Alternative Matrices for Cocaine, Heroin, and Methadone In Utero Drug Exposure Detection

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Introduction: Drug determination in biological matrices from the mother and the newborn is an objective measure of maternal and fetal drug exposure. The aim of this study was to compare maternal hair, meconium, umbilical cord, and placenta for detecting in utero drug exposure to cocaine, opiates, methadone, and amphetamines.

Method: Maternal hair, meconium, umbilical cord, and placenta were collected from 175 mother–newborn dyads. Maternal hair (segmented in trimesters) and meconium specimens were analyzed for cocaine, opiates, methadone, and amphetamines. If either maternal hair or meconium tested positive, umbilical cord and placenta were analyzed. Analyses were performed by liquid chromatography tandem mass spectrometry.

Results: In hair, 24 participants tested positive; 21 for cocaine [cocaine 20–50,605, benzoylecgonine (BE) 17–46,668 pg/mg], 7 for methadone (76–26,845 pg/mg), 2 for opiates (morphine 298–2398 pg/mg, codeine 65–914 pg/mg, 6-acetylmorphine 1635–15,657 pg/mg), and 1 for amphetamines (amphetamine 1990 pg/mg, 3,4-methylenedioxyamphetamine 30 pg/mg, 3,4-methylenedioxymethamphetamine 294 pg/mg). In meconium, 6 were positive; 5 for methadone [methadone 88–3752, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) 642–25,179 ng/g], 3 for cocaine (cocaine 7, BE 79, hydroxybenzoylecgonine 5–135, ecgonine–methyl ester 2–56 ng/g), and 2 for opiates (morphine 152–1025, morphine-3-glucuronide 22–23, codeine 4–34 ng/g). Placenta and umbilical cord were positive in 5 and 6 cases, respectively; 5 for methadone in placenta (methadone 7–543, EDDP 10–51 ng/g) and cord (methadone 3–183, EDDP 2–109 ng/g); 1 for cocaine in placenta (cocaine 7, BE 2 ng/g) and cord (BE 6 ng/g); and 1 for opiates in placenta (morphine 6, morphine-3-glucuronide 48 ng/g), and 2 in cord (morphine 2, morphine-3-glucuronide 15–38, morphine-6-glucuronide 5 ng/g). Meconium, placenta, and umbilical cord only tested positive if hair concentrations were greater than Society of Hair Testing cutoffs.

Conclusions: Maternal hair is the most sensitive specimen to detect drug consumption during pregnancy. Placenta and umbilical cord could be alternatives to meconium for detecting high in utero drug exposure.

Key Words: placenta, umbilical cord, meconium, hair, drugs of abuse

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INTRODUCTION

Drug-of-abuse consumption throughout pregnancy may negatively affect pregnancy itself (spontaneous abortion, premature labor) and embryo/fetus development. Prenatal exposure to drugs of abuse has been associated with deleterious short-term and long-term effects in exposed children, such as the neonatal abstinence syndrome and neurological disorders.[1,2] Therefore, the consumption of drugs of abuse during pregnancy constitutes a major public health concern.

Depending on the trimester of pregnancy, the harm produced by drugs of abuse may be different. Heavy drug use in the first trimester often results in spontaneous abortion.[3] Also, in this period, the fetus is most susceptible to teratogens.[1] In the second and third trimesters, the main harmful fetal effects due to drug abuse are related to fetal growth and maturation. Preterm low birth weight newborns have a higher morbidity and mortality than do older heavier fetuses.[1]

In the United States, 5% of the pregnant women aged 15–44 years were current illicit drug users in 2010–2011.[4] Unfortunately, European data in this issue are scarce; a study conducted in Barcelona (Spain) showed an 11% positivity rate to drugs of abuse in meconium.[5]

The method employed in many clinical settings to detect drug consumption by pregnant women is the personal interview. This approach is easy and economic; however, it is often not reliable. Mothers underreport because of the fears to the legal and social consequences that would follow if they confessed their addiction problems.[6,7] The determination of drugs and/or metabolites in biological specimens

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from the mother (urine, sweat, oral fluid, hair), from the newborn (urine, meconium, hair, umbilical cord) and from the maternal–fetal unit (placenta) offers an objective measure of this exposure. Several previously published reviews discussed the main advantages and disadvantages for some of these matrices.[8–10]

Although maternal monitoring is not a direct marker of in utero exposure, it allows the clinician to be aware of the problem before delivery and to take treatment decisions in advance. Among the different maternal matrices, hair shows the longest window of detection. The detection time frame in oral fluid is hours, in urine days, in sweat a week (depending on the time the patch is worn), and in hair, it may be several months. Hair grows at an accepted rate of 1 cm/mo.[11] If the lock of hair is long enough, and through the use of segmented analysis, hair can provide a detailed account of drug exposure in the 3 different trimesters of pregnancy.

Specimens collected from newborns show the actual fetal exposure to these substances, and each matrix has different advantages and disadvantages. Newborn urine, hair, and meconium have been extensively studied.[8–10] Newborn urine is easy to analyze; however, its collection is difficult, and its window of detection is just a few days. Neonatal hair and meconium have a longer window of detection; they reflect in utero drug exposure from the third trimester.[12–14] However, neonatal hair is often not available or in very little amount, or the parents reject its collection because of cosmetic or cultural reasons.[8] In the case of meconium, its expulsion could take up to 5 days after delivery, or it could be passed in utero, making this matrix unavailable for drug testing.

Recently, placenta[15–20] and umbilical cord tissue[21–25] have been used as alternative matrices to monitor drugs of abuse during pregnancy. Some of these studies compared meconium and placenta[15,16,18–20] and meconium and umbilical cord,[21–23] and only one compared the mother's hair and placenta from abortions at the 12th week of gestation.[17] The main advantages of the placenta and the umbilical cord are their easy and noninvasive collection, the abundant amount of specimen, readily available at the time of delivery, and the fact that they are considered waste products. In utero drug exposure cases are complex, and very often, not all the biological matrices are available. The knowledge of the window of detection and metabolic profile in each of the neonatal matrices, and the correlation between the degree of maternal drug use and newborn exposure, will substantially help us in the interpretation of the results, to predict the clinical outcomes of these fragile patients, and to take intervention and treatment decisions.

The aim of this study was to compare maternal hair, meconium, umbilical cord, and placenta at term pregnancies (n = 175) for detecting in utero drug exposure to cocaine, opiates, methadone, and amphetamines. The maternal locks of hair were divided into 3 different segments to study drug exposure in each trimester. These results were compared with those found in the meconium, umbilical cord, and placenta, to study the window of detection and the sensitivity of these matrices. Sixteen drugs and metabolites were analyzed in the meconium, placenta, and umbilical cord to compare their different drug disposition and metabolic profiles.

MATERIALS AND METHODS

Participants

The participants were pregnant women who delivered at the University Hospital of Vigo, Spain, from May to July 2011. They were informed about the study both in writing and orally after the delivery, and they gave written consent. The subjects were not paid for participation. The study was approved by the Ethics Committee of the University of Santiago de Compostela, Spain. Two hundred and ten pregnant women were screened; one refused to participate, one or more specimens could not be collected in 34 cases, and all the specimens (maternal hair, meconium, placenta, and umbilical cord) were collected in 175 cases.

Specimens

The entire term placenta and umbilical cord specimens were collected at delivery, and meconium specimens were collected up to 3 days after. These specimens were stored at −20°C until analysis. Maternal hair specimens were collected after delivery. A lock of about 100 hairs was oriented (tied up at the root), cut in vertex posterior as close as possible from the scalp, and stored at room temperature until analysis.

All maternal hair (segmented in trimesters) and meconium specimens were analyzed for cocaine, opiates, methadone, and amphetamines. Umbilical cord or placenta specimens were analyzed if either maternal hair or meconium specimens tested positive for any drug.

Maternal Hair Analysis

Maternal hair specimens were analyzed by using a previously published liquid chromatography tandem mass spectrometry (LC–MSMS) method.[26] This method allowed the determination of 35 licit and illicit drugs in 50 mg of hair, including the drugs of interest in this study (cocaine, opiates, methadone, and amphetamines). Each lock of hair was measured, and divided into 3 segments corresponding to the 3 trimesters: segment 1, 2 cm, from 0 (root) to 2 cm; segment 2, 3 cm, from 3 to 5 cm; and segment 3, 3 cm, from 6 to 8 cm. If the amount of hair specimen was not enough for segmentation, only 1 segment up to 8 cm long was analyzed.

Hair decontamination was performed with dichloromethane and incubation in 2 mL of acetonitrile at 50°C overnight. The extraction procedure was performed in 2 steps, first liquid–liquid extraction with hexane:ethyl acetate (55:45, vol/vol) at pH 9, followed by solid phase extraction (Strata-X cartridges, Phenomenex, Torrance, CA). Chromatographic separation was performed in Atlantis T3, 2.1 × 100 mm, 3 μm, column (Waters, Milford, MA), acetonitrile and ammonium formate pH 3 as the mobile phase and a 32-minute total run time.

The method was specific (no endogenous interferences, n = 9); the limit of detection (LOD) was 0.2–50 pg/mg; and the limit of quantification (LOQ) was 0.5–100 pg/mg; linearity ranged from 0.5–100 to 2000–20,000 pg/mg; imprecision <15%; analytical recovery 85%–115%; and extraction efficiency was 4.1%–85.6%. Twenty-seven analytes showed ion suppression (up to −86.2%), 4 ion enhancement (up to 647.1%), and 4 no matrix effect; compounds showed good stability 24–48 hours in an autosampler.

Placenta and Umbilical Cord Analysis

Placenta and umbilical cord were analyzed by LC–MSMS for the determination of morphine, morphine-3-glucuronide, morphine-6-glucuronide, codeine, 6-acetilmorphine (6 AM), amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), cocaine, benzoylecgonine (BE), ecgonine methyl ester (EME), hydroxybenzoylecgonine (OH-BE), methadone, and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). Briefly, 1 ± 0.02 g of placenta or umbilical cord was homogenized in 5 mL of water with the Ultra-Turrax T8 dispenser (IKA, Staufen, Germany). After the addition of 50 μL of 10% formic acid and centrifugation, the supernatants were submitted to solid phase extraction (OASIS MCX cartridges, Waters). Chromatographic separation was performed in an Atlantis T3, 2.1 × 100 mm, 3 μm, column (Waters), acetonitrile and 0.1% formic acid as the mobile phase, and 18 minutes of total run time.

The methods were specific (no endogenous interferences, n = 10); the LOD was 0.5–2.5 ng/g in placenta, and 0.5–1 ng/g in umbilical cord. The LOQ was 0.5–5 ng/g in placenta and 1–2.5 ng/g in umbilical cord; linearity ranged from LOQ to 100–500 ng/g; imprecision <15.3%; analytical recovery 87.1%–114%; and extraction efficiency 16.3%–154%. Due to the complexity of the matrices, most of the compounds showed a matrix effect (n = 10); ion suppression (up to −59.1% in placenta, −75.7% in umbilical cord); or enhancement (up to 375.2% in placenta, 147.4% in umbilical cord). The use of deuterated internal standards compensated for these effects.

Meconium Analysis

Meconium was analyzed by LC–MSMS for the determination of morphine, morphine-3-glucuronide, morphine-6-glucuronide, codeine, 6 AM, amphetamine, methamphetamine, MDA, MDMA, cocaine, BE, EME, OH–BE, methadone, and EDDP. Briefly, 0.5 ± 0.01 g of meconium was pretreated with 2 mL of methanol + 0.01% formic acid, and mixed in a rotor for 30 minutes. After centrifugation, the supernatant was evaporated to dryness and reconstituted in 200 μL methanol and 2 mL of 0.1% formic acid. This solution was submitted to solid phase extraction (OASIS MCX cartridges, Waters). Chromatographic separation was performed in an Atlantis T3, 2.1 × 100 mm, 3 μm, column (Waters), acetonitrile and formic acid 0.1% as mobile phase, and 18 minutes of the total run time.

The method was specific (no endogenous interferences, n = 10); the LOD was 0.5–2.5 ng/g. The LOQ was 5 ng/g; linearity ranged from LOQ to 500 ng/g; imprecision <16.8%; analytical recovery 83.1%–113%; and extraction efficiency 23.1%–69.5%. To compensate for the ion enhancement detected for all compounds (n = 10), we employed deuterated analogs as internal standards.

RESULTS

Maternal Hair Results

Hair specimens were analyzed from 175 participants. Depending on the amount and length of hair available for the analysis, the lock of hair was divided into 3 segments of 2, 3, and 3 cm, respectively (n = 76), in 2 segments of 2 and 3 cm each (n = 3), or just on a segment of 7–8 cm (n = 96). Segment 1 (2 cm) corresponded to the third trimester, and segments 2 and 3 (3 cm each) corresponded to the second and first trimesters, respectively. If only 1 segment was analyzed (7–8 cm), this reflected drug consumption throughout all pregnancy.

Twenty-four participants out of 175 (13.7%) tested positive for ≥1 of the studied drugs; 17 cases were positive for cocaine only; 2 cases for methadone only; 2 cases for opiates, methadone, and cocaine; 1 case for methadone and cocaine; and 1 case for amphetamines and cocaine. Cocaine concentrations ranged from 20.2 to 50,604.8 pg/mg and BE from 17.1 to 46,668 pg/mg. For methadone, the concentrations were 75.7–26,844.8 pg/mg. In the case of the opiates, morphine concentrations were 298–2398 pg/mg, codeine concentrations were 64.8–913.7 pg/mg, and 6 AM concentrations were 1634.9–15,657.1 pg/mg. Only 1 participant was positive for amphetamines, and the concentrations were amphetamine 1989.9 pg/mg, MDA 30 pg/mg, and MDMA 294.1 pg/mg.

Meconium Results

Meconium specimens were collected from 179 newborns (171 single births, and 4 pairs of twins), and analyzed for cocaine, amphetamines, opiates, and methadone. Six specimens, all from single births, were positive; 2 specimens were positive for methadone, 1 for cocaine, 1 for cocaine and methadone, 1 for opiates and methadone, and 1 for opiates, cocaine, and methadone.

In the 5 meconium specimens positive for methadone, methadone concentrations ranged from 88.2 to 3751.7 ng/g and EDDP concentrations ranged from 642.3 to 25,179 ng/g. Cocaine was positive in 3 cases; cocaine itself and BE were detected in 1 case (6.6 and 79 ng/g, respectively), OH-BE in 2 cases (5.2 and 134.8 ng/g), and EME in 3 cases (1.6–56 ng/g). In the 2 cases positive for opiates, morphine concentrations were 152.1–11,024.5 ng/g, codeine 4.4–33.7 ng/g, and morphine-3-glucuronide 21.5–22.6 ng/g.

Placenta and Umbilical Cord Results

Placenta and umbilical cord were collected from 175 participants (175 placentas and 179 umbilical cords). These specimens were analyzed if and when maternal hair or meconium was positive for cocaine, amphetamines, opiates, or methadone. A total of 24 placenta and umbilical cord specimens from single births were analyzed. Five placenta and umbilical cord specimens were positive to ≥1 compounds.

Three placenta specimens were positive for methadone, 1 for cocaine and methadone, and 1 for opiates and methadone. Methadone was detected in 5 cases (6.9–542.8 ng/g) and EDDP in 3 cases (10.3–51.4 ng/g). Only 1 case was positive for cocaine group (cocaine 7.1 ng/g, EME 1.9 ng/g), and another one for opiates (morphine 5.8 ng/g and morphine-3-glucuronide 47.5 ng/g).

Three umbilical cord specimens were positive for methadone, 1 for opiates and methadone, and 1 for opiates, cocaine, and methadone. Methadone and EDDP were detected in 5 cases (3.2–182.6 and 2.1–109.1 ng/g, respectively). Opiates

were positive in 2 cases; morphine and morphine-6-glucuronide were detected in 1 case (1.5 and 5.2 ng/g, respectively), and morphine-3-glucuronide in 2 cases (15–38.3 ng/g). Only 1 case was positive for cocaine group, and the analyte detected was BE at 6.4 ng/g.

Matrix Comparison

To compare the different matrices, we considered maternal hair results as the "gold standard." For frequent exposure, we used the cutoff values recommended by the Society of Hair Testing (SoHT) for opiates (morphine or 6 AM at 200 pg/mg), for cocaine (cocaine at 500 pg/mg and BE at 50 pg/mg), and for amphetamines (amphetamine, methamphetamine, MDA, or MDMA at 200 pg/mg). In the case of methadone, we established the cutoff at 200 pg/mg. For low-exposure cutoff, we used the method's LOQ (20 pg/mg for all compounds included in the study). Therefore, we classified the cases according to the maternal hair concentrations in 3 groups: nonexposure (segment was negative), low exposure (segment concentrations were between method's LOQ and SoHT cutoffs), and high exposure (segment concentrations were equal or greater than the SoHT cutoffs).

Twenty-one participants showed at least 1 hair segment positive for cocaine. In 12 cases, the lock of hair could be segmented (segments 1–3), and in 9 cases, only 1 segment of 8 cm was analyzed. The 12 segmented cases showed that if cocaine exposure was low (n = 9), even if this happened throughout all pregnancy, meconium, umbilical cord, and placenta specimens were negative for cocaine. In 1 case, the exposure was high during the 3 trimesters, and the 3 specimens were positive. In 2 cases, the exposure was high during the first and second trimesters, and low in the third trimester, and only 1 meconium was positive to EME at method's LOD. In 9 cases, only 1 segment of 8 cm was analyzed. In 7 out of these 9 cases, the exposure was low, and the 3 specimens, meconium, umbilical cord, and placenta, were negative. In 2 cases, the exposure was high, and only 1 meconium was positive at low concentrations (EME at LOD, and OH–BE 5.2 ng/g). Table 1 summarizes these results.

Seven participants were positive for methadone in hair. We observed that if methadone concentrations in hair indicated high exposure (n = 5), meconium, placenta and umbilical cord were also positive. If the exposure was low (n = 2), the 3 matrices were negative. Two participants showed high exposure to opiates throughout pregnancy. In these 2 cases, meconium and umbilical cord were positive for opiates, and placenta was positive in just 1 case. Only 1 case, for which the lock of hair was not segmented, was positive for amphetamines in maternal hair. Although the exposure was high, none of the 3 matrices was positive for amphetamines. Table 2 shows methadone, opiates, and amphetamine results. In Table 3, we summarized the concentrations detected in meconium, placenta, and umbilical cord for opiates, cocaine, and methadone.

TABLE 1. Comparison of Maternal Hair, Meconium, Placenta, and Umbilical Cord for the Detection of In Utero Exposure to Cocaine

		Hair						
Case	N Segments	Third Trimester	Second Trimester	First Trimester	Pregnancy	Meconium	Umbilical Cord	Placenta
1	3	LE	LE	LE		NEG	NEG	NEG
5	3	LE	LE	LE		NEG	NEG	NEG
52	3	NE	LE	LE		NEG	NEG	NEG
62	3	LE	LE	LE		NEG	NEG	NEG
70	3	NE	LE	LE		NEG	NEG	NEG
73	3	LE	LE	LE		NEG	NEG	NEG
97	3	NE	NE	LE		NEG	NEG	NEG
106	3	NE	LE	LE		NEG	NEG	NEG
144	3	NE	NE	LE		NEG	NEG	NEG
2	3	HE	HE	HE		POS	POS	POS
51	3	LE	HE	HE		POS*	NEG	NEG
216	3	LE	HE	HE		NEG	NEG	NEG
116	1				LE	NEG	NEG	NEG
131	1				LE	NEG	NEG	NEG
149	1				LE	NEG	NEG	NEG
150	1				LE	NEG	NEG	NEG
162	1				LE	NEG	NEG	NEG
183	1				LE	NEG	NEG	NEG
190	1				LE	NEG	NEG	NEG
199	1				HE	POS	NEG	NEG
211	1				HE	NEG	NEG	NEG

*Only EME was detected at the method's LOD.
HE, high exposure; LE, low exposure; NE, nonexposure; NEG, negative; POS, positive.

TABLE 2. Comparison of Maternal Hair, Meconium, Placenta, and Umbilical Cord for the Detection of In Utero Exposure to Methadone, Opiates, and Amphetamines

			Hair						
Drug Group	Case	N Segments	Third Trimester	Second Trimester	First Trimester	Pregnancy	Meconium	Umbilical Cord	Placenta
Methadone	1	3	NE	NE	LE		NEG	NEG	NEG
	2	3	HE	HE	HE		POS	POS	POS
	29	3	HE	HE	HE		POS	POS	POS
	80	3	HE	HE	HE		POS	POS	POS
	216	3	HE	HE	HE		POS	POS	POS
	148	1				LE	NEG	NEG	NEG
	199	1				HE	POS	POS	POS
Opiates	2	3	HE	HE	HE		POS	POS	NEG
	216	3	HE	HE	HE		POS	POS	POS
Amphetamines	131	1				HE	NEG	NEG	NEG

HE, high exposure; LE, low exposure; NE, nonexposure; NEG, negative; POS, positive.

DISCUSSION

In this study, we compared maternal hair, meconium, umbilical cord, and placenta for the detection of in utero exposure to cocaine, methadone, opiates, and amphetamines. A total of 175 mother–newborn dyads were included in the study. All maternal hair and meconium specimens were analyzed for the drugs of interest. If maternal hair or meconium tested positive for any drug, the umbilical cord and placenta were analyzed. Only 1 location of placenta and umbilical cord specimens was studied because the homogenous distribution of different kinds of compounds, including methadone, EDDP, morphine, cocaine, and BE, was previously reported in these tissues.[15,16,21,22]

Maternal hair has been used to describe drug consumption during pregnancy. However, only the proximal segment, the 1 closest to the scalp, has been reported by most of the authors.[7,27,28] This segment represents the exposure just during the last months of pregnancy. Ostrea et al[6] collected the proximal segment at the end of each trimester, and compared it with the results from the maternal interview. In that study, hair analysis showed higher sensitivities for detecting perinatal use of cocaine and opiates. Maternal hair has been compared with hair and meconium matrices in newborns.[6,27,29,30] Only 1 article compared maternal hair and placenta specimens,[17] and none compared maternal hair and umbilical cord. Falcon et al[17]

TABLE 3. Comparison of Meconium, Placenta, and Umbilical Cord Concentrations Measured in the Drug Positive Cases for These 3 Matrices

Case	Specimen	Morph (ng/g)	M-3-G (ng/g)	M-6-G (ng/g)	Cod (ng/g)	EME (ng/g)	OH-BE (ng/g)	BE (ng/g)	Coc (ng/g)	EDDP (ng/g)	MTD (ng/g)
2	Mec	1025	21.5	ND	33.7	56	134.8	79	6.6	5957	325.8
	Plac	ND	ND	ND	ND	1.9	ND	ND	7.1	ND	6.9
	UC	ND	15	1	ND	ND	ND	6.4	ND	6.4	17.1
29	Mec	ND	ND	ND	ND	ND	ND	ND	ND	25,179	3752
	Plac	ND	ND	ND	ND	ND	ND	ND	ND	51.4	332
	UC	ND	ND	ND	ND	ND	ND	ND	ND	109.1	182.6
51	Mec	ND	ND	ND	ND	LOD	ND	ND	ND	ND	ND
	Plac	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	UC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
80	Mec	ND	ND	ND	ND	ND	ND	ND	ND	6213	2824
	Plac	ND	ND	ND	ND	ND	ND	ND	ND	10.3	542.8
	UC	ND	ND	ND	ND	ND	ND	ND	ND	4.7	125.8
199	Mec	ND	ND	ND	ND	LOD	5.2	ND	ND	6423	1417
	Plac	ND	ND	ND	ND	ND	ND	ND	ND	11.4	133.7
	UC	ND	ND	ND	ND	ND	ND	ND	ND	2.7	49.5
216	Mec	1521	22.6	ND	4.4	ND	ND	ND	ND	1197	88.2
	Plac	5.8	47.5	ND	ND	ND	ND	ND	ND	ND	64.9
	UC	1.5	38.3	5.2	ND	ND	ND	ND	ND	2.1	3.2

OH-BE, hydroxybenzoylecgonine; Coc, cocaine; Cod, codeine; LOD, limit of detection; Mec, meconium; Morph, morphine; M-3-G, morphine-3-glucuronide; M-6-G, morphine-6-glucuronide; MTD, methadone; ND, not detected; Plac, placenta; UC, umbilical cord.

compared the proximal 4-cm maternal hair segment to placenta and fetal remains from abortions at the 12th week of gestation. In this study, we collected just 1 hair sample at delivery, and through hair segmentation, we could describe the mother drug history during pregnancy. This information was compared with matched newborn specimens (meconium, umbilical cord, and placenta) to study their sensitivity and specificity.

Maternal hair was collected after delivery and, whenever it was possible, it was divided in 3 segments that corresponded to each of the trimesters (segmented hair n = 79; nonsegmented hair n = 96). The first segment was just 2 cm long (2 months of third trimester), and the second and third segments were 3 cm long (second and first trimesters, respectively). The first 1-cm segment of the collected hair typically corresponds to hair formed 1.3 ± 0.2 to 2.2 ± 0.4 months ealier.[31] This is because it takes about 2 weeks for newly formed hair in the follicle to reach the scalp, and also because the length of hair remaining at the sample area after cutting next to the scalp could be around 0.8 cm, depending on the expertise of the collector.[31]

A total of 7 participants tested positive for methadone, 21 for cocaine, 2 for opiates, and 1 for amphetamines, in at least 1 of the maternal hair segments. In the case of meconium, 5 specimens were positive for methadone, 3 for cocaine and 2 for opiates. Five placenta and umbilical cord specimens were positive for methadone, 1 for cocaine, and 1 placenta and 2 umbilical cords for opiates. Our results showed that if drug exposure was low or sporadic (maternal hair concentrations < SoHT cutoffs), even if it was happening throughout the whole pregnancy, meconium, placenta, and umbilical cord tested negative. In all cases, maternal hair concentrations had to be above SoHT cutoffs, which means a frequent drug use by the mother, to be able to detect drugs and metabolites in the newborn matrices, meconium, placenta, and umbilical cord. In the case of cocaine, meconium (3 positive specimens) was more sensitive than were placenta and umbilical cord (1 positive specimen); however, meconium concentrations were below the LOQ of the method in the 2 negative placenta and umbilical cord cases. For opiates, 2 matched meconium and umbilical cord tested positive, and only 1 placenta; and for methadone 5 meconium, placenta, and umbilical cord were positive. All meconium, placenta, or umbilical cord specimens were negative for amphetamines.

With regard to methadone, 5 participants showed high concentrations in hair throughout pregnancy, and matched meconium, umbilical cord, and placenta also were positive to methadone and its metabolite. Concentrations found in meconium (methadone 88.2–3751.7 ng/g, EDDP 642.3–25,179 ng/g); in placenta (methadone 6.9–542.8 ng/g, EDDP 10.3–51.4 ng/g); and in umbilical cord (methadone 3.2–182.6 ng/g, EDDP 2.1–109.1 ng/g) were lower than those reported by de Castro et al[15,21] (meconium methadone 2184–21,902 ng/g and EDDP 6375–80,503 ng/g; placenta methadone 308–2647 ng/g and EDDP 35.9–517.4 ng/g; umbilical cord methadone 29.7–262.2 ng/g and EDDP 8.2–240.8 ng/g). In these studies,[15,21] specimens were collected from pregnant women enrolled in a methadone treatment program with a mean daily dose of 30–102.5 mg. Unfortunately, dosing information was not available in this study. In accordance with the findings of de Castro et al,[15,21] we observed the same metabolic profile for methadone and EDDP in meconium, placenta, and umbilical cord. In meconium, the predominant metabolite was EDDP; in placenta, it was methadone; and in umbilical cord, methadone and EDDP showed similar concentrations. In both studies, concentrations in placenta and umbilical cord were much lower than in meconium.

Falcon et al[17] compared maternal hair with placenta and fetal remains from abortions at the 12th week of gestation. For cocaine, positive hair test results above SoHT cutoffs were confirmed in 25% of placenta specimens (BE 37.8–531.9 ng/g, cocaine 58.9–149.8 ng/g). We detected only 1 placenta positive for cocaine (EME 1.9 ng/g, cocaine 7.1 ng/g) out of 21 positive hair participants (5%). However, if we selected only the hair specimens that were equal or greater than the SoHT cutoff (5 specimens), the percentage of placenta–hair agreement would be 20%, similar to the one obtained by Falcon et al[17] in interrupted pregnancies. De Castro et al[15] only could confirm 1 placenta positive to cocaine (BE 458.9 ng/g, cocaine 7.3 ng/g) out of 11 matched meconium (OH–BE 12.2–254 ng/g, BE 2.4–969 ng/g, cocaine 4.4–233 ng/g). In this study, placenta was negative in 2 positive meconium cases where cocaine metabolite concentrations were low (case 51, EME at LOD; case 199, EME at LOD and OH–BE 5.2 ng/g), and positive in a case with higher meconium concentrations (case 2, EME 56 ng/g, OH–BE 134.8 ng/g, BE 79 ng/g, and cocaine 6.6 ng/g).

In our study, we observed similar results for cocaine in umbilical cord and in placenta. Case 2 also yielded umbilical cord–positive result for cocaine (BE 6.4 ng/mL), whereas cases 51 and 199, with low cocaine concentrations in meconium, were negative. In case 51, high maternal hair concentrations were found in the first and second but not in the third trimester. This may indicate that the higher positivity rate in meconium was due to a longer window of detection compared with that in placenta and umbilical cord. For case 199, there were not enough data available to support this statement, as only one 8-cm segment could be analyzed and the consumption pattern could not be chronologically established. De Castro et al[21] could only confirm 1 umbilical cord (BE 442.2 ng/g) out of 11 matched cocaine positive meconium (OH–BE 12.2–254 ng/g, BE 2.4–969 ng/g, cocaine 4.4–233 ng/g). On the other hand, Montgomery et al[23] reported a high percentage of agreement in cocaine results between meconium and umbilical cord (99.2%). However, most of the analyzed specimens in that study were negative (114 out of 118 pair of specimens). Moore et al[24] reported BE at 1200 ng/g in umbilical cord from 1 cocaine user, and Winecker et al[25] determined BE (up to 1237 ng/g) and EME (up to 52 ng/g) in 28 umbilical cords from self-reported cocaine users. No data on drug consumption amount or timing were available. Reducing the LOD of the method and monitoring additional cocaine metabolites such as norcocaine could increase the placenta and umbilical cord sensitivity for cocaine.[32]

In this study, 2 participants were high opiates users throughout pregnancy, and meconium and umbilical cord were able to confirm this result. Placenta tested positive in just 1 of these cases. Falcon et al[17] reported that any placenta could confirm a positive opiate hair result (n = 6). De Castro et al[15,21] analyzed 8 matched placenta, umbilical cord, and meconium specimens. None of the 8 meconium opiate positive specimens could be confirmed in any of the matched placenta or umbilical cord specimens. In these

3 studies,[15,17,21] morphine–glucuronide metabolites were not monitored in placenta or umbilical cord. In this study, morphine-3-glucuronide was the predominant metabolite detected in placenta (47.5 ng/g) and umbilical cord (15–38.3 ng/g) specimens, and morphine was just detected in 1 umbilical cord at low concentrations (1.5 ng/g). Montgomery et al[23] reported good correlations between umbilical cord and meconium results for opiates (94.9%). Unfortunately, no data were reported on metabolites analyzed or measured concentrations. In a previous study, the authors[16,22] also observed that, in the case of buprenorphine, glucuronide metabolites accumulated in the placenta and in umbilical cord. The free analytes, buprenorphine and norbuprenorphine, were the predominant compounds in meconium, whereas in the umbilical cord and placenta the glucuronides were in higher concentrations than the free forms.

Meconium, placenta, and umbilical cord were negative to amphetamine, methamphetamine, MDA, and MDMA in the only positive maternal hair participant. Hair concentrations were amphetamine 1989.9 pg/mg, MDA 30 pg/mg, and MDMA 294.1 pg/mg. Segmented hair analysis could not be performed in this case due to the low amount of specimen available. Falcon et al[17] could not confirm 3 maternal hair specimens positive to MDMA (200–2500 pg/mg) in placenta specimens from abortions at the 12th week of gestation.

We found that placenta and umbilical cord concentrations were much lower than meconium concentrations for methadone, cocaine, and opiates. These differences in concentrations among matrices have been previously reported for buprenorphine, methadone, cocaine, and opiates,[15,16,21,22] and may suggest a higher accumulation of the drugs in meconium compared with placenta or umbilical cord. However, for other compounds such as alprazolam,[18] theobromine and caffeine,[19] and arecoline,[20] placenta and meconium concentrations were similar or even higher in placenta than in meconium. The physicochemical properties of the biomarkers, placental diffusion and metabolism, fetal metabolism, maternal dosing, and timing may influence differential accumulation in these matrices.

Additional research with a larger cohort is needed to get statistically significant results. This study is underpowered due to the low number of positive specimens. Twenty-four hair specimens, 6 meconium specimens, and 5 placenta and umbilical cord specimens out of 175 cases tested positive to $\geq$1 of the studied drugs. However, the data presented are unique in assessing these different matrices, maternal hair, meconium, placenta, and umbilical cord to detect in utero drug exposure.

CONCLUSIONS

The data presented in this study indicate that maternal hair is the most sensitive specimen to detect drug consumption during pregnancy. Through segmental analysis, we could study the pattern of consumption in each trimester. Meconium detected high in utero drug exposure, and placenta and umbilical cord could be employed as alternative matrices. In these alternative matrices, placenta and umbilical cord, the drugs are present in very low concentrations, and therefore, high sensitivity techniques are required.

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