K18 Simultaneous LC-MS/MS Quantification of Opiate, Cocaine, and Metabolites in Urine of Pregnant Substance-Abuse Treatment Participants

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After this presentation, attendees will be knowledgeable about opiate, cocaine and metabolites concentrations in human urine after illicit opioid and cocaine use by pregnant women.

The simultaneous LC/MS/MS analysis of 26 opiate and cocaine analytes in urine demonstrated that this technology was useful for monitor- ing multiple biomarkers of illicit drug use in opiate and cocaine dependent pregnant women. This presentation will impact the forensic science community by presenting data that will be evaluated to determine potential correlations with opioid and cocaine concentrations in meconium from infants of the women and with neonatal outcome measures.

Methadone maintenance is the only currently recognized pharma- cotherapy for opiate dependency during pregnancy. Urine testing is an integral component of drug treatment, is a deterrent to drug use and is the most objective measure of drug use and effectiveness of new drug treatments. Urine drug testing provides a long detection window for drug abuse, from several days for opiates and cocaine, up to a month for chronic cannabinoid use.

Fifteen pregnant heroin dependent women from the Center for Addiction and Pregnancy (CAP) at the Johns Hopkins Bayview Medical Center (JHBMC) participated while enrolled in methadone maintenance treatment. Eleven African American and four Caucasian first-time drug treatment seekers had a mean ± SD age of 29.5 ± 6.7 years (range 19-40 years) and were between 8 and 28 weeks of gestation. Throughout gestation, participants received daily methadone (mean dose 75 ± 17 mg/day; range splitless injection mode. The Deans switch valve was programmed to divert 45-110 mg/day), weekly individual and group counseling and specialized prenatal care. The protocol was approved by the JHBMC and the National Institute on Drug Abuse Institutional Review Boards. Participants provided written informed consent and earned vouchers for negative urine tests as part of behavioral contingency management.

Participants joined the study as early as eight weeks estimated gesta- tional age (mean number of weeks on study 17.0 ± 5.8; range 8.2-27.2 weeks) and visited the clinic seven days per week. A variety of biological speci- mens, including urine, oral fluids, sweat and hair, were collected at fixed times during the study, under direct observation by trained staff. Urine samples were collected three times a week and stored at -20°C until analysis. The number of specimens collected was dependent on the enrollment period. A total of 284 urine specimens were collected from fifteen participants with a mean pH of 6.7 ± 0.8 (range 4.3-8.8). LC-APCI-MS/MS analyses were performed using an LCQ Deca XP ion trap mass spectrometer, equipped with an orthogonal APCI source, and interfaced to a Surveyor HPLC system. 100 µL of urine was fortified with deuterated internal standard working solution, briefly vortex-mixed and centrifuged to remove large particles (5 min at 510 *g*). Ten µL of supernatant were injected onto the LC-MS/MS. Pre-concentration during sample preparation was not required based on the sensitivity achieved. Urine specimens were analyzed for heroin and metabo- lites (morphine, normorphine, 6-acetylmorphine, codeine, acetylcodeine, norcodeine, noscapine and papaverine) and concurrently for cocaine and metabolites [ecgonine, ecgonine methyl ester (EME), ecgonine ethyl ester (EEE), anhydroecgonine methyl ester (AEME), *p*-hydroxybenzoylecgonine (*p*-OHBE), *m*-hydroxybenzoylecgonine (*m*-OHBE), benzoylecgonine (BE), benzoylnorecgonine (BNE), *p*-hydroxycocaine (*p*-OHCOC), *m*-hydroxyco- caine (*m*-OHCOC), and norcocaine based on selected reaction monitoring.

Opiates were detected in 149 (52.5%) of urine specimens. Of fifteen participants, one had no opiate positive results, five had less than 20%, eight between 20% and 90%, and two more than 90% positive tests for opiates, often with high concentrations of morphine-3-glucuronide. Morphine, normorphine, 6-acetylmorphine (6-AM), codeine and norcodeine were the other primary opiates identified in urine specimens. 30 % of opioid positive specimens contained 6-acetylmorphine, a specific biomarker of heroin use. 165 (58%) specimens from all 15 participants tested positive for one or more cocaine analytes. Seven subjects had between 20 and 50% cocaine positive specimens, 5 between 50 and 80%, 3 more than 80%, and cocaine was found in all urine specimens of one subject, often with high concentrations. EME and BE were the primary cocaine analytes in urine specimens. EME was detected in 123 (43.3%) urine specimens with a median concentration of 115 ng/mL (range 51-33002 ng/mL), and BE in 84 (29.6%) with a median concentration of 47 (10-73758).

Opiate, Cocaine, LC-MS/MS

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