

Comparing Bile and Urine Samples as Morphine Detectors in Corpses

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Abstract

Introduction: The present study aims to compare bile and urine samples of a corpse for detection of morphine. **Methods:** In this cross-sectional study, forensic examination of the bodies placed 59 of the 366 referred corpses at the high suspicion of opioid overdose. Urine and bile samples of the bodies were analyzed for morphine using thin layer chromatography. **Results:** All samples were taken from male bodies. There were no urine samples in four corpses (7%) and two urine samples (3%) produced negative results. In the remaining 53 cases, 14(24%) turned out 1+, 29(49%) turned out 2+, 8(14%) bodies turned out 3+ and 2(3%) corpses produced 4+. On the other hand, all bile samples produced positive results, in which 3(5%) turned out 1+, 20(34%) turned out 2+, 22(37%) bodies turned out 3+ and 14(24%) corpses produced 4+. The Spearman coefficient for positive urine or bile samples was 0.377($P = 0.005$). **Conclusion:** The results of the present study show that the concentration of detected morphine in bile is greater than that of urine. Therefore, a bile sample can contribute to the detection of opioid in the corpses, especially when urine samples are not available or are negative.

Keywords: Opioid, Morphine, Urine, Bile, Corpse

Introduction

Drug abuse is one of the foremost factors spreading the seeds of crime, family violence and fatal or disabling incidences with significant physiological and psychological effects on individuals while committing a crime ^(1, 2). In fact, the majority of criminals constitute drug addicts and deliberate or inadvertent drug overdose may lead to the death of the abuser ^(3, 4). Today, drug facilitated crime has come to the fore and addressed extensively. This type of crime includes an extensive range of offences such

as theft, rape, money extortion and even murder ⁽⁵⁾.

Despite the fact that evidence collection and crime scene investigation might play significant roles in resolving criminal cases at forensic departments, one problem faced in solving such drug facilitated crime mysteries is proving the trace of drugs in the evidence pieces remaining at the crime scene ⁽⁵⁾. Accordingly, numerous studies have been conducted on the detection methods of drug in biological samples such as blood, plasma, hair, cerebrospinal fluid and urine ⁽⁶⁻¹⁰⁾. Bile is one of these samples which can

prove useful in postmortem drug sampling because it easily intensifies drugs and their metabolites and is easily obtainable. In some studies, bile and blood samples were compared in terms of drug detection facility and concentration levels accruing the result that drugs are more detectable in higher concentration in bile than in blood ⁽¹¹⁻¹⁷⁾. Yet, no such a study has compared bile and urine samples obtained at the autopsies for detection of drugs. So the aim of this study is to compare bile and urine samples of corpses for detection of morphine.

This cross-sectional study was conducted from February 2008 to July 2009 at the Reference Laboratory of the Forensic Medicine Department of the Province of Markazi, Iran. Forensic examination of the bodies placed 59 of the 366 referred corpses at the suspicion of opioid overdose. Urine and bile samples of the bodies were analyzed using thin layer chromatography (TLC) for detection of opioid abuse (morphine). The present study and its relevant experiments were conducted in compliance with Paragraphs I and II of the Declaration of Helsinki and its 2008 Seoul supplement and the 26 codes of ethics mandated by the Research Guidelines of the Iranian Ministry of Health. In addition, an ethical approval was earned from the Ethical Committee of Arak University of Medical Sciences. The study was performed in full observation of religious and conventional requirements pertaining to the privacy of the corpses and their families and the confidentiality of the test results.

Morphine was extracted through liquid-liquid extraction method. In this method, we used 10 cc HCL 10% to acidize 10 cc urine and then added 10-15 cc distilled water to the solution. The solution was then direct-heated for 20 minutes and then ammoniac was gradually added to basify the solution (at a desired pH of 8.5-9). Next, we added 250 cc of

chloroform:isopropanolol compound (4:1, v/v) and shook the resultant solution for 20 minutes. We then separated the lower phase containing opioid, poured it into a beaker and boiled it in a bain-marie to be evaporated. The deposit left was used in staining in TLC.

A silica gel with fluorescent indicator UV245 plate with Ar Number 805023 was used for the TLC process. We added 3 cc methanol to each sample and started staining using a capillary tube. We would stop for a stain to dry before forming another stain. Staining for each sample was performed 20 times. The maximum diameters of the stains were kept at 5 mm. The distance between the stains was kept at a minimum of 2 cm. Since we used two chromatography tanks with different solvents, staining for each sample was performed on two different TLC plates. Once the stains were dried and there were no scratch, artifact or extra stains on the plates, the plates were immersed into the tanks. Two glass tanks measuring 22×9.5×21.5 cm with flat walls and even bottoms were used for chromatography. The solvent in the first tank included ethyl acetate: chloroform, dioxane, ammonia (10:25:60:5, v/v) and the solvent in the second tank included benzene, dioxane, methanol, ammonia (50:40:5:5, v/v). Once the solvents were prepared, we poured them into the respective tanks and agitated them for the solution to mix well. The solvent remained for 10 minutes sealed in the tanks to saturate. The stain plates were then slipped into the tanks and remained there sealed. Once the solvent reached the top of the plate and the criterion point, the plates were removed from the tanks to dry and be ready for spraying.

UV and iodoplatinate indicators were used to detect and spot the stain on the TLC plates. The formed stains were compared with the standard drug stains (the narcotic contained in the opium which is used by

the Reference Laboratory of Forensic Diagnosis and Laboratory of Iran and confirmed by the GC-MASS) from four perspectives: RF (Rate of Flow), the shape of the formed stain in that RF, the size of the stain, and its color. The results prove opioid positive if the stains in the foregoing aspects are exactly similar to the standard stain and also if the results of the TLC plate stains used in the two tanks are similar as well. The degree of positivity was determined on the basis of the color concentration of stain.

The descriptive statistics for the analysis of data included frequency and relative frequency (percent). Spearman coefficient was calculated to examine the relationship between the positivity of urine and bile in terms of morphine concentration. Significance level was set at $P < 0.05$.

Results

The sample included all male decedents ranging in ages of 19-83 years with a mean (SD) of 32 ± 11 years. There were no urine samples in four (7%) corpses and two (3%) samples produced negative results. In the remaining 53 cases, 14 (24%) samples turned out 1+, 29 (49%) turned out 2+, 8 (14%) turned out 3+ and 2 (3%) produced 4+. On the other hand, all bile samples produced positive results, in which 3 (5%) turned out 1+, 20 (34%) turned out 2+, 22 (37%) turned out 3+ and 14 (24%) produced 4+. Table 1 compares the analysis results between bile and urine samples. As can be observed, the bile samples show a higher concentration of drug than the urine samples. Where the urine samples turned out to be 1+, 36% of the bile samples turned out to be 3+ or 4+. And in cases where the urine samples were 2+ or 3+, the bile samples showed to be 3+ or 4+ in 72% and 75% of the cases, respectively. Spearman coefficient for positive urine or bile samples was 0.377 ($P = 0.005$).

Table 1. Results of morphine test of bile and urine samples of corps

Urine Sample	Bile Sample				Total
	1+	2+	3+	4+	
No specimen	-	1 (25%)	3 (75%)	-	4
Negative	2 (100%)	-	-	-	2
1+	1 (7%)	8 (57%)	4 (29%)	1 (7%)	14
2+	-	8 (28%)	14 (48%)	7 (24%)	29
3+	-	2 (25%)	1 (12%)	5 (63%)	8
4+	-	1 (50%)	-	1 (50%)	2
Total	3	20	22	14	59

Discussion

The comparison results between the bile and urine samples showed that bile can show a higher concentration of Morphine in the body than urine. Therefore, a bile sample can contribute to the detection of these drugs in the corpse, especially when urine samples are not available from the corpses or when they are drug negative. Bile is a major way for the release of many types of substances. The liquidity of bile causes the secretion of solvents into the water. Moreover, bile acids can solve lipophilic compounds. Bile is an important way for the disposal of certain drugs and their metabolites from the body. The drugs disposed through bile may enter *enterohepatic cycle* and be reabsorbed through the digestive system. Accordingly, the pharmacological effects of certain medicines or their metabolites will extend ⁽¹⁸⁻²⁰⁾. In addition, bile and the gallbladder function as storage for the accumulation of certain drugs ⁽¹⁴⁻¹⁶⁾. As a result, bile can be a revealing sample for the detection of many substances and also liquid sampling ⁽²¹⁾. Numerous studies have shown a higher concentration of substances such as morphine, codeine and cocaine in bile compared with blood ^(11-17, 22).

So, bile as a postmortem sample is recommended for detection of drugs such as morphine, buprenorphine, tramadol and benzodiazepines ⁽²¹⁾. In addition, bile in some cases may test drug positive where other samples, such as blood or urine, may fail to do so as a study on heroin overdose ⁽²³⁾ proved so. Our study also showed that the concentration of morphine was higher in bile than urine in corpses suspicious of drug abuse. In addition, urine samples could not be obtained from four corpses where the obtained bile samples were positive.

Some studies assert that the enterohepatic cycle contributes to the maintenance of morphine at blood and tissue levels and its metabolites in the long-term use ⁽²⁴⁾. Therefore, morphine concentration in the bile can be used to detect morphine and heroin overdose or chronic abuse ⁽²⁵⁻²⁷⁾. Nevertheless, high concentration of morphine in bile could also be due to hot shots ⁽²¹⁾. Since various studies have shown a higher concentration of certain drugs in bile compared to blood, a pathologist is recommended to analyze the gallbladder and its content besides autopsy samples. In addition, since in some cases bile has been shown to detect drug abuse where a blood sample has failed to do so, it is strongly recommended to utilize bile as a routine process along other biological fluids and samples in forensic medicine. In this way, bile could function as a supplementary test ⁽¹⁴⁾. Some researchers even assert that bile should be referred to where blood sample tests turn out drug negative ⁽¹³⁻¹⁵⁾.

Conclusion

The results of the present study show that the concentration of detected drug is higher in bile than urine and that a bile sample could be used for detection of drug abuse in a corpse, especially when urine samples are not available or turn out drug negative.

Therefore, bile samples are strongly recommended to be applied in detection of Morphine in body.

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