Toxicology results of bile samples: Are they valuable in determining the cause of death?

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Background: Various samples are taken from a deceased body for toxicological analysis, with the number of samples varying based on the individual's background, to determine the cause of death. The primary objective is to ascertain the connection between the toxicology findings from bile and other samples in determining the cause of death.

Method: In a descriptive and cross-sectional study, two methods were utilized: high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS).

Result and conclusion: The findings indicate that the toxicology results from bile were either similar to the other main samples or different. In a few cases, there were varied results that were not related to the cause of death. The study used highly sensitive analytical methods such as GC–MS and HPLC, and qualitative results from toxicology labs in legal medicine. These results suggest that toxicology results from bile samples are not considered crucial in determining causes of death. They play a minor role and are typically only examined when blood samples are not available.

Keywords: bile, toxicology testing, cause of death, forensic toxicology

Introduction

To determine the cause of death, different samples used for toxicology were collected from different sources. The number of samples either differed according to the individual's background or was limited to blood and tissue samples [14]. Historically, samples of bile were gathered even though their benefits are limited [14, 15]. In the past, bile used to be very valuable because it has a high density of conjugated drugs, especially morphine and other medicines (such as benzodiazepine, colchicine, and buprenorphine), which are likely to be found in bile rather than in blood. The concentration of medicines found in the blood is 1000 times less than the amount found in bile [8, 10, 14, 15]. In addition, researchers have found that medicines are more commonly found in bile than in blood, and the ratio of medicines in bile rather than blood is 1 (for paracetamol and amphetamine) and 2000 (for desmethyl clobazam) [16]. Most forensic medicines accumulate in the bile, particularly those medicines that have been conjugated, such as opioids, benzodiazepines, and cannabinoids [14]. Bile may also be used for chronic heavy metal poisoning [14]. Bile is also regarded as a suitable solution for evaluation when urine samples are not available (for opioids, benzodiazepines, and colchicine) [10]. The International Association of Forensic Toxicologists (TIAFT) recommends that bile samples be collected under certain circumstances [8]. Some researchers agree that the concentration in bile after the individual's death could be used for the estimation of blood concentration [8]. Additionally, the academic literature on toxicology has reported the blood and bile concentrations, suggesting that the concentration in bile is a valid factor [8]. Nevertheless, in a 2017 research project, England researchers showed that for most medicines, the ratio of medicine consternation in bile is high. Therefore, there is a small correlation and connection between the concentration of poison and drug in bile and blood [8]. Moreover, given that the concentration of poison in bile is more frequent than in blood, bile samples could be used for qualitative investigations, especially for cases where the concentration of the poison is less than what could be diagnosed [8]. Like urine, bile is a waste liquid, and the relation between the concentration of medicines in bile and blood is negative, except for ethanol [10]. Based on a review article (1970-2015), it was stated that bile could be simply used along

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Table I *The frequency cases accurding to gender, addiction and death cause*

Parameter		Frequency	Percentage
Gender	Males	82	82
	Females	18	18
Record of Addiction	No	82	82
	Yes	18	18
Death Cause	The pressure over the vital organs of the neck	30	30
	Stabbed with sharp objects	24	24
	Stabbed with hard objects	20	20
	Being shot	7	7
	Aluminum phosphide poisoning	4	4
	Drug intoxication	5	5
	Opioid intoxication	3	3
	Unidentified	2	2
	Carbon monoxide poisoning	2	2
	Advanced internal diseases	1	1
	Rubble	1	1

with other samples, including blood and stomach contents, in an autopsy, especially when there is no blood for the examination or sampling [2]. Because of the presence of fat and salt in bile, the extraction of medicines from this matrix is complicated; consequently, it demands extensive extraction, preparation approaches, and filtering. Additionally, there may be interference with bile salts [14]. Additionally, there is a limited amount of reference data, and immunoassays and other sensitive techniques are extensively used; therefore, the use of bile as a sample sounds less valuable when compared with the past [10, 14].

Methods

This descriptive and cross-sectional study aimed to examine samples from 100 deceased individuals at the Tehran legal medicine autopsy section in early 2016, employing two methods: HPLC and GC-MS. Following formal protocols, essential data were gathered using a checklist that included gender, age, cause of death, toxicology, and addiction history, which were subsequently analyzed.

Results

Based on the data presented in **Table I**, out of the 100 samples analyzed, 82% were males and 18% were females. Among the cases, 58% were in the 21-30 age range, while 10% were in the 41-50 age range. The 21-30 age range was predominantly represented by males, and all the females fell within this age range. The table also indicates that 18% of the cases were drug addicts, all of whom were males. Furthermore, the age range of 31-40 had the highest number of addiction cases (8), while the 41-50 age range had the lowest (4). **Table I** also reveals that the main causes of death were pressure on vital neck organs (30%), stabbing with sharp objects (24%), and stabbing with hard objects (20%).

Regarding the analysis of bile, stomach contents, tissue, and urine for positive cases, the results were reported separately in **Table II.** In all deceased individuals, amphetamine, methamphetamine, and morphine were found in the urine samples, while these substances were absent from other samples (tissue, stomach contents, and bile). In two cases, only the urine sample results were used to determine the cause of death, as the results from other samples were not relevant. This underscores the importance of the urine sample. Additionally, in some positive cases, the urine sample tested negative, while the results for tissue and stomach contents were positive. This discrepancy was due to the less sensitive TLC approach used for the urine sample, as opposed to the GCMS and HPLC approaches.

1. Discussion

In postmortem toxicology, alternative matrices to blood are useful in cases of limited, unavailable, or unusable blood samples and suspected postmortem redistribution [2, 6]. Bile is one sample that may be collected postmortem and submitted to the forensic and toxicology laboratory for analysis [3]. Bile is a complex sample secreted from liver cells [2]. Because bile is an excretion product of the liver, it can be used for screening purposes and to determine what drugs an individual used

Table II *A cross-comparison of bile samples with other samples*

Number	Death Cause	Addiction Record	Bile Result	Urine Result	Tissue Result	Stomach Contents Result	Approaches
1	Drug intoxication	No	Tramadol	Negative	Tramadol	Tramadol	TLC, HPLC
2	Drug intoxication	Yes	Tramadol	Negative	Tramadol	Tramadol	TLC, HPLC
3	Opioid intoxication	No	Tramadol	Negative	Tramadol	Tramadol	TLC, HPLC
4	Stabbed with sharp objects in chest	No	Ketamine	Ketamine	Ketamine	Ketamine	GCMS
5	Drug overdose	Yes	Aspirin	Nothing	Midazolam	Midazolam	GCMS
6	Stabbed with sharp objects in chest	No	Tramadol	Negative	Tramadol	Tramadol	TLC, HPLC
7	Stabbed with sharp objects in chest	No	Tramadol	Negative	Tramadol	Tramadol	TLC, HPLC
8	The pressure over vital organs of the neck	No	Hexyl Salicylate	Nothing	Midazolam	Midazolam, Diphenhydramine	TLC, GCMS
9	Drug overdose	No	Ketamine, Tramadol	Ketamine and Metabolite, Tramadol and Metabolite	Ketamine, Tramadol	Ketamine and Metabolite, Tramadol	TLC. GCMS
10	Stabbed with sharp objects in chest	No	Tramadol	Negative	Tramadol	Metabolite, Tramadol	TLC. GCMS
11	Pressure over the vital organ of neck	Yes	Tramadol	Negative	Negative	Metabolite, Tramadol	TLC. GCMS
12	Stabbed with sharp objects	No	Ketamine, Tramadol	Ketamine, Metabolite	Ketamine, Tramadol	Ketamine, Metabolite, Tramadol	TLC. GCMS
13	Drug poisoning	No	Hexyl Salicylate	Nothing	Midazolam	Midazolam and Diphenhydramine	TLC. GCMS

or was exposed to prior to death [3]. Bile is a useful specimen for the detection of drugs and can even be used as a screening fluid when urine samples are not available [5]. Very few studies have reported data on bile samples [1]. Drug concentrations in bile often exceed those in the blood [8]. Bile concentrations were usually lower than urine concentrations for caffeine, amphetamines and methylephedrine but were usually equal to or higher for other drugs [15]. Bile may be a useful sample for the detection of benzodiazepines and their metabolites to be collected at autopsy, as one study reported [9]. For determination of postmortem ethanol concentration, although there was a positive correlation between blood and bile ethanol levels [11], the wide range of ethanol concentrations makes it undesirable to use to predict blood ethanol concentrations [18]. The relationship between ethanol levels in blood specimens and corrected levels in bone marrow was better than that between blood and bile, vitreous humor or urine [17]. Bile samples are a useful means of disclosing overdoses where blood specimens are not available or of confirming blood tests when postmortem redistribution (PMR) is suspected [7]. Bile can be used in toxicological analysis and be complementary to the other specimens because the concentrations of drugs are commonly several-fold higher than blood concentrations [16]. The accumulation of 3-hydroxy-2-oxoquazepam in bile may be due to enterohepatic circulation and offers a long detection window [19, 20].

The data analysis involved 30 out of 100 cases where toxicology analysis showed positive results.

In 10 out of the 30 cases, toxicology analysis for bile was similar to other main toxicology samples such as stomach contents and tissue. In 17 out of the 30 cases, toxicology for bile was reported as negative. For a few cases, different results were obtained, but these did not impact the cause of death. Consistent with findings from a prior article review, it can be stated that bile, along with other samples like blood and stomach contents, may serve as an additional specimen in the autopsy process. The investigation results indicated that in all examined cadavers, urine samples contained amphetamine, methamphetamine, and morphine (12 out of 30 cases). However, these substances were not detected in other samples, including tissue, stomach content, and bile. Tominaga et al. asserted that the concentrations of amphetamine, caffeine, and methyl ephedrine in bile are low as opposed to urine[15]. One study reported a case of fatal colchicine poisoning in which the drug was detected in urine, although it was not detected in bile [4]. Although cocaine, clobazam, codeine, northiaden, 7-acetamidoflunitrazepam, and MDA were detected in a blood sample, they were not found in bile [16]. Bile specimen analysis requires specific drug extraction techniques to prevent interference and to isolate drugs [12].

Therefore, based on the reference toxicology, the number of samples could vary according to the individual's record or be limited to blood and tissue [14]. This could also be found in common samples for recent deaths—blood, vitreous body, stomach contents, and at least one tissue (generally liver) [14]. The book "An Introduction to Sampling and Preparation For Laboratory of Legal Medicine" points out that in corpora that have not yet decayed or have undergone a low degree of decay, samples of blood, stomach content, urine, vitreous body, and at least one sample of tissue (generally liver) could be used for toxicology [13]. Furthermore, the presence of medications in bile may lead to the generation of inaccurate data, as bile is a waste substance and does not accurately reflect the concentration of medication in the body or the effects of medications on the body. Given that the study utilized highly sensitive and complex techniques such as GC–MS and HPLC, and considering the qualitative results obtained in toxicology laboratories, it can be concluded that bile should not be considered a contributing or complementary factor in determining the cause of death.

The cases were not compared with the blood test results, as the blood sample is not commonly used for toxicology screening in Iranian legal medicine organizations. Quantitative test results for blood and other materials, including bile, are also not conducted for toxicology screening in Iranian legal medicine organizations. Based on these findings, it is recommended to conduct further research and develop new protocols for the effective use of bile samples and to avoid overuse of bile samples.

2. Conclusion

Overall, because the study employed highly sensitive analytical methods such as GC–MS and HPLC and qualitative results in the toxicology laboratories of legal medicine, the results suggest that the toxicology results of the bile sample cannot be counted as a contributing factor for the determination of death cause. Bile is an alternative specimen for screening proposed when limited blood and urine samples are available. Bile is not considered to be of major importance and it only plays a subordinate role and is generally only examined when blood samples are not available.

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