Chula Med J. July - August 2025;69(4): 243 - 253 **DOI : 10.56808/2673-060X.**5575

Original article

Comparison of blood and urinary ketamine profiles among different causes of death in Thai postmortem cases

Supanida Leungsukjaroen[a, b], Sunitda Khawthong[b], Peerayuht Phuangphung[b, *]

[a]*King Narai Hospital, Ministry of Public Health, Lopburi, Thailand*
[b]*Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand*

Abstract

Background: Ketamine is a commonly abused substance, especially among young adults. Blood and urinary ketamine profiles are important for the interpretation of the cause of death.
Objectives: This study aimed to compare the blood and urine ketamine profiles between the different causes of death in Thai postmortem cases and to develop predictive equations for ketamine metabolism in urine.
Methods: A cross-sectional study was performed in 40 Thai postmortem cases whose urine samples tested positive for ketamine. Sex, age, cause of death, manner of death, blood alcohol concentration, and concomitant drugs found with ketamine in the subjects were recorded for each case. Blood and urinary ketamine and its two metabolites (norketamine and dehydronorketamine (DHNK)) were analyzed using liquid chromatography quadrupole time-of-flight mass spectrometry. Statistical analysis was performed using the Mann–Whitney U test and Kruskal–Wallis H test.
Results: The study subjects included 11 females and 29 males with a mean age of 29.6 years. The causes of death were classified into three groups, namely the road traffic injury (RTI), drug intoxication, and non-RTI groups. The blood ketamine concentration in the non-RTI group was significantly higher than that in the RTI and drug intoxication groups. The two ketamine metabolite concentrations in the urine samples of the non-RTI group were significantly higher than those in the RTI and drug intoxication groups. Male subjects had significantly higher concentrations of blood ketamine and its metabolites than female subjects. Linear regression curves were established between urinary ketamine and norketamine (Log (urinary norketamine) = 1.175(Log (urinary ketamine)) + 0.659; $r^2 = 0.684$) and between urinary norketamine and DHNK (Log (urinary DHNK) = 0.045(Log (urinary norketamine)) + 1.147; $r^2 = 0.773$).
Conclusion: This study developed predictive equations for ketamine metabolism in urine, demonstrating strong correlations between ketamine and its two metabolites. The differences in the ketamine concentrations among the different causes of death and sex potentially indicate drug use patterns rather than direct causal relationships.

Keywords: Drug intoxication, ketamine, postmortem, road traffic injury (RTI), Thai.

Ketamine is an anesthetic agent used for anesthetic induction and maintenance during minor and major surgical procedures. In addition, ketamine can produce dissociative symptoms, analgesia, and amnesia. [1] The dissociative symptoms of ketamine are associated with its antagonistic effect on the N-methyl-D-aspartate receptor in the brain. This effect has led to the abuse of ketamine as a recreational drug and in drug-facilitated sexual assault.[1, 2] It has been reported that ketamine's dissociative symptoms and other adverse effects, including headache, vertigo, and nausea, occur to a higher degree in females than males, and this potentially supports its exploitation in drug-facilitated sexual assault. [2] In a study conducted in the United States of America (USA), cocaine,3,4-methylenedioxy methamphetamine (MDMA), and ketamine were found to be the top three illicit drugs detected in hair obtained from people who were at least 18 years old entering nightclubs and dance festivals. [3] Moreover, in a study in Thailand, it was reported that 16.6% of Thai adolescents aged 10–18 years had experienced

***Correspondence to:** Peerayuht Phuangphung, Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
E-mail: peerayuht.phu@mahidol.ac.th
Received: February 4, 2025
Revised: May 27, 2025
Accepted: June 20, 2025

some kind of illicit drug use, with ketamine being one of the top five drugs abused by Thai adolescents. [4] Moreover, the proportion of Thai adolescents who used ketamine was higher among the age group 15 years than the other age groups considered. [4] Regarding Thai postmortem cases, it was found that ketamine was among the top 10 drugs of abuse detected in medico-legal cases, thus leading the Office of the Narcotics Control Board, Ministry of Justice, Thailand, to formulate a policy that ketamine should be on the drug screening panel in Thai medico-legal cases. [5]

When ketamine is administered into the body, it is mainly metabolized into norketamine, dehydronorketamine (DHNK), and hydroxynorketamine (HNK). [6] A previous study indicated that three cytochrome P450 (CYP) enzymes, namely CYP2B6, CYP3A4, and CYP2C9, were involved in ketamine metabolism, and the elimination half-life of ketamine was 1.5–5 h. [6] Another study reported that when ketamine was excreted via the kidney, it was in the form of unmetabolized ketamine (2.0%), norketamine (2.0%), DHNK (16.0%), and conjugated forms of HNK with glucuronic acid (80.0%). [7] According to this metabolic profile, approximately 20.0% of ketamine and its metabolites are excreted in their free form, while 80.0% are excreted in a conjugated form. In addition, the concentrations of ketamine and its metabolites (norketamine and DHNK) in urine samples obtained from living patients who were suspected of being drug abusers were found to be 6–7,744 ng/mL for ketamine, 7–7,986 ng/mL for norketamine, and 37–23,239 ng/mL for DHNK. [7] Furthermore, the mean urinary ratios of norketamine/ketamine and DHNK/ketamine were 2.2 and 7.0, respectively. [7] Thus, considering the profiles of ketamine and its metabolites may be valuable for interpreting a history of ketamine use.

There is currently no information regarding the ketamine metabolic profiles within the Thai population. Therefore, this study aimed to determine the concentrations of ketamine and its metabolites in blood and urine samples obtained from Thai postmortem cases with different causes of death, but whose urine samples tested positive for ketamine. Furthermore, the urinary ketamine metabolic profiles and urine metabolic ratios were compared with the findings reported by Moore, *et al.* [7] In addition, other drugs that were concomitantly present with ketamine were detected to ascertain whether they have an impact on the ketamine concentration profiles, as it has been found that ketamine-related deaths usually involve intoxication with multiple drugs, such as illegally manufactured fentanyl, methamphetamine, and cocaine. [8] The second objective was to develop predictive equations for urinary ketamine metabolism in Thai postmortem cases based on the determined urinary ketamine profiles. This information should provide fundamental data for further studies on ketamine metabolic profiles within the Thai population.

Materials and methods

Study design and data collection

A cross-sectional study was performed considering the medico-legal cases sent to the Department of Forensic Medicine, Siriraj Hospital, Mahidol University, for autopsy. The inclusion criteria were deceased Thai individuals who were at least 15 years old at the time of death, sent for autopsy between May 1, 2024, and November 8, 2024, and whose urine samples tested positive for the presence of ketamine. The exclusion criteria were decomposed bodies and bodies with extensive trauma leading to the inability to collect blood and urine samples. This study was reviewed and approved by the Siriraj Institutional Review Board, Faculty of Medicine, Siriraj Hospital, Mahidol University (COA no. Si 928/2023, SIRB protocol no. 915/2566 (IRB2)).

Chemicals and reagents

Ketamine (1 mg/mL) was purchased from Cerilliant, while norketamine (1 mg/mL) and DHNK (100 µg/mL) were obtained from LGC Standards Ltd. Ketamine-d_4 (100 µg/mL) was sourced from Sigma-Aldrich. Liquid chromatography-mass spectrometry (LC-MS)-grade acetonitrile and methanol were obtained from Duksan Pure Chemicals Co., Ltd. Phosphoric acid, ammonium acetate, and formic acid were purchased from Merck Ltd., while sodium formate was obtained from Honeywell Fluka. All other chemicals and reagents were provided by U&V Holding Co., Ltd. (Thailand). The deionized water (dH_2O) utilized in this study was produced using a Merck Millipore Direct-Q® 3 UV-R Water Purification System.

Instrumentation

The analysis of ketamine, norketamine, and DHNK was performed by electrospray ionization liquid chromatography quadrupole time-of-flight mass

spectrometry (ESI-LC-QTOF/MS) using a Thermo Scientific Dionex Ultimate 3000 high-performance liquid chromatography system equipped with a Maxis Impact Bruker Daltonics QTOF-MS instrument. Ketamine analysis was performed using a Phenomenex Luna C18 column (10 cm × 2 mm × 3 µm, 100 A). The mobile phase consisted of 0.1% formic acid and 5 mM ammonium acetate in dH_2O (A) and acetonitrile (B). Chromatographic separation was achieved under gradient elution at a flow rate of 0.3 mL/min. The gradient condition began with a mobile phase of 90.0% A:10% B, followed by increasing mobile phase B to 30.0% in 7.5 min, then increasing mobile phase B further to 90.0% in 0.5 min, holding at 10.0% A:90.0% B for 3 min, before returning to 90.0% A:10% B within 0.1 min; and finally continuing this condition for 4 min for a total run time of 15 min. The injected sample volume was 5 µL, and the column temperature was set at 40°C.

Mass spectrometry was performed in the positive ESI mode, with the ESI parameters set as follows: capillary voltage 2,900 V, nebulizer gas 2.0 bar, drying gas 8.0 L/min, and drying temperature 180°C. The broadband collision-induced dissociation (bbCID) mode was utilized with a mass range of 50–1,500 m/z. The collision energy for the MS and MS/MS (bbCID) modes were set at 4.0 and 35.0 eV, respectively. Moreover, the retention times for ketamine, norketamine, and DHNK were 4.6, 4.3, and 3.5 min, respectively. The multiple reaction monitoring transitions for ketamine, norketamine, and DHNK were 238.1 > 125.0/238.1 > 220.1, 224.1 > 125.0/ 224.1 > 207.1, and 222.1 > 142.1/222.1 > 205.0, respectively, with the quantitation ions underscored.

Sample collection and extraction

Blood and urine samples from each case were obtained from the femoral vein in a capped blood tube and a plastic bottle filled with 30 mL of urine, respectively. The blood and urine samples were stored at 4°C in the laboratory and analyzed for ketamine and its metabolites the following day by ESI-LC-QTOF/MS.

For the analysis experiments, sample preparation was performed using solid-phase extraction (SPE). To prepare the blood samples, 1 mL of blood was placed into a test tube, followed by the gradual addition of 1.5 mL of acetonitrile, with subsequent shaking for 30 s to facilitate protein precipitation. The mixture was then centrifuged at 3,000 rpm for 5 min. After centrifugation, the supernatant was carefully transferred into a new test tube, and 3 mL of 0.1 M phosphate buffer (pH 6) was added to the supernatant for subsequent extraction via SPE. Urine samples were prepared by placing 1 mL of urine into a test tube and then adding 1 mL of 1.0% (v/v) phosphoric acid solution to the urine sample. The mixture was then thoroughly mixed using a vortex mixer for 30 sec before sample extraction via SPE.

SPE was performed using Waters Oasis HLB® cartridges (60 mg, 3 mL). The SPE cartridges were preconditioned with methanol and dH_2O. The prepared sample was loaded into the cartridge, followed by washing with 2 mL of 10% methanol in dH_2O to remove any impurities. After drying the SPE cartridges for 5 min, the target analytes were eluted with 2 mL of methanol. The collected eluent was evaporated under a nitrogen stream at 40°C. The residue was then reconstituted in a mobile phase mixture (90.0% Phase A: 10.0% Phase B) and subsequently injected into the ESI-LC-QTOF/MS system for analysis. All the samples were analyzed in duplicate, and the average values of the samples were used for statistical analysis. The sex, age, cause of death, manner of death, blood alcohol concentration (BAC), and concomitant drugs detected by ESI-LC-QTOF/MS in a drug screening panel for each case were recorded for statistical analysis.

Method validation

Method validation was performed according to the Standard Guidelines for Method Validation in Forensic Toxicology. [9] The method validation protocol for blood was performed using expired whole blood obtained from the Department of Transfusion Medicine, Siriraj Hospital, Mahidol University, while the method validation protocol for urine was performed using synthetic urine. A complete chromatographic separation of ketamine and its metabolites from the baseline noise was achieved through interference studies. In addition, other drugs of abuse, including methamphetamine, amphetamine, MDMA, 3, 4-methylenedioxyamphetamine, cocaine, benzoylecgonine, 6-acetylmorphine, codeine, morphine, fentanyl, methadone, tramadol, cannabis and its metabolites, mitragynine, lysergic acid diethylamide, phencyclidine, and psilocybin, were tested to ascertain that they did not produce interference during ketamine analysis. The limit of detection (LOD) and lower limit of quantitation (LLOQ) for ketamine, norketamine, and DHNK were

evaluated in the blood and urine samples. Specifically, the LOD and LLOQ were determined from the lowest concentrations that generated a signal-to-noise (S/N) greater than 3 and 10 times, respectively. The LOD and LLOQ for combined ketamine, norketamine, and DHNK were 10 and 25 ng/mL, respectively.

As this analysis was performed using ESI-LC-QTOF/MS, it was necessary to assess the possible matrix effects. The matrix effects of ketamine, norketamine, and DHNK were evaluated at three concentrations (75, 150, and 750 ng/mL). The method validation guidelines suggest that the matrix effect should not exceed ± 25.0% for all target analytes. The matrix effects for ketamine, norketamine, and DHNK in blood were -3.9% to -13.0%, -4.3% to -14.3%, and -5.6% to -18.0%, respectively. Meanwhile, the matrix effects for ketamine, norketamine, and DHNK in urine were -3.0% to -11.6%, -3.2% to -13.2%, and -5.2% to -14.5%, respectively.

The linearity ranges for ketamine, norketamine, and DHNK in blood and urine samples were obtained at concentrations of 25, 50, 100, 200, 500, and 1,000 ng/mL. The calibration curves for ketamine, norketamine, and DHNK were established using the Bruker Daltonics Compass for OTOF Series 1.7 Software®. For acceptance of the linearity criteria, a coefficient of determination (r^2) ≥ 0.99, an accuracy of each calibrator within ± 15.0% (LLOQ ± 20.0%), and a %coefficient of variation (%CV) ≤ 15.0% should be achieved. The average calibration curve parameters for ketamine, norketamine, and DHNK are shown in **Table 1**.

Accuracy and precision were assessed using spiked blood and urine samples at low, medium, and high quality control (QC) concentrations (75, 150, and 750 ng/mL, respectively). The accuracy for each QC concentration should be within ± 15.0%, and the precision evaluated by the %CV should be ≤ 15.0%. The accuracy and precision of these three concentrations of ketamine and its metabolites in the blood and urine are shown in **Table 2 – 3**, respectively.

Table 1. Calibration curve parameters for ketamine, norketamine, and DHNK.

Sample	Analytes	Concentration range (ng/mL)	Linear regression equation (n = 5)		r^2
			Slope	Intercept	
Blood	Ketamine	25 – 1,000	1.215335	+0.018994	≥0.99
	Norketamine	25 – 1,000	0.514983	+0.052081	≥0.99
	DHNK	25 – 1,000	0.648196	+0.008511	≥0.99
Urine	Ketamine	25 – 1,000	1.206594	-0.028953	≥0.99
	Norketamine	25 – 1,000	0.549445	+0.046061	≥0.99
	DHNK	25 – 1,000	0.550417	-0.001836	≥0.99

DHNK, dehydronorketamine.

Table 2. Accuracy and precision of three QC concentrations in blood samples.

QC concentration (ng/mL)		Accuracy (%) (n = 5)	Precision (n = 5)	
			Intraday (%)	Interday (%)
Ketamine	75	87.4 – 103.7	≤ 8.3	≤ 8.9
	150	89.3 – 114.3	≤11.2	≤10.7
	750	89.5 – 103.4	≤ 7.1	≤ 6.6
Norketamine	75	87.8 – 108.7	≤ 7.9	≤ 9.2
	150	89.0 – 113.7	≤10.3	≤ 9.9
	750	89.7 – 103.3	≤ 5.4	≤ 5.5
DHNK	75	87.3 – 105.2	≤ 7.7	≤ 9.1
	150	89.4 – 114.6	≤ 13.2	≤11.1
	750	89.0 – 107.0	≤ 6.4	≤ 4.3

DHNK, dehydronorketamine.

Table 3. Accuracy and precision of three QC concentrations in urine samples.

QC concentration (ng/mL)		Accuracy (%) (n = 5)	Precision (n = 5)	
			Intraday (%)	Interday (%)
Ketamine	75	85.7 – 104.1	≤ 9.8	≤ 7.9
	150	94.1 – 113.3	≤ 6.9	≤ 8.3
	750	88.0 – 104.8	≤ 8.0	≤ 6.4
Norketamine	75	87.1 – 105.4	≤ 8.0	≤ 9.7
	150	92.7 – 114.2	≤13.0	≤10.3
	750	87.7 – 106.2	≤ 8.2	≤ 7.2
DHNK	75	88.1 – 103.2	≤ 8.9	≤ 8.2
	150	89.8 – 114.2	≤ 11.0	≤ 10.9
	750	87.0 – 105.8	≤ 4.3	≤ 5.5

DHNK, dehydronorketamine.

Table 4. Distribution of age groups among the causes of death.

Cause of death	N	Mean ± SD (years old)	Range (years old)
RTI (motorcycle riders)	19	29.3 ± 6.9	20 – 49
Drug intoxication	13	28.2 ± 6.2	19 – 38
Non-RTI deaths (including hanging, gunshot wound, fall from height, and electrocution)	8	32.8 ± 9.3	21 – 51
Total	**40**	**29.6 ± 7.2**	**19 – 51**

Statistical analysis

Statistical analysis was performed using IBM SPSS® Statistics for Windows version 25. Descriptive statistics, including the mean, median, and standard deviation(SD), were calculated and reported. The Kolgomorov–Smirnov test was analyzed to determine normality for the concentrations of ketamine and its metabolites in the blood and urine samples, and it was found that they were not normally distributed. Thus, comparisons of the concentrations of ketamine and its metabolites in the blood and urine samples were performed using the Mann–Whitney U test and Kruskal–Wallis H test. Bivariate correlation and linear regression analysis were performed to determine the association between urinary ketamine concentrations and its metabolites. $P < 0.05$ was considered statistically significant.

Results

A total of 40 subjects were included in this study, comprising 11 females (27.5%) and 29 males (72.5%). The mean age of the subjects at death was 29.6 years (range 19–51 years), and 34 cases (85.0%) were aged 19–35 years. The distribution of the age groups among the causes of death is described in **Table 4**, and there was no significant difference in age groups among the different causes of death **(Table 4).** The causes of death in this study were divided into three groups, namely RTI (all of them were motorcycle riders), drug intoxication, and non-RTI groups. All cases of drug intoxication were accidental deaths. The non-RTI cases consisted of five suicide cases (hanging, gunshot wound, and fall from height), two homicide cases (gunshot wound), and one accident case (electrocution).

Table 5. Comparison of the blood and urine ketamine profiles among the three causes of death.

Ketamine parameters		Mean ± SD, Median (Range) (ng/mL)			*P*-value
		RTI	Drug intoxication	Non-RTI	
Blood	Ketamine	916.6 ± 2,106.5 273.5 (ND – 9,176.7)	1,272.8 ± 2,186.1 269.5 (29.3 – 7,564.5)	3,217.27 ± 3,481.1 1,914.1 (120.8 – 9,266.9)	0.019*
	Norketamine	404.3 ± 454.9 117.5 (ND – 1,207.0)	987.9 ± 1,656.0 225.2 (20.6 – 5,210.8)	1,353.2 ± 1,252.3 956.7 (41.3 – 3,513.4)	0.092
	DHNK	50.8 ± 76.5 26.2 (ND – 296.1)	367.2 ± 1,090.0 53.8 (ND – 3,984.8)	256.3 ± 332.2 88.5 (ND – 919.5)	0.174
Urine	Ketamine	3,316.1 ± 6,414.9 1095.1 (33.8 – 25,715.4)	2,441.1 ± 4,546.2 1112.5 (172.7 – 16,929.7)	6,310.0 ± 5,701.2 3239.3 (158.9 – 14,175.1)	0.056
	Norketamine	2,654.5 ± 3,911.0 865.3 (158.6 – 15,103.2)	2,426.5 ± 3,171.4 954.8 (175.1 – 9,784.1)	6787.9 ± 4,172.2 6,683.2 (388.5 – 13,578.3)	0.047*
	DHNK	12,426.2 ± 22,156.5 1,863.5 (101.5 – 91,979.6)	9,518.4 ± 11,549.6 3,635.0 (305.7 – 33,906.5)	32,814.4 ± 17,850.5 32,238.9 (3,646.9 – 58,739.5)	0.005*

DHNK, dehydronorketamine; *ND, not detected (less than the LOD); RTI, road traffic injury.

Table 6. Comparison of the blood and urine ketamine profiles between the female and male subjects.

Ketamine parameters		Mean ± SD, Median (Range) (ng/mL)		*P*-value
		Female	Male	
Blood	Ketamine	451.5 ± 793.8 120.8 (ND–2,430.4)	1,887.3 ± 2,866.3 712.0 (ND–9,266.9)	0.029*
	Norketamine	348.5 ± 568.9 41.3 (ND–1,550.0)	948.8 ± 1,300.7 598.3 (ND–5,210.8)	0.049*
	DHNK	44.8 ± 98.7 ND (ND–330.3)	251.6 ± 744.2 39.3 (ND–3,984.8)	0.038*
Urine	Ketamine	1,325.0 ± 935.7 1,662.5 (158.9–2,713.6)	4,505.0 ± 6,559.0 1,250.8 (33.8–25,715.4)	0.617
	Norketamine	2,584.6 ± 3,023.1 826.4 (265.4–8,170.2)	3,719.1 ± 4,354.4 1,316.5 (158.6–15,103.2)	0.477
	DHNK	11,767.3 ± 13,886.6 3,646.9 (305.7–33,906.5)	16,997.0 ± 22,009.6 4,405.7 (101.5–91,979.6)	0.774

DHNK, dehydronorketamine; *ND, not detected (less than the LOD).

Table 5 shows a comparison of the blood and urine ketamine profiles among the three causes of death groups. It was found that the blood ketamine concentration in the non-RTI group was significantly higher than that in the RTI and drug intoxication groups ($P < 0.05$), whereas the two ketamine metabolites were not significantly different among the groups. However, the norketamine and DHNK concentrations in the urine from the non-RTI group were significantly greater than in the RTI and drug intoxication groups ($P < 0.05$ and $P < 0.01$, respectively), whereas the concentration of ketamine itself did not exhibit a significant difference among the groups.

Table 6 shows a comparison of the blood ketamine profiles between the female and male subjects. These results showed that the concentrations of ketamine and its two metabolites in the blood of the male subjects were significantly higher than those in the female subjects ($P < 0.05$). However, the ketamine profiles in the urine of male subjects were not significantly different from those of the female subjects.

The ratios between the two ketamine metabolites and ketamine in the urine were determined. The average norketamine/ketamine ratios in the RTI, drug intoxication, and non-RTI groups were 2.9 ± 5.1, 1.6 ± 1.2, and 1.6 ± 0.7, respectively. Meanwhile, the average DHNK/ketamine ratios in the RTI, drug intoxication, and non-RTI groups were 8.3 ± 8.9, 6.2 ± 5.9, and 10.6 ± 8.7, respectively, and the average DHNK/norketamine ratios in the RTI, drug intoxication, and non-RTI groups were 3.5 ± 2.6, 4.7 ± 4.0, and 6.1 ± 3.0, respectively. A comparison of these three ratios among the causes of death did not reveal a significant difference ($P = 0.699$, 0.481, and 0.088, respectively). When logarithm was applied for the urinary concentrations of ketamine and its two metabolites, it was found that Spearman's correlation (r) between log (ketamine) and log (norketamine) was 0.858 ($P < 0.001$) and between log (norketamine) and log (DHNK) was 0.855 ($P < 0.001$). Linear regression analysis was performed to determine the relationship between ketamine and norketamine and between norketamine and DHNK in the urine. These two equations are presented below, and scatter plots with trend lines for these two equations are shown in **Figure 1 – 2,** respectively:

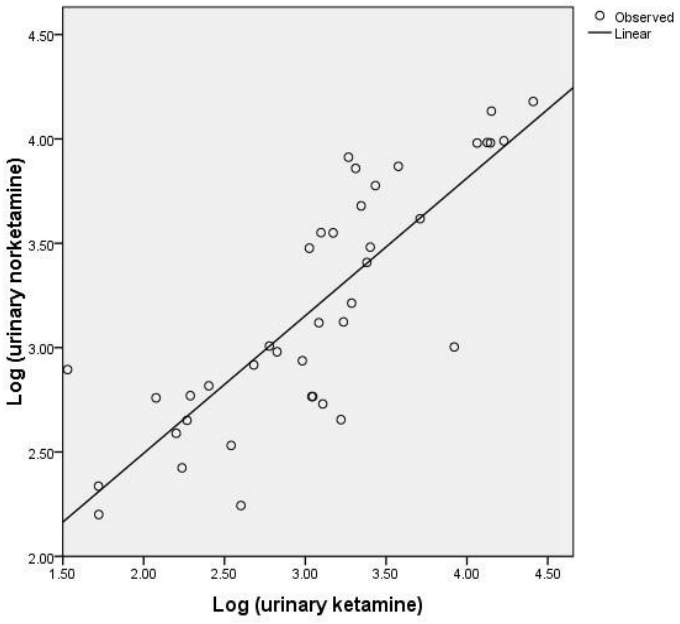

Figure 1. Scatter plot with trend line for the relationship between Log (urinary norketamine) and Log (urinary ketamine).

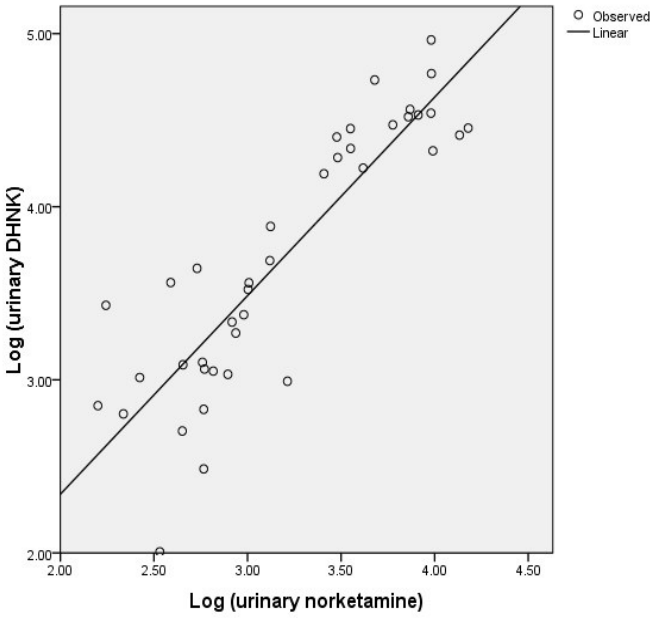

Figure 2. Scatter plot with trend line for the relationship between Log (urinary DHNK) and Log (urinary norketamine).

Table 7. Concomitant drugs detected with ketamine classified by the causes of death.

Concomitant drugs	RTI	Drug intoxication	Non-RTI	Total
Antihistamine	10	10	5	25 (62.5%)
Mitragynine	12	8	4	24 (60.0%)
Methamphetamine	8	3	3	14 (35.0%)
MDMA	7	4	1	12 (30.0%)
Benzodiazepine	4	6	1	11 (27.5%)
Tramadol	2	5	1	8 (20.0%)
Cannabis	3	2	2	7 (17.5%)
Heroin	1	5	0	6 (15.0%)
Antidepressant	0	1	2	3 (7.5%)

MDMA, 3,4-methylenedioxy methamphetamine; RTI, road traffic injury.

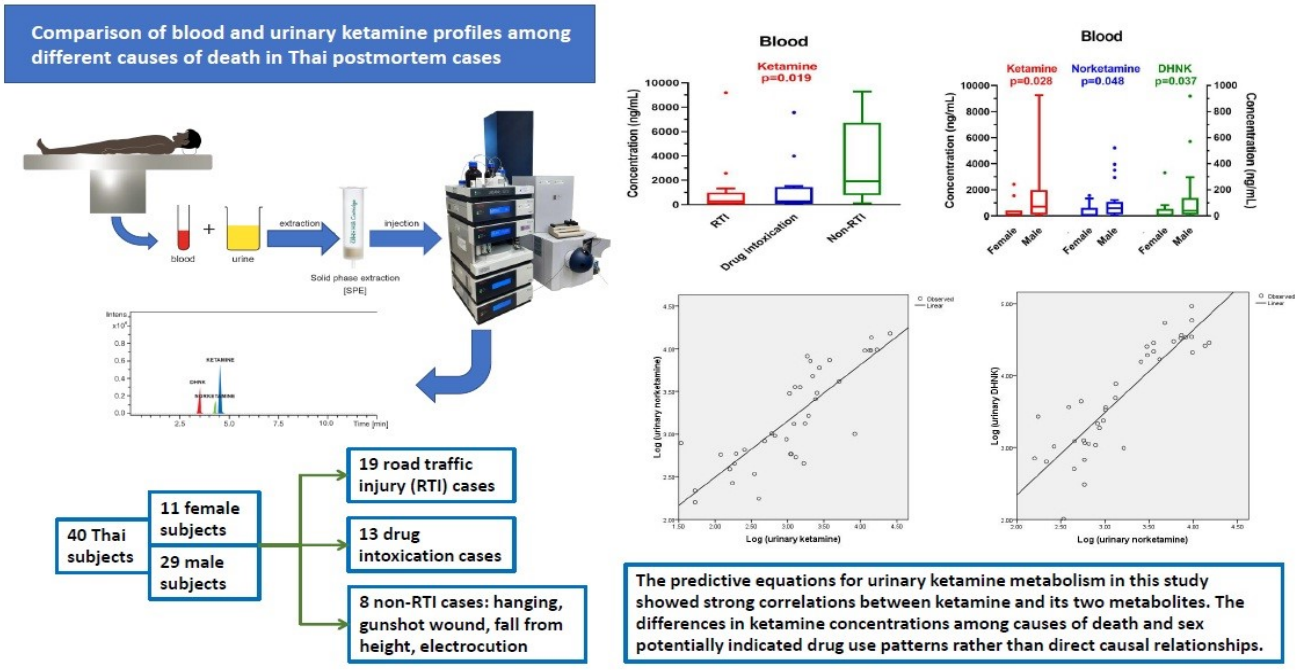

Figure 3. Summary of overall results in this study.

Log (urinary norketamine) = 1.175(Log (urinary ketamine)) + 0.659; $P < 0.001$; $r^2 = 0.684$
Log (urinary DHNK) = 0.045(Log (urinary norketamine)) + 1.147; $P < 0.001$; $r^2 = 0.773$

When the BAC was considered, only five RTI subjects (5/19, 26.32%) were found to have a positive BAC, whereas all subjects who died from drug intoxication and non-RTI unnatural causes had a negative BAC. Among the five subjects who tested positive for BAC, one subject had a BAC < 50 mg/dL (27.2 mg/dL), while the other four subjects had a BAC ranging from 82.2 to 204.5 mg/dL. Furthermore, the concomitant drugs detected in the subjects in this study revealed that only seven subjects (17.5%) used ketamine alone. The percentage of subjects using ketamine alone in the RTI, drug intoxication, and non-RTI groups was 21.1% (4/19), 15.4% (2/13), and 12.5% (1/8), and there was no significant difference among these ($P = 0.842$). The total number of drugs detected in the subjects in this study ranged from 1 to 10 drugs, and 20 subjects (50.0%) had drug profiles that included 3–5 drugs. The average number of drugs present in the RTI, drug intoxication, and non-RTI groups was 3.7 ± 1.9, 5.2 ± 2.9, and 3.9 ± 2.3, respectively, and this did not exhibit a significant difference ($P = 0.286$). The most common drugs found in the subjects were antihistamines, followed by mitragynine, methamphetamine, MDMA, and benzodiazepines, as shown in **Table 7**. The results of this study are summarized in **Figure 3.**

Discussion

This study investigated the ketamine profiles in blood and urine samples from Thai postmortem cases. It was found that the cause of death and the subject's sex were the two main factors that influenced the ketamine concentration profiles in the blood and urine samples of the Thai subjects in this study. Regarding the cause of death, the blood ketamine concentrations and the urinary concentrations of both ketamine metabolites were significantly higher in the non-RTI group than in the RTI and drug intoxication groups. There is no data available from previous research related to blood and urine ketamine profiles in this group, as most previous studies related to ketamine abuse focused on drug intoxication. However, the authors hypothesized that the cause of death might have partially played an important role in the ketamine profiles in the non-RTI group because seven out of the eight cases in the non-RTI group were non-accidental deaths [five cases (62.5%) were suicide and two cases (25.0%) were homicide], whereas the RTI and drug intoxication groups in this study were accidental deaths. Further studies should be conducted with a focus on ketamine profiles in non-accidental deaths, such as suicidal and homicidal cases, to expand on and elucidate these findings.

Another factor that might have affected the higher ketamine profiles in the non-RTI group was the effect of drug combinations. The majority of subjects in this study used ketamine combined with other drugs (33/40 subjects, 82.5%). This finding is consistent with previous studies that presented figures for poly-drug use in ketamine abuse in the range of 53.0–97.4%. [8,10-12] This combination of drug use might affect the ketamine concentration profiles, especially in the blood samples. Although the pattern of poly-drug use in this study did not produce any significant differences among the cause of death groups considered, it is notable that 26.3% of subjects in that RTI group combined ketamine with alcohol, while the drug intoxication group presented a higher average number of subjects that exhibited ketamine use in combination with other drugs. This combined effect of alcohol and poly-drug use might be related to the significantly lower blood ketamine concentrations in these two groups compared with the non-RTI group.

The main cause of death in this study was death related to RTI, whereas the majority of ketamine-related deaths in other studies were accidental drug intoxication, and the proportion of RTI deaths in previous studies was < 10.0%. [11, 12] This study found that the mean and median blood ketamine concentrations in the RTI cases were 916.6 and 273.5 ng/mL, respectively. A previous study focusing on ketamine in oral fluid (reflecting blood ketamine levels 1–2 h after exposure) and urine in drivers reported mean and median oral fluid ketamine concentrations of 1,055 and 271 ng/mL, respectively. [13] These concentration profiles are comparable to the blood ketamine profiles in the RTI cases in this study. In addition, this previous study indicated that ketamine concentrations in oral fluid > 300 ng/mL and urine >900 ng/mL showed signs of driving impairment, as determined by a field impairment test. [13] These cut-off levels should be studied further in the Thai population.

For ketamine-related drug intoxication, most ketamine-related deaths were found to be accidental intoxication, which is consistent with the findings of this study.[8, 10-12] Corkery, *et al.* reported that the mean blood ketamine concentration in ketamine-related deaths where ketamine was the only compound was 4,757 ng/mL (107–32,000 ng/mL), while the mean blood ketamine concentration in poly-drug use cases was 1,418 ng/mL (11–24,730 ng/mL). [11] As drug intoxication from using ketamine alone in this study only related to 15.4% of the cases (2/13), the mean blood ketamine concentration in the drug intoxication cases in this study, calculated from the total drug intoxication cases, was 1,272.8 ng/mL (29.3–7,564.5 ng/mL). This is consistent with the study of Corkery, *et al.,* where poly-drug use with ketamine was considered. [11]

The subject's sex was found to be an important factor that contributed to the concentration of ketamine and its metabolites, especially in the blood. Male subjects presented with significantly higher blood ketamine, norketamine, and DHNK concentrations than females. However, the urinary ketamine profiles in males did not differ significantly from those in females. According to a previous study, it was found that sex did not affect the pharmacokinetic parameters of ketamine regardless of the administration of either a single dose of esketamine or a racemic mixture of ketamine. [14] Therefore, this finding potentially resulted from the patterns of ketamine use, whereby the male subjects had different patterns of use from those of females regarding the form, amount, and frequency of ketamine use, as well as the route of

ketamine administration. According to the study design and statistical analysis, the difference in blood and urinary ketamine profiles among the causes of death and between male and female subjects might only reflect the drug use patterns in Thai individuals rather than direct causal relationships. Therefore, the history of ketamine use should be explored further for the interpretation of ketamine profiles in biological samples.

The patterns of poly-drug use in ketamine abuse in this study were different from those in other studies. The three most common drugs found with ketamine in this study were antihistamine, mitragynine, and methamphetamine. However, the study by Darke, *et al.* found that opiates and opioids, sedative-hypnotic drugs, and stimulants (such as methamphetamine, MDMA, and cocaine) were the three most common drug groups found in ketamine-related deaths in Australia. [12] Corkery, *et al.* reported that illicit stimulants, opioids, and anxiolytics/hypnotics were common concomitant substances found in ketamine-related deaths in the UK. [11] Vivolo-Kantor, *et al.* reported that illegally manufactured fentanyl, methamphetamine, and cocaine were commonly detected in ketamine-related deaths in the USA. [8] These findings reflected the different patterns of poly-drug use with ketamine, revealing that this often depends on the geographical location. Furthermore, this study revealed that the mean urinary ratios of norketamine/ketamine ranged from 1.6 to 2.9, and the mean ratios of DHNK/ketamine ranged from 6.2 to 10.6. These results were consistent with a previous study. [7] In addition, there was no significant difference in the urinary ratios of norketamine/ketamine and DHNK/ketamine among the different causes of death in this study. Thus, urinary ratios of ketamine and its metabolites might not be related to the causes of death. Moreover, this study presented the linear regression curves between the logarithms of the urine concentrations of ketamine/norketamine and norketamine/DHNK. These two equation models presented relatively high r^2 values and could be used for the prediction of ketamine and its two metabolites in urine. This predictive data may be employed with a history of ketamine exposure to assist in the interpretation of the timeframe and the effect of ketamine exposure. Furthermore, this may provide a quantitative framework for forensic toxicologists to interpret ketamine exposure and metabolism in postmortem cases.

This study has some notable limitations. First, the sample size in this study was relatively small, especially the groups related to drug intoxication and unnatural non-RTI deaths. Second, the majority of subjects included in this study were male, which may have affected the comparisons with the female ketamine profiles. These two factors might have had an effect on the average concentrations of ketamine and its metabolites in the blood and urine samples. Thus, future research should include more diverse sample populations, especially female subjects, as well as cases of drug intoxication, suicide, and homicide, to confirm this result. Finally, the majority of subjects in this study exhibited poly-drug use, which might have directly affected the ketamine profiles in the biological samples. Therefore, further studies should be performed on subjects who use ketamine alone to determine their blood and urinary ketamine profiles.

Conclusion

The RTI and drug intoxication groups in this study exhibited significantly lower blood ketamine concentrations and urinary concentrations of the two main ketamine metabolites than the non-RTI group. In addition, the blood samples from male subjects had significantly higher concentrations of ketamine and its metabolites than those of the female subjects. Moreover, this study developed predictive equations for urinary ketamine metabolism, demonstrating strong correlations between the ketamine, norketamine, and DHNK levels. These equations provide a forensic framework for interpreting ketamine concentrations in postmortem cases. While differences in ketamine concentrations were observed across causes of death and sex, these differences likely reflected drug use patterns rather than direct causal relationships.

Acknowledgments

The authors gratefully thank Asst. Prof. Dr. Chulaluk Komoltri for her invaluable assistance and advice for the statistical analysis. This research project was supported by the Siriraj Research Development Fund (Grant No. (IO) R016731045), Faculty of Medicine, Siriraj Hospital, Mahidol University.

Conflicts of interest

All authors have completed and submitted the International Committee of Medical Journal Editors Uniform Disclosure Form for Potential Conflicts of Interest. None of the authors had any conflict of interest to disclose.

Data sharing statement

All data generated or analyzed in this study are included in this published article. Further details are available for non-commercial purposes from the corresponding author on reasonable request.

References

1. Gitlin J, Chamadia S, Locascio JJ, Ethridge BR, Pedemonte JC, Hahm EY, et al. Dissociative and analgesic properties of ketamine are independent. Anesthesiology 2020;133:1021–8.
2. Lynam M, Keatley D, Maker G, Coumbaros J. The prevalence of selected licit and illicit drugs in drug facilitated sexual assaults. Forensic Sci Int Synerg 2024;9:100545.
3. Palamar JJ, Salomone A. Trends and correlates of discordant reporting of drug use among nightclub/festival attendees, 2019-2022. Clin Toxicol (Phila) 2023;61:665–73.
4. Kanato M, Leyatikul P, Wonguppa R. Substance use among Thai teenagers, 2022. ONCB J 2022;39:33–44.
5. Worasuwannarak W, Sribanditmongkol P, Aramrattana A, Junkuy A, Chaijaruwanich J, Kummaraka U, et al. Trends in substance abuse detection in unnatural deaths in Thailand, 2007-2018. ONCB J 2019;35:44–51.
6. Schep LJ, Slaughter RJ, Watts M, Mackenzie E, Gee P. The clinical toxicology of ketamine. Clin Toxicol (Phila) 2023;61:415–28.
7. Moore KA, Sklerov J, Levine B, Jacobs AJ. Urine concentrations of ketamine and norketamine following illegal consumption. J Anal Toxicol 2001;25:583–8.
8. Vivolo-Kantor AM, Mattson CL, Zlotorzynska M. Notes from the field: ketamine detection and involvement in drug overdose deaths - United States, July 2019-June 2023. MMWR Morb Mortal Wkly Rep 2024;73:1010–2.
9. Scientific Working Group for Forensic Toxicology. Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. J Anal Toxicol 2013;37:452–74.
10. Chaves TV, Wilffert B, Sanchez ZM. Overdoses and deaths related to the use of ketamine and its analogues: a systematic review. Am J Drug Alcohol Abuse 2023;49:141–50.
11. Corkery JM, Hung WC, Claridge H, Goodair C, Copeland CS, Schifano F. Recreational ketamine-related deaths notified to the National Programme on Substance Abuse Deaths, England, 1997-2019. J Psychopharmacol 2021;35:1324–48.
12. Darke S, Duflou J, Farrell M, Peacock A, Lappin J. Characteristics and circumstances of death related to the self-administration of ketamine. Addiction 2021;116:339–45.
13. Cheng WC, Ng KM, Chan KK, Mok VK, Cheung BK. Roadside detection of impairment under the influence of ketamine-evaluation of ketamine impairment symptoms with reference to its concentration in oral fluid and urine. Forensic Sci Int 2007;170:51–8.
14. Wang J, Huang J, Yang S, Cui C, Ye L, Wang SY, et al. Pharmacokinetics and safety of esketamine in Chinese patients undergoing painless gastroscopy in comparison with ketamine: a randomized, open-label clinical study. Drug Des Devel Ther 2019;13:4135–44.