Drug related deaths in Tehran, Iran: Toxicological, Death and Crime Scene investigations

Maryam Akhgari1*, Farzaneh Jokar2, Afshar Etemadi Aleagha2

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ABSTRACT
Background: To determine the role of drugs in fatal overdose, analyses were done on samples obtained from autopsy examination and death and crime scene investigations. The aim of this study was to evaluate the usefulness of toxicology analyses of postmortem biological and non-biological samples belonging to a subject in determining the forensic cause of death.

Methods: Toxicological analysis were done on 31 cases of fatal drug poisoning accompanied with scene evidence that had been referred to Legal Medicine Organization (LMO) of Iran, Tehran, over 12 months, starting from March 2008. Drugs and ethanol were detected in biological and non-biological samples through toxicological analyses.

Results: Overall, 64.52% of the cases showed the same positive analytical results in both biological and non-biological samples. Opiates were indicated in 65% of the deaths. About two thirds (64.51%) of the cases indicated the involvement of more than one drug (active ingredient). With a sex ratio of 4.16, men composed 80.65% of the study cases. The mean age for male and female victims (mean ± SD) was 34.07±11.93 and 32.67±8.04, respectively, and the majority of the cases (77.42%) were below the age of 40.

Conclusion: There are many important parameters in determining the forensic cause of death, among them autopsy reports, toxicological findings, police information and circumstances surrounding the death are important.

Keywords: Cause of Death, Crime Scene Investigation, Drugs, Forensic Toxicology Analysis.

INTRODUCTION

Determining the forensic cause of death in fatal drug poisoning is difficult and depends on several factors, such as medical history of the victim, autopsy reports, death and crime scene investigations, police reports, and toxicological analyses results (1-3). Forensic toxicology is the use of toxicology science and other analytical methods to aid making decisions for medicolegal investigations and clearing-out the cause of death; poisoning and drug use(4). Medicolegal autopsies, including toxicological examinations provide useful information on drug related deaths, drug policy, and drug problems in a country(6). Death among drug or substance users is an important problem in Iran (7). This study is a descriptive research on fatal drug poisoning concerning death and crime scene investigations in Tehran, Iran for the 2008-2009 period. Biological and non-biological samples from 31 cases were analyzed from a toxicological point of view. The aim of the present study was to assess the usefulness of qualitative and quantitative toxicological analyses in determining the forensic cause of death in drug or substance users accompanied with drugs found in the scene investigation and autopsy examination.

MATERIALS AND METHODS

The material consisted of all deaths from drug or substance poisoning accompanied by

1. Department of Forensic Toxicology, Scientific and Educational Research Center of Legal Medicine Organization (LMO), Tehran, Iran.
2. Tehran University of Medical Sciences, Faculty of Medicine, Amir Alam Hospital, Tehran, Iran.
*Corresponding Author: Email: akhgari1349@yahoo.com
evidence collected at death scene investigations or autopsy findings submitted to toxicological analyses at the Forensic Toxicology Department of Legal Medicine Organization of Iran, Tehran in a one-year period. Fatal drug poisoning was supported by autopsy reports and toxicological findings.

**Chemicals**

Standard samples of drugs were obtained from pharmaceutical factories. Solvents such as methanol, ethanol, ammonia, ethyl acetate, acetonitrile, water, and HPLC grade prepared from Merck Chemical Co. (Germany) were used as the analytical chemicals.

**Biological and non-biological samples**

Autopsies were performed on suspicious cases (suicide, homicide, robbery, addiction, and body packing). Biological samples (liver, urine, femoral vein blood, bile, vitreous humor, and stomach content) and drug packets in the gastrointestinal tract were collected by experienced staff supervised by a forensic medicine specialist. Also, non-biological samples, such as pills, syringes, and food and beverage residues, belonging to victims were referred to forensic toxicology laboratory by police.

**Toxicological analysis**

Pre-analytical preparation of biological matrices was done by liquid-liquid extraction (LLE) of urine, blood, liver, stomach content, and bile. Cleavage of drugs and metabolites from conjugates, extraction of unknown analytes, and clean-up steps were done by pH adjustment and use of suitable solvents, such as chloroform, methanol, isopropanol, and diethyl ether. Femoral vein blood (containing 1% W/V sodium fluoride) and vitreous humor needed no preparation prior to analysis for detection of ethanol with headspace gas chromatography (HSGC). For preliminary qualitative analysis, thin layer chromatography (TLC) technique was used to screen drugs, poisons, and opioid alkaloids in extracts from biological samples. The samples were analyzed with more sensitive and specific instrumentations. High Performance Liquid chromatography (HPLC) (Knauer, Germany) with a diode array detector (DAD) (Knauer DAD 2700, Germany) plus a quaternary pump (Knauer pump 1000, Germany) and a gas chromatography/mass spectrometry (GC/MS) (Agilent 7890A, USA) with a mass detector (5975C) were used to confirm positive results in this study. Blood alcohol concentration (BAC) and vitreous humor alcohol concentration (VHAC) were routinely determined using a quantitative HSGC (Agilent 6890N, USA) equipped with Flame Ionization Detector (FID). Headspace injections were performed using an automatic sampler. Colorimetric (prussian blue) and voltametric/polarographic methods were used for cyanide detection.

Pharmaceutical dosage forms, such as tablets, were analyzed directly on TLC without prior extraction or purification. Unknown suspicious powders and tablets were mixed with organic solvents like chloroform or methanol to dissolve probable analytes. Suspicious food and beverage residues were prepared using LLE method to extract analytes. Syringes with or without clotted blood were washed with distilled water. Acidic and basic drugs and opioid alkaloids were extracted by LLE method. In this study, TLC method was used as a screening test. All results were confirmed by HPLC and GC/MS instrumentations.

**RESULTS**

Men formed 80.65% of the study cases with a sex ratio of 4.16 (25 men and 6 women). The mean age of the victims was 34.07±11.93 years (mean±SD) in men and 32.67±8.04 years in women. As it is shown in Figure 1, victims were divided into 6 groups according to the forensic investigations. Positive toxicological results were detected in biological samples obtained from 21 cases through routine toxicological testing. Detectable intoxicating drugs or substances were not found in postmortem samples of 10 cases. All blood and vitreous humor (VH) samples referred to toxicology laboratory were tested for alcohols. Ethanol was found in both blood and VH of 16.13% (5) cases. Table 1 shows the results of ethanol analysis in victims.

Deaths due to opioid overdose numbered 20, comprising 64.51% of the study population. Results from toxicological analysis of
biological and non-biological samples in opioid addicts are summarized in Table 2.

Body packers numbered 6, comprising 19.35% of all fatal drug poisonings in this study. All of the body packers were male and 5 cases had Iranian Crack (heroin plus adulterants) packs in their stomach. Table 3 includes information about this group.

The cause of death of 3 (9.68%) cases was homicide. Two cases were female and drugs and poisons were not found in biological samples obtained from them. The cause of death of one of the homicide cases was drug overdose following the ingestion of ice cream which contained drugs. Suspicious cases in homicide category are shown in Table 4.

Only one case in our study population was a suicidal death. Overdose following drug abuse was the cause of death in 5 (16.13%) cases. Toxicology results of drug abusers are shown in Table 5. The cause of death of 9.68% (3) cases was undetermined.

In 20 (64.51%) of the study cases, more than one drug (active ingredient) was detected in biological samples. The most common drug categories detected are shown in Figure 2.

Table 1. History of ethanol use with other drugs

<table>
<thead>
<tr>
<th>Manner of Death</th>
<th>BEC (mg/dL)</th>
<th>VHEC (mg/dL)</th>
<th>Drugs in Biological Samples</th>
<th>Drugs in Non-biological Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suicide</td>
<td>125</td>
<td>130</td>
<td>None</td>
<td>Mefenamic acid capsules in stomach content</td>
</tr>
<tr>
<td>Drug Abuse</td>
<td>196</td>
<td>154</td>
<td>None</td>
<td>Tramadol and buprenorphine in victim's pocket</td>
</tr>
<tr>
<td>Opioid Overdose</td>
<td>22</td>
<td>10</td>
<td>Morphine, codeine, diazepam</td>
<td>A glass containing brown viscous liquid (morphine, codeine, noscapine, papaverine) &amp; diazepam tablets besides him</td>
</tr>
<tr>
<td>Drug Abuse</td>
<td>125</td>
<td>149</td>
<td>None</td>
<td>Naproxen tablets in stomach content</td>
</tr>
<tr>
<td>Homicide</td>
<td>90</td>
<td>30</td>
<td>Phenobarbital, amitriptyline, phennmetrazine, ephedrine, dextromethorphan, propranolol</td>
<td>A glass containing ice cream besides the victim (Phenobarbital, amitriptyline, phennmetrazine, ephedrine, dextromethorphan, propranolol, clonazepam, biperiden, morphine &amp; codeine)</td>
</tr>
</tbody>
</table>

BEC: Blood ethanol concentration
VHEC: Vitreous humor ethanol concentration
6MAM: 6-Monoacetylmorphine

Table 2. History of drug use in opioid addicts

Table 3. Drug Intoxication in Body Packers

<table>
<thead>
<tr>
<th>Drugs in Biological Samples</th>
<th>Drugs in Non-biological Samples</th>
<th>Autopsy Reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine, codeine</td>
<td>Heroin, morphine, codeine, caffeine</td>
<td>41 packs in victim’s stomach</td>
</tr>
<tr>
<td>Morphine, codeine</td>
<td>Iranian Crack, dextromethorphan</td>
<td>2 packs in stomach</td>
</tr>
<tr>
<td>Morphine, codeine</td>
<td>Heroin, acetylcodeine, 6MAM</td>
<td>40 role packs in stomach</td>
</tr>
<tr>
<td>Morphine, codeine</td>
<td>Heroin, 6MAM</td>
<td>A packet containing wet white powder in stomach</td>
</tr>
<tr>
<td>Morphine, codeine</td>
<td>Heroin, morphine, codeine, papaverine</td>
<td>A packet containing wet creamy powder in stomach</td>
</tr>
</tbody>
</table>
**Table 4.** Toxicological Analysis In Suspicious Homicide Cases

<table>
<thead>
<tr>
<th>Drugs in Biological Samples</th>
<th>Drugs in Non-biological Samples</th>
<th>Autopsy Reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>Victim was found with deep seizures in neck and both wrists, a cup of sticky white material was found besides her bed</td>
</tr>
<tr>
<td>Phenobarbital, amitriptyline, phenmetrazine, ephedrine, dextromethorphan, propranolol, ethanol</td>
<td>Phenobarbital, amitriptyline, phenmetrazine, ephedrine, dextromethorphan, propranolol, clonazepam, biperiden, morphine &amp; codeine</td>
<td>A cup of ice cream besides the victim</td>
</tr>
<tr>
<td>None</td>
<td>Iranian Crack, dextromethorphan</td>
<td>Multiple trauma, scalp fracture and brain hemorrhage with a pack of white powder beside the victim</td>
</tr>
</tbody>
</table>

**Table 5.** Drug Intoxication in Drug Abusers

<table>
<thead>
<tr>
<th>Drugs in Biological Samples</th>
<th>Drugs in Non-biological Samples</th>
<th>Autopsy Reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine, codeine, lidocaine, alprazolam</td>
<td>Lidocaine (vial), alprazolam (tablets)</td>
<td>drugs were found besides victim's bed</td>
</tr>
<tr>
<td>None</td>
<td>Methadone</td>
<td>Tablets were found in victim's pocket</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Naproxen</td>
<td>Tablets were found in stomach content</td>
</tr>
<tr>
<td>Methadone</td>
<td>Methadone</td>
<td>A bottle containing 10 mL of transparent liquid was found in his room</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Buprenorphine and methadone</td>
<td>27 buprenorphine and 3 methadone tablets in victim's pocket</td>
</tr>
</tbody>
</table>
DISCUSSION

Careful scene investigation is very important and has a fundamental role in detecting the forensic cause of death. In this study, we focused on the correlation between the biological and suspicious non-biological samples obtained from scene investigations and autopsy examinations to detect drugs and poisons through systematic toxicological analysis (STA) of the samples. Opioid (particularly heroin) users in Iran tend to be poly drug users, and they use sedative hypnotics and other central nervous system (CNS) depressants together (7). None of these drugs can be a substitute for opioids, but they have additive and synergistic pharmacological effects (8). In effect, 16.13% of our study population had shown positive results for ethanol detection in blood and VH. As it is shown in Table 2, in two cases, alcohol was found with other drugs. The actual underlying cause of death in these cases is not ethanol or drug alone but the additive CNS depression with concurrent use of them can contribute to fatal overdose and death. This finding is in accordance with previous studies which stated that the use of drugs with alcohol is associated with fatal poisoning (9,13). Abuse of opioids is widespread and leads to many difficulties in communities. About half of our cases (52%) were opioid addicts and the cause of death of 8 cases was heroin and its adulterants overdose. Also, the role of opioid poisoning in increasing the mortality rate was reported by other studies (14). Heroin (diacetylmorphine or diamorphine) is a semi-synthetic opiate which is highly addictive. It can be made of morphine acetylation (15). In black market, heroin does not come in as pure heroin, and it contains many other drugs as adulterants (16). In recent years in Iran, heroin with high purity is adulterated by acidic and basic drugs that have pharmacological effects on CNS. These drugs include benzodiazepines, barbiturates, antihistamines, caffeine, and, opium alkaloids and their acetylated derivatives. This adulterated form of heroin is called "crack" which appears as a white, creamy, or brown powder that differs from white crystalline cocaine that is known as crack in other countries. According to the estimate of United Nations Office on Drugs and Crime (UNODC) in 1999, the annual prevalence of opiates use was 2.8% in 15-64 year olds in Iranian population. In 2008, Iran led all countries by reporting 23% of all heroin seizures in the world due to its geographical and situation on drug trafficking routes. Deaths due to heroin abuse can be secondary to the effects of other drugs and impurities. When these drugs are used in conjunction with heroin, a normally tolerated dose of heroin may be toxic or fatal. The effect of heroin purity on fatal heroin overdose was discussed by Darke et al (9). Also, death among drug addicts was shown by Karlovsek (18). The presence of other drugs in illegal street heroins in Iran is worrying since they can modify or intensify the signs and symptoms of intoxication, fatal drug overdose, and misjudgment in assessing the cause of death without toxicological analysis.

In this study, 6 male victims were body packers. Toxicological analysis of biological samples and packets in their stomach was
positive and confirmed each other in 5 cases. The drug traffickers who swallow packets of illicit drugs for transport are body packers. Toxicity occurs when the drug leaks from these packets into the stomach and absorption takes place (19, 20). Toxicological analyses in both types of samples (biological and packets in the bowel) were negative in one case in our study. The cause of death in this case is not clear, but other probable factors such as peritonitis and bowel obstruction caused by two large packets in small intestine can be the cause of death of this 45 year old male body packer. These findings are in accordance with Wetli et al’s study (19).

In our study, there were three suspicious homicide cases (one male and two females). Toxicological analyses revealed positive results in both biological and non-biological samples of a 30 year old man who was found dead with a cup containing ice cream with a teaspoon beside him. The cause of death of the victim was the synergistic pharmacologic effects (CNS depressant) of alcohol and drugs. The use of drugs for incapacitating persons and committing drug-facilitated crimes, such as robbery, sexual assaults, and homicide was discussed by other studies (21, 22). According to Stewart et al’s study in 2004, drug misusers may be experienced in committing criminal act (23). The presence of alcohol, drugs, and opioids in homicide victims is supported by previous studies (24-26). Consumption of alcohol in male-on-male homicides by perpetrator or victim was reported by Shaw et al (27).

Concomitant use of drugs and alcohol in suicidal attempts and drug abuse is a common issue. The suicidal case was a 20 year old soldier who attempted suicide by any drug or substance accessible. Ethanol was found in blood and VH samples, and mefenamic acid capsules were found in his gastric content. BEC and VHAC were 125 and 130 mg/dL, respectively, which showed non-lethal alcohol concentrations. The interval between alcohol ingestion and death (6 hours in this study) and rapid metabolism of ethanol should be considered in interpreting toxicological results and assessing the cause of death (28). The use of drugs by suicide victims has been confirmed by previous studies (4, 29). Fanton et al discussed the use of drugs and alcohol in suicide due to falling from heights (30). Alcohols and drugs were detected postmortem in suicide cases in Shaw et al’s study (27). The influence of alcohol and psychoactive drugs such as antidepressants, benzodiazepines, opioids on committing suicide was discussed in a 10-year retrospective study (5). Also, Lahti and Vouri stated that single and multiple toxicant poisonings can play significant roles in accidental and suicidal death (31). Association of alcohol abuse and suicidal behavior was shown by other studies (22, 33). Suicide can be categorized as a manner of death that many factors can contribute to. Some of these factors are social and financial problems, depression, alcoholism, and so forth. The cause of death of the suicidal case in this study (a soldier with a suicide note) can be the interaction of psychosocial factors in the decedent’s wish to end his life.

Of our study population, 16.13% (2 females, 3 males) had a history of drug abuse. Drug abuse has different definitions in different countries according to the social status. However, it can be viewed as the intentional use of a drug for non-medical purposes, such as altering consciousness (6). Drug abuse remains a significant problem facing Iran and some other countries. Drug abusers suffer a higher risk of premature death. As it was indicated in previous studies, the mortality rate in heroin injecting drug users was 13 times greater than that in the general population (34). IV drug use can result in serious health and social problems, including HIV and hepatitis C infections, suicide, and drug overdose (35). Also, the cause of death of drug abusers may be HIV/AIDS (36). However, the cause of death in drug abusers in this study is drug related deaths.

As it is shown in this study, the prevalence of death as a result of opioid overdose is high in Iran. This finding is in agreement with Paulozzi’s which stated that opioid analgesics contribute to fatal drug poisoning in the USA. Illicit drugs, such as heroin in black market are not pure and contain
variable percentages of other psychoactive drugs. Multiple drug intoxications may be the cause of death in addicts or victims who use these illegal substances. Also, ethanol is known to potentiate the toxicity of these chemicals.

We have to indicate that the results of our study must be interpreted in the context of several important limitations. Some vital clinical information about our study cases, such as the survival time, signs and symptoms of poisoning upon admission or according to their family reports, clinical management (hemodialysis or gut decontamination) before death, the quantity of drug intake, and medical history were not actually included in our study due to the inaccessibility of the related records. However, more attempts are required to pave the way for future studies on more samples.

**CONCLUSION**

In determining the cause and manner of death, many factors should be taken into account. Scene investigation, systematic toxicological analysis of biological, and suspicious non-biological samples, history of drug abuse, police report, and circumstances surrounding death are of utmost importance.

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**REFERENCES**