CASE REPORT

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Detection of Drugs-of-Abuse in Meconium of a Stillborn Baby and in Stool of a Deceased 41-Day-Old Infant

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ABSTRACT: When blood or urine is unavailable, postmortem meconium or stool from infants or stillbirths can be used to detect drugs-of-abuse, thus providing datum in assessing drug-abuse exposure.

Two case reports illustrate how drugs-of-abuse findings in postmortem specimens were used to substantiate exposure prior to death or a history of maternal drug abuse. The first, a congenital hydrocephalus, born to a non-drug abusing mother, expired at the age of 41 days, had opiates in the stool by screening method, enzyme multiplied immunoassay technique, confirmed by gas chromatographic/mass spectrometric (GC/MS) analysis. Investigation revealed that morphine had been administered for three days prior to death. The second was a stillbirth infant born to a drug abuser. Almost equal amounts of benzoylecgonine were found in different bowel segments, a finding consistent with admitted cocaine use throughout pregnancy.

KEYWORDS: toxicology, meconium, drugs-of-abuse, cocaine, morphine, enzyme multiplied immunoassay technique (EMIT), gas chromatography/mass spectrometer

Substance abuse is a major social problem in the United States. In the first half of 1989, Los Angeles County Department of Health Services reported more than 4000 abusers of cocaine, heroin/morphine, phencyclidine (PCP), and amphetamines treated in its emergency rooms [*1*]. Annually, since 1986, the Los Angeles County Office of Chief Medical Examiner-Coroner (LAC CMEC) has reported approximately 2000 drug-related deaths [*1*].

Additionally, drug abuse during pregnancy is a serious problem and of great concern for obstetricians, and pediatricians [*2,3*]. There is a high incidence of (a) perinatal complications, for example, stillbirths, abruptio placentae and fetal stress with meconium-stained amniotic fluid [*4*], and (b) increased newborn mortality and morbidity, for example, prematurity, low birth weight, asphyxia, pneumonia, congenital malformations, cerebral infarction and drug withdrawal [*5–10*].

Screening of neonates born to suspected drug abusers is essential to prompt intervention and follow-up care. Los Angeles County + University of Southern California Medical Center (LAC + USC MC) reported 16 to 20% of babies, born to suspected drug abuse mothers, tested positive for one or more drugs [*11*]. The LAC CMEC reported that 40% of neonates dying within two days with no apparent cause of death had cocaine and/or benzoylecgonine in their blood [*12*].

Therefore, screening stillbirth infants for drugs-of-abuse is important, especially when the cause of death is not apparent. However, for stillbirth infants, it is often difficult to obtain adequate urine or blood samples. Organ tissue can be used, but the procedures are more difficult and tedious. Also, biological fluids and tissues may be negative if there was maternal abstinence for a few days before delivery.

Meconium has been used as an alternative specimen in neonatal drug abuse screening [*13–20*]. Meconium begins to accumulate at approximately 16 weeks of gestation and is not normally excreted until birth [*21*]. Our experience does not indicate that meconium is better than urine in the screening of drugs-of-abuse in newborn infants, it can be useful when urine is not available, when urine results are negative but there is a strong suspicion of maternal drug usage during pregnancy [22]. In stillbirth infants and infants that died after birth, it may be the specimen of choice for drugs-of-abuse testing because of the difficulty in obtaining uring specimens or using organs.

In this communication, we report two cases in which the positive drug findings in meconium or stool identified therapeutic use of morphine not apparent from the medical records or cocaine use throughout pregnancy as supported by the mother's history.

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Case Reports

Case 1

A 41-day-old male congenital hydrocephalus (Dandy Walker Syndrome) was born to a 24-year-old Hispanic multipara after 41 weeks gestation following an uncomplicated pregnancy and delivery at LAC + USC MC. The mother denied usage of any drugs-of-abuse or alcohol during pregnancy and was not screened for drugs-of-abuse. Soon after birth, nasogastric tube feedings were instituted due to vestigial epiglottis. Tracheal intubation and tracheostomy were performed due to poor respiratory effort and tracheal atresia respectively. During the shunting treatment for hydrocephalus, phenobarbital, diazepam, meperidine, epinephrine, and antibiotics were administered.

Autopsy showed normal development (weight 5.10 kg, length 59.3 cm), congenital hydrocephalus status post ventriculoperitoneal shunt placement, vestigial epiglottis, and tracheal atresia. The bowels contained moderate amounts of yellow brown stool and no meconium. Microscopic examination revealed no remarkable congestion or edema. Stool analysis revealed the presence of 1340 ng/g of morphine and no codeine.

Case 2

A stillbirth baby girl (weight 2.06 kg, length 42 cm), 31 weeks of gestation, was born to a 32-year-old black multipara with normal pregnancy and delivery with no clinical evidence of abruptio placentae. The mother admitted regular alcohol and cocaine abuse, and using cocaine one day prior to delivery. Maternal urine tested positive for benzoylecgonine and the serum negative for alcohol. The clinical cause of death was thought to be intrauterine demise due to maternal cocaine use.

Gross and microscopic autopsy findings only showed maceration. The bowels were separated into 5 segments as follows: large intestine, 2 equal segments; small intestine, 3 equal segments. The meconium in all 5 segments contained approximately the same concentration of benzoylecgonine (Table 1).

TABLE 1—*Segmental analysis of meconium from a baby still born to mother strongly suspected of cocaine abuse during pregnancy.*

Segments[a]	Cocaine metabolite by EMIT screening		Benzoylecgonine by GC/MS
	Delta ABS[b]	Presumptive Result	ng/g
Large intestine			
Lower part	199	Positive	1910
Upper part	193	Positive	2240
Small intestine			
Lower part	195	Positive	1860
Middle part	197	Positive	1880
Upper part	201	Positive	2210

[a]Large intestine was divided into 2 equal segments and small intestine into 3 equal segments.

[b]Meconium was considered to be presumptive positive for cocaine metabolite when the delta absorbance (that is, absorbance rate of the specimen minus the absorbance rate of a negative control) exceeded 40 units [22]. This corresponded to a cutoff concentration of 250 ng benzoylecgonine per gram of meconium.

Analytical Methods

Specimens

The meconium/stool specimens were stored at −15°C until use.

Screening with Enzyme Multiplied Immunoassay Technique (EMIT)

Meconium specimens were processed for screening and confirmation as previously described [22]. For EMIT screening, half a gram of meconium was combined with 2 mL of water and 50 to 100 mg of sodium bicarbonate/sodium carbonate (5/1) powder in a glass test tube. The mixture was vortex-mixed for 3 minutes with 6 mL of 3:1 chloroform/isopropanol and centrifuged at 2500 rpm for 5 min. Five mL of the organic phase were transferred to a glass test tube. A drop of concentrated HCl was added and the mixture was evaporated to dryness under a gentle stream of nitrogen at 50 to 70°C. The residues were reconstituted with 0.5 mL of pH 6.0 buffer and mixed with 0.5 mL of ethyl acetate for 10 s to remove the lipids. The aqueous phase was removed and analyzed for amphetamine/methamphetamine, cocaine metabolites, opiates and phencyclidine by EMIT using the SYVA®ETS®-PLUS SYSTEM. Meconium was presumed positive when the delta absorbance (that is, reaction rate for the specimen minus the reaction rate for the negative control) exceeded 40 units for cocaine metabolite and 30 units for opiates [22].

Gas Chromatographic/Mass Spectrometric (GC/MS) Confirmation of Benzoylecgonine and Opiates

Drugs were extracted from 1 g of meconium using the above procedure with the addition of 0.2 mL of internal standard (2 μg/mL of D3-benzoylecgonine in methanol or 2 μg/mL of nalorphine in distilled water). Benzoylecgonine and opiates were extracted from 3 mL of the aqueous phase using 10 mL Bond Elut Certify® columns (Analytichem International Inc., Harbor City, CA) according to the manufacturer's procedures. The eluates were evaporated to dryness under a gentle stream of nitrogen gas at 40°C.

Benzoylecgonine was derivatized with 50 μL of N-methyl-N-trimethylsilytrifluoro-acetamide (MSTFA) at 70°C for 15 min. A 1 μL aliquot of the sample was injected into the GC/MS. The injector temperature was 250°C. The column temperatures were as follows: 150°C for 2 min, increased at 25°C/min to 280°C and maintained for 5.4 min. Selected ion monitoring (SIM) was performed on m/z 82, 240, and 361 for benzoylecgonine and on m/z 243, 364, and 349 for D3-benzoylecgonine. The sensitivity of the GC/MS confirmation method for benzoylecgonine in meconium was 50 ng/g.

Opiates were derivatized with 0.1 mL of trifluoroacetic anhydride (TFA) at 70°C for 15 min. The mixture was evaporated to dryness under a gentle stream of nitrogen at 40°C. The residues were reconstituted with 0.1 mL of chloroform and 1 μL was injected into the GC/MS. The injector temperature was 250°C, and the column temperatures were as follows: 140°C for 0.5 min, increased at 26°C/min to 250°C, then at 8°C/min to 287°C, and at 35°C/min to a final temperature of 300°C. Mass spectral analysis was performed in the scan mode for masses from 50 to 500 atomic mass units (amu). Quantitation was achieved on SIM mode using ions m/z 364, 477, and 365 for morphine, m/z 395, 282, and 396 for codeine, and m/z 390, 503, and 391 for nalorphine. The sensitivity of the GC/MS confirmation for morphine and codeine were 30 ng/g.

The GC/MS was consisted of a Hewlett Packard (Lawndale, CA) GC 5890 Series II equipped with a Hewlett Packard Injector 7673, a HP-1 capillary column (5% methyl silicone gum, 25 mX0.02 mm), and a Hewlett Packard Mass Selective Detector 5970. The helium flow rate was 0.65 mL/min. The MS detector was operated at 70eV and interface temperature of 280°C.

Results and Discussion

We screened the autopsy stool specimens of 31 consecutive perinatal cases at LAC + USC MC from 8/10/92 to 1/11/93 for amphetamine/methamphetamine, cocaine metabolites, opiates and PCP using EMIT. Of these 31 cases, two were positive for drugs-of-abuse: 1 for opiates and 1 for cocaine metabolites. This positivity rate (6.5%) is low when compared with the 34.4% positivity rate for newborns born to mothers suspected of drug abuse.

In Case #1, the stool from the 41-day-old infant with no apparent history of morphine administration tested positive for opiates by EMIT. GC/MS confirmation and quantitation using the SIM mode indicated the presence of only morphine (1340 ng morphine per gram of stool) and no codeine. The presence of morphine in the stool was puzzling. It could not be attributed to maternal drug use during pregnancy because even though meconium has been used to document drug exposure during pregnancy, meconium could not be retained in the bowel for 41 days. The stool recovered from this infant contained no visible or significant amount of meconium. Assimilation from maternal milk [*23–25*] was impossible because the infant was isolated from birth and fed infant formula. Therefore, in order to eliminate artifactual identification of morphine by the SIM mode, a mass spectral analysis of the trifluoroacetic acid derivatized extract was obtained. The retention time and mass spectrum of the presumed morphine peak matched well with those for the morphine standard. This prompted a more thorough review of the medical records, which revealed that morphine, 2.43 mg, was administered the last three days before death.

In Case #2, the meconium from all five intestinal segments contained benzoylecgonine in approximately the same concentration (Table 1). With the current knowledge about the accumulation of drugs-of-abuse in meconium, our findings are consistent with maternal cocaine use throughout the pregnancy.

In our previous study of neonatal necropsies, we concluded that meconium appears to be no better a specimen than urine for general drugs-of-abuse screening [*22*]. In these two cases, stool and meconium successfully documented drug exposure. These findings strongly support the contention that meconium and stool can be valuable specimens for documenting drug exposure or for providing a glimpse into the maternal history of drug abuse, especially when blood or urine is unavailable.

Perinatal hair has been used for ascertaining maternal history of drug abuse [*26*]. However, it is difficult to obtain sufficient hair for analysis, and the high demand on the sensitivity of the method [*27*].

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