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

B Bar-Oz, J Klein, T Karaskov, G Koren

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. In the United States, 10–45% of the women cared for at urban teaching hospitals use cocaine during pregnancy. 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 placental abruption and premature labour, and as women using illicit drugs 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.
Detection of in utero exposure to drugs

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 immuno-
sorbent 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 super-
natant was diluted 1:5 with phosphate buffered saline, and an
aliquot was analysed for cocaine, benzoylcegonine, opiates,
and/or cannabinoids. Standards were prepared in blank meco-
nium extract similarly to the hair samples. Similar immu-
noassays 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 sensi-
tivity of the test was calculated for each drug, with the rate of
positive hair plus meconium in neonates estimated to

Table 1 Proportion of positive/negative tests for cocaine, benzoylcegonine, opiates, and cannabinoids in neonatal hair and meconium

<table>
<thead>
<tr>
<th></th>
<th>Cocaine</th>
<th>Benzoylcegonine</th>
<th>Opiates</th>
<th>Cannabinoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ meconium, + hair</td>
<td>41</td>
<td>35</td>
<td>19</td>
<td>33</td>
</tr>
<tr>
<td>+ meconium, – hair</td>
<td>10</td>
<td>15</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>– meconium, + hair</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total positive sample</td>
<td>53</td>
<td>53</td>
<td>27</td>
<td>55</td>
</tr>
<tr>
<td>– meconium, – hair</td>
<td>120</td>
<td>119</td>
<td>109</td>
<td>86</td>
</tr>
<tr>
<td>Total samples tested</td>
<td>173</td>
<td>172</td>
<td>136</td>
<td>141</td>
</tr>
</tbody>
</table>

**RESULTS**

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

**Cocaine testing**

The total number of positive samples for cocaine (hair, meco-
nium or both) was 53, of which 51 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).

The total number of positive samples for benzoylcegonine
was 53, of which 50 were positive in meconium (sensitivity
of 95%), and 38 were positive in hair (sensitivity of 78%).

There was a significant correlation between hair and meco-
nium levels of cocaine ($r = 0.83$, $p < 0.001$) and benzoylcego-
nine ($r = 0.56$, $p < 0.001$).

**Opiate testing**

The total number of positive samples was 27, of which 23 were
positive in both or one of the matrices studied (sensitivity
87%). There was a highly significant correlation between hair
and meconium levels ($r = 0.69$, $p < 0.0001$).

**Cannabis testing**

The total number of positive samples was 55, of which 54 were
positive in meconium and 34 were positive in hair. The calcu-
lated sensitivity for cannabis in meconium was 98%, and for
hair it was 71%. There was a highly significant correlation between
hair and meconium measures ($r = 0.73$, $p < 0.0001$).

**Miscellaneous testing**

Two hair-meconium pairs were positive for benzodiazepines in
both matrices, methadone was positive in hair and in
meconium in two pairs tested, and one pair was positive for
barbiturates (table 3).

**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 posi-
tive meconium test can reflect maternal use of illicit drugs.
from the second trimester of pregnancy onwards. Only meconium collected during the first 3 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, benzoylegonine, opiates, and cannabis. For cocaine, benzoylegonine, 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 positive 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 al.11–21 have shown a concentration-response effect of cocaine, as measured in maternal hair, on newborn head circumference and abnormalities in muscle tone, movement, and posture. Our study is the first to document significant correlation between meconium and hair levels of different 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, possibly causing oral clefts. 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.10

Both meconium and hair analysis have advantages and disadvantages. 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 production 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 implications for neonatal care. Some parents resist hair cutting, whereas meconium is a discarded material. Equally as important, 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.22–24

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, rendering 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.

ACKNOWLEDGEMENTS

This work was supported by a grant from The Canadian Institute for Health Research (CIHR), and the Research Leadership in Better Pharmacotherapy During Pregnancy and Lactation. BB-O was a recipient of a Fellowship award by the Research Training Center, The Hospital for Sick Children. GK is a Senior Scientist of CIHR.

Authors’ affiliations

B Bar-Oz, J Klein, T Karaskov, G Koren, The Motherisk Program, Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children and University of Toronto, Toronto, Canada

B Bar-Oz, Department of Neonatology, Hadassah Medical Center and The Hebrew University, Jerusalem, Israel

REFERENCES