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Forensic Toxicology

Analysis of fatalities involving amphetamine in Jazan, Saudi Arabia

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ABSTRACT

Amphetamine use is associated with high tendence of homicidal or suicidal deaths and fatalities making amphetamine a persistent area of concern. This study analyzed fatalities associated with amphetamine use in Jazan city, Saudi Aarabia from 2018 to 2020 and investigated the postmortem tissue distribution of amphetamine. The fatalities associated with the use of amphetamine and other drugs were increased from 18% in 2018to 52% in 2019 and to 80% in 2020 compared to all fatalities associated with amphetamine alone. Suicidal people had the highest average amphetamine blood concentrations with a 90th percentile concentration of 7.6 mg/L. In those who use amphetamine in combination with other drugs, suicidal and homicidal deaths are more common than those who use amphetamine alone. The results demonstrate the need to raise the awareness of the increasing number of deaths associated with amphetamine use in combination with other drugs in health care providers.

Introduction

Amphetamine abuse has become a major concern facing the people of Saudi Arabia. It is frequently abused for its euphoric and stimulant effects. Due to its abusability, dependency and toxicity, the clinical use of different pharmaceutical forms of amphetamine is restricted and strictly regulated. Therefore, its abuse as a mean of increasing alertness and wakefulness in students, drivers and night workers, thus should be discouraged. Overdose or chronic excessive use of amphetamine results in tachycardia, hypertension, agitation, and psychosis. Also, chronic use of amphetamine is associated with high tendencies for homicidal or suicidal deaths, particularly in person with psychopathic personality. Severe poisoning is seen after illicit use of high doses and cause hyperthermia, dehydration, severe hypertension, myocardial infarction, cerebral vascular accidents, seizures, and sudden cardiac death. Also, severe acidosis, multiorgan failure, and death can occur [1,2].

The acute lethal dose of amphetamine has been reported to be 20–25 mg/kg. However, tolerance develops with chronic user, who uses up to 15 gm/day without lethal result. Postmortem concentrations in fatalities due to an overdose of amphetamine ranged between 0.5 and 41 mg/l, 2.8–3 mg/l, 4.3–74 mg/l, 3.2–52 mg/l, and 25–700 mg/l in blood, brain, liver, kidney and urine, respectively [3,4]. These variations could be attributed to the drug physiochemical, properties, such as volume of distribution, pka, lipophilicity, solubility and size of molecule or could be due to other factors that include different, route of drug administration, the site of sample collection, the time interval between death and postmortem specimen collection, bacterial degradation of the drugs and their metabolites [5]. In addition, several factors contribute to the variation in postmortem drug redistribution include age, body fat composition, nutritional status, degree of decomposition, body handling, body temperature, body position and time interval since death [6].

Amphetamine (C9 H13 N; MWt: 135.21) ia a lipophilic molecule with high solublity in lipid with pKa of 9.9 (basic) and volume of distribution of 3.2–5.6 l/kg. Knowledge of pKa allows us to know how pH affect the movement of amphetamine across tissue membranes. For example, drugs with volume of distribution greater than 3 l/kg which is basic with high lipophilic properties might undergo postmortem redistribution [7]. Although this hypothesis is probably not completely accurate with amphetamine, but it provides a view of what might happen to amphetamine in postmortem cases. Amphetamine levels can be assayed in biological media by liquid chromatography with mass

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spectrometry (LC-MS) [4].

Amphetamine use is associated with high risk of toxicity particularly in person with psychopathic personality or a history of homicidal or suicidal tendencies. Chronic use of amphetamines is associated with multiorgan toxicities such as cardiomyopathies and intracranial hemorrhages, which lead to sudden death [1]. A recent Australian study found that amphetamine (the major active metabolite of methamphetamine) was present in 79.7% of all methamphetamine related deaths. It was also detected in 84.8. 79.2, 78.9% of suicide, homicide, and accidental drug toxicity of methamphetamine-related deaths, respectively. Furthermore, amphetamine was responsible for more than 79% of all unnatural causes of methamphetamine-related deaths [8]. Another important Danish study found that amphetamine users have a higher mortality rate than non-users [9].

Amphetamine deaths are due to toxic effects on cardiovascular and central nervous system, in which postmortem findings often include organ congestion and hemorrhage [4]. Analyses of fatalities involving amphetamines are important in order to design preventative approaches. Thus, the purposes of this study were to a) analyze fatalities involving amphetamine in Jazan city in Saudi Arabia and b) explore the distribution of amphetamine in postmortem tissues.

Methods

All fatal cases reported to the Poison Control & Medical Forensic Chemistry Center (Jazan, Saudi Arabia) between January 2018 to December 2020 were evaluated retrospectively. All information regarding to the toxicological study results and accident summary, including the manner of death involving amphetamine was obtained from Electronic OTARR System (OTARR) utilizing a data collection form. The manner of death is classified as following: suicidal, homicidal, accidental, and undetermined. The toxicological analysis and quantification results were collected and analyzed, and the percentage of amphetamine associated fatalities was determined. Initial toxicology analysis included immunoassays for drug of abuse screening, gas chromatography-flame ionization detector GC-FID for alcohol screening, gas chromatography-mass spectrometry (GC-MS) for general unknown screening, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for confirmation and quantification of amphetamine.

Blood sample was mixed with 1 ml phosphate buffer (pH 6). Blood and tissue homogenate samples were extracted by solid phase extraction (SPE) technique using cartridges HYPERSEP VERIFY CX cartridges (Thermo Scientific, USA) according to the manufacture's instruction. For instant, cartridges were used conditioned by 3 ml methanol followed by 3 ml deionized water and then equilibrated by 1 ml phosphate buffer (pH 6). Approximately 2 ml of each sample was loaded and allowed to pass slowly, and then, cartridges were washed with 3 ml deionized water followed by 1 ml of 0.1 M acetic acid and allowed to dry for 15 min under high flow of air. First elution was collected by adding 2 ml of ethyl acetate:hexane (50:50, v/v). Thereafter, cartridges were washed with 3 ml methanol and 2 ml of the second elution (dichloromethane/ isopropanol/ammonium hydroxide; (78/20/2,v/v) was dried under nitrogen. All samples were reconstituted with methanol (100 μl) for the GC–MS analysis.

The quantification analysis was carried out using LC-MS/MS system consisted of a LCQ Fleet Single quadrupole Ion Trap Mass spectrometer (Thermo Scientific, USA) equipped with Thermo Finnigan Surveyor MS Pump.

For amphetamine identification and quantification, tissue samples were homogenized by stomacher and centrifugation (3000 rpm) for 15 min and there were extracted using solid phase extraction. The quantification analysis was carried out using LC-MS/MS system consisted of a LCQ Fleet Single quadrupole Ion Trap Mass spectrometer (Thermo Scientific, USA) equipped with Thermo Finnigan Surveyor MS Pump. 1 ml of blood and 1 gm of organ tissue were added to 1 ml deionized water (1:1 ratio).

Liquid Chromatography Conditions: 10 μl of the sample was injected and the analytes were separated on a Hypersil GOLD column (150 × 3 mm i.d.: 5 μm, Thermo Scientific, USA). The compounds were eluted by isocratic mobile phase made from 85% of 10 mmol ammonium formate buffer and 15% of 0.1% formic acid in acetonitrile (B). The run time was 7 min with a flow rate of 0.3 ml/min.

Mass Spectrometry Conditions: after chromatographic separation, amphetamine and internal standard (amphetamine d5) reached the Electrospray Ionization (ESI) interface and positively charged. The ESI conditions were 5 kV spray voltage, 275 ºC capillary temperature, 50 capillary voltage, 110 tube voltage and 30 arb flow rate of nitrogen sheath gas. The analysis was performed in the scanning mode, monitoring the following transitions: m/z 136 → 119 and m/z 136 → 91 for amphetamine and m/z 141 → 124 for amphetamine d5. Helium gas was used as fragmentation gas in the Collision-induced decompositions (CID). The CID value was 19 for amphetamine and 20 for amphetamine d5. The above method was validated for amphetamine quantitation in Jazan Poison Control Center with LOD of 0.05 μg/ml and LOQ of 0.1 μg/ml.

Statistical analysis

All variables were categorized and tabulated using descriptive statistics. Means, standard error of mean (SEM), median, and 10^{th}–90^{th} percentiles were presented. All data were investigated and calculated using SigmaPlot 11 for Windows.

Result

The toxicological analysis of fatalities involving amphetamine in blood and postmortem tissues was investigated and summarized (Table 1). According to the manner of death, suicidal cases represent the highest average levels of amphetamine in blood and urine, followed by accidental, homicidal, and undetermined. All average blood concentrations of amphetamine are within the lethal range (0.5–41 mg/l) in all decedents, while less in undetermined decedents. Whereas, the postmortem average blood concentrations of amphetamine were 2.32, 0.8, 1.8, and 0.24 mg/l in suicidal, homicidal, accidental, and undetermined decedents, respectively. The 10^{th}–90^{th} percentiles of amphetamine blood concentrations were 0.13–7.6 mg/l in suicidal decedents, 0. 2–2.3 mg/l in homicidal decedents, 0.1–4.5 mg/l in accidental decedents, and 0.02–0.4 mg/l in undetermined decedents. These results show 90% of the homicidal and suicidal decedents having concentrations more than 0.13 and 0.2 mg/l, respectively. The highest 90^{th} percentile blood concentrations (7.6 mg/l) were seen in suicidal decedents (n = 9).

The abundance of fatalities (per year) involving amphetamine alone and in combination with other drugs is demonstrated in Fig. 1. The abundance of fatalities involving amphetamine with other drugs increased from 18% in 2018 of total fatalities involving amphetamine to 52% and 80% in 2019 and 2020, respectively. Whereas the fatalities involving amphetamine alone was decreased from 82%, 48%, and 20% in 2018, 2019, and 2020, respectively.

In addition, the occurrence of amphetamine related fatalities in different manner of death cases was presented in Fig. 2. Among amphetamine related fatalities, the occurrence of suicidal and homicidal intoxications was the most common with high proportion of detections in fatalities involving amphetamine with other drugs compared with fatalities involving amphetamine alone (Fig. 2). Moreover, the percentage of amphetamine related fatalities according to the manner of death by age group was presented in Fig. 3. Interestingly, more than 68% of the suicidal and homicidal was occurred in age group of 16–25 year and these percentage decreased with increased the age (Fig. 3).

Furthermore, the percentage of amphetamine with other drugs in fatalities involving amphetamine was presented as pie chart in Fig. 4. The results represents that 52% of fatalities involved amphetamine plus

Table 1
Summary of toxicological analysis of fatalities involving amphetamine by manner of death.

Manner of death	Age group	N. of Samples		Amphetamine concentrations (mg/l)						Other detected drugs
				Brain	Liver	Kidney	Stomach	Blood	Urine	
Suicidal (N = 12)	16–25	4	Mean±SEM	0.96 ± 0.2	1.03 ± 0.2	0.6 ± 0.1	0.75 ± 0.4	2.32 ± 1	10.2 ± 6.4	Cathinone, Cathine, THC, Methamphetamine, Ethanol
	26–35	5	Median	0.85	1.14	0.625	0.18	1.2	2.8	
	> 35	3	10 – 90 Percentile	0.21 – 2.02	0.31 – 1.88	0.14 – 0.9	0.14 – 2.8	0.13 – 7.6	1.4 – 38.6	
Homicidal (N = 19)	16–25	7	Mean±SEM	0.93 ± 0.3	1.17 ± 0.3	0.7 ± 0.2	1 ± 0.4	0.8 ± 0.3	8.8 ± 3	Cathinone, Cathine, THC, Methamphetamine, Ethanol
	26–35	5	Median	0.5	1.5	0.5	0.3	0.11	10	
	> 35	7	10 – 90 Percentile	0.01 – 2.5	0.08 – 2.4	0.04 – 2.1	0.006 – 2.9	0. 2 – 2.3	0.13 – 17.15	
Accidental (N = 8)	16–25	2	Mean±SEM	1.5 ± 0.8	1.3 ± 0.7	0.7 ± 0.3	0.4 ± 0.1	1.8 ± 0.9	2.6 ± 1.1	Cathinone, Ethanol
	26–35	3	Median	1.1	0.3	0.3	0.3	1.2	2.1	
	> 35	3	10 – 90 Percentile	0.07 – 3.6	0.06 – 3.3	0.1 – 2.1	0.07 – 0.8	0.1 – 4.5	1 – 4.8	
Undetermined (N = 19)	16–25	3	Mean±SEM	0.87 ± 0.2	1.2 ± 0.4	0.2 ± 0.04	0.3 ± 0.2	0.24 ± 0.1	2.6 ± 0.9	Cathinone, THC, Methamphetamine, Ethanol
	26–35	5	Median	0.65	0.61	0.1	0.1	0.3	2.1	
	> 35	11	10 – 90 Percentile	0.05 – 2.2	0.2 – 3.3	0.05 – 0.3	0.06 – 1.2	0.02 – 0.4	0.3 – 4.8	

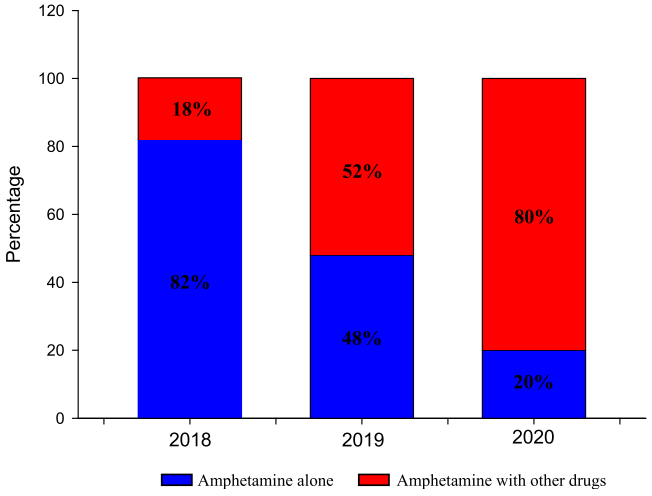

Fig. 1. Stacked bar chart of the abundance of fatalities per yearinvolving amphetamine alone and amphetamine with other drugs.

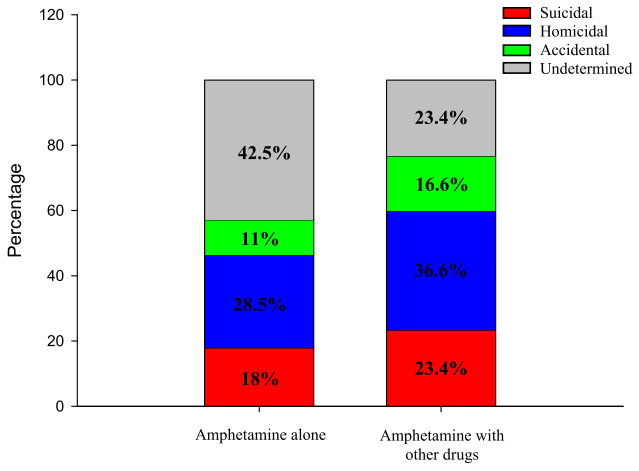

Fig. 2. Stacked bar chart of occurrence of the manner of death according to fatalities involving amphetamine alone or amphetamine with other drugs.

THC and ethanol, while 48% of fatalities involved amphetamine plus cathine, cathinone, and methamphetamine. In fatalities involving amphetamine and methamphetamine, the amphetamine may actually be a reflective of methamphetamine usage, as amphetamine is present as a metabolite rather than being consumed alone.

Discussion

Globally, the rate of fatalities involving amphetamines increased nearly 5-fold; from 0.8 to 3.9 in 2012 through 2018 [10]. They are the fourth largest cause of death among illegal drug users, according to the Global Burden of Disease Report from 1990 to 2017 [11]. In Saudi Arabia, the rate of fatalities involving amphetamine use increased by two-fold, from 0.02 per 100,000 to 0.04 per 100,000 [11]. According to the findings of the current study, fatalities involving amphetamines combined with other drugs were the major cause of amphetamine involving fatalities, representing more than 4-fold increases between 2018 and 2020. The increase in the percentage of fatalities involving amphetamine combined with other drugs could be linked to an increase in overdose mortality [2]. A recent study has demonstrated that 14.9%

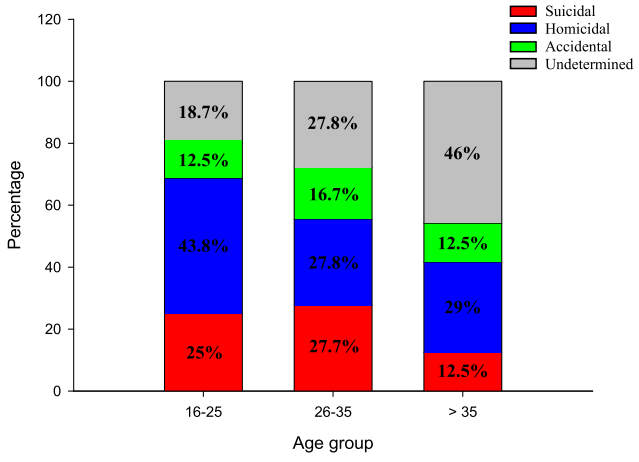

Fig. 3. Stacked bar chart of occurrence of the manner of death according to age group.

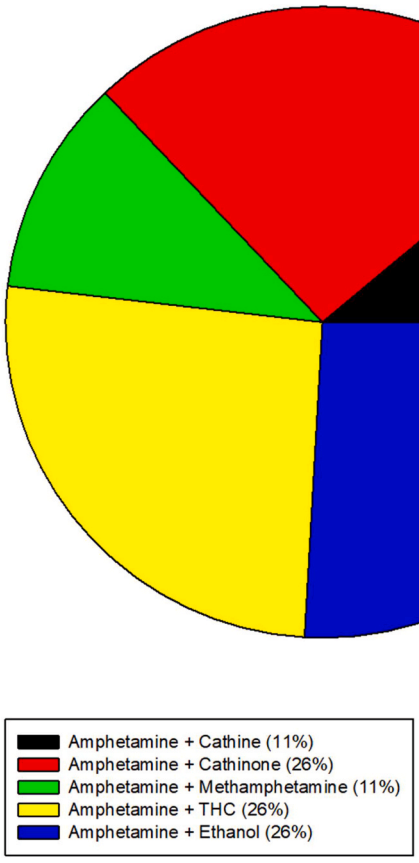

Fig. 4. Percentage of amphetamine with other drugs in fatalities involving amphetamine.

of road traffic accident fatalities in the Eastern region of Saudi Arabia between between 2015 and 2019 was attributed to the abuse of amphetamine combined with other drugs [12].

In comparison to all fatalities involving amphetamine over the last three years, the number of those involving amphetamine in combination with other drugs has proportionately increased from 18% in 2018 to 55% in 2019 and to 80% in 2020. In addition, the number of suicide and homicidal deaths caused by amphetamine combined with other drugs is bigger than the number of deaths caused by amphetamine alone. Furthermore, the suicidal group's average amphetamine concentrations in blood and urine were higher than the other groups. Suicide, homicide, and accidental deaths all had amphetamine blood concentrations within the previously established lethal range (0.5-41 mg/l) [4], but undetermined deaths had amphetamine blood concentrations less than 0.5 mg/l. A thorough death investigation, including autopsy findings and cause of death, is required to determine whether amphetamine poisoning was the cause of death.

Amphetamine usage has been linked to aggression, violence, and suicidality, [13,14], and these effects can be exacerbated when amphetamine is combined with other drugs.. Approximately, 78% of all amphetamine fatalities involved ethanol, THC, and cathinone in addition to amphetamine. In accordance with previous papers, polydrug use was commonly reported as risk factors that leads to fatal drug overdose [15,16]. These findings indicate that combination of amphetamine with other drugs continues to be a large concern in clinical and forensic toxicology.

As a result, it is necessary to consider the abuse of amphetamine in combination with other drugs in younger individuals (16–25 years old) who are more likely to commit suicide and have higher average blood and urine amphetamine concentrations.

These findings emphasize the critical importance of increasing healthcare provider awareness of amphetamine use, as well as the additive toxic effect of amphetamine use in combination with other drugs on the occurrence of suicidal and homicidal deaths.

Conclusion

The higher percentage of fatalities involving amphetamines and other drugs was found to be proportionally linked to the rate of suicidality and homicides and average blood amphetamine concentration, especially among those under 35 years old. Ethanol, THC, and cathinone are the most commonly used substances in combination with amphetamine. This study also raises awareness to the rising numbers of fatalities linked to the use of ethanol, THC, and cathinone in combination with amphetamine. Further research is needed to determine the relationship between tissue amphetamine concentrations and the manner of death, as well as the effect and proper treatment options for combining amphetamine with ethanol, THC, and cathinone.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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