Hair analysis

New laboratory ability to test for substance use

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The detection of drugs of misuse in hair has been a technical possibility for several decades. A single hair test gives information covering a period of several weeks or months, compared with the day or two covered by a single urine test. Hair analysis is increasingly employed commercially in employment screening for drugs of misuse. However, until recently, the technology required for hair analysis has been relatively complex, expensive and beyond the scope of most clinical laboratories. Doubts also exist about the possibility of false positives arising from environmental contamination of hair, and false negatives caused by cosmetic hair treatments such as colouring and perming. Thus, hair analysis is viewed with more caution than the more everyday procedure of urine analysis, and some applications of the technique have little, if any, direct clinical relevance. These include the post-mortem findings of arsenic in the hair of Napoleon Bonaparte and laudanum in that of the poet, Keats (Smith *et al*, 1962; Lyon, 1986), and the detection of cocaine in the hair of pre-Columbian and Egyptian mummies that were thousands of years old (Cartmell *et al*, 1991; Balabanova *et al*, 1992). Nevertheless, such demonstrations illustrate the extraordinary durability of the hair matrix, the ability of hair to concentrate and retain drugs, and the startling capacity of modern analytical methods to detect even minute traces of chemicals. We will review recent advances in addressing the technical and theoretical obstacles to the routine use of hair analysis. This powerful new tool should now be more widely employed in clinical practice and in psychiatric research.

TECHNICAL ASPECTS OF HAIR TESTING

Collecting and preparing samples

Human hair grows at approximately 1 cm per month, though there may be periods of non-growth for each individual hair. The rich capillary bed in the hair follicle transfers drugs from the blood to the hair bulb by unknown mechanisms. Diffusion from the capillary network into growing cells has been proposed as an explanation (Cone, 1996). From the cells of the hair bulb, drugs become incorporated into the hair shaft. As the hair grows, deposited drugs are carried along in a relatively fixed position. Thus, the hair acts like a physiological 'tape recording', with the linear position of drug deposits along the hair giving an estimate of when the drug was taken (Nakahara, 1995). Once bound in the hair matrix, drugs are resistant to removal by ordinary hygiene measures. While washing, dying, applying permanent wave solutions and bleaching may reduce the amounts of drugs present in hair, the drugs remain present in detectable quantities (Welch *et al*, 1993; Marsh *et al*, 1995).

Drugs may be found in axillary, beard and pubic hair (Mangin & Kintz, 1993), though most sampling takes place at the posterior vertex of the scalp, where the rate of growth shows least variability and the proportion of growing hairs is 85% or greater. At any time, around 15% of hairs are in a resting phase and have ceased to grow or are ready to fall out (Harkey, 1993). During this period, no new drugs are incorporated into the hair, though they may permeate its outside via sweat or sebum (Blank & Kidwell, 1993; Skopp *et al*, 1997). Where an attempt to demonstrate the timing or pattern of drug use is contemplated, it is advisable to gently tug or comb the hairs in the sampling area to remove all but actively growing hairs. Although hair analysis has been performed on single hairs (Suzuki *et al*, 1984), a laboratory usually requires 50–100 hairs, weighing at least 10 mg, cut next to the scalp. Such a sample is cosmetically insignificant. Because the bulb of the hair is not sampled using this approach, the sample provides no information on drug use over the preceding week or so. Once the sample reaches the laboratory, it is washed to remove possible external contaminants. After washing, the hair undergoes physical degradation by a variety of methods, including digestion with enzymes or dissolution in acid, alkaki and organic solvents. This process releases the drugs bound in hair. A liquid extract is prepared and subjected to the next stage, that of analysis. Some laboratories retain wash solutions to assist with interpretation of an equivocal result: high concentrations of drug in the wash solutions compared with the subsequent extract suggest external contamination (Baumgartner & Hill, 1993).

Sample analysis

The methods available for analysing aqueous extracts from hair samples are similar to those used to analyse urine. The two most common analytic methods are immunoassays, using specific antibodies to various drugs or drug classes, and gas chromatography linked to mass spectroscopy (GC–MS).

Immunoassays are very widely used in urine testing. Some require relatively little equipment and may be employed on hospital wards or in police stations, with minimal training. However, the unit cost of each test is high, especially if a full drug screen is required, as one kit is usually required for each drug or drug class tested for (Anonymous, 1987). With antibody testing, there is a possibility of false positive results. These are mainly due to cross-reactivity of the antibody with other compounds, including minor analgesics, cold remedies and antipsychotic drugs. It is therefore advisable to perform GC–MS confirmation tests in the event of positive results if absolute proof is required. Assays based on similar principles are available for hair testing, but the procedures of hair testing require trained laboratory staff, and safety precautions where radioactive materials are employed in radioimmunoassays. The same limitations regarding false positive results apply and a confirmation test is usually required.

GC–MS, when properly conducted, is regarded as indisputable evidence of the presence of a drug. This procedure volatilises molecules before fragmenting them and separating them in a magnetic field, according to their electrical properties and mass. Each molecule has a highly characteristic pattern of physical properties and breakdown products which identify it precisely, even in the presence of other very similar compounds. Thus, parent compounds, such

as morphine, and their immediate precursors or metabolites, such as 6-monoacetyl morphine and diamorphine, can be distinguished and quantified, as can other groups of closely related chemicals such as dexamphetamine, methamphetamine and methylenedioxymethamphetamine (ecstasy). Theoretically, there is no limit to the number of drugs which can be tested for in this way, provided their mass spectra are known. The chief limitation of GC–MS is the high purchase cost of the machine and the high level of technical skill required to operate it. However, modern GC–MS machines can be configured to detect a range of illicit drugs extracted from the same sample in a single analysis, a feature that increases the speed of the procedure and reduces costs (Cone *et al*, 1993; Moeller *et al*, 1993). Such advances may permit the adoption of GC–MS as a one-step procedure for mass screening in the future. Some international guidelines for the standardisation of laboratory procedures in hair testing have already been proposed (Sachs, 1997).

INTERPRETATION OF RESULTS

'True' false positives and false negatives

As described above, positive results on immunoassay usually require confirmation, with GC–MS, or a similar procedure such as tandem mass spectrometry (Uhl, 1997). 'True' false positives are rare with GC–MS and occur because of violations of laboratory procedures. Machine defects are avoided by regularly calibrating the machine against a known standard and by regular submission of known drug-free samples. Inter-laboratory comparisons, where split samples are processed blindly using independently derived thresholds for detection, have reported high concordance between the results of radioimmunoassay and of GC–MS (Mieczkowski *et al*, 1997). Such studies also show that hair tests are generally able to employ lower cut-off points for each drug than urine tests and are thus more sensitive (Kintz, 1996). An exception is with cannabis, where the cut-off point in hair is similar to that in urine. False negatives occur where the amount of drug consumed is extremely small. They are more likely in urine testing than in hair testing, and with radioimmunoassay than with GC–MS.

'Evidentiary' false positives

A hair sample may occasionally test positive, while the subject contends that they must have unknowingly contaminated their hair without actually ingesting a drug. Here, recent developments have improved the diagnostic capabilities of hair testing. GC–MS can measure the relative quantities of parent drug and metabolites, or detect unique metabolites found only when a drug has passed through the human body. This approach circumvents the theoretical objection that heavy environmental contamination with powders, vapours or solutions of drugs may cause 'evidentiary' false positives. Thus, the presence of delta-9-tetrahydrocannabinol, a metabolite of cannabis, is considered diagnostic of actual use (Baumgartner & Hill, 1993; DuPont & Baumgartner, 1995). 6-monoacetyl morphine is a marker for heroin use (Cone *et al*, 1993) and cocaethylene and norcocaine indicate ingestion of cocaine (Cone *et al*, 1991).

PSYCHIATRIC USES

There are two main areas where hair analysis has already been employed; in the clinical treatment of those dependent on drugs and in the detection of comorbid substance misuse among those with major mental illnesses. Strang *et al* (1993) have observed that hair analysis is potentially well-suited to research, where self-report can be potentially inaccurate and where repeated urine testing would be required to detect intermittent substance misuse. It is also possible to quantitatively detect antipsychotic drugs, such as haloperidol, in hair (Sato *et al*, 1989). Therefore, hair analysis might be a relatively economical way to measure medication compliance in the treatment of mental illness.

Treatment of substance dependence

It is sometimes assumed that once a person is identified as suffering from substance dependence, they will accurately report their drug-taking because there is no need to conceal it further. In practice, this is often far from being the case, as the patients may fear the imposition of sanctions if non-prescribed drug use is detected in a treatment programme: indeed, disciplinary action or expulsion have historically been common consequences of non-sanctioned drug use by a resident of a drug-free residential programme (De Leon, 1984; Donellan & Toon, 1986; Toon & Lynch, 1994). Loss of privileges or dose reductions have been specifically incorporated into reward and punishment systems in 'contingency contracting' with patients attending methadone maintenance programmes (Stitzer *et al*, 1983, 1986; McCarthy & Borders, 1985; Calsyn *et al*, 1991). Furthermore, for other patients, continuing abstinence may be a precondition of continuing employment, the return of a driving licence, the return of children and resumption of parental responsibilities following 'ward of court' proceedings or non-custodial sentencing for drug-related criminal offences. Thus, there may be considerable incentives for ostensibly abstinent subjects to conceal any drug use at all, and for patients prescribed maintenance drugs, such as methadone, to conceal any additional drug use. For patients entering a methadone maintenance programme, a key outcome measure in the short-term is abstinence from use of, or a substantial reduction in use of, other drugs. Drug use is usually monitored with regular urine screening, but such tests can be falsified in a number of ways, ranging from substitution of the sample to adulteration with a variety of substances designed to interfere with an antibody test. Even where GC–MS of urine is used to overcome these problems, it is not proof against 'tactical' use of drugs, for example at the weekend, when a urine test is unlikely to be requested.

Urine testing is not considered a quantitative method of analysis because the concentration of a drug in urine is critically dependent on factors such as the timing of the taking of the sample and the state of hydration of the subject. It may also be altered markedly by a change in the acidity of the urine being produced. By contrast, although inter-individual variations in concentrations of drugs found in hair are large, with steady prescribing, hair segments demonstrate quite close concentrations of drug when compared over time. Thus, drugs in hair can be measured in a semi-quantitative manner, so that a marked increase or decrease in the amount of drug intake over months can be demonstrated (Strang *et al*, 1990; Moeller *et al*, 1993; Marsh *et al*, 1995). Finally, in the substance misuse setting, hair testing has been reported to be widely acceptable to patients and superior to urine testing in its ability to detect intermittent drug use (Brewer, 1993). Thus, the potential applications for

hair analysis in this context include the detection of misuse of unreported drugs at initial presentation, and the monitoring of maintenance therapy and any covert use of additional medication.

Comorbid substance misuse

The epidemiology of substance misuse among psychiatric patients has been largely based on studies using questionnaires only. There has been general agreement that psychiatric patients may be at least as prone to substance misuse as their peers, and that comorbid substance misuse is associated with poor outcome in terms of symptoms, course and prognosis of the primary psychiatric illness (Linszen *et al*, 1994; Menezes *et al*, 1996). However, investigations based on questionnaire reports have revealed widespread use of cannabis but very low rates of current or recent stimulant use and relatively weak associations between substance misuse and adverse illness outcome (Menezes *et al*, 1996). Early results obtained from urine and hair analysis suggest that relatives and mental health professionals remain largely unaware of the majority of substance misuse among psychiatric patients, particularly the use of stimulant drugs (Shaner *et al*, 1993; McPhillips *et al*, 1997). These drugs are of particular interest because they are very extensively used among young people, and increasingly so, and because of their action in raising the amount of dopaminergic activity in the brain. This property is directly antagonistic to the proposed therapeutic action of most antipsychotic drugs.

Preliminary studies using biological methods of detection in psychiatric populations show links between stimulant misuse and adverse outcome (Shaner *et al*, 1993) but the direction of causality has not been established. Of possible relevance here is the self-medication hypothesis, which holds that those with more disturbing and distressing symptoms and/or medication side-effects may seek some relief with drugs of misuse (Khantzian, 1985; Schneier & Siris, 1987). Prospective studies of first-episode cohorts will be required to disentangle the relationship between substance misuse and the course and outcome of major mental illness.

Apart from its usefulness as a tool in epidemiological studies of comorbidity, there are a number of further potential applications of hair analysis in general psychiatry. These include: the diagnosis of undisclosed substance misuse in the months before the onset of an illness (for example, in the differential diagnosis of a first psychotic episode); the detection of substance misuse as a complicating factor where a subject appears to fail to respond adequately to treatment; and the assessment of the contribution of substance misuse to psychotic relapse or an episode of violence. In these situations, the fact that a hair sample covering the period of interest may be taken weeks or even months after an acute psychotic episode is a major advantage, as it is often difficult to collect urine samples from acutely psychotic patients.

CONCLUSIONS

Technological improvements in the design and capabilities of GC–MS machines have brought very highly specified machines within financial reach of more hospital laboratories. Such machines are now capable of automated processing of large numbers of extracted samples and of sequentially screening for different compounds within the same sample. These improvements, combined with a steady increase in the range of drugs and drug metabolites which can be analysed from hair, offer the prospect of routine screening of hair samples at a comparable cost to that of a standard antibody-based urine screen, though 'one-off' requests would remain expensive. GC–MS may make it possible to say whether a drug has been ingested and to discern whether other drugs from the same chemical class have been taken simultaneously. This depth of analytical penetration is also possible with GC–MS urinalysis but not with the recently introduced immunoassay kits.

The longer period that can be scrutinised with a single hair test offers important advantages over urinalysis: retrospective information on substance use, which would be impossible to obtain by urine testing, is obtained and there is a significant increase in the likelihood of detecting unreported use of stimulant or opiate drugs. A further advantage is that, in contrast with urine screening, a hair test can readily demonstrate abstinence from drugs over a lengthy period. The use of segmental hair analysis may allow escalating or decreasing drug use to be detected. By contrast, a urine test has the advantage of being able to confirm very recent use (or abstinence from) a particular drug. Further, the most commonly misused illegal drug, cannabis, may be detectable in urine for rather longer than stimulant drugs are, and is less easily detected in hair than opiates and stimulants are.

The interpretation of the results of hair analysis must take into account the problems discussed above in respect of false positives and false negatives, particularly if GC–MS confirmation is not available. Further, the clinical situations in which hair analysis can be considered the current method of choice for obtaining evidence of drug use are presently limited by the lack of general availability of the technique. Nevertheless, hair analysis has already yielded findings of clinical relevance in investigations of outcome in substance dependence, particularly methadone maintenance treatment, and in the investigation of comorbid substance use in schizophrenia. In these settings, it has proved to be as acceptable to subjects as urine testing. Other potential clinical applications such as the monitoring of compliance with antipsychotic therapy still await proper exploration.

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