Rates of Fetal Polydrug Exposures in Methadone-Maintained Pregnancies from a High-Risk Population

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Abstract

Methadone maintenance treatment (MMT) is the standard of care during pregnancy for opioid-dependency, showing efficacy in improving prenatal care and reducing risk of relapse. By design, however, MMT is only intended to prevent withdrawal thus facilitating cognitive behavioural interventions. In order to maximize the benefits of MMT, it is essential that methadone is both properly prescribed and that additional addiction treatment is concurrently administered. This study aims to determine the effectiveness of MMT engagement in high-risk pregnant women in reducing polydrug use by objective laboratory examination of neonatal meconium.

Patients and Methods: Over a 29-month period, the Motherisk Laboratory at the Hospital for Sick Children in Toronto analyzed meconium samples as per request by social services and hospitals for drugs of abuse.

Results: Of the 904 meconium samples received, 273 were tested for methadone with 164 positive and 109 negative for methadone. Almost half of the methadone positive samples (46.34%) were also positive for at least one other opioid compound, which did not differ statistically from the methadone-negative control samples (46.79%; Chi square test, p=0.94). No differences were found between the methadone positive and negative groups in rates of concurrent amphetamines, cocaine, cannabis, and alcohol use indicating a similar risk of polydrug use between pregnant women taking or not taking methadone in this population.

Discussion: The high rates of additional opioid and other drug use in the MMT group, suggest that MMT is failing this population of patients. It is possible that methadone doses during pregnancy are not appropriately adjusted for changes in pharmacokinetic parameters (e.g. blood volume, renal function) during the second and third trimesters. This may result in sub-therapeutic dosing creating withdrawal symptoms leading to additional substance use. Alternatively, these results may be demonstrating a substantial lack in delivery of addiction support services in this vulnerable population.

Introduction

Over the past decades, there has been an increase in the prevalence of substance abuse among young women, with up to 90% being of reproductive age [1],[2]. This general increase has been paralleled with a marked increase in the prevalence of opioid abuse during pregnancy [3]. The long-acting opioid, methadone, is currently the first line of treatment for opioid dependence for both pregnant and non-pregnant individuals. Methadone maintenance treatment (MMT) provides more stable opioid blood concentrations, resulting in decreased withdrawal symptoms. Prevention of withdrawal is intended to

decrease illicit use of other opioids and facilitate addiction treatment interventions. Pregnant women in MMT have been found to take better care of their health and be less likely to relapse during pregnancy when compared to non-MMT, drug-using women [4].

Polydrug use continues to be a serious issue in the drug-using community. The rates of polydrug use in pregnancy are relatively unknown, as maternal self-reports tend to be an unreliable source of information [5]. In contrast to self-reports, the use of biological markers in meconium analysis allow for an objective analysis of drug use during the third, and possibly second trimesters of pregnancy [6],[7]. With the use of

meconium analysis for drugs of abuse, the effectiveness of MMT can be estimated using rates of polydrug use during pregnancy as a surrogate marker for clinical progress.

Meconium is evacuated by neonates over their first few bowel movements; it begins to form in the fetus at around 12 weeks of gestation, when fetal swallowing of amniotic fluid is initiated [8],[9]. Xenobiotics and their metabolites are deposited into meconium by the biliary route or through fetal swallowing [8]. While meconium starts to form at the beginning of the second trimester, evidence suggests that meconium likely reflects third trimester fetal exposures [10]. Meconium is considered a highly sensitive matrix, demonstrating a window of detection far exceeding that afforded by traditional urinalysis and therefore, serves as an optimal matrix for population-based studies examining gestational exposures [11],[12].

The aim of the present study was to examine a population of high-risk, social services-involved women and determine if rates of gestational polydrug use were lower in women on MMT.

Methods

Ethics Statement

The study was approved by the Hospital for Sick Children Research Ethics Committee. As approved by the Research Ethics Committee, all results from the Motherisk Laboratory are available for retrospective analysis. The Research Ethics Committee waived written consent from sample donors as database results are anonymized.

Sample Analysis

This study is a retrospective, observational assessment of meconium toxicology performed on samples referred by physicians, primarily at the request of social services, over a 29-month period. Meconium samples were shipped frozen from sites of collection to the Motherisk Laboratory at the Hospital for Sick Children and stored at -80°C until analysis.

Briefly, 0.300 g of meconium was thawed at room temperature, homogenized and transferred into a 13mL test tube (Sarstedt; Montreal, QC). One mL of methanol was added to each sample, vortexed for 30 seconds then centrifuged for 15 min at 3,500 rpm at room temperature. The supernatant for each sample was then decanted into a 5mL test tube. Samples were then screened by ELISA (Immunalysis, Pomona CA) for the following drugs and metabolites: cocaine, benzoylecgonine, opiates (including codeine, morphine, 6-monoacetylmorphine, hydrocodone and hydromorphone), oxycodone (with cross-reactivity to oxymorphone), amphetamine, methamphetamine, delta-9-tetrahydroxycannabinol (THC), benzodiazepines, barbiturates, meperidine, and methadone. One hundred µL aliquots of the methanol extract are taken for each individual ELISA test and 400µL of phosphate buffered saline (PBS) is added. Samples are then added to the ELISA plate, 100µL of enzyme conjugate added, then incubated for 60 min at room temperature in the dark. Wells are then washed with ddH$_2$O and 100µL of substrate reagent is added to each well and then incubated again for 30 min at room temperature in the dark. Stop solution is added and absorbance is then measured at a dual wavelength of 450nm and 650nm using a SUNRISE Absorbance Reader (Tecan Systems Inc, San Jose CA). Samples are then deemed positive or negative, if the absorbance is below or above the cut-off standard respectively.

THC analysis was performed on ELISA only; all other screen-positive ELISA samples were subsequently confirmed through GC-MS analysis using previously published methods for drugs of abuse and alcohol metabolites [13],[14]. The drugs of abuse GC-MS method is capable of simultaneously detecting and quantifying cocaine, benzoylecgonine, norcocaine, cocaethylene, 6-monoacetylmorphine, morphine, codeine, oxycodone, oxymorphone, hydrocodone, hydromorphone, meperidine, methadone, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, and methylenedioxyamphetamine. Deuterated internal standards of the 17 analytes and 20% methoxiamine solution is added to an aliquot of the previously described methanol extract from each sample, vortexed and incubated at room temperature for 1 hour. Two mL of 0.1M phosphate solution is then added to each sample, vortexed and incubated for 15 min at room temperature. Samples then undergo automated SPE extraction (Gilson ASPEC GX-274 Automated SPE extractor; Middleton, WI, USA), transferred to a 10mL SPME vial (Supelco; Bellefonte, PA) and then dried under nitrogen at 35°C. Once dried, 20 µL of derivatizing mixture (BSTFA and MSTFA 3:1) is added and then samples are analyzed via GC-MS on a Shimadzu QP2010 Plus (Shimadzu Scientific Inc; Columbia, MD).

For quality control procedures, quality control samples are analyzed after every 20 samples. These quality control samples are blank meconium specimens, which are spiked with a known amount of the tested compounds. Any discordance between ELISA and GC-MS analyses results in reanalysis of the sample.

Data Analysis

Meconium results were dichotomized into two groups, positive and negative for methadone. Number and proportions of positive drug classes (cocaine, amphetamines, opioids, cannabinoids, benzodiazepines and alcohol) were calculated. As some of the compounds tested are both metabolites of a drug of abuse and drugs of abuse themselves, the source of these compounds cannot be determined, thus requiring the calculation of minimum and maximum number of positive drugs. To further elaborate, morphine is itself a drug of abuse as well as a metabolite of both codeine and heroin. Hydromorphone and oxymorphone are also drugs of abuse but also metabolites of hydrocodone and morphine, and oxycodone respectively. Finally, amphetamine is both a metabolite of methamphetamine and a drug of abuse itself. This apparent overlap must be taken into consideration when determining the number of positive drugs detected in the meconium samples.

For statistical analyses, Chi Square and Fisher's Exact tests were performed, where appropriate, to compare the rates of positivity of individual drugs and drug classes between methadone positive and methadone negative samples. Mann Whitney U test was performed to compare the mean number of

Table 1. Rates of positivity for six drug classes.

	Rate of Positivity (%) (n)		
	Methadone Positive	Methadone Negative	
Drug Class	(N=164)	(N=109)	P value
Amphetamines	4.27 (7)	2.75 (3)	0.744[&]
Cocaine	30.49 (50)	26.61 (29)	0.488[^]
Opioids	46.34 (76)	46.79 (51)	0.942[^]
Cannabinoids*	55.10 (54)	52.17 (48)	0.686[^]
Benzodiazepines#	14.29 (4)	3.23 (2)	0.073[&]
Alcohol+	5.88 (4)	6.67 (4)	1[&]

[^]Pearson Chi-square test performed; [&]Fisher's Exact test performed; *Methadone positive n=98, Methadone negative n=92; #Methadone positive n=28, Methadone negative n=62; +Methadone positive n=68, Methadone negative n=60

doi: 10.1371/journal.pone.0082647.t001

Table 2. Rates of positivity for individual compounds.

	Rate of Positivity (%) (n)		
Compound	Methadone Positive	Methadone negative	P value
Amphetamine*	5.31 (6)	1.09 (1)	0.132[&]
Methamphetamine#	3.57 (4)	3.26 (3)	1[&]
MDMA$	0	2.17 (2)	0.204[&]
Meperidine%	4.55 (1)	1.09 (1)	0.440[&]
Cocaine=	23.46 (38)	21.30 (23)	0.677[^]
Cocaethylene+	2.47 (4)	0.94 (1)	0.651[&]
Benzoylecgonine[a]	30.86 (50)	27.78 (30)	0.586[^]
Norcocaine[a]	12.30 (21)	14.81 (16)	0.665[^]
Codeine[a]	11.73 (19)	16.67 (18)	0.248[^]
Morphine[a]	29.63 (48)	20.37 (22)	0.089[^]
6-MAM[a]	0.62 (1)	0.93 (1)	1[&]
Hydromorphone[a]	11.73 (19)	11.11 (12)	0.876[^]
Oxycodone[a]	17.28 (28)	22.22 (24)	0.313[^]
Oxymorphone[a]	12.96 (21)	16.67 (18)	0.396[^]

[^]Pearson Chi-square test performed; [&]Fisher's Exact test performed; *Methadone positive n=113, Methadone negative n=92; #Methadone positive n=112, Methadone negative n=92; $Methadone positive n=111, Methadone negative n=92; %Methadone positive n=22, Methadone negative n=66; =Methadone positive n=162, Methadone negative n=108; +Methadone positive n=162, Methadone negative n=106; [a]Methadone positive n=162, Methadone negative n=108

doi: 10.1371/journal.pone.0082647.t002

positive drugs overall and mean number of positive opioids between the two groups. All statistical tests were performed using IBM SPSS Statistics 20.0.

Results

Of the 273 meconium samples analyzed for methadone, 164 were found to be positive and 109 were negative. Fifty-eight (35.37%) of methadone-positive samples were positive, at minimum, for one additional opioid and 18 (10.98%) were positive for 2 additional opioids. In regards to maximum number of positive opioids, 39 (23.78%), 19 (11.59%), 14 (8.54%), 3 (1.83%) and 1 (0.61%) were positive for 1, 2, 3, 4 and 5 additional opioids respectively.

No statistically significant differences were found with regards to proportions of illicit drug use between the methadone-positive and negative groups for any drug class (Table 1); although a trend towards higher rates of amphetamine (drug) and benzodiazepine (class) use was noted (Table 2). As well, no statistical difference was found for mean minimum number of positive drugs (methadone positive 1.293, SD = 0.997; methadone negative 1.431, SD= 1.141; p=0.477). Also, no statistical difference was found for mean maximum number of positive drugs (methadone positive 1.567, SD = 1.348; methadone negative 1.716, SD = 1.441; p=0.451).

When looking specifically at opioids, no statistical difference was found for either mean minimum or maximum number of positive opioids (min: methadone positive 0.580, SD = 0.685; methadone negative 0.602, SD = 0.723; p=0.907; max: methadone positive 0.840, SD=1.114; methadone negative 0.880, SD=1.174; p=0.913).

Discussion

Individuals are expected to be engaged in MMT with the clear clinical goal of replacing the need for other opioids. While in most programs, routine urine tests are conducted to verify that indeed this is the case; sample tempering is a major issue [15],[16]. Our results, based on meconium analysis, which yields an integral of fetal exposure to drugs over at least the

last trimester of pregnancy, indicate that at least one third of pregnant women on MMT continue to use at least one additional opioid narcotic. When comparing them to meconium with no detected methadone, indicating that the mother was not on MMT, no significant difference was found in proportion of opioid or other illicit drug use between the methadone positive and negative meconium samples, indicating similar patterns of substance use during late pregnancy. This alarming finding of a lack of an apparent decrease in opioid use for those positive for methadone means ineffective MMT, and increased burden of illicit drug exposure to the fetus.

With such a large proportion of the methadone positive meconium samples also being positive for at least one other opioid, the efficacy and effectiveness of methadone is brought into question. Changes in clearance rate and volume of distribution during pregnancy may affect the efficacy of methadone. Wolff et al. (2005) found that weight-adjusted methadone clearance increased between the first and third trimesters [17]. Dickmann and Isoherranen (2012) observed increased CYP2B6 activity in human hepatocytes due to estradiol induction, which would increase methadone metabolism via CYP2B6 [18]. Additionally, Jarvis et al (2008) determined, in a population of methadone dependent pregnant females, that the half-life of methadone was almost one half that of non-pregnant methadone users [19]. Absorption of methadone may also be affected during pregnancy, as gastric acid secretions decrease, inhibiting gastrointestinal absorption [19]. Overall, these pharmacokinetic changes may render the prescribed dose of methadone subtherapeutic. The unstable

exposure to opioids in utero may induce fetal stress, increase the risk of abortion and preterm birth [20].

Polydrug use was found in both methadone positive and negative cohorts with cannabinoids, opioids and cocaine as the most common drug classes detected in the samples for both groups. This highlights the numerous compounds the fetus is exposed to, and the increased risk of fetal stress and adverse outcomes.

The population assessed in this study is high-risk; almost all women whose neonates were tested are actively involved with social service organizations and demonstrate ongoing child protection concerns. This study assumes that methadone present in meconium is the result of MMT, however, methadone is used illicitly as well and it should be considered that a proportion of the methadone-positive group may not be actively engaged in MMT. While the characteristics of the study population limit the generalizability of these findings to the MMT population at large, the lack of effectiveness of MMT in this particular population underscores current limitations in the delivery of effective addiction treatment services to this highly vulnerable group. Despite substantial mobilization of government resources to this population, they remain under-served in meeting their addiction treatment needs. While comprehensive strategies for addressing substance addiction in pregnancy have been extensively reported in the literature [21],[22],[23],[24]; these data demonstrate that the current model of MMT delivery does not address the addiction treatment needs of women who have already been identified as high-needs.

This study demonstrates a need for closer clinical monitoring of pregnant women in MMT to ensure that treatment is effective in preventing maternal or neonatal withdrawal symptoms, continued opioid or polydrug use, and adverse neonatal outcomes. The high rate of polydrug use in the methadone-positive cohort demonstrates that the current level of care provided to these women is ineffective and should be augmented with consideration given to: i) therapeutic drug monitoring of methadone to evaluate necessity for dose increases due to altered pharmacokinetics in pregnancy, ii) alternative therapies, such as buprenorphine, that can provide a more stable maintenance treatment regime and result in potentially better neonatal outcomes [25], iii) ensuring accessibility of complementary non-pharmacological interventions (e.g. cognitive behavioural therapy, etc.) that are an established requirement to successful addiction treatment.

Acknowledgements

Author Contributions

Conceived and designed the experiments: KD JG GK. Performed the experiments: KD. Analyzed the data: KD. Wrote the manuscript: KD JG GK.

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