

Post-mortem Distribution of Morphine in Cadavers' Body Fluids

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Abstract

Purpose: We aimed to compare morphine in urine and other body fluids, including cerebrospinal fluid (CSF), bile, pericardial fluid (PCF), and vitreous humor to determine the most reliable fluid for detection of postmortem morphine.

Methods: In this cross-sectional study on 87 cadavers of Kahrizak Forensic Autopsy Center of Iran, cadavers with a maximum of 72 hours after death with positive urine morphine rapid strip test were included. Morphine was evaluated with thin layer chromatography (TLC) test in urine, bile, CSF, PCF, and vitreous samples. The presence of morphine in these fluids was compared to urine samples. Data were analyzed by SPSS software, version 21.0.

Result: Mean±SD age of the cadavers was 44.5±4.1 (range: 22–67) years consisting of 85 (97.7%) men and 2 (2.29%) women. From 87 cadavers with positive urine morphine Rapid Strip Test, only 42 urine samples (48.3%) had positive TLC results, among which TLC was positive in 24 cases (27.6%) of bile,

9 cases (10.3%) of PCF, 5 cases (5.7%) of CSF, and 2 cases (2.3%) of vitreous sample. There was a statistically significant relationship between urinary and biliary morphine (Kappa=0.527, P<0.001), PCF (Kappa=0.22, P<0.001), and CSF morphine (Kappa=0.123, P=0.017), but the relationship between urinary and vitreous morphine was not statistically significant (P=0.139).

Conclusion: The moderate agreement between urine TLC and bile TLC reveals bile sample as the most reliable fluid for morphine detection, when a urine sample is not accessible.

Key words: Morphine; Bile; Cerebrospinal Fluid; Pericardial Fluid; Vitreous Body; thin layer Chromatography; Post-mortem

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Introduction

Opioids are frequently used as drug abuse and in clinical practice for acute and severe pain management (1). It alleviates pain at different levels, including raising the threshold at the spinal level, attenuating the perception of pain, and influencing the emotional and hormonal conditions at the limbic system; they act as full-agonists for μ receptor and a weak agonist for δ and κ receptors (2).

The major metabolites of morphine include morphine-3-glucuronide (M3G), and morphine-6-glucuronide (M6G), which are metabolized in different organs, such as liver, brain, and the kidneys (3). The ultimate aim of drug metabolism is to facilitate its urinary excretion (4); thus, urine samples are considered an appropriate method for measurement of drugs, like opioids (5). But the clinical measurements are different from postmortem methods, as the drug concentrations may be redistributed according to the anatomical site of sampling and time after death, known as postmortem redistribution (PMR) (6). As far as peripheral blood samples are suggested to have lower concentrations than the central samples and peripheral samples are not always available in cadavers after a few hours (7).

Thin layer chromatography (TLC) is an easy and inexpensive method to isolate or assess the purity of a compound in a mixture with high sensitivity and good reproducibility (8) that is used to detect drugs in biological materials, including measurement of urinary morphine (9). The distribution of drug metabolism and the PMR phenomenon make plasma, whole blood, urine, bile, and cerebrospinal fluid (CSF) as appropriate sampling sites for detection of morphine in cadavers (10). When central blood samples, like femoral vein samples, and urinary samples (as gold standard sampling sites) are not available, other body fluids can be used (11), yet, the difference in measurement accuracy of different sampling sites has to be further studied. We aimed to compare morphine in urine and other body fluids, including cerebrospinal fluid (CSF), bile, pericardial fluid (PCF), and vitreous humor (VH) to determine the most reliable fluid for detection of postmortem morphine.

Materials and Methods

Study design

In this cross-sectional study, 87 cadavers who referred to Kahrizak Forensic Autopsy Center, Tehran, Iran were recruited. The protocol of the study was approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran. Before recruitment of cadavers into the study, the design and objectives of the study were explained to their family and written informed consent was obtained. All principles of Helsinki's guideline were met throughout the whole steps of the present study.

The sample size was calculated to be 85 cases, based on the frequency of positive morphine in body fluids (12), with an accuracy of 10%, and $\alpha=0.05$. The eligible cadavers were included using convenient sampling method. The inclusion criteria consisted of cadavers with a maximum of 72 hours after death with positive urinary morphine, documented by rapid strip test. Participants' age and sex were recorded and morphine was evaluated with thin layer chromatography (TLC) test in urine, bile, CSF, PCF, and vitreous samples. The presence of morphine in the fluids was compared to urine samples.

Statistical analysis

The results were reported by descriptive analysis, including mean \pm standard deviation (SD), and frequency (percentage) and inferential statistics, including independent sample T test and chi-square test. The associations of variables were tested by Kappa. For the statistical analysis, SPSS software, version 21.0 for Windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant.

Results

Mean \pm SD age of the cadavers was 44.5 \pm 4.1 (range: 22–67) years consisting of 85 (97.7%) men and 2 (2.29%) women. From 87 cadavers with positive urinary morphine (Rapid Strip Test), only 42 samples (48.3%) had positive TLC results, among which TLC was positive in 24 cases (27.6%) of bile, 9 cases (10.3%) of PCF, 5 cases (5.7%) of CSF, and 2 cases (2.3%) of vitreous sample. Comparison of positive and negative cases detected by urinary morphine than other fluids (sensitivity and specificity) are shown in (Table and Figure 1 - next page).

There was a statistically significant relationship between urinary and biliary morphine, PCF ($P<0.001$), and CSF morphine ($P=0.017$), but the relationship between urinary and vitreous morphine was not statistically significant ($P=0.139$). Measurement of agreement showed moderate correlation (Kappa=0.527) between urinary and biliary morphine, and weak agreement between urinary and PCF morphine (Kappa=0.22); also, there was a weak agreement between urinary and CSF morphine (Kappa=0.123).

Discussion

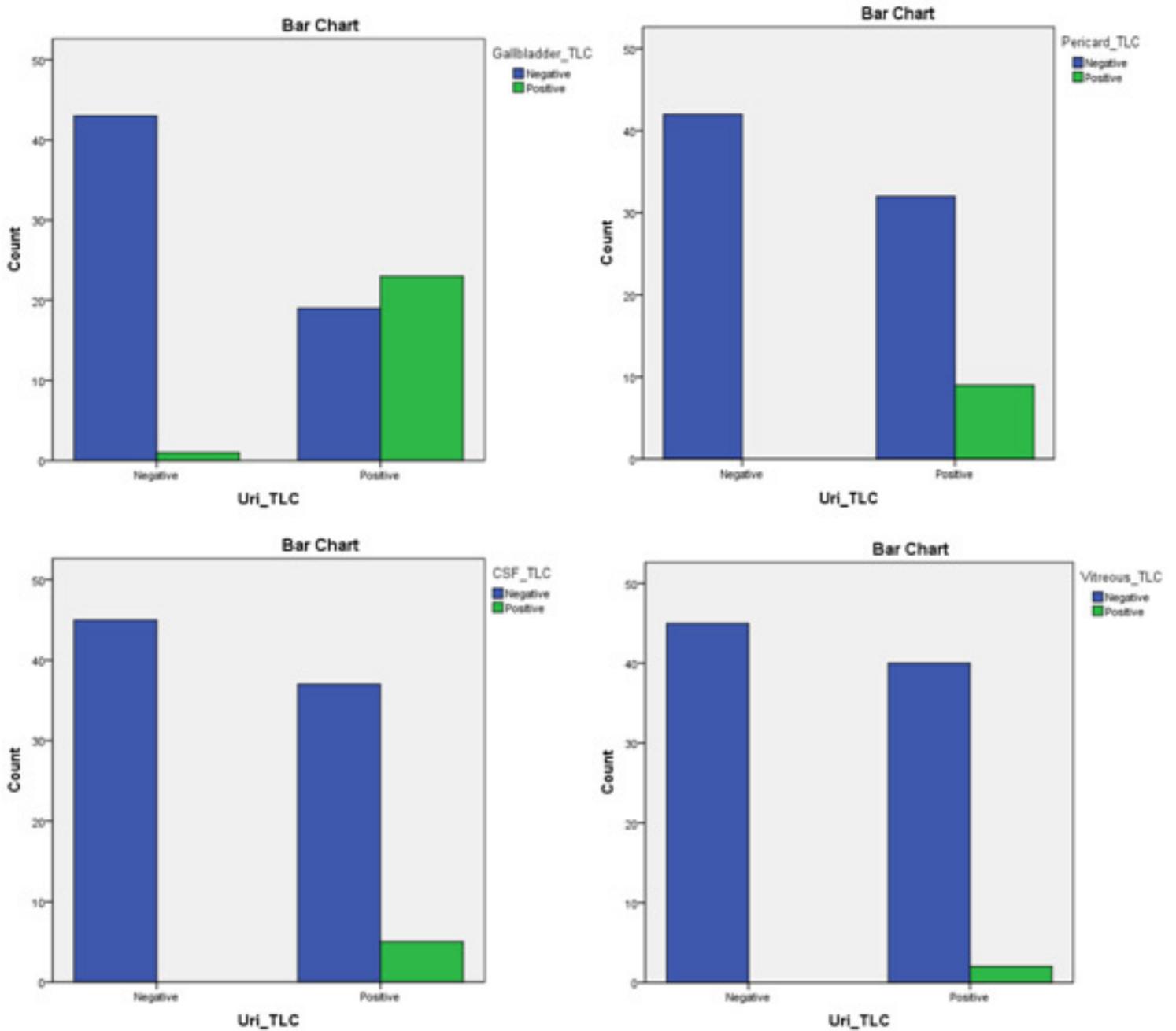
The results of the present cross-sectional study on 87 cadavers indicated the statistically significant association between urinary morphine and biliary morphine, PCF, and CSF morphine with moderate agreement between urinary morphine and biliary morphine, and a weak agreement between urinary morphine and PCF, and CSF morphine.

There are various reasons that a cadaver must be studied for the presence of drugs, for instance, toxicity of opioids and blood samples are the gold standard sampling site (13). But in cases where blood samples are not available or accessible, other specimens should be selected, including urine, bile, CSF, and VH (14).

Table 1. Comparison of positive and negative cases detected by urinary morphine than other fluids (sensitivity and specificity)

Urinary morphine	Biliary morphine		Pericardial morphine		CSF morphine		Vitreous morphine	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Negative	97.7%	2.3%	100%	0%	100%	0%	100%	0%
Positive	45.2%	54.8%	78%	22%	88.1%	11.9%	95.2%	4.8%
Total	72.1%	27.9%	89.2%	10.8%	94.3%	5.7%	97.7%	2.3%

Figure 1. Frequency of positive and negative cases detected by urinary morphine than other fluids



There are few studies that have evaluated the postmortem concentration of morphine in different body fluids and most studies have only focused on the comparison of one or two methods. One study showed higher drug concentrations in bile analysis than blood samples obtained from different sites (15), which is in line with the present study. Therefore, biliary specimens are an appropriate sample for assessment of morphine in cadavers. Also, other studies have indicated similar concentrations in PCF and blood samples for most drugs, especially morphine and its metabolites, and suggested PCF as a useful material for forensic toxicological assessment, when blood samples are not available (16, 17), which is consistent with the results of the present study, as there was a statistically significant association between PCF and urinary morphine, although the correlation was weak. Wyman and colleagues demonstrated highest morphine levels in liver, blood, CSF, and VH, respectively (12), which is similar to the results of the present study, indicating a statistically significant association between urinary and CSF samples, while this association was not statistically significant for VH. In another study, morphine and its metabolites was positive (>1 ng/ml) in 89% of urine samples, 68% of CSF samples, and 75% of VH cases (18), which was higher than the present study, indicating positive morphine in 48.3%, 5.7%, and 2.3% of urine, CSF, and VH samples. This difference can be due to the differences in the sampling technique, and measurement method. Holmgren and partners showed a significant difference between the concentrations in the VH and femoral blood for 23 substances and suggested VH an alternative specimen when blood samples are not available (19), while the results of the present study did not depict VH as an appropriate specimen, as there was no statistically significant association with urinary morphine, although in the study by Holmgren and colleagues, it was compared with blood sample, and was not specifically for morphine, which can justify the discrepancy between the results of the studies. The results of the present study on VH might be due to the inappropriateness of TLC method for analysis of morphine in VH, as studies have shown disposable pipette extraction (DPX) a fast, reliable, and easy to perform method for detection of drug abuse in VH with satisfactory sensitivity, precision, and accuracy (72–91%) (20), although TLC method, used in the present study, is an appropriate tool for forensic medical analysis of urinary opioids (21). In addition to the issues raised above, the interval between death and sampling also plays a significant role in the concentration of the drug, due to PMR phenomenon (6), which can justify the discrepancies among studies, as well. Thus, it is suggested that specimens should be selected individually for each case, based on the history and availability, while the procedures should be performed with proper quality (22), and post-mortem tissue/samples should be carefully selected, stored, preserved and utilized (23). Other studies have also assessed the tissue distribution of morphine and its metabolites in forensic medicine (24, 25), while the present research could suggest body fluids as an easy access method, especially bile, although further research is required on a comparison of the diagnostic accuracy of tissue versus fluids.

The main strength of the present study was a comparison of different sampling sites in one study in a referral Forensic Center that enables researchers with an appropriate spectrum, while most studies have only evaluated one or two methods (12, 16, 19). On the other hand, the present study had several limitations, such as limited sample size and cases of one Forensic Center that limits the generalizability of the results. In conclusion, the results of the present study showed that biliary measurement of morphine by TLC method could be an appropriate alternative for morphine detection in cadavers less than 72 hours after death when the urine sample is not accessible.

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