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Case Report

High risk of misinterpreting hair analysis results for children tested for methadone

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ABSTRACT

The major problem after testing the hair of a child for drugs is the interpretation of the findings. In 2016, the laboratory received several hair specimens with the request to verify if there was any evidence of previous methadone exposure by the donor of the sample. Case 1 was a child admitted to the Emergency Unit for intense sedation and breathing difficulties. Cases 2–4 involved children found dead at home. In all cases, methadone and EDDP, its metabolite, were found in blood. After decontamination, the hair was analysed with LC–MS/MS for methadone and EDDP. The LOQ for both drugs was 10 pg/mg. Concentrations were in the range 60–1590 and <10–220 pg/mg for methadone and EDDP, respectively. In all of the cases, segmental analysis revealed approximately the same amount of drug along the hair lock. As a consequence, contamination was considered as an issue and interpretation of the results was a challenge that deserves particular attention.

It must be considered that the amount of hair from children, available for analysis, can be low, particularly when several drugs have to be tested. This has consequences on the limit of quantitation and the identification of the metabolite(s). It must be also noted that hair from children is finer and more porous in comparison with adult (risk of higher contamination by sweat versus adults). It is very difficult to put any window of detection when testing for drugs in young children as hair growing is asynchronous. It is even more complicated as it has been demonstrated that drugs can be incorporated during pregnancy in the hair of the foetus, which will contribute to the positive findings after delivery. Several weeks or months after delivery, identification of a drug in hair can indicate: 1, in-utero exposure, or 2, exposure after delivery, or 3, a mix of both situations. Whereas the detection of drugs in a child's hair unambiguously shows drug handling in the environment of the child, it is difficult to distinguish between systemic incorporation into hair after ingestion or inhalation and external deposition into hair from smoke, dust, or contaminated surfaces. However, the interpretation of hair results with respect to systemic or only external exposure is particularly important in case of children for a realistic assessment of the toxic health risk. Practising scientists have the responsibility to inform the child protection authorities, courts, etc about these limitations.

1. Introduction

By providing information on exposure to drugs over time, hair analysis may be useful in verifying self-reported histories of drug use in any situation in which a history of past rather than recent drug use is desired. Hair analysis can also provide a retrospective calendar of an individual's drug use. For this, multi sectional analysis is required and involves taking a length of hair and cutting it into sections to measure drug use during shorter periods of time. The hair must be cut as close as possible to the scalp and particular care is also required to ensure that the individual hairs in the cut-off tuft retain their original orientation. Segmental hair analysis can be used to verify both previous drug history and recent enforced abstinence. To verify abstinence or absence of consumption, the lowest drug concentration has to be found in the segments nearest to the root [1].

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A critical question about hair analysis that remains controversial is the possibility to obtain a positive hair test due to environmental contamination without deliberate ingestion/injection of a particular drug. The most crucial issue facing hair analysis is the avoidance of false-positive results caused by passive exposure to the drug or environmental contamination. To avoid false positive results due to external contamination, the Society of Hair Testing (SoHT) [2] and the European Workplace Drug Testing Society (EWDTS) [3] have proposed criteria for a positive result, including cut-offs. These cut-offs, along with the identification of a metabolite and the implementation of a decontamination procedure associated to the analysis of the washes were supposed to facilitate the interpretation of a hair test result. In some instances, the positive cut-offs are not applied, where low levels of consumption or even a single exposure have to be demonstrated in some specific situations. This is particularly true in cases of child survey. To qualify for a positive hair test result for methadone, the drug must be detected at a positive cut-off of 0.2 ng/mg and EDDP (at 0.05 ng/mg), its metabolite, must be present.

In children, it is difficult to distinguish between systemic incorporation into hair after ingestion from external contamination [4]. However, the discrimination is particularly important for the evaluation of the health risks. In the recent years, several papers [5–11] have demonstrated that the interpretation of hair test results from children is very complicated and must involve multiple sources of data.

We present here a series of hair tests for methadone where the conclusive interpretation was almost impossible to establish.

2. Materials and methods

During 2016, 4 forensic cases were received. The request was to verify if there was any evidence of previous methadone exposure by the donor of the sample. The items were received sealed and the chain of custody was intact. The samples were logged onto the system and processed at the laboratory. These samples were obtained after admission to the local hospital for intense sedation and dyspnoea (1 case) or after autopsy (3 cases). Medical staff or the Prosecutor wanted to verify potential drug administration to the children. All the parents denied any (repetitive) administration to their child.

2.1. Case histories

2.1.1. Case 1

A 4-year old boy was admitted to the Emergency Unit for intense sedation and breathing difficulties (dyspnoea) after a stay with his mother. Blood screening for general unknowns revealed the presence of methadone at 70 ng/ml. EDDP, the metabolite, was also identified at 10 ng/ml. The medical staff requested the testing of a hair sample for any evidence of previous methadone administration. A lock of hair that was 4 cm in length and blonde in colour was collected the day of admission. Eight weeks after the event, another hair lock (5 cm) was collected.

2.1.2. Case 2

A 2-year old boy died of methadone overdose (femoral blood tested at 354 and 72 ng/ml for methadone and EDDP respectively). A history of use was required to assist the police in assessing if it was a single dose or had it been given on more than one occasion. A lock of hair that was 3 cm in length and light brown in colour was collected.

2.1.3. Case 3

A 18-month old girl was found dead at home. Both parents were reported to have been under methadone therapy. Analysis of central blood tested positive for methadone and EDDP at 285 and 102 ng/ml, respectively. The police has requested the testing of a hair sample to assess if there was any historical evidence that the mother or the father may have administered the drug to the child on more than one occasion. A lock of hair that was 5 cm in length and brown in colour was collected.

2.1.4. Case 4

Parents were suspected of administering methadone to a 16-month old child, found dead at home. The circumstance of the death was first listed as sudden infant death but the police investigations revealed that the father was sometimes using opiate drugs. Analysis of femoral blood tested positive for methadone and EDDP at 245 and 61 ng/ml, respectively. The police requested the testing of a hair sample for any evidence of previous methadone administration. A lock of hair that was 6 cm in length and blonde in colour was collected.

2.2. Toxicological analyses

Methadone and EDDP were tested in hair using a previously described method [4]. Briefly, hair locks were decontaminated twice, using methylene chloride (5 ml, 2 min) and then segmented. Each segment was cut into small pieces (<1 mm). About 20 mg were incubated overnight in 1 ml of ammonium chloride buffer at pH 9.5, in the presence of 10 ng of methadone-d_3 and EDDP-d_3 used as internal standards (IS), at 40 °C without agitation. After a liquid–liquid extraction with 5 ml of a mixture of dichloromethane/isopropanol/*n*-heptane (25/10/65) and evaporation to dryness, the residue was reconstituted in 50 μl of ammonium formate buffer adjusted at pH 3. Chromatography was achieved using a Waters Acquity HSS C18 column (150 × 2.1 mm × 1.8 μm) maintained at 50 °C in a thermostatically controlled oven. A gradient elution was performed using formate buffer adjusted to pH 3 (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The flow rate was 0.4 ml/min. The initial gradient was 87% phase A and the final gradient, at 15 min, was 5% phase A. An injection volume of 10 μl was used in all cases. A Xevo TQD triple quadrupole mass spectrometer was used for the detection of the molecule. Ionization was achieved using electrospray in the positive ionization mode (ES+). The following conditions were found to be optimal for the analysis of methadone, EDDP and the internal standards: capillary voltage at 1.5 kV; source block temperature at 149 °C; desolvation gas nitrogen heated at 600 °C and delivered at a flow rate of 1000 l/h. MassLynx 4.1 software was used for quantification.

Linearity was observed for methadone and EDDP concentrations ranging from 10 to 5000 pg/mg, with a correlation coefficient >0.999. QC samples (100 pg/mg and 500 pg/mg), analysed in duplicate in six independent experimental assays, were used for determination a coefficient of variation for precision and accuracy. These CVs were lower than 20%. The limit of detection and the lower limit of quantification were 2 pg/mg and 10 pg/mg respectively. Under the used chromatographic conditions, there was no interference with the analytes by chemicals or any extractable endogenous materials present in hair. The lack of matrix effect (<20%) was verified with spiked methadone and EDDP at 500 pg/mg in 15 blank hair samples.

3. Results and discussion

In the more recent years, it has been observed an increase of situations were a drug was used just to obtain sedation, particularly in cases involving children. The interpretation of the findings can be an issue, as the pharmacology of drugs is different from adults. Like other types of domestic violence, knowledge