

Original article

Determination of central-to-peripheral blood diazepam concentration ratio (C/P ratio) in Thai postmortem cases: a postmortem redistribution study

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Abstract

Background: Interpretation of postmortem blood diazepam concentrations is important in establishing the cause of death. However, this may be complicated by postmortem redistribution (PMR). Currently, there is limited information regarding PMR of diazepam in the Thai people.

Objective: To compare cardiac and femoral blood diazepam concentrations to examine PMR in Thai postmortem cases.

Methods: A prospective study was performed on medico-legal cases sent for autopsy at the Department of Forensic Medicine, Siriraj Hospital. Thai individuals who were 18 years of age or older and had received diazepam during hospitalization were recruited. Cardiac and femoral blood samples were obtained during autopsy. Blood diazepam concentrations were analyzed, and the cardiac-to-femoral blood ratio (C/P ratio) was calculated. Statistical analysis was performed using the Wilcoxon signed-rank test, and the Mann-Whitney *U* test.

Results: Forty two subjects were recruited for this study. The median/mean diazepam concentrations in cardiac and femoral blood were 52.0/83.1 ng/mL and 53.6/69.9 ng/mL, respectively. The median/mean C/P ratio of diazepam was 1.0/1.2. Diazepam concentrations in cardiac blood were not significantly different from those in femoral blood ($P = 0.385$). Although no significant correlation between the C/P ratio and body mass index, postmortem interval, or hospitalization time was observed, the C/P ratio was significantly higher in cases with chest injury than in cases without chest injury ($P = 0.007$).

Conclusion: The median/mean C/P ratio of diazepam in Thai postmortem cases was 1.0/1.2. Chest injury was the only factor that had an impact on the C/P ratio of diazepam.

Keywords: Cardiac blood, diazepam, femoral blood, postmortem redistribution (PMR), Thai

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Diazepam is one of the benzodiazepine drugs that has been commonly detected in medico-legal cases, including suicide, drug intoxication, driving under the influence of drugs, and drug-facilitated crime.⁽¹⁾ Although it is relatively safe, it can produce a synergistic effect when combined with some central nervous system suppressants, such as opioids, and may lead to an increased risk of respiratory depression and death.⁽²⁾ Therefore, interpreting postmortem blood concentrations of diazepam is important for determining the cause of death. However, drug concentrations in blood after death can be altered due to a process known as postmortem redistribution (PMR).⁽³⁾ PMR is a complex phenomenon that produces variations in drug concentrations at different sampling sites and times of blood collection and leads to alterations in drug concentrations after death.^(4, 5) The degree of PMR can be influenced by multiple factors, including the drug's volume of distribution (Vd), physical properties of drugs such as molecular size and their ionization state (acidic/basic), which can be affected by blood pH, and lipophilicity.⁽⁶⁾ These pharmacokinetic properties cause variability in the extent of PMR observed among different drugs, affecting the interpretation of postmortem drug concentrations.

Current standard recommendations for postmortem blood collection involve obtaining blood samples from both the heart and the femoral vein.⁽⁷⁾ Cardiac blood is more easily accessible and typically available in larger volumes; however, it is more prone to PMR due to passive diffusion of drugs from surrounding tissues such as the liver, lungs, and gastrointestinal tract, in the central compartment.⁽⁴⁾ In contrast, femoral venous blood is less susceptible to PMR; therefore, the interpretation of postmortem drug concentrations is commonly based on concentrations found in femoral blood.⁽⁸⁾ However, in cases where femoral venous blood cannot be obtained, interpretation based on cardiac blood concentrations may become necessary. Thus, the study of PMR of various drugs—particularly those that are widely used—can provide information for estimating drug concentrations and contribute to the determination of the cause of death.

Previous studies on PMR of various drugs have typically involved comparing drug concentrations in blood or fluids collected from different parts of the body. One of the most common parameters in PMR studies is the central-to-peripheral blood concentration

ratio (C/P ratio), which compares the drug concentration in central blood (from the heart) to that in peripheral blood (usually from the femoral vein).^(4, 5, 9) Although diazepam is a widely used drug, studies on its PMR are relatively limited. Previous studies have reported median C/P ratios for diazepam of 0.9⁽⁵⁾, 1.1⁽⁸⁾, and 1.2⁽¹⁰⁾, respectively. Additionally, there was only one study that investigated factors affecting changes in diazepam concentrations after death, such as cause of death, postmortem interval (PMI), and body mass index (BMI).⁽⁸⁾ Currently, there is no data on PMR of diazepam in the Thai population.

Therefore, this research aimed to study the PMR of diazepam in Thai postmortem cases by comparing its concentrations in cardiac and femoral blood using the C/P ratio. In addition, this study also focused on factors that potentially influence the C/P ratio of diazepam. These findings will provide fundamental data for the interpretation of diazepam concentrations in Thai postmortem cases and will serve as a basis for further PMR studies in the Thai population.

Materials and methods

Study design and participants

A prospective in vitro laboratory-based study was conducted in this research. All subjects were recruited from medico-legal cases that were sent for autopsy at the Department of Forensic Medicine, Siriraj Hospital, Mahidol University, between 20th January 2025 and 30th September 2025. The postmortem blood samples were obtained from the femoral vein and the left side of the heart during the autopsy procedure (**Figure 1**). The inclusion criteria for this study were Thai individuals who were 18 years of age or older, had been involved in traffic accidents, and had a medical history of receiving diazepam during hospitalization. All subjects had no history of using benzodiazepines prior to hospitalization. The exclusion criteria were Thai individuals who had extensive trauma and decomposed bodies. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the Faculty of Medicine, Siriraj Hospital, with COA No. Si 713/2024 (SIRB Protocol no. 656/2567 (IRB), approval date: 13th September 2024, renewed approval date: 13th September 2025).

All blood samples were collected in sodium fluoride blood tubes. After collection, blood samples were stored in a refrigerator at 4°C and analyzed for diazepam within one week after collection. The

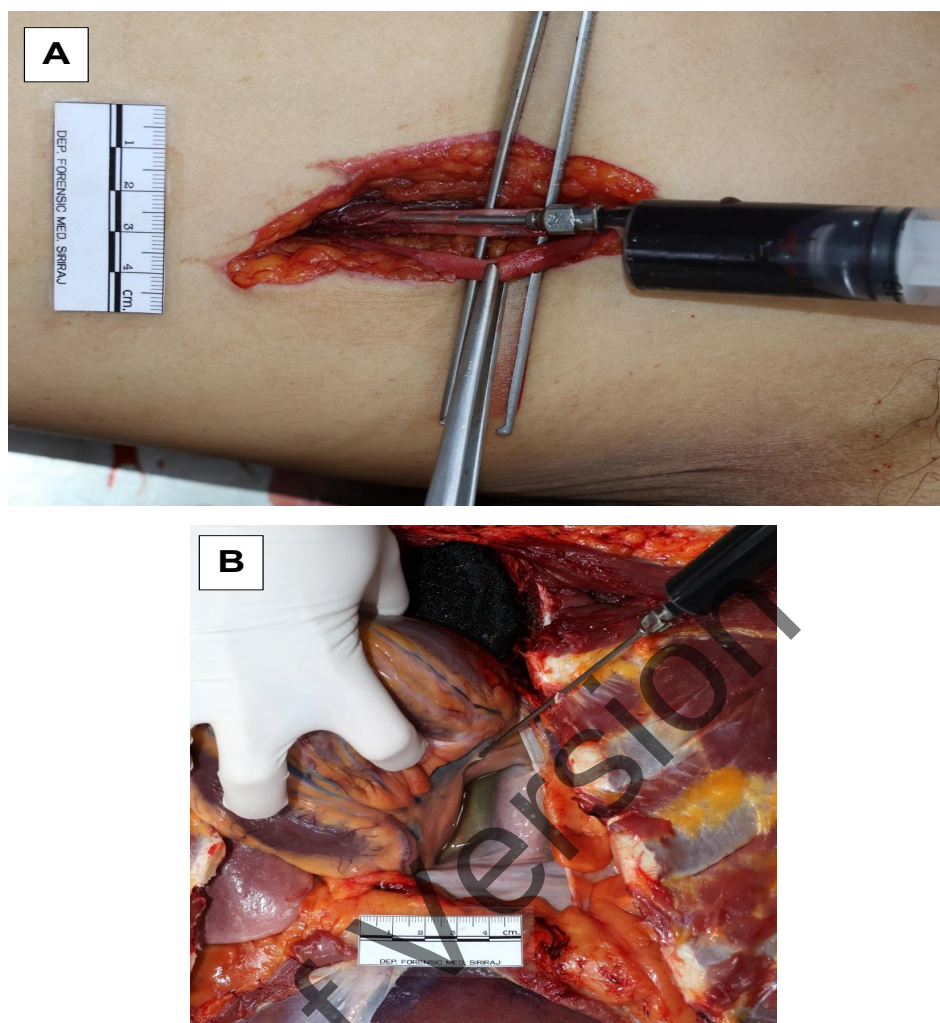


Figure 1. Demonstration of the collection of postmortem blood from the femoral vein (A); and the left side of the heart (B).

analysis was performed in duplicate for both cardiac and femoral blood samples, and the average diazepam concentrations were determined for further statistical analysis. The C/P ratio was then calculated for each case. Case data, including sex, age, weight, height, the postmortem interval (PMI) between the time of death and the time of specimen collection, time of hospitalization, cause of death, and the presence or absence of chest injury (rib fracture, hemothorax, or pulmonary contusion), were recorded. Body mass index (BMI) was calculated using weight divided by the square of height in meters. These data and the C/P ratio were employed for statistical analysis.

Instrumentation

The analysis of diazepam in blood was conducted using electrospray ionization liquid chromatography–quadrupole Time-of-flight mass spectrometry (ESI–LC–QTOF–MS). The condition was adapted from the analytical method described in a previous study.⁽¹¹⁾ Chromatographic separation was performed using a Phenomenex Luna C18 column (10 cm x 2 mm x 3

μm, 100 Å). The gradient elution was carried out with a flow rate of 0.3 mL/min using 5 mM ammonium acetate with 0.1% formic acid in deionized water (dH₂O) (mobile phase A) and acetonitrile (mobile phase B). The gradient program was initiated with mobile phase 75.0% A: 25.0% B, then increasing mobile phase B to 75.0% in 8 minutes, increasing mobile phase B to 90.0% in 0.1 minute and holding 10.0% A: 90.0% B for 3 minutes, back to 75.0% A: 25.0% B within 0.1 minutes and continuing with this condition for 4 minutes. The total run time was 15 minutes. The column temperature was set at 40 °C. The injection volume was 5 μL. Mass spectrometry parameters, broad band collision-induced dissociation (bbCID) mode, and collision energy were set as described in the previous study.⁽¹¹⁾ Multiple reaction monitoring (MRM) transitions for diazepam were 285 > 193 (quantitation) and 285 > 154 (qualifier) whereas MRM transition for diazepam-d₅ was 290 > 198. The retention time for diazepam was 6.9 minutes. The chromatograms of diazepam and diazepam-d₅ are shown in **Figure 2**.

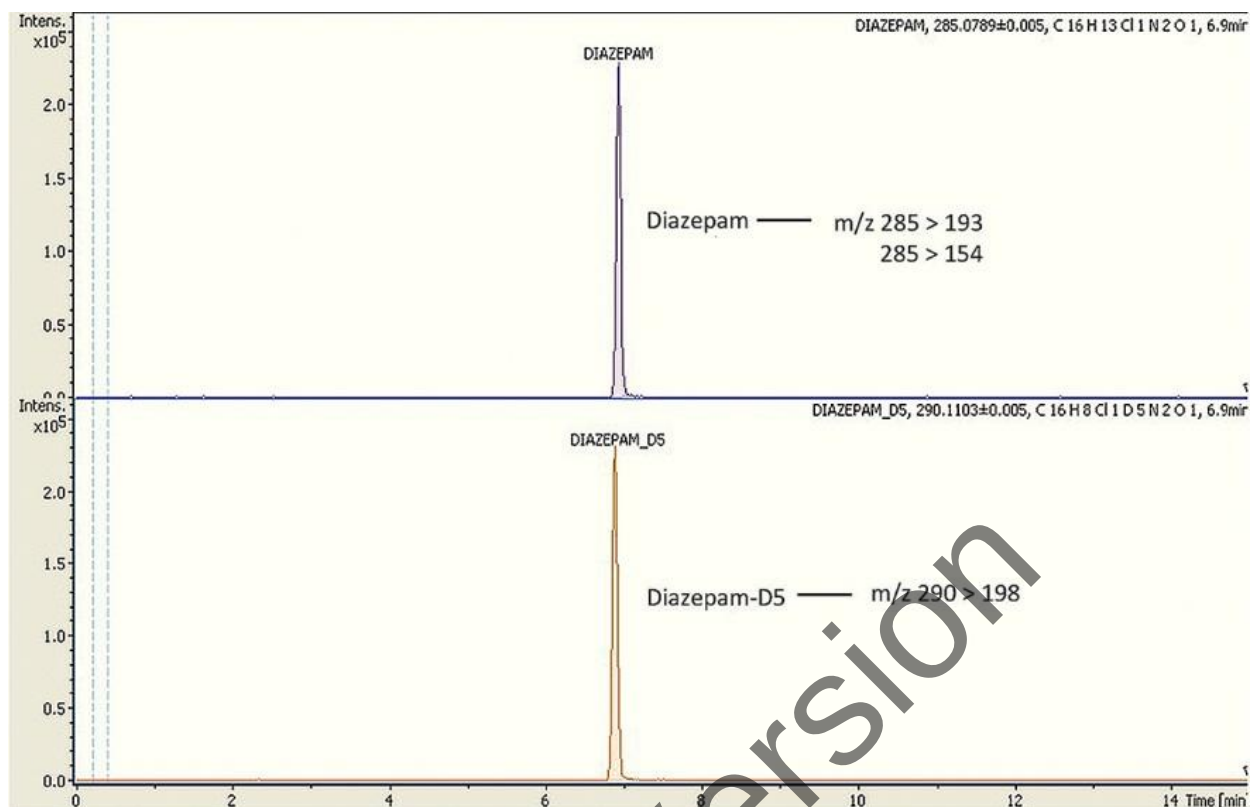


Figure 2 Chromatograms of diazepam and diazepam-d₅.

Sample preparation

Sample preparation for postmortem blood was carried out following the previous study.⁽¹¹⁾ Briefly, 1 mL of blood sample was transferred into a test tube and protein precipitation was performed using 1.5 mL of cold acetonitrile. The supernatant was collected and 0.1 M phosphate buffer (pH 6.0) was added. The sample was then extracted by solid-phase extraction (SPE) using Oasis HLB[®] SPE cartridges. The SPE extraction protocol was performed following the previous study.⁽¹¹⁾ After elution, the extracted sample was collected and evaporated under a nitrogen stream at 40° C. Finally, the sample was reconstituted with 75.0%: 25.0% mobile phase A : B and injected into LC–QTOF–MS.

Method validation

Method validation was performed following the *Standard Practices for Method Validation in Forensic Toxicology* issued by the Scientific Working Group for Forensic Toxicology (SWGTOX) (2013).⁽¹²⁾ Linearity range was established from 10 ng/mL to 500 ng/mL. The limit of detection (LOD) and lower limit of quantitation (LLOQ) for diazepam in this study were 5 ng/mL and 10 ng/mL, respectively.

Statistical analysis

Statistical analysis was conducted using SPSS for Windows[®], version 25. Descriptive statistics, including mean, median, and standard deviation (SD), were analyzed. After the Kolgomorov–Smirnov test indicated a non-normal distribution of blood diazepam concentration data, nonparametric tests, including the Wilcoxon signed-rank test, Spearman's rank correlation, and the Mann–Whitney *U* test, were performed where appropriate. A *P* < 0.05 was considered statistically significant.

Results

A total of 42 postmortem cases were recruited for this study, comprising five female and 37 male subjects, with an overall mean age of 40.6 years (age range = 18.0 – 83.0 years old). The mean BMI was 22.8 kg/m² (BMI range = 16.3 – 32.7 kg/). The mean PMI between the time of death and the time of specimen collection was 15.3 hours (range = 4.0 – 24.0 hours), and the mean time of hospitalization was 3.3 days (range = 0.1–19.0 days), as shown in **Table 1**.

Table 1. Descriptive data of all subjects and blood diazepam concentrations.

Category	Parameter	Mean	Median	Range
Subject characteristics	Age (years)	40.6	39.0	18.0–83.0
	BMI (kg/m ²)	22.8	21.5	16.3–32.7
	PMI (hours)	15.3	15.4	4.0–24.0
	Hospitalization time (days)	3.3	2.0	0.1–19.0
Diazepam concentration	Cardiac blood (ng/mL)	83.1	52.0	12.4–335.9
	Femoral blood (ng/mL)	69.9	53.6	14.7–316.8
	C/P ratio	1.2	1.0	0.4–3.3

Table 2. Correlation between the C/P ratio of diazepam and age, BMI, and hospitalization time.

Correlation	Parameter	N	Correlation coefficient	P-value
C/P ratio vs	Age (years)	42	–0.029	0.854
	BMI (kg/m ²)	42	0.064	0.685
	Hospitalization time (days)	42	–0.215	0.171

Table 3. Comparison of the C/P ratio of diazepam across different groups of PMI between the time of death and the time of specimen collection, and between subjects with and without chest injury.

Parameter	Group	N	C/P ratio		P-value
			Median (mean)	Range	
PMI between the time of death and the time of specimen collection	< 12 hours	14	1.1 (1.3)	0.7–2.7	0.171
	≥ 12 hours	28	0.9 (1.2)	0.4–3.3	
Chest injury	Presence	22	1.1 (1.4)	0.7–3.3	0.007
	Absence	20	0.9 (1.0)	0.4–2.0	

The median/mean diazepam concentrations in cardiac and femoral blood were 52.0/83.1 ng/mL (range = 12.4–335.9 ng/mL) and 53.6/69.9 ng/mL (range = 14.7–316.8 ng/mL), respectively. The median (mean) C/P ratio for diazepam was 1.0 (1.2), with a range of 0.4–3.3. Using the Wilcoxon signed-rank test, diazepam concentrations of cardiac blood samples were not significantly different from those in femoral blood samples ($P = 0.385$).

Using Spearman's rank correlation, no significant correlation between age, body mass index, PMI between the time of death and the time of specimen collection, time of hospitalization, and the C/P ratio was observed, as shown in **Table 2**. After categorizing the cases into two groups based on their postmortem interval (less than 12 hours, $n = 14$; and 12 hours or more, $n = 28$) and performing the Mann–Whitney u test, no significant difference in the C/P ratio between the groups was observed ($P = 0.171$). However, when dividing the subjects into two groups according to the presence of chest injuries—including rib fractures, hemothorax, and pulmonary contusion ($n = 22$ with chest injuries; $n = 20$ without chest injuries)—a statistically significant difference in the C/P ratio for diazepam was found between those with and without chest injuries, with median C/P ratios of 1.1 and 0.9, respectively ($P = 0.007$), as shown in **Table 3**.

Discussion

To the best of our knowledge, this study is the first PMR study in the Thai population focusing on diazepam concentrations in blood. The results of this study revealed that the median and mean C/P ratios of diazepam were 1.0 and 1.2, respectively, with a range of 0.4–3.3, and with no significant difference between diazepam concentrations in cardiac and femoral blood samples. This result was consistent with previous studies that reported median C/P ratios (ranges) for diazepam of 0.9 (0.5–2.1)⁽⁵⁾, 1.1 (0.4–3.9)⁽⁸⁾, and 1.2 (0.4–2.5)⁽¹⁰⁾, respectively. As the C/P ratio of diazepam in this study was also close to 1, corresponding with other previous studies^(5, 8, 10), this suggests that diazepam concentrations in Thai cadavers were less likely to be susceptible to PMR. A previous study hypothesized that this may result from some physicochemical properties of diazepam, because diazepam is a weakly basic drug and has a low V_d .⁽⁵⁾ Previous studies have indicated that drugs that are basic and have a high V_d are likely to be susceptible to PMR.^(4–6, 13) This suggests that when a femoral blood sample is unavailable, a cardiac blood sample might be an alternative sample for determining blood diazepam concentration in Thai postmortem cases.

This study showed no significant correlation between the C/P ratio and other factors, including age, BMI, and hospitalization time. These results were consistent with findings from previous research.⁽⁸⁾ When the PMI between the time of death and the time of specimen collection was divided into two groups (< 12 hours and ≥ 12 hours), it was also found that the C/P ratio between these two groups did not show a significant difference. Although this finding was consistent with the previous study⁽⁸⁾, this study revealed that the C/P ratio in the ≥ 12 hours group tended to be lower than that in the < 12 hours group, resulting in a *P*-value of 0.171. Compared with the previous study, median C/P ratios of diazepam at PMIs of 0–24 hours, 24–48 hours, 48–72 hours, and > 72 hours were 0.9, 1.0, 1.1 and 1.0, respectively, and there was no significant difference among these four groups.⁽⁸⁾ According to these results, the previous study showed that the C/P ratio of diazepam was relatively stable across increasing PMI, but our study suggested that the C/P ratio of diazepam tended to decrease with increasing PMI. The tendency of the decrease of blood diazepam concentration in this study may be related to the stability of diazepam in postmortem blood. Stability is an important factor to consider because the previous study showed that diazepam is prone to hydrolysis under aqueous acidic conditions, which occur in postmortem blood.⁽¹⁴⁾ After diazepam had undergone hydrolysis, its structure was altered to other chemical structures other than their conventional metabolites leading to the reduction of blood diazepam concentration.⁽¹⁴⁾ This behavior of instability may differ between femoral blood and cardiac blood because cardiac blood was closed to body central compartment including small and large intestine and prone to be affected by bacterial translocation. It could be possible that cardiac blood was more susceptible to alteration to acidic condition and the decline of blood diazepam concentration than femoral blood and this may have an effect on the trend of C/P ratio following the increasing PMI. Therefore, further studies should be conducted to determine the difference in the C/P ratio of diazepam between these two sample types.

In this study, chest injury was the only factor that significantly affected the C/P ratio of diazepam. This result contrasts with the previous study, which indicated no significant difference in the C/P ratio of diazepam between trauma and non-trauma groups.⁽⁸⁾ The previous study found that trauma led to an increased C/P ratio for amphetamine but not for

diazepam and its metabolites, or midazolam.⁽⁸⁾ However, our study differed from the previous study because chest injury was specifically focused on and considered as a factor of interest. Chest injury in this study included rib fractures, hemothorax, or pulmonary contusion, and all of these injuries led to tissue and vascular destruction, particularly in lung tissue. This may result in tissue ruptures in the thoracic cavity, causing membrane and barrier disruption.⁽⁸⁾ This effect may lead to subsequent diffusion of diazepam from surrounding tissues, causing mixing of bodily fluids.⁽⁸⁾ This mechanism may help explain the higher diazepam concentrations in cardiac blood. Therefore, interpretation of blood diazepam concentrations in subjects with chest injury should be performed carefully.

This study has some limitations. First, the sample size was relatively small, which may limit the ability to detect a clear relationship between such variables and the C/P ratio of diazepam, because the PMI between the time of death and the time of specimen collection, as well as the time of hospitalization in this study, had wide ranges, which may affect the power of the statistical analysis. In addition, the previous study stated that higher BMI was associated with a higher C/P ratio of midazolam compared with lower BMI⁽⁸⁾, but this study indicated that BMI did not affect the C/P ratio of diazepam. Thus, a larger sample size should be recruited in further study to elucidate this finding for benzodiazepine groups. Second, with only five female subjects, the data were insufficient to evaluate the association between sex and the C/P ratio. Lastly, this study did not measure some factors, such as the dosage, number of doses, and timing of diazepam administration during hospitalization, which may affect the C/P ratio. Thus, this study showed that chest injury had an effect on C/P ratio of diazepam in Thai postmortem cases and this should be concerned by forensic pathologists for interpretation of blood diazepam concentrations in Thai postmortem cases. In addition, due to relatively small sample size in this study, using C/P ratio for interpretation of blood diazepam concentrations in Thai postmortem cases should be carefully performed.

Conclusion

The median and mean C/P ratios of diazepam in Thai postmortem cases were 1.0 and 1.2, respectively. This finding suggested that diazepam concentrations are only slightly affected by PMR. Chest injury was the only factor that significantly affected the C/P ratio of diazepam in Thai subjects in this study.

Author contributions

Benya Pornwattanakul and Peerayuht Phuangphung contributed substantially to the concept and design of this study, acquiring the data, reviewing the literature, and its analysis and interpretation. Benya Pornwattanakul and Sunitda Khawthong contributed substantially to acquiring the data. Benya Pornwattanakul contributed to drafting the manuscript. Peerayuht Phuangphung edited the manuscript critically for important intellectual content.

All authors approved the final version submitted for publication and take responsibility for statements made in the published article.

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Conflicts of interest

The authors have no conflicts of interest to report.

Data sharing statement

All data that were generated and analyzed for supporting the findings of this study are available from the corresponding author upon reasonable request.

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