we propose that morphine concentrations in hair from 5 to 15 ng/mg indicate medium severity of consumption. Morphine concentrations above 15 ng/mg hair indicate high severity of drug consumption.

Hair analysis is a powerful tool for the diagnosis of poisoning [17] associated with opiate abuse and of the state of addiction itself. The validation of hair testing has revealed its strengths and limitations up to now. The unresolved scientific issues should not obstruct the admissibility of strongly positive test results. Specific questions need to be answered (e.g., toxicomania) and the laboratory results may give strongly positive (no doubt of external contamination, other factors, etc.) but also may not be explanatory for the entire case.

The court seeks the truth and in the case of toxicomania the truth is the occurrence or absence of addiction. The exact value of hair morphine level is not necessary in order to conclude that a person falls in the category of heavy heroin users and is addicted (if sectional hair testing confirms such levels of 6-MAM for period a several months). The values 15, 30, or 50 ng/mg (morphine in hair) confirm a person's daily intake of heroin (possible quantities from 0.3 to 1.5 g daily).

Addiction is associated with chronic increase of abuse and tolerance. Hair drug time profiles are only objective evidence to confirm chronic increase of abuse (addiction). Sectional hair testing can prove systematic, chronic abuse of drugs. Each laboratory in the field should create its own bank of data of the home population (investigate individual and external parameters at site). Each case should be considered as a unique case in the context of laboratory-owned set of data. Cases with strongly positive results should be distinguished from less clear-cut cases. The data from sectional analysis of the samples obtained from the imprisoned abusers (Table 1) and from heavy heroin users (Fig. 1) strongly support the possibility of applying hair testing (pubic, head and axillary) to evaluate the systematic drug use of heroin, even when considerable time has passed with obligatory refrain from use.

Clinical applications

The laboratory results showed a reduction on CBZ and PHT concentrations from the 1st to the 5th hair segments. This is due to drug degradation over time or to drug extraction from the hair by washing with hair cosmetics. Figure 4 exhibits valproic acid (VPA) concentration hair profiles for 8 patients. The mean and the SD value for the data of hair sections concentrations of 40 patients are also presented in Fig. 4 [18]. On the other hand, VPA concentrations increased from the proximal to the distal hair segments. Probably, this was due to "passive" drug incorporation from the sweat (external contamination).

In the slightly acidic environment of sweat, the drugs bind through hydrophobic interactions, so only drugs with similar hydrophobicities to VPA bind well. CBZ and PHT hair concentrations showed higher values than VPA hair concentrations (Fig. 5).

These drugs are mainly incorporated into hairs from the bloodstream during the growth phase. In the slightly alkaline environment of blood, CBZ and PHT are found as positive ions. On the other hand, VPA is negatively charged. It has been reported that negatively charged substances, such as hair care products, bind poorly to hair, whereas positively charged materials bind tightly. The concentration of CBZ, PHT, and VPA in hair segments of patients (the first segment close to the root) is illustrated in Fig. 5.

There were no significant differences in hair drug levels of patients based on their sex [14] nor in hair drug levels between patients, receiving only one of the test drugs and those that were receiving one or two other drugs. Lower values of drugs in blond, brown, and dyed hair than in black hair, despite similar drugs dosages were observed. The hair drug levels of CBZ and PHT were found to be dependent

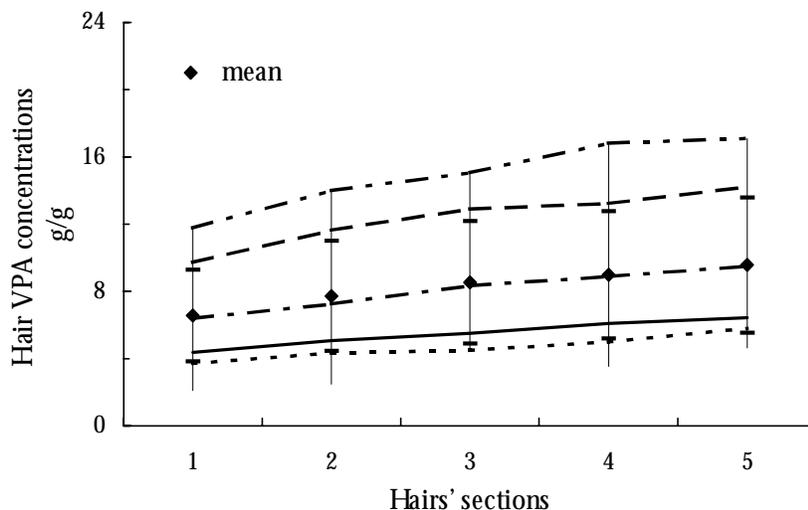


Fig. 4 VPA hair profiles from 8 patients in comparison with the mean \pm SD values from the data of 40 patients.

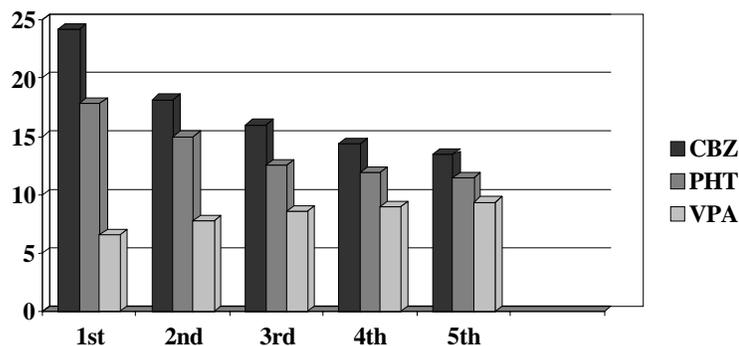


Fig. 5 Comparison between hair sections CBZ (N = 40), PHT (N = 60) and VPA (N = 40) concentrations (the 1st close to the root).

on the dosages of drugs (Fig. 6) [14]. It was also found that the duration of the treatment only slightly affected CBZ and PHT hair levels.

The hair drug levels increased ($p \leq 0.05$) with increasing doses of drugs administered to the patients (Fig. 6). The drugs' "profiles" were determined from the measured concentrations in hair segments. In the majority of cases, the CBZ and PHT hair profiles determined from the measured concentrations in hair sections fell within the range of the standard deviation. These cases represented good compliance with therapy. Some cases showed abnormal drug hair profiles and were attributed to polytherapy and to lack of standard drug dosage (numerous doses were omitted).

Although there are still many issues to be studied and clarified, sectional hair testing may be used as a marker of the dosage history and the compliance of patients under long-term treatment with carbamazepine and phenytoin.

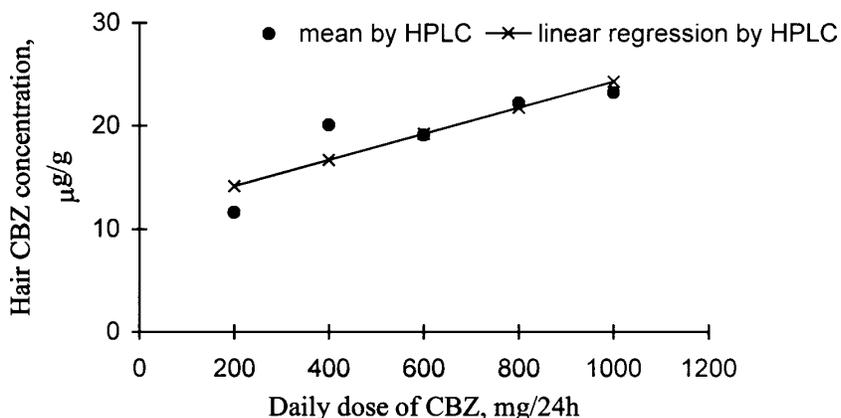


Fig. 6 Daily dose of drug and CBZ concentration by HPLC from the first hair section.

ACKNOWLEDGMENTS

The authors express their appreciation to Dr. Manolis Frangoulis, Medical Examiner of the Ministry of Justice in Crete, for his comments concerning legal cases (toxicomania) and his assistance in the collection of hair samples and to Dr. Thanasis Psillakis for providing data from cases concerning clinical application of hair testing.

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