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ORIGINAL ARTICLE

Infant With *In Utero* Ketamine Exposure: Quantitative Measurement of Residual Dosage in Hair

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Background: The drug ketamine is frequently abused for recreational use in Asia, but few studies in humans have focused on the effects of ketamine exposure during pregnancy on the health of neonates. Here, we report a neonate whose mother was suspected of ketamine abuse during pregnancy. The case was confirmed by testing hair samples of the neonate.
Methods: Hair samples of the neonate were taken on the first day of referral. Levels of common drugs of abuse in Asia were measured in the hair sample by gas chromatography-mass spectrometry using our previously reported method with modifications. This method was developed and validated to simultaneously quantify levels of amphetamine, ketamine and opiate in human hair.
Results: The neonate was a female baby, born full term, with a low birth weight of 2250 g. Very high levels of ketamine were detected in the neonate's hair, even though the mother stated that she had stopped abusing ketamine during the early stage of pregnancy. The neonate suffered from general hypotonia; moderate cerebral dysfunction was found by electroencephalography. Fortunately, her hypotonia improved gradually within 21 days.
Conclusion: This is the first report of ketamine exposure during late pregnancy detected by hair testing. We noted several clinical features in this case, including the infant being small for gestational age, intrauterine growth retardation, remarkable hypotonia, and poor reflex responses. Although the mother denied the use of ketamine during the late stage of her pregnancy, significant amount of ketamine and norketamine was still found in hair samples (only 2 cm long and 25 mg) from the infant.

1. Introduction

Ketamine [2-(o-chlorophenyl)-2-(methylamino)-cyclohexanone or K], an anesthetic primarily used in animals, was initially commercialized by Parke, Davis and Company in 1962 to replace phencyclidine and provide a safer anesthetic alternative which avoided hallucinations, neurotoxicity and seizures.[1,2] In terms of its pharmacology, ketamine is an N-methyl D-aspartete (NMDA) receptor antagonist, but at high

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and full anesthetic doses, it also binds to opioid μ and σ receptors.[2,3] One of the many "club" recreational drugs, ketamine is classified as a schedule III drug in Taiwan's Drugs Hazard Control Act introduced in 1997. It has become one of the most popular recreational drugs abused by teenagers and young adults in rave subcultures in Taiwan.[4] A comparison study with a parallel-group design that included volunteer participants showed that acute exposure to ketamine causes a broad spectrum of cognitive impairments, remarkable dissociative effects and schizotypal symptomatology. Moreover, the dissociated effects and schizotypal symptomatology have been reported to last for at least 3 days. In addition, persisting impairments of semantic memory have been reported to exist among users 3 days after administration.[5]

Ketamine has been found to induce acute pathomorphological changes in specific brain neurons. It also induces neuronal brain apoptosis and disrupts spontaneous activities and learning processes in animal studies.[6] *In vitro*, chronic exposure to low and subanesthetic doses of ketamine, while not affecting survival of neural cells, could still impair neuronal morphology and lead to neural dysfunctions.[7] Ketamine-induced NMDA receptor hypofunction is associated with early-occurring memory impairments followed by specific schizophrenia-like symptoms in humans.[8] However, ketamine was reported to decrease "behavioral despair" in the forced swim test, a widely used animal model of antidepressant drug action. Intriguingly, it also produced anxiolytic-like effects at lower doses, without affecting general locomotion measures. In another neurophysiological model of anxiolytic drug action, ketamine reduced the frequency of reticular-activated ϑ oscillations in the hippocampus.[9]

There is scarce research on the influence of ketamine exposure during pregnancy on the health of the nervous system in neonates. Most of the related studies were performed in animals. Of note, local cerebral glucose utilization was found to be impaired in fetal sheep.[10] Meanwhile, taste recognition memory response and taste-mediated conditioned motor response were reported to be weakened or even blocked in rats exposed to ketamine *in utero*.[11,12] It has been shown that ketamine administered to immature rodents triggered widespread apoptotic neurodegeneration throughout the developing brain during the period of synaptogenesis, which corresponds to the sixth month of gestation to several years after birth in humans. Thus neurons of an immature central nervous system are prone to commit suicide if exposed to intoxicating concentrations of NMDA antagonists.[13,14] Consequently, to provide greater insight into the effects of ketamine abuse during pregnancy, there is a need for more research in humans in various settings. Here, we report an infant whose mother stated that she stopped the recreational use of ketamine during the early stage of her pregnancy. However, we identified ketamine exposure in the neonate's hair after birth. We suspect that the mother was still exposed to ketamine as late as 2 months before delivery.

2. Materials and Methods

2.1. Case presentation

The case is a full-term female infant, born to a 30-year-old mother at the gestational age of 38^{+6} weeks via cesarean section because of breech presentation. Apgar scores were 8 at 1 minute and 9 at 5 minutes after birth. The infant's birth weight was 2250 g ($<3^{rd}$ percentile), body length was 47 cm (10^{th} percentile), and head circumference was 33 cm (3^{rd} percentile), with a ponderal index of 2.16%. The mother did not undergo any prenatal examination until 29 weeks of gestation. In addition, she continued smoking an average of five cigarettes per day during pregnancy. She declared that she had quit recreational ketamine use during the first term of her pregnancy (at around 8 weeks of gestation).

The infant was admitted into our neonatal intensive care unit immediately after her birth through referral from primary medical care because of general hypotonia. Hypotonia was predominant. Other general physical findings of this newborn were unremarkable. She also had primitive reflex. Electroencephalography revealed moderate cerebral dysfunction. Her auditory brainstem response testing was normal. Brain ultrasonography displayed a normal brain anatomical structure. Heart, liver and spleen ultrasonography were also normal. Her vital signs were stable from the first day of admission. Blood culture, cerebral spinal fluid culture, toxoplasmosis, rubella, cytomegalovirus and herpes simplex survey findings were unremarkable. Chromosome analysis showed 46, XX. She started to receive oral feeding gradually at 7 days old, and her hypotonia improved progressively, within 21 days.

2.2. Methods

Hair samples of the neonate were taken on the first day of referral. Hair was cut with the length of 1–2 centimeters from sites behind the ears and on occipital bone across an area of approximately 5 cm^2. Levels of common drugs of abuse in Asia were then measured in the hair by gas chromatography-mass spectrometry (GC-MS) using our previously reported method.[15] A simultaneous assay was developed and validated with some modifications. This method

can simultaneously quantify amphetamines (amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxy methamphetamine, and 3,4-methylenedioxyethylamphetamine), ketamine (ketamine, norketamine), and opiates (morphine, codeine and 6-acetylmorphine) in human hair.

In brief, hair samples (25 mg) were washed, cut and incubated overnight at 25ºC in methanol, then extracted by solid-phase extraction, and derivatized using heptafluorobutyric acid anhydride (HFBA) at 70ºC for 30 minutes. Finally, the derivatives were analyzed by GC-MS in electron ionization mode with full scan or selected ion monitoring mode (SIM). GC-MS analyses were performed with an Agilent 6890 GC interfaced to a 5973 MS in electron ionization mode, and a 6890 series automatic injector (Agilent Technologies, Palo Alto, CA, USA). Separation was achieved with a capillary column (DB-5 MS, 5% phenyl methyl siloxane, 30 m length × 0.25 mm inner diameter, 0.25 μm film thickness; Agilent J & W Scientific, Folsom, CA, USA) at a flow rate of 1 mL/min.

3. Results

Hair testing can be used to construct a gestational drug exposure profile and study its potential link with the appearance of neonatal withdrawal syndrome. Although the hair of neonates is often very short, it still gives enough information when tested by GC-MS. For 25 mg of hair samples, the limits of detection and quantification were 10 pg/mg and 20 pg/mg, respectively, for amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxy methamphetamine and 3,4-methylenedioxyethylamphetamine, and 20 pg/mg and 50 pg/mg for ketamine, norketamine, morphine, codeine and 6-acetylmorphine. Calibration curves for 10 analytes were established in the concentration range 0.05–5 ng/mg with high correlation coefficients ($r^2 > 0.997$) using 200 pg/mg of deuterated analogs of the analytes as internal standards for quantification. All quantitative analyses were performed in the SIM mode, with a dwell time of 20 ms for each ion (Figure 1).

In this case, the neonate hair was about 2 cm long and it could be used to detect the drugs abused by the mother during the last 2–3 months of pregnancy. The hair testing revealed that ketamine was the only drug abused by the mother, and the concentrations of ketamine and its metabolite norketamine were 141 pg/mg and 63 pg/mg of hair, respectively. The GC/MS SIM chromatogram of the drugs after derivatization with HFBA recorded norketamine-HFBA and ketamine-HFBA at 5.19 minutes and 6.01 minutes, respectively (Figure 2). The target ions monitored for ketamine and norketamine in SIM mode were quantization ion (m/z) 356, 360, 362 and 366 for norketamine, norketamine-d4, ketamine and ketamine-d4; and qualification ions (m/z) were 340 and 384 for norketamine, 388 for norketamine-d4, 236 and 370 for ketamine, and 374 for ketamine-d4.

Although the mother declared that she had stopped recreational use of ketamine during the first term of her pregnancy (at around 8 weeks of gestation), the results show ketamine and its metabolite were present, and were authentically determined using deuterated analogs of the analytes as internal standards and high correlation coefficient calibration curves were obtained. Thus we concluded that the mother had used ketamine within at least 2 months before birth.

4. Discussion

In recent years, ketamine has become one of the most commonly used recreational drugs and its abuse is a public health issue in Hong Kong, China,

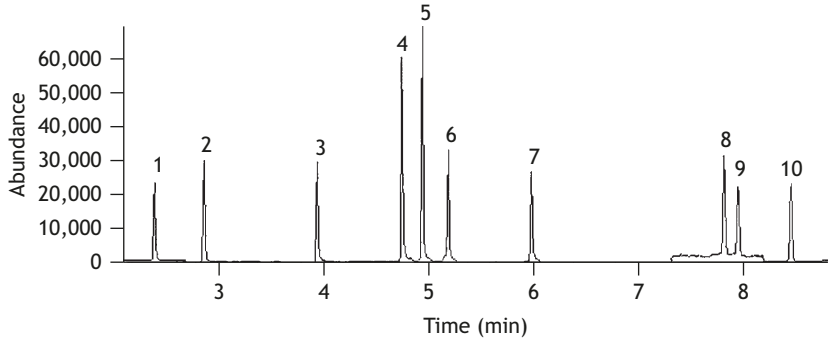

Figure 1 Gas chromatography-mass spectrometry (selected ion monitoring mode) chromatogram of samples spiked with 5 ng/mg of the target drugs after derivatization with heptafluorobutyric acid anhydride. 1 = amphetamine; 2 = methamphetamine; 3 = 3,4-methylenedioxyamphetamine; 4 = 3,4-methylenedioxy methamphetamine; 5 = 3,4-methylenedioxyethylamphetamine; 6 = norketamine; 7 = ketamine; 8 = morphine; 9 = codeine; 10 = 6-acetylmorphine.

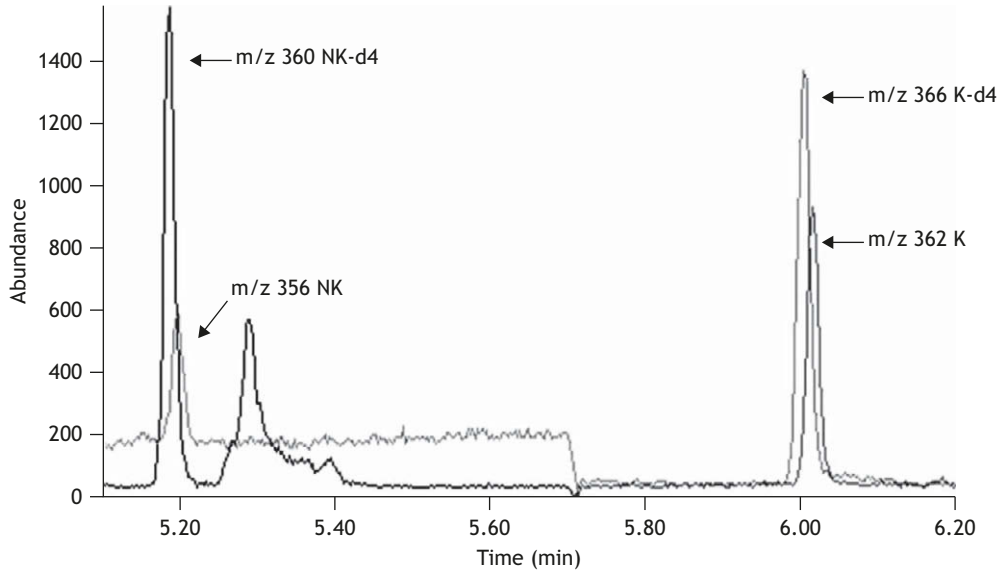

Figure 2 Gas chromatography-mass spectrometry (selected ion monitoring mode) chromatogram of a neonatal hair sample with ketamine (K) and norketamine (NK), and K-d4 and NK-d4 as internal standards.

Singapore and Taiwan.[16] To our knowledge, this is the first case report of ketamine abuse during late pregnancy with full clinical and laboratory evidence. Although ketamine has not been reported as an environmental agent for neonatal malformation (unlike alcohol, hydantoin, valproate, warfarin, aminopterin/methotrexate, retinoic acid, varicella, and hyperthermia),[17] it is notable that the case presented as small for gestational age, with intrauterine growth retardation, remarkable hypotonia, and poor reflex responses.

Drug testing provides objective information regarding an individual's use of or exposure to illicit drugs. Blood and urine testing are the most commonly used approaches, but these are only applicable for exposure within the last few days. Hair differs from other biological specimens used for toxicological analysis because of its substantially longer detection window (months to years), enabling retrospective investigation of chronic and past consumption. In addition, hair gives additional particular advantages; it can be easily obtained, it is difficult to adulterate, and it can be stored and transported without specific precautions because of its stability. Moreover, segmental hair analysis may help determine the time of drug exposure. Therefore, hair testing provides broader applications for evaluating clinical toxicology and clinical chemistry.

Epidemiologic studies have revealed that drug abusers frequently use multiple recreational drugs.[18] We recently developed a GC-MS hair testing method to simultaneously detect several common drugs of abuse in Asia. This simultaneous method is based on an isotope dilution mass spectrometry method that can eliminate the principal source of measurement bias and inaccuracy, and has been successfully used to assess different drug classes, including amphetamines, ketamine, opiates and their metabolites. Although liquid chromatography/tandem mass spectrometry offers great potential for hair testing, GC-MS is less expensive and easier to operate, and is thus often used to test for drug abuse.

Therefore, it was confirmed that this case was only exposed to a single recreational drug, ketamine, during pregnancy. Although urine is the most commonly used sample to detect ketamine exposure—because of the convenience of collection—the half-life of ketamine in urine is only 2–3 hours.[19] Thus the pharmacokinetics make it difficult to detect the recreational use of ketamine during pregnancy. Instead, as reported in this study, we can infer the duration from the last use of ketamine based on the distance of the hair sample to skin. Since the rate of hair growth differs between people (usually 0.96–1.38 cm per month),[20] we used the average rate of 1.2 cm per month to estimate the last instance of ketamine exposure of the mother and detected ketamine and norketamine at concentrations of 141 pg/mg and 63 pg/mg hair, respectively. Thus we concluded that the mother had used ketamine within at least 2 months before birth. Infants with intrauterine methamphetamine exposure often present with neurobehavioral symptoms of decreased arousal, increased stress, and poor quality of movement, or even abnormalities of brain structure,[21] whereas the clinical expressions of our case differed from these. Accordingly, the case reported here provides a practical demonstration for clinical practice.

Gaillard and Pepin[22] reported that the amount of norketamine was 7–12% of that of ketamine, based

on their analysis of hair segments from a deceased woman.[22] Leong et al[16] also reported the ratio of norketamine to ketamine in hair segments (84 out of 91 hair segments) ranged from 0.05 to 0.84, with a mean of 0.33. In our case, the ratio was within these ranges, but higher than the mean. This finding could be because the body composition of women during pregnancy includes greater fat content. Unfortunately, in the present study, because of the small number of samples, which did not allow for statistical analysis, the relationship between hair concentrations or the appearance in urine or blood samples, the severity of neonatal withdrawal syndrome could not be established.

Based on studies of humans and animal models, it is necessary to continue to follow up cases exposed to ketamine *in utero*, focusing on brain development, recognition, and learning,[6] specifically for cognitive impairments, dissociative effects and schizotypal symptomatology,[5] motor responses,[23] and memory impairments.[8] The process of laboratory examination for detecting ketamine exposure level in hair is well established for neonates, and is even more feasible and precise than blood and urine samples for long-term exposures.[15]

With a broader view in public health, the consequence of ketamine abuse during pregnancy, for instance, intimate partner violence toward women with substance abuse during pregnancy has gradually become increasingly important in psychology and sociology.[24] Thus it is critical to characterize substance abusing pregnant women to identify vulnerable groups and their demographic and psychosocial characteristics, and provide them with appropriate medical care.[25] In addition, parental substance abuse is a major risk factor placing their children at higher risk of substance abuse later in life. Children of addicted parents are at greater risk of later dysfunctional behaviors and need assistance to help prevent intergenerational transmission of drug abuse.[26]

5. Conclusions

In conclusion, ketamine is a drug that is widely used as an anesthetic. Most clinical reports have focused on its use in cesarean or pediatric anesthesia.[27] However, ketamine has now become one of the most commonly abused recreational drugs worldwide making it an emerging topic of clinical interest for pediatricians and neonatologists.[28] Unfortunately in the present study, the limited amount of samples did not allow for segmental analysis, the exact time and frequency of abuse could not be established. However, results obtained with neonatal hair show that it is possible to confirm fetal drug exposure and corresponding it to the appearance of neonatal withdrawal syndrome. We believe the use of hair for drug testing will grow because of its ability to detect maternal drug use, evaluate drug exposure during gestation, and aid in the assessment of neonatal withdrawal syndrome. Further studies, including clinical observations of new cases and long-term follow-up for neurobehavioral developments, are needed to expand our knowledge on this issue.

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References

1. Sinner B, Graf BM. Ketamine. *Handb Exp Pharmacol* 2008: 313–3.
2. Annetta MG, Iemma D, Garisto C, Tafani C, Proietti R. Ketamine: new indications for an old drug. *Curr Drug Targets* 2005;6:789–94.
3. Bergman SA. Ketamine: review of its pharmacology and its use in pediatric anesthesia. *Anesth Prog* 1999;46:10–20.
4. National Bureau of Controlled Drugs. *Current Status and Future Trends of Drug Abuse in Taiwan*. Taiwan: National Bureau of Controlled Drugs, 2006.
5. Curran HV, Morgan C. Cognitive, dissociative and psychotogenic effects of ketamine in recreational users on the night of drug use and 3 days later. *Addiction* 2000;95:575–90.
6. Olney JW, Labruyere J, Price MT. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science* 1989;244:1360–2.
7. Vutskits L, Gascon E, Potter G, Tassonyi E, Kiss JZ. Low concentrations of ketamine initiate dendritic atrophy of differentiated GABAergic neurons in culture. *Toxicology* 2007; 234:216–26.
8. Beneyto M, Meador-Woodruff JH. Lamina-specific abnormalities of NMDA receptor-associated postsynaptic protein transcripts in the prefrontal cortex in schizophrenia and bipolar disorder. *Neuropsychopharmacology* 2008;33:2175–86.
9. Engin E, Treit D, Dickson CT. Anxiolytic- and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models. *Neuroscience* 2009;161:359–69.
10. Abrams RM, Cooper RJ. Effect of ketamine on local cerebral glucose utilization in fetal sheep. *Am J Obstet Gynecol* 1987;156:1018–23.
11. Mickley GA, Remmers-Roeber DR, Crouse C, Peluso R. Ketamine blocks a taste recognition memory in fetal rats. *Pharmacol Biochem Behav* 2000;67:575–81.
12. Mickley GA, Remmers-Roeber DR, Crouse C, Peluso R. Ketamine blocks a taste-mediated conditioned motor response in perinatal rats. *Pharmacol Biochem Behav* 2000;66: 547–52.
13. Olney JW, Farber NB, Wozniak DF, Jevtovic-Todorovic V, Ikonomidou C. Environmental agents that have the potential

to trigger massive apoptotic neurodegeneration in the developing brain. *Environ Health Perspect* 2000;108(Suppl 3): 383–8.
14. Young C, Jevtovic-Todorovic V, Qin YQ, et al. Potential of ketamine and midazolam, individually or in combination, to induce apoptotic neurodegeneration in the infant mouse brain. *Br J Pharmacol* 2005;146:189–97.
15. Wu YH, Lin KL, Chen SC, Chang YZ. Integration of GC/EI-MS and GC/NCI-MS for simultaneous quantitative determination of opiates, amphetamines, MDMA, ketamine, and metabolites in human hair. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008;870:192–202.
16. Leong HS, Tan NL, Lui CP, Lee TK. Evaluation of ketamine abuse using hair analysis: concentration trends in a Singapore population. *J Anal Toxicol* 2005;29:314–8.
17. Jones KL. *Smith's Recognizable Patterns of Human Malformation*. Philadelphia: WB Saunders, 2006.
18. Degenhardt L, Dunn M. The epidemiology of GHB and ketamine use in an Australian household survey. *Int J Drug Policy* 2008;19:311–6.
19. Wieber J, Gugler R, Hengstmann JH, Dengler HJ. Pharmacokinetics of ketamine in man. *Anaesthesist* 1975;24:260–3.
20. Pragst F, Balikova MA. State of the art in hair analysis for detection of drug and alcohol abuse. *Clin Chim Acta* 2006; 370:17–49.
21. Smith LM, Lagasse LL, Derauf C, et al. Prenatal methamphetamine use and neonatal neurobehavioral outcome. *Neurotoxicol Teratol* 2008;30:20–8.
22. Gaillard Y, Pepin G. Evidence of polydrug use using hair analysis: a fatal case involving heroin, cocaine, cannabis, chloroform, thiopental and ketamine. *J Forensic Sci* 1998; 43:435–8.
23. Mickley GA, Remmers-Roeber DR, Dengler CM, Kenmuir CL, Crouse C. Paradoxical effects of ketamine on the memory of fetuses of different ages. *Brain Res Dev Brain Res* 2001;127: 71–6.
24. Martin SL, Beaumont JL, Kupper LL. Substance use before and during pregnancy: links to intimate partner violence. *Am J Drug Alcohol Abuse* 2003;29:599–617.
25. Hans SL. Demographic and psychosocial characteristics of substance-abusing pregnant women. *Clin Perinatol* 1999; 26:55–74.
26. Johnson JL, Leff M. Children of substance abusers: overview of research findings. *Pediatrics* 1999;103:1085–99.
27. Lois F, De Kock M. Something new about ketamine for pediatric anesthesia? *Curr Opin Anaesthesiol* 2008;21:340–4.
28. Mickley GA, Kenmuir CL, McMullen CA, et al. Long-term age-dependent behavioral changes following a single episode of fetal N-methyl-D-aspartate (NMDA) receptor blockade. *BMC Pharmacol* 2004;4:28.