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Comparison of Blood and Urinary Cannabis Profiles Between Road Traffic Injury (RTI) and Other Causes of Death in Thai Postmortem Cases

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ABSTRACT

OBJECTIVE This study aims to compare blood and urinary cannabis profiles between road traffic injury (RTI) and other unnatural causes of death in the Thai population.

METHODS A cross-sectional study was conducted of Thai postmortem cases where the individual who died was 15 years old or over. Sex, age, cause of death, manner of death, blood alcohol concentration (BAC), and concomitant drugs found were documented for each case. Blood and urinary concentrations of delta9-tetrahydrocannabinol (THC) and its two metabolites, 11-hydroxy-delta9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-delta9-tetrahydrocannabinol (THC-COOH), were analyzed using gas chromatography tandem mass spectrometry (GC-MS/MS). Statistical analysis was performed using the Mann-Whitney U test and Kruskal-Wallis H test.

RESULTS A total of 80 subjects were included in this study, comprising 43 RTI and 37 non-RTI subjects. All the RTI cases were motorcycle riders. Blood concentrations of THC, 11-OH-THC, and THC-COOH in the RTI group were significantly higher than those in the non-RTI group ($p < 0.01$). The number of subjects who had recent cannabis exposure in the RTI group was significantly higher than in the non-RTI group ($p< 0.05$). Furthermore, the blood concentrations of THC, 11-OH-THC, and THC-COOH in subjects who used cannabis without other drugs of abuse or medications were significantly higher than those in subjects who used cannabis with other drugs of abuse or other medications ($p < 0.05$).

CONCLUSIONS Blood THC, 11-OH-THC, and THC-COOH concentrations in RTI cases were significantly higher than in cases with other unnatural causes of death.

KEYWORDS cannabis, road traffic injury (RTI), motorcycle rider, Thai

INTRODUCTION

Cannabis is one of the most common drugs of abuse used by adolescents and adults worldwide. According to figures from the United States (US), an estimated 48 million people 12 years old or over had experience of cannabis use in 2019 (1). In 2019 the Thai government initiated a public policy to authorize cannabis use for medical purposes and has allowed cannabis use at home as well as cannabis home cultivation since 2022 (2). A previous study in Thailand suggested that the prevalence of lifetime cannabis use in Thai

people in 2011 was 5.05%, while the prevalence of cannabis use in the previous year was 0.20% (3). Subsequently, following amendments to government policy related to cannabis, the number of users has risen, with a recent study reporting that approximately 15.00% of Thai people had used cannabis (around 7.5 million people 20 years old or over) (4). In addition, a 2024 study focusing on chronic pain patients found that the prevalence of active cannabis use was 15.00% in chronic cancer pain cases and 3.10% in non-cancer pain cases (5). Moreover, the prevalence of cannabis use in Thai people who were 18-65 years old has also increased, from about 4.00% in 2021 to about 25.00% in 2022 (2). These figures suggest that the current trend of cannabis use in Thailand is increasing.

Cannabis contains an important psychoactive substance called delta9-tetrahydrocannabinol (THC). When THC enters the bloodstream, it is metabolized into two main metabolites: 11-hydroxy-delta9-tetrahydrocannabinol (11-OH-THC) and 11-Nor-9-carboxy-delta9-tetrahydrocannabinol (THC-COOH) (6). The presence of blood THC concentrations greater than 2-3 ng/mL and a ratio of THC-COOH/THC > 1 suggests recent cannabis exposure (within the past 6-8 hours) (6). Additionally, it has been suggested that chronic cannabis users could have residual blood THC concentrations of <2 ng/mL at 12 hours after cannabis use (6). The presence of THC and/or 11-OH-THC in blood and/or urine could also be used as a marker of recent cannabis use (6). Thus, the concentrations of THC, 11-OH-THC, and THC-COOH in blood and urine are important for estimating the time of cannabis exposure, where confirmation of such exposure could be used for implying clinical impairment.

According to legislation in the US, Canada, and the United Kingdom (UK), THC concentrations of 2 to 5 ng/mL are used to indicate driving under the influence of cannabis (7). A previous study found that the mean and median whole blood THC concentrations in all drivers arrested for driving under the influence of drugs (DUID) were 4.9 and 3 ng/mL, respectively, while the mean and median whole blood THC concentrations in all drivers who died while driving with cannabis in their postmortem blood were 11.7 and 4.5 ng/mL, respectively (8). That study suggested that most DUID cases were recent cannabis users, and reported that the whole blood THC concentrations in postmortem cases were higher than those in drivers arrested for DUID (8). Another study found that the majority of motorists tested who were suspected of DUID had whole blood THC concentrations between 1-6 ng/mL. This finding also suggests that most of the drivers had recently used cannabis (9).

Currently, there is still a lack of information about the cannabis concentration profiles in Thai people. Consequently, the present study aimed to investigate the concentration profiles of THC, 11-OH-THC, and THC-COOH in blood and urine samples obtained from road traffic injury (RTI) cases, focusing on motorcycle riders, who comprise the majority of RTI cases in Thailand, and comparing that with samples obtained from Thai people who had other unnatural causes of death. This information was collected to elucidate the levels of cannabis exposure in these two groups. It is expected that the study findings and data will be useful for further research on cannabis exposure in the Thai population.

METHODS

Study design and data collection

A cross-sectional study was conducted of medico-legal cases sent for autopsy at the Department of Forensic Medicine, Siriraj Hospital, Mahidol University. Thai postmortem cases that were at least 15 years old at the time of death and which had been sent for autopsy between May 1, and October 31, 2024, were recruited. Individuals who were dead at the scene and who had undergone the autopsy procedure within 24 hours after death were included. All cases that were positive for THC and/or its two metabolites in either blood or urine were included. The exclusion criteria were decomposed bodies and bodies with extensive injuries, such as injuries from being run over by a motor vehicle. All cases that were suspected of being cannabis-related sudden cardiac death or cannabis intoxication were excluded.

This study was approved by the Siriraj Institutional Review Board, Faculty of Medicine, Siriraj Hospital, Mahidol University (COA No. Si 937/2023, SIRB protocol No. 910/2566 (IRB2)).

Chemicals and reagents

THC, 11-OH-THC, and THC-COOH at a concentration of 1 mg/mL were purchased from LGC (LGC Standards, Teddington, England) and THC-COOH-d3 at a concentration of 100 µg/mL was also sourced from LGC. LC-MS-grade methanol, acetonitrile, ethyl acetate, and n-hexane were acquired from Duksan Pure Chemicals Co., Ltd. (Gyeonggi-do, South Korea.) Acetic acid was purchased from vWR, Radnor, PA, USA. N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Solid phase extraction (SPE) Oasis® MAX cartridges (60 mg, 3 mL) were obtained from Waters (Waters Oasis, Wexford, Ireland). All other chemicals and reagents were provided by U&V Holding (Thailand) Co., Ltd., Nonthaburi, Thailand. The deionized water (dH_2O) used in this study was produced using a Merck Millipore Direct-Q® 3 UV-R Water Purification System (Burlington, MA, USA).

Instrumentation

Gas chromatography-triple quadrupole tandem mass spectrometry (GC-MS/MS) was performed using a Thermo Scientific™ TSQ™ 9000 Triple Quadrupole GC-MS/MS system (Waltham, MA, USA), with the GC utilizing an HP5-MS (30 m × 0.25 mm ID coated with a 0.25 µm film) capillary column. The GC conditions were set according to the temperature program. The initial temperature was 150°C, for 3 minutes, then the temperature was elevated to 220°C at a rate of 40°C/minute, followed by an increase to 300°C at a rate of 7°C/minute and then holding there for 5 minutes. Helium was used as the carrier gas at a flow rate of 1.3 mL/minute. The injector and transfer line temperatures were set at 150°C and 250°C, respectively. The mass analyzer was operated in the electron ionization mode at 70 eV and the ion source temperature was programmed at 230°C. The selected reaction monitoring (SRM) mode was applied, as described in Table 1.

Sample preparation and extraction procedure

Blood samples were collected from the femoral vein, while urine samples were aspirated from the urinary bladder during the autopsy for collection. Then, 10 mL of blood was put into a red-capped blood tube while 30 mL of urine was put into a plastic bottle. All the blood and urine samples were stored at 4°C in the laboratory and then analyzed for THC and its metabolites the next day by GC-MS/MS.

Solid phase extraction (SPE) was achieved using Waters Oasis® MAX cartridges (60 mg, 3 mL) (Waters Oasis, Wexford, Ireland). The SPE cartridges were preconditioned with methanol and dH_2O. Then, the prepared samples was loaded into the cartridges. Next, a washing step was performed with 2 mL of 50% methanol followed by 2 mL of n-hexane. After drying the SPE cartridges for 5 minutes, the target analytes were eluted using 2 mL of an elution solvent mixture (49% n-hexane: 49% ethyl acetate: 2% acetic acid). Then the eluent was dried under a nitrogen stream at 40°C. Next, the derivatization process was performed by adding 50 µL of BSTFA containing 1% TMCS into the residue. The incubation process was performed at 70°C for 40 minutes, then the cartridges were left to cool at room temperature at the end of the process of derivatization. Finally, the derivatized product was injected into a GC-MS/MS system for analysis.

Method validation

Method validation was conducted following the Standard Guidelines in Forensic Toxicology (10). The expired whole blood used in the method validation for blood was obtained from the Department of Transfusion Medicine, Siriraj Hospital,

Table 1. Selected reaction monitoring (SRM) mode for THC and its metabolites

Analytes	Quantifier ion	Qualifier ion 1	Qualifier ion 2	Collision energy (eV)	Retention time (min)
THC	371.2 > 289.2	371.2 > 305.2	371.2 > 265.2	15 > 10 > 10	10.04
11-OH-THC	371.2 > 289.2	371.2 > 305.2	371.2 > 265.2	15 > 10 > 10	12.20
THC-COOH	371.2 > 289.2	371.2 > 305.2	371.2 > 265.2	15 > 10 > 10	13.62
THC-COOH-d_3	374.3 > 292.2	374.3 > 308.2	374.3 > 268.2	15 > 10 > 10	13.60

THC, delta9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-delta9-tetrahydrocannabinol; THC-COOH, 11-Nor-9-carboxy-delta9-tetrahydrocannabinol

Mahidol University, while synthetic urine was used for the method validation for urine. A complete chromatographic separation of THC and its metabolites from the endogenous baseline noise was carried out during the interference studies. In addition, other drugs of abuse, including methamphetamine, amphetamine, 3,4-methylenedioxy methamphetamine (MDMA), 3,4-methylenedioxy amphetamine (MDA), cocaine, benzoylecgonine, 6-acetylmorphine, codeine, morphine, fentanyl, methadone, tramadol, ketamine, mitragynine, lysergic acid diethylamide (LSD), phencyclidine (PCP), and psilocybin were tested to ascertain whether they would interfere with the target analysis during the THC analysis. The limit of detection (LOD) and lower limit of quantitation (LLOQ) were evaluated for THC, 11-OH-THC, and THC-COOH in both the blood and urine samples. Finally, the LOD and LLOQ for combined THC, 11-OH-THC, and THC-COOH were set at 0.1 and 0.2 ng/mL, respectively.

The linearity ranges for THC, 11-OH-THC, and THC-COOH in the blood and urine samples were obtained at concentrations of 0.2, 0.5, 1, 2, 5, 10, and 20 ng/mL. Calibration curves for THC, 11-OH-THC, and THC-COOH were obtained using Thermo ScientificTM (Waltham, MA, USA) TraceFinderTM 5.0 SP1 Software®. For the linearity range criteria, a coefficient of determination (r2) ≥ 0.99, an accuracy of each calibrator within ±15% (LLOQ ± 20%), and a % coefficient of variation (%CV) ≤ 15% were achieved. A summary of the criteria of the linearity ranges for THC and its two metabolites in blood and urine in this study is shown in Table 2.

Accuracy and precision were evaluated for the spiked blood and urine samples at three quality control (QC) concentrations, 0.6, 6, and 15 ng/mL. The accuracy of each QC concentration should be within ± 15% and the precision evaluated by the %CV should also be ≤ 15%. The obtained accuracy and precision of these three concentrations of THC, 11-OH-THC, and THC-COOH in blood and urine are shown in Table 3 and Table 4, respectively. Moreover, the dilution integrity was evaluated for a dilution factor of 1:5 for THC and THC-COOH and 1:50 for THC-COOH due to the high concentrations of THC-COOH in the authentic samples. Accuracy and precision testing was also performed and all the results, both in the blood and urine samples, passed the criteria of acceptability of an accuracy within ± 15% and precision evaluated by a %CV ≤ 15%.

Statistical analysis

Statistical analysis was conducted using IBM SPSS® Statistics for Windows version 25. Descriptive statistics, including the mean, median, and standard deviation (SD), were calculated. The Kolgomorov-Smirnov test was used for normality testing of the blood and urinary profiles of cannabis and its metabolites. It was found that the blood and urinary cannabis and its metabolites were not normally distributed. Thus, comparisons of the blood and urinary concentrations of cannabis and its metabolites were performed using the Mann-Whitney U test and Kruskal-Wallis H test where appropriate. Contingency table chi-square analysis was used for comparison of the number of subjects between the recent and non-recent cannabis exposure cases and for comparison of the number of subjects who had used other drugs with cannabis in the RTI and non-RTI groups, respectively.

Table 2. Summary of the linearity ranges for THC, 11-OH-THC, and THC-COOH in blood and urine

Analyte	Range (ng/mL)	Linear regression equation		r^2
		Slope	Intercept	
Blood				
THC	0.2-20	0.047998	+0.017198	≥0.99
11-OH-THC	0.2-20	0.030840	+0.026120	≥0.99
THC-COOH	0.2-20	0.012535	+0.075585	≥0.99
Urine				
THC	0.2-20	0.032106	+0.016848	≥0.99
11-OH-THC	0.2-20	0.033484	+0.054792	≥0.99
THC-COOH	0.2-20	0.013934	+0.030306	≥0.99

THC, delta9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-delta9-tetrahydrocannabinol; THC-COOH, 11-Nor-9-carboxy-delta9-tetrahydrocannabinol

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

QC concentration (ng/mL)	Accuracy (%) (n=5)	Precision (n=5)	
		Intra-day (%)	Inter-day (%)
THC			
0.6	90.33-111.67	≤12.47	≤11.67
6	89.17-112.67	≤12.58	≤11.84
15	86.67-110.33	≤11.33	≤10.18
11-OH-THC			
0.6	88.33-113.67	≤12.74	≤13.63
6	87.67-112.17	≤13.62	≤12.37
15	90.58-113.83	≤11.81	≤12.53
THC-COOH			
0.6	89.33-112.33	≤11.67	≤13.05
6	87.17-111.33	≤11.94	≤12.83
15	88.58-113.08	≤10.42	≤13.17

THC, delta9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-delta9-tetrahydrocannabinol; THC-COOH, 11-Nor-9-carboxy-delta9-tetrahydrocannabinol

Table 4. Accuracy and precision of three QC concentrations in urine samples

QC concentration (ng/mL)	Accuracy (%) (n=5)	Precision (n=5)	
		Intra-day (%)	Inter-day (%)
THC			
0.6	87.67-113.33	≤13.67	≤12.85
6	91.83-114.33	≤13.58	≤11.45
15	91.67-113.42	≤10.17	≤9.64
11-OH-THC			
0.6	86.33-112.33	≤13.33	≤12.57
6	88.83-113.17	≤12.17	≤12.47
15	93.58-109.08	≤10.08	≤11.64
THC-COOH			
0.6	88.33-111.33	≤12.33	≤13.12
6	89.67-112.67	≤11.63	≤12.67
15	93.33-113.75	≤12.83	≤13.04

THC, delta9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-delta9-tetrahydrocannabinol; THC-COOH, 11-Nor-9-carboxy-delta9-tetrahydrocannabinol

Table 5. Comparison of the causes of death and age ranges between the RTI and non-RTI groups

Group	N	Mean ± SD, median (years old)	Range (yrs old)	*p*-value
RTI	43	27.14±10.06, 25.00	15-66	0.020
Non-RTI	37	34.81±14.75, 31.00	15-79	
Total	80	30.69±12.95, 27.00	15-79	

RTI, road traffic injury

RESULTS

Overall, 80 subjects were included in this study, comprising 43 RTI cases (53.75%) and 37 non-RTI cases (46.25%). There were only 3 females (3.75%) and 77 males (96.25%). The mean age of all the subjects at death was 30.69 ± 12.95 years (range = 15-79). All the RTI cases were motorcycle riders. The age of the subjects in the RTI group was significantly lower than that of the non-RTI group according to the Mann-Whitney U test (p = 0.020), as shown in Table 5. The non-RTI group consisted of cases of hanging, gunshot wounds, stab wounds, drowning, falls from height, electrocution, drug intoxication, carbon monoxide poisoning, and homicidal blunt head trauma. Information on the non-RTI group in this study is provided in Table 6. Comparison of the age of the subjects among three manners of death in the non-RTI group did not show any statistically significant difference according to the Kruskal-Wallis H test (p = 0.340).

Comparison of the cannabis profiles in blood and urine samples from the RTI and non-RTI groups found that the concentrations of THC and its two metabolites in blood in the RTI group were significantly higher than in the non-RTI group, whereas the concentrations of THC and its two metabolites in urine in these two groups did not show any statistically significant difference (Table 7). In addition, when classifying the cannabis profiles as recent and non-recent cannabis exposure based on the presence of THC and/or 11-OH-THC in blood and/or urine, it was found that 90.70% (39/43) of the subjects in the RTI group had recent cannabis exposure, compared to 72.97% (27/37) of the subjects in the non-RTI group. Contingency table chi-square analysis showed that the number of subjects who had recent cannabis exposure in the RTI group was significantly higher than in the non-RTI group (p = 0.044).

As the urinary cannabis profiles did not show any significant differences, the blood cannabis profiles were further analyzed for comparison among four groups: RTI, suicide, homicide, and other accidental deaths. The Kruskal-Wallis H test showed that there were significant differences in the blood THC, 11-OH-THC, and THC-COOH among these four groups (Table 8). Furthermore, when cut-off concentrations of blood THC at ≥2 ng/mL and ≥5 ng/mL were applied, it was found that 76.74% (33/43) and 58.14% (25/43) of the subjects in the RTI group had blood THC concentrations ≥2 ng/mL and ≥5 ng/mL, respectively.

Table 6. Information on the non-RTI group

Non-RTI group		N	Age	
Manner of death	**Cause of death**		**Mean ± SD, median (years old)**	**Range (years old)**
Suicide	Hanging, gunshot wound, fall from height, carbon monoxide poisoning	14	35.14±16.76, 30.50	17-79
Homicide	Gunshot wound, stab wound, blunt head trauma	9	29.00±11.59, 30.00	15-46
Other accident	Drowning, fall from height, electrocution, drug intoxication	14	38.21±14.26, 37.00	21-66
Total		37	34.41±14.95, 30.00	15-79

RTI, road traffic injury

Table 7. Comparison of the cannabis profiles in blood and urine between the RTI and non-RTI groups

Cannabis profiles	Mean ± SD, median (Range) (ng/mL)		*p*-value
	RTI	**Non-RTI**	
Blood			
THC	9.50±9.40, 7.39 (ND-40.92)	5.31±8.05, 1.38 (ND-35.91)	0.004*
11-OH-THC	1.98±2.16, 1.34 (ND-11.92)	0.84±1.37, 0.42 (ND-7.19)	0.001*
THC-COOH	39.34±35.26, 33.06 (0.63-168.91)	14.29±21.16, 7.04 (ND-109.67)	<0.001*
Urine			
THC	0.15±0.43, ND (ND-2.21)	0.13±0.40, ND (ND-1.76)	0.427
11-OH-THC	0.69±2.98, 0.25 (ND-19.67)	0.28±0.38, ND (ND-1.22)	0.988
THC-COOH	237.82±282.59, 91.25 (1.45-931.78)	139.89±200.52, 60.24 (3.76-860.19)	0.071

*ND, not detected; RTI, road traffic injury, THC, delta9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-delta9-tetrahydrocannabinol; THC-COOH, 11-Nor-9-carboxy-delta9-tetrahydrocannabinol

Table 8. Comparison of the blood cannabis profiles among four manners of death

Blood cannabis profiles	THC (ng/mL)	11-OH-THC (ng/mL)	THC-COOH (ng/mL)
RTI group			
Mean±SD	9.50±9.40	1.98±2.16	39.34±35.26
Median	7.39	1.34	33.06
RTI group			
Mean±SD	8.07±10.29	1.05±1.88	12.14±12.31
Median	4.95	0.53	8.60
Homicide			
Mean±SD	4.57±8.06	0.84±0.93	23.37±34.90
Median	1.16	0.46	14.15
Other accident			
Mean±SD	3.04±4.42	0.66±1.03	10.59±16.19
Median	0.99	0.30	3.29
p-value	0.015*	0.010*	<0.001*

RTI, road traffic injury

When considering the combination of use of alcohol and cannabis, the BAC ranges in the RTI and non-RTI groups were 0-229.85 mg/dL and 0-387.66 mg/dL, respectively, while the mean ± SD BAC in the RTI and non-RTI groups were 39.66 ± 38.36 mg/dL and 38.36 ± 93.02, respectively. The BAC in the RTI group was not significantly different from the BAC in the non-RTI group (p = 0.605). Interestingly, it was found that the majority of subjects in both groups had negative BAC results (72.09% (31/43) of RTI cases and 75.68% (28/37) of non-RTI cases). In addition, focusing on the RTI group, it was found that the median blood THC, 11-OH-THC, and THC-COOH concentrations in the subjects who had a BAC greater than 50 mg/dL were 8.72, 1.66, and 29.28 ng/mL, while the median blood THC, 11-OH-THC, and THC-COOH concentrations in the subjects who had a negative BAC were 5.24, 0.98, and 34.14 ng/mL, respectively. There was no significant difference in blood THC, 11-OH-THC, or THC-COOH concentrations between the subjects who had a BAC greater than 50 mg/dL and subjects who had a negative BAC (p = 0.731, 0.742, and 0.920, respectively).

Of the subjects using cannabis with other drugs, 28.75% (23/80) had used cannabis with other drugs. In the two groups, 18.60% (8/43) of the RTI subjects and 40.54% (15/37) of the non-RTI subjects had used other drugs with cannabis. Contingency table chi-square analysis showed that the number of subjects who had used other drugs with cannabis in the RTI group was significantly lower than that in the non-RTI group (p = 0.031). The mean ± SD and median blood THC concentrations of the subjects who had used cannabis without other drugs or medication were 11.04 ± 9.72 ng/mL and 8.72 ng/mL, while the mean ± SD and median blood THC concentrations of the subjects who had used cannabis with other drugs or medication were 2.77 ± 2.75 ng/mL and 2.68 ng/mL in the RTI group, showing that the blood THC concentrations of the subjects who had used cannabis without other drugs or medication were significantly higher than those of the subjects who had used cannabis with other drugs or medication (p = 0.010). The number of other drugs used with cannabis ranged from 1 to 8 drugs. The most common drugs found with cannabis in this study were mitragynine (13.75%), antihistamine (12.50%), ketamine (11.25%), benzodiazepine (10.00%), and methamphetamine (10.00%). Details of concomitant drugs found with cannabis in this study are shown in Table 9. Although statistical analysis could not be performed because some cells in Table 9 had less than 5 observations, it was found that mitragynine was predominant in the RTI group whereas heroin and benzodiazepine were predominant in the other accident groups (particularly drug intoxication).

Comparing the cannabis profiles in the blood and urine of the subjects who had used cannabis without drugs of abuse or medication with those of the subjects who had used cannabis with drugs of abuse or medication, it was again found that the concentrations of THC and its two metabolites in the subjects who had used cannabis without drugs of abuse or medication were significantly higher than those in the subjects who had used cannabis with drugs of abuse or medication (Table 10). However, comparison of the concentrations of THC and its two metabolites in urine between these two groups did not show any statistically difference (Table 10).

Table 9. Details of concomitant drugs found in subjects positive for cannabis

Drugs	RTI group	Suicide	Homicide	Other accident	Total percentage
Mitragynine	5	2	2	2	13.75% (11/80)
Antihistamine	4	1	2	3	12.50% (10/80)
Ketamine	4	1	1	3	11.25% (9/80)
Methamphetamine	4	1	1	2	10.00% (8/80)
Benzodiazepine	1	0	1	6	10.00% (8/80)
Heroin	2	0	1	4	8.75% (7/80)
Tramadol	1	1	2	3	8.75% (7/80)
Other drugs	1	1	1	4	8.75% (7/80)

RTI, road traffic injury

Table 10. Comparison of the cannabis profiles in blood and urine between the RTI and non-RTI groups

Cannabis profiles	Mean ± SD, median (range) (ng/mL)		*p*-value
	Cannabis without drugs	Cannabis with drugs	
Blood			
THC	9.40±9.79, 7.06 (ND-40.92)	3.03±4.04, 1.16 (ND-13.33)	0.001*
11-OH-THC	1.78±2.13, 0.94 (ND-11.92)	0.65±0.81, 0.30 (ND-2.82)	0.005*
THC-COOH	30.73±33.27, 18.81 (0.63-168.91)	20.38±27.86, 4.45 (ND-109.67)	0.045*
Urine			
THC	0.15±0.43, ND (ND-2.21)	0.12±0.38, ND (ND-1.63)	0.516
11-OH-THC	0.64±2.59, 0.25 (ND-19.67)	0.16±0.29, ND (ND-1.22)	0.075
THC-COOH	210.11±260.19, 93.95 (1.45-931.78)	148.93±227.62, 54.23 (3.76-860.19)	0.108

*ND, not detected; RTI, road traffic injury, THC, delta9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-delta9-tetrahydrocannabinol; THC-COOH, 11-Nor-9-carboxy-delta 9-tetrahydrocannabinol

DISCUSSION

To the best of our knowledge, this is the first study that reports and compares the concentrations of THC and its two metabolites (11-OH-THC and THC-COOH) in blood and urine samples from Thai postmortem cases. As THC and its two metabolites are highly lipophilic molecules, postmortem blood concentrations of THC and its two metabolites are prone to postmortem distribution leading to limitation of interpretation of cannabis profiles in postmortem blood samples (6). For that reason, recent cannabis exposure in this study was interpreted mainly based on the presence of THC and/or 11-OH-THC in blood and/or urine. This study showed that the blood concentrations of THC in RTI cases were significantly higher than those in cases with other causes of death. A previous study reported mean and median blood THC concentrations in fatal RTI cases of 11.7 and 4.5 ng/mL, respectively (8). These figures are comparable to our study, with mean and median blood THC concentrations in the RTI cases of 9.50 and 7.39 ng/mL, respectively. In addition, the present study found that the majority of fatal RTI cases were recent cannabis users which is consistent with previous studies (8, 9). A previous study suggested that drivers who were recent cannabis users and who had a blood THC concentration of 8.2 ng/mL and 13.1 ng/mL showed driving impairment similar to BACs of 50 mg/dL and 80 mg/dL, respectively (11). In addition, a blood THC concentration of 5 ng/mL plus BAC of 50 mg/dL have been reported to produce driving impairment similar to a BAC of 80 mg/dL (11). Drummer, OH et al. reported adjusted odd ratios (OR) for injured drivers with THC levels of 1-4.9, ≥ 5, and ≥ 10 ng/mL of 1.6, 3.2, and 10, respectively, while the adjusted OR for injured drivers with a BAC of 50-100 mg/dL was 5.7 (12). Thus, a common cut-off for blood THC concentrations of 2 ng/mL and 5 ng/mL is applied in many countries in Europe and North America. Based on this finding, it could be implied that the majority of fatal RTI cases in this study were under the influence of cannabis because 76.74% and 58.14% of the subjects in the RTI group had a blood THC concentration of ≥ 2 ng/mL and ≥ 5 ng/mL, respectively. However, additional study should be performed to further elucidate the risk of driving while using cannabis in the Thai population. Currently, investigation of cases of driving under influence in Thailand is mandatory only for alcohol and methamphetamine. This result shows that investigation of driving under influence of cannabis in Thai people should be required because the majority of Thai RTI cases in this study had blood THC concentrations that were comparable to fatal RTI cases in previous studies (8, 9). The best indicator of driving under influence of cannabis was blood samples (7). Oral fluid can be useful because

it is easier to collect, but THC concentration in oral fluid is not closely associated with blood THC concentration, limiting the use of oral fluid for the investigation of driving under influence of cannabis (7). Additionally, blood THC concentrations rapidly decline after exposure, leading to difficulty with blood THC determination. Thus, timing of blood collection is crucial for interpretation and further research should be conducted to elucidate this finding. In addition, the effect of cannabis exposure on driving performance in living Thai people should be studied based on the results of previous reports (11, 12). Such research is fundamental for establishing the association between blood THC concentration and driving performance, and could lead to determination of a cut-off blood THC concentration for driving under influence of cannabis in Thai population.

This study shows that Thai people who used cannabis with other drugs or medication had blood THC, 11-OH-THC, and THC-COOH concentrations significantly less than Thai people who used cannabis without other drugs or medication. There is only limited data about the comparison of blood THC and its metabolite concentrations between cannabis abusers using other drugs or medication and cannabis abusers not using other drugs or medication because most data are focused on common conventional medications that are known to affect cannabinoid compounds (13). Ho, JJY et al.'s review reported that morphine did not have an effect on blood THC concentration (14). Thus, it could be possible that the presence of other drugs of abuse or medication might not directly affect blood THC and its metabolite concentration. However, the use of other drugs of abuse or medication might have an effect in that people may not need to use a high dose of cannabis because of the effect of other drugs of abuse or medication. Further study should be conducted to investigate the interaction between cannabis and other drugs of abuse or medication.

Previous studies have reported that marijuana users are prone to using other drugs, including hallucinogens, inhalants, prescription drugs (pain relievers and sedative-hypnotic drugs), and drugs of abuse (particularly cocaine and methamphetamine) (15, 16). However, the majority of subjects in this study had used cannabis without other drugs of abuse or medication. Kalayasiri, R. et al. reported that kratom (Mitragyna speciosa) leaves and kratom cocktails (which commonly contain antihistamine, tramadol, and benzodiazepine) are often consumed by cannabis users in Thailand (2). This study found that drugs commonly used with cannabis by Thai people include mitragynine (an alkaloid compound from kratom leaves) and antihistamine, consistent with findings from Kalayasiri, R. et al. (2). This result suggests that concomitant drug use with cannabis might depend on different peoples' behavior and geographical area.

Although there was significant difference in blood concentrations of THC and its two metabolites between RTI cases and non-RTI cases, there was no statistically significant difference in urinary concentrations of cannabis profiles between RTI and non-RTI cases. This result could be explained by the pharmacokinetics of cannabis. THC and its two metabolites are lipophilic molecules which can be distributed into adipose tissue and then released for excretion (6). Thus, urinary concentrations of THC and its two metabolites can result from both acute cannabis exposure and from accumulation from chronic cannabis exposure leading to longer than usual detection times of cannabis excretion in urine (6). This indicates that the urinary concentrations of THC and its two metabolites in this study did not come exclusively from acute cannabis exposure before death but also from past cannabis exposure. Desrosiers, NA, et al. reported that occasional cannabis smokers can test positive for THC and its two metabolites in blood for 6-30 hours, whereas frequent cannabis smokers can test positive for THC and its two metabolites in blood for more than 30 hours (17). Compared with urinary cannabis profiles, previous studies have shown that light cannabis smokers can test positive for urinary THC or its two metabolites for 24-120 hours, (18) whereas chronic cannabis users who come to a health facility for rehabilitation can present with positive detection for urinary THC-COOH for up to 30 days (19). When urinary cannabis profiles are affected by the accumulation from the past cannabis use, it can lead to a finding of no significant difference in urinary concentrations of cannabis profiles between RTI and non-RTI cases. Thus, using urinary cannabis profiles for medicolegal interpretation should be done cautiously,

particularly when THC-COOH is found only in urine because THC-COOH can be found for up to 30 days after cannabis exposure in cases of chronic cannabis abuse (19). This suggests that urinary cannabis profiles may not be suitable for proving acute physical impairment, including impaired driving capability. According to current Thai legislation, driving under the influence is compulsory only for the detection of blood alcohol concentration and methamphetamine in urine. Thus, if the government plans to enact new legislation related to driving under influence of cannabis, the use of blood samples should be considered for detection of THC and/or its two metabolites rather than using only urine for investigations.

The main limitation of this study is the disproportionately low number of females, with only three subjects included in this study. Thus, comparison of the blood and urinary cannabis profiles between female and male subjects could not be performed. In addition, the number of subjects who used cannabis with alcohol and drugs of abuse or medication were relatively small compared with the number of subjects who used cannabis without alcohol or drugs of abuse or medication which might have had an effect on the non-parametric statistical analysis. Further studies should be performed which include more female subjects.

CONCLUSIONS

Blood THC, 11-OH-THC, and THC-COOH concentrations in RTI cases are significantly higher than those in cases of other unnatural causes of death. The mean and median blood THC concentrations in the RTI cases were 9.50 and 7.39 ng/mL, respectively. The majority of fatal RTI cases in this study were recent cannabis users. In addition, blood THC, 11-OH-THC, and THC-COOH concentrations in subjects that used cannabis without drugs of abuse or medication were significantly higher than those in subjects who used cannabis with drugs of abuse or medication.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

ADDITIONAL INFORMATION

Author's contribution

P.C.: conceptualization, literature review, methodology, data curation, data analysis, writing - original draft preparation; S.S.: method validation, data curation, data analysis; PP: conceptualization, literature review, methodology, review & editing, supervision. All authors have read and approved the final version of this manuscript that was submitted for publication.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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