**ORIGINAL ARTICLE**

Comparison of meconium and neonatal hair analysis for detection of gestational exposure to drugs of abuse

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Background: Meconium and hair are two biological markers of in utero exposure to illicit drugs. Objective: To compare the sensitivity of the two tests for different drugs. Setting: Motherisk laboratory which tests in utero drug exposure in Toronto. Methods: Cocaine, benzoylecgonine, opiates, cannabis, benzodiazepines, methadone, and barbiturates were measured in pairs of hair and meconium samples from the same neonates. Results: Meconium was marginally more sensitive than neonatal hair for detection of cocaine and cannabis, possibly because it may detect second trimester exposure whereas hair grows only during the third trimester of pregnancy. There was a significant correlation between hair and meconium concentrations of cocaine, cannabis, and opiates. Conclusion: In cases of clinical suspicion and a negative neonatal urine test, both meconium and hair are effective biological markers of in utero illicit drug exposure. Meconium may be more sensitive, but neonatal hair is available for three months whereas meconium is available for only one or two days. In contrast, the use of meconium, being a discarded material, is more acceptable to some parents than hair testing, which entails cutting scalp hair from the newborn.

During the past two decades, illicit drug use has reached epidemic proportions in North America.1 In the United States, 10–45% of the women cared for at urban teaching hospitals use cocaine during pregnancy.2 As women of reproductive age constitute a large segment of the drug using population, the effects of their drug use on the fetus has been studied extensively. Prenatal cocaine use has been associated with increased rates of low birth weight, microcephaly, congenital anomalies, and necrotising enterocolitis.3 However, because of multiple other reproductive risk factors in women using illicit drugs, it is possible that many of the adverse effects attributed to drugs are caused by other factors.4 Similarly, it is not clear whether cocaine per se, or other risk factors, lead to adverse neurobehavioural effects.5 Of importance, maternal addiction itself is a determinant of serious postnatal risk for the infant. Newborns exposed to opioids, barbiturates, benzodiazepines, or alcohol in utero may experience withdrawal symptoms, often requiring treatment.6–9

Estimated rates of infants exposed prenatally to cocaine range between 2.6% and 11% of all live births.10 A prevalence study of cocaine use during pregnancy conducted by our group in 1990–1991 in three Metropolitan Toronto hospital nurseries (one inner city, two suburban) found 37 out of 600 (6.25%) infants tested positive for cocaine.11 In the Metropolitan Toronto area, there has been a steady increase in the number of newborns affected by maternal drug use.11

It has been shown that maternal reporting of drug use is far from accurate.12–14 Fearing legal consequences and embarrassment from admitting illicit substance use, most users tend to deny or to under-report drug consumption. A major problem in studying the adverse effects of illicit drugs is the lack of standardised techniques to ascertain fetal exposure. The validity of blood and urine tests depends on the elimination half life of the compound in question. In the case of cocaine, which has a short elimination half life of less than one hour, the drug and its metabolites are not likely to be detected for more than a few days in either blood or urine.15 Other drugs, such as cannabis and opioids, have longer elimination half lives, but even these drugs can be detected for only a maximum of three to four weeks after use.16

These facts have highlighted an urgent need for a biological marker which will still be sensitive weeks after the end of exposure and which may yield a cumulative reflection of long term exposure to illicit drugs. In 1989, we first reported the use of hair analysis as a biological marker for gestational cocaine exposure in seven neonates whose mothers were known cocaine users.17 This test is widely used at present. Meconium testing has proved to be another very effective tool for verifying gestational drug exposure.18 No formal comparison of the sensitivities of meconium and hair analysis for different drugs of abuse has as yet been conducted.

The aim of this study was to estimate the sensitivity and correlation between neonatal hair and meconium testing in 185 infants suspected of being exposed in utero to one or more illicit substances, namely cocaine, opiates, and cannabis.

**SUBJECTS AND METHODS**

Between 1999 and September 2001 the Motherisk laboratory at the Hospital for Sick Children in Toronto, Canada received thousands of neonatal hair and meconium samples for analysis. Among them were 185 pairs of hair and meconium samples collected from the same babies in various hospitals in Ontario. Based on clinical suspicion of maternal drug abuse, the testing of hair and/or meconium was requested by either a doctor or the Children’s Aid Societies. Most of the requests were for analysis for cocaine, opiates, and cannabinoids. We report on measurements of cocaine, its metabolite benzoylecgonine, heroin, morphine, cannabis, methadone, benzodiazepines, and barbiturates.

**Hair testing**

Hair was analysed by well established methods.19 It was not washed before testing unless external contamination was suspected. If washing of the hair sample was included in the procedure, a previously described method was followed.20 Briefly, 2–5 mg finely cut hair, unwashed or previously washed, was sonicated in 1 ml methanol/5 M HCl (20:1, v/v)
for 30 minutes and incubated overnight at 45°C. On the next day, the methanol was pipetted off, and the hair rinsed briefly with an additional 1 ml methanol. After evaporation of the methanol at 40°C under a stream of nitrogen, 200 µl phosphate buffered saline at pH 7.0–7.4 was added, and the individual drugs were analysed by enzyme linked immunosorbent assay using kits manufactured by Immunalysis (San Diego, California, USA).

For quantification, standards were prepared in blank hair extract to control for matrix effect. Different blank hair extracts were used to match the age and hair colour of the subject. The limit of detection for each drug was 0.2 ng/mg hair when 2 mg hair was used. Positive results were confirmed using gas chromatography/mass spectrometry with the mass selective detector operating in selective ion monitoring mode.

**Meconium testing**

For meconium testing, approximately 0.2 g wet meconium was extracted with methanol. After centrifugation, the supernatant was diluted 1:5 with phosphate buffered saline, and an aliquot was analysed for cocaine, benzoylecgonine, opiates, and/or cannabinoids. Standards were prepared in blank meconium extract similarly to the hair samples. Similar immunosassays were used to those for the hair analysis described above. Here too, positive results were confirmed by gas chromatography/mass spectrometry. The limit of detection for each drug was 50 ng/g meconium when 0.2 g meconium was used for testing. The coefficient of variation of these tests in our laboratory is less than 5%.

**Statistical analysis**

Assuming a false positive rate of zero for all the drugs analysed—that is, a specificity of 100%—the estimated sensitivity for cocaine, benzoylecgonine, opiates, and/or cannabinoids in neonatal hair and meconium in neonates estimated to be 96% in meconium and 84% for hair (table 2).

**RESULTS**

Of 185 pairs of hair and meconium samples assayed, 75 were negative for all the drugs analysed. Table 1 shows the distributions of cocaine, benzoylecgonine, opiates, and cannabis in the positive hair and meconium samples. A total of 173 pairs were tested for cocaine, 172 pairs for benzoylecgonine, 136 pairs for opiates, and 141 pairs for cannabis. Additional tests were performed for benzodiazepines (two pairs), methadone (two pairs), and barbiturates (one pair).

**Cocaine testing**

The total number of positive samples for cocaine (hair, meconium or both) was 53, of which 50 were positive in meconium whereas 43 were positive in hair. The calculated sensitivity for cocaine testing was 96% in meconium and 84% for hair (table 2).

**DISCUSSION**

As illicit drug use reaches epidemic proportions, protecting the wellbeing of the fetus and offspring of drug users is a serious challenge for health professionals and social services. A positive meconium test can reflect maternal use of illicit drugs...
from the second trimester of pregnancy onwards. Only meco-
nium collected during the first 1 or 2 days of life or for the first
three stools can be used to document in utero drug exposure.
In contrast, neonatal hair, which grows during the third
 trimester, may reflect exposure of drugs during the last
 trimester of pregnancy, and can stay positive for up to three
 months after birth.

This study shows the correlation between meconium-hair
 pairs for cocaine, benzoylecgonine, opiates, and cannabis. For
cocaine, benzoylecgonine, and cannabis, meconium testing
seems to be more sensitive (95% and above) than hair testing.
This may be partly explained by the earlier formation of
meconium compared with hair (roughly the second trimester
compared with the third trimester). The limitation of using
meconium for routine testing is the narrow time frame for
obtaining the sample. In all the cases in which meconium
tested negative whereas the hair tested positive, although the
documents specified the sample to be meconium, in fact it was
a mixture of meconium and stool, confirming that, for the test
to be accurate, meconium has to be collected not later than one or
two days after birth.

Chiriboga et al21–24 have shown a concentration-response
effect of cocaine, as measured in maternal hair, on newborn
head circumference and abnormalities in muscle tone, move-
ment, and posture. Our study is the first to document signi-
ficant correlation between meconium and hair levels of differ-
ent illicit drugs, which enables the use of either meconium or
neonatal hair for assessment of the magnitude of exposure
and therefore the expected neurological impairment to the
exposed newborn. It is possible that a combination of negative
hair test and positive meconium test reflects second trimester
exposure to drugs, with no third trimester exposure.

Benzodiazepines are thought to be human teratogens, pos-
sibly causing oral clefts.25 Although meconium forms only in
the second trimester, it is important to document the pattern
of benzodiazepine use in cases of oral cleft. Both meconium
and hair can be used for this purpose.

Maternal abuse of barbiturates can cause abstinence
syndrome in the newborn infant. Sampling either meconium
or hair, or both, can help to establish the diagnosis when the
maternal history is not accurate or available.26

Both meconium and hair analysis have advantages and dis-
advantages. Meconium may be more sensitive. However, it is
available for only two days after birth, whereas hair may be
available for up to three months. Because meconium produc-
tion begins in weeks 14–16, meconium testing may detect
second trimester exposure to drugs, whereas the hair present
at birth only develops in the third trimester. Although this
may increase the sensitivity of the meconium test, third
trimester exposure, evidenced by hair testing, reflects drug
abuse long after pregnancy was detected and hence is
diagnostic of maternal addiction, which has important impli-
cations for neonatal care. Some parents resist hair cutting,
whereas meconium is a discarded material. Equally as impor-
tant, some babies are born with very little hair or no hair at all.
Another problem with hair analysis is that hair levels of drugs
are affected by the amount of melanin in the shaft.21 The
dose-response characteristics of deposition of drugs in hair
and meconium have been documented.20–24

To improve the yield of both matrices involved, we propose
to initiate clinical investigations with urine testing in
suspected cases. If the urine test is negative, meconium or hair
testing will be used depending on postnatal age. In cases with
a high index of suspicion, both matrices should be used, ren-
dering higher sensitivity. Because of the strong correlation
between their measured levels, both matrices can be used to
estimate the extent and timing of fetal exposure and the
resulting neurological impairment.

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