Original Research Article

Cardiopathology in Methamphetamine Poisoning-Related Deaths in Chiang Mai Thailand

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Abstract

Background: Blood Methamphetamine levels have been utilized to assess methamphetamine exposure and its toxicity. Heart is a major target organ of methamphetamine intoxication. In some autopsy cases heart pathologies have been revealed at low level of methamphetamine and extensively to be understand a relationship between the blood methamphetamine level and heart pathology.

Aim: The aim of this study was to assess the relationship between blood methamphetamine level and heart pathology by using postmortem cases.

Methodology: One hundred and twenty medico-legal cases were included and blood methamphetamine or amphetamine levels in whole blood along with heart pathological finding were determined.

Results: Coronary atherosclerosis, myocardial fiber hypertrophy, and fibrosis of the left ventricular myocardium were highly frequency findings in methamphetamine intoxication. Interestingly, forensic cases revealed myocardial fiber hypertrophy in chronic methamphetamine users.

Conclusion: The levels of methamphetamine and amphetamine associated with myocarditis, cardiomyopathy and dystrophic calcification mitral valve. Evaluation of methamphetamine and amphetamine levels are key biomarkers for predicting the seriousness of heart-related pathological conditions.

Key words: Methamphetamine, Amphetamine, heart pathology, Arteriosclerosis, BloodMethamphetamine level.

Introduction

Methamphetamine (Meth) is a synthetic drug and stimulate at central nervous system. It has emerged as a significant public health concern due to its widespread use and the myriad of adverse effects it exerts on various organ systems[1,2]. One of the critical

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areas of concern is its impact on cardiovascular health, particularly its association with heart pathology. Meth abuse has been linked to a range of cardiovascular complications, including hypertension, arrhythmias, and structural damage to the heart[3]. Understanding the intricate interplay between Meth use and heart pathology is essential for healthcare professionals, researchers and policymakers to develop effective strategies for prevention, intervention and treatment.

Meth use is growing globally resulting in significant morbidity and mortality exacerbated by a poorly understood increase in multiple forms of cardiovascular disease[4,5].Meth use is associated with cardiovascular disease through two main mechanisms: catecholamine toxicity and direct effects on cardiac and vascular tissue[6].In vivo models with long-term Meth exposure, histopathological examinations have presented cardiac lesions including necrosis of the myocytes, atrophy, mitochondrial degeneration, inflammation, interstitial oedema and fibrosis[7]. The lesions could be reversible after Meth cessation[8]. In post-mortem examination, the heart presented concentric myocardial hypertrophy, extensive myocardial remodeling with perivascular and interstitial fibrosis and myocardial scarring due to infraction[9].

Normally, cytochrome P450 2D6 (CYP2D6) is an essential enzyme in drug metabolism, especially addictive substances, Meth, codeine, fentanyl, and methadone[10].The enzyme CYP2D6 plays the most crucial role in the transformation of methamphetamine and the ratio of Am/ Meth reflected to CYP2D6 activity[11]. Meth is metabolized by CYP2D6 to give amphetamine (Am).Meth was excreted unchanged form in the urine, while only 37-54% was excreted as Am[12]. Polymorphism in CYP2D6 might be related the heart failure in Meth intoxication[13].

There still is uncertainty about the percentage of patients that develop heart failure or other cardiac pathologies due to Meth abuse. Exploration of the intricate relationship between Meth use and heart pathology, delving into the physiological mechanisms, clinical manifestations, and broader implications for public health. In many cases where the level of a drug is present in the body at a quantity below lethal doses, death may still occur. The presence of the drug in the bloodstream or urine, even in small amounts, may not serve as a reliable indicator of the cause of death. The objective of this study was to study correlation between Meth level and cardiopathology using postmortem cases. It might be contributed to a more comprehensive understanding of the challenges posed by Meth abuse on cardiovascular well-being and inform efforts to mitigate its impact on individuals and communities.

Materials and methods

Study design:

This study investigated medico-legal cases where autopsies were performed at the Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, between 2017 and 2022. Urine samples were screened for methamphetamine using an immunoassay kit. Blood and heart samples were collected to confirm the presence of methamphetamine and to investigate; heart pathology. The case selection process is depicted in Figure 1. This study received ethical approval from the Research Ethics Committee, Faculty of Medicine, Chiang Mai University (Study code: FOR-2565-0069, Research ID: 0069).

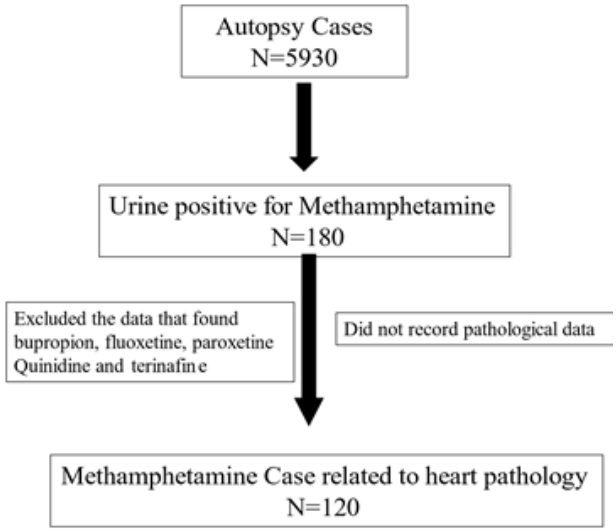

Figure 1: Flow chart of inclusion criteria for case selection

Methamphetamine and amphetamine analysis

Methamphetamine (Meth) and amphetamine (Am) were analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS) by applied the method of Nakashima[14]. In brief, whole blood samples were transferred to microcentrifuge tubes

and spiked with saturated sodium tetraborate and internal standards Meth-D5 and Am-D5. The mixture was extracted with 1-chlorobutane, followed by centrifugation at 13,000 rpm for 5 minutes at room temperature. The organic layer was collected and evaporated under nitrogen gas. The residue was reconstituted with a 9:1 mixture of 5 mM ammonium formate and 0.1% formic acid. The final extract was analyzed by LC-MS/MS following the operating conditions presented in Table 1.

Table1: Instrument parameter for methamphetamine and amphetamine with LC-MS/MS

HPLC-Agilent 1290 Infinity	Details
Autosampler	Agilent G4226A
Column	Zorbax Eclipse C18 rapid resolution HT 2.1 × 100 mm 1.8 μm 600 bar
Guard column	Zorbax Eclipse Plus-C18 Narrow Bore Guard column 2.1 × 12.5 mm 5 μm
Column temperature	40 °C
Flow rate	0.3 mL/ min
Mobile phase	A: 5 mM ammonium formate with 0.1% formic acid in ultrapure water B: 0.1% formic acid in acetonitrile
Gradient solvent (%B, min)	Initial, 5%; 3 min, 30%; 2 min, 90%; 6 min, stop time 11 min, post run 4 min
Injection volume	5 µL
MS-Agilent 6490 Triple Quad	
Capillary (V)	3500
Gas temperature (°C)	320
Gas flow (L/min)	9
Nebulizer (psi)	45
Nozzle voltage (V)	500
Sheath gas Temp (°C)	350
Sheath gas flow (L/min)	11
Ion source	ESI positive mode
Scan type	MRM

Histological examination

The heart was carefully removed and weighed. Selected heart tissues were fixed in a 4% paraformaldehyde solution, dehydrated through an alcohol series, cleared in xylene, and finally embedded in paraffin wax. These paraffin blocks were sectioned at 5 µm thickness using a microtome and stained with hematoxylin and eosin (H&E) following the method of Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai Thailand. Upon histological examination, various cardiopathological findings were observed, including myocardial fiber hypertrophy, fibrosis of the left ventricle (LV) myocardium, contraction band necrosis within the ventricular myocardium, myocardial infarction, cardiomyopathy, endocarditis, myocarditis, dystrophic calcification of both the aortic and mitral valves, and coronary atherosclerosis.

Statistical analysis

Quantitative variables were presented as mean ± standard deviation (SD), while qualitative variables were expressed as percentages. Descriptive statistics were employed to summarize the data. Spearman correlation analysis was conducted to assess the relationships between the levels of methamphetamine (Meth), amphetamine (Am), the Am/Meth ratio, and various pathological findings. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant.

Results

A total of 120 cases were included from 5930 autopsied cases that tested positive for methamphetamine in blood and urine samples. The subjects comprised 107 males (89.2%) and 13 females (10.8%). The age of the subjects ranged between 14 years and 77 years. The mean age for males and females was 39 years each. Methamphetamine blood levels ranged from 0.01 to 12.50 µg/mL (with a mean of 0.45 ± 1.23 µg/mL), while amphetamine levels ranged from 0 to 1.10 µg/mL (with a mean of 0.06 ± 0.14 µg/mL). The amphetamine/methamphetamine ratio ranged from 0 to 1.50 (with a mean of 0.20 ± 0.21), and the weight of the heart ranged from 144 to 950 grams (with a mean of 386.86 ± 129.23 grams).The results are presented in Table 2.

Table 2: The demographic data of the subjects.

Parameters	Male	Female	p-values
Numbers (n=120)	89.2%	10.8%	ND
Age (years)	38.9± 11.5 range 14-77	38.6±9.5 Range 26-60	0.847
Methamphetamine levels (ng/ mL)	0.45±1.29 Range 0.01-12.5	0.47±0.58 Range 0.01-2.20	0.225
Amphetamine level (ng/mL)	0.06±0.15 Range 0-1.10	0.06±0.06 Range 0-0.20	0.143
Amphetamine/ methamphetamine ratio	0.20±0.21 Range 0-1.14	0.22±0.39 Range 0-1.5	0.368
Heart weight (g)	392.08±129.54 Range 144-950	344.69±123.37 Range 205-590	0.171

Meth intoxication was found in male more than female cases about 8 times. However, the blood level of Meth, Am and Am/ Meth in male and female were similar. The retention time of Meth, Meth-D5, Am and Am-d5 showed 5.63, 5.64, 5.43 and 5.40 min, respectively. The limit of detection and quantitationof Meth presented 1 and 10 ng/ mL. Similarly, Am showed 5 and 10 ng/ mL, respectively. The LC-MS Chromatogram of Methamphetamine and amphetamine is presented in Figure 2.

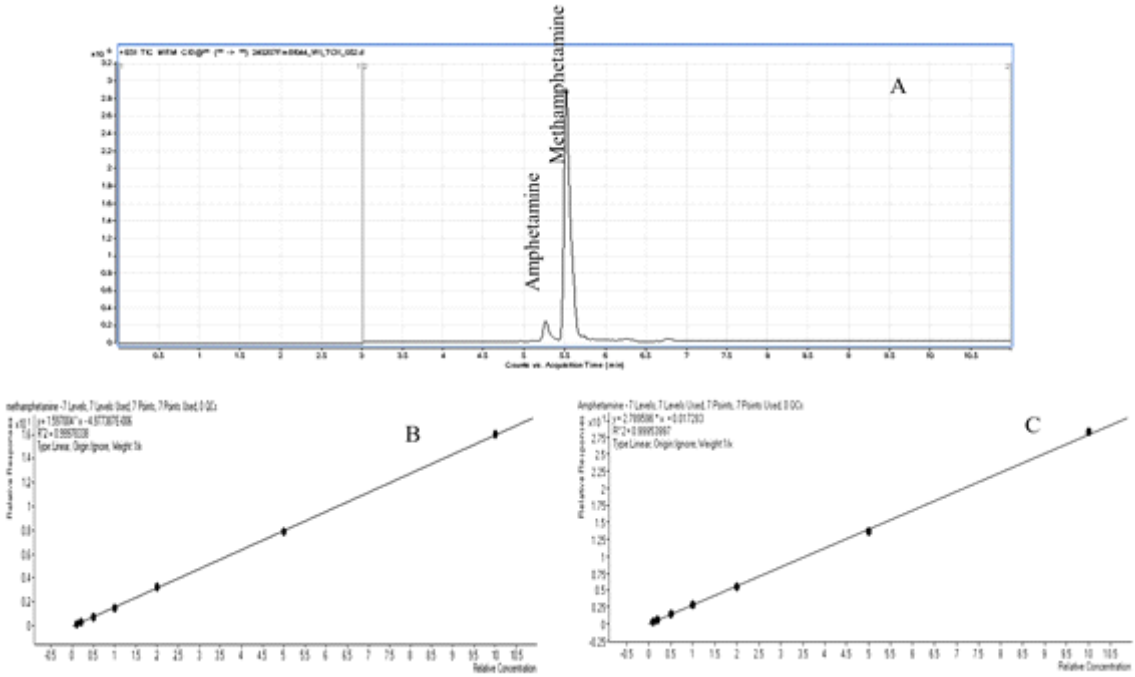

Figure 2: LC-MS Chromatogram of Methamphetamine and amphetamine (A), standard curve for methamphetamine (B) and amphetamine (C)

The range of Meth and Am concentrations presented 0.01-12.5(mean 0.45) and 0-1.10ng/ mL (mean 0.06), respectively. Am is a major metabolite of Meth that can be found in blood and samples. Ratio of Am/ Meth is reflected for CYP2D6 activity and the results revealed 0-1.14 and 0-1.50 for male and female, respectively. For autopsy cases, the CYP2D6 activity showed low the ratio value and could be assumed as

poor metabolizer[11]. CYP2D6 is an enzyme found in the liver that is responsible for metabolizing Meth[15]. People who have certain variations (polymorphisms) in the CYP2D6 gene metabolize Meth differently. Those with two reduced-function alleles (poor metabolizers) metabolize Meth more slowly, which can lead to higher levels of the drug in the bloodstream and increased risk of toxic effects[16]. Meth is a stimulant drug that can cause a variety of harmful effects, including addiction, psychosis, heart damage, and stroke. Heart wight of male and female who exposed to Meth were similar, however the heart wight in the Meth intoxication were higher than normal[17].

Pathological findings

Pathology of the heart can be categorized and presented in Table 3. Coronary atherosclerosis, myocardial fiber hypertrophy, and fibrosis of the left ventricular (LV) myocardium were highly frequent findings in Meth intoxication. Exposure to Meth is associated with acute vascular constriction and vasospasm[3]. It induces inflammation and increases T cells and macrophages, activating proinflammatory signaling and the fibrosis process[18]. Chronic Meth use induces endothelial damage and pulmonary hypertension[3]. Our results showed that coronary atherosclerosis (55%), myocardial fiber hypertrophy (35.8%), and fibrosis of the LV myocardium (25%) were very frequent pathological findings. Interestingly, forensic cases revealed myocardial fiber hypertrophy in chronic Meth users. The results are presented in Table 3 and the pathological findings are shown in Figure 3.

Table 3: Characteristics of heart pathology that found in postmortem related with Methamphetamine.

Characteristic of Heart Pathology	Amount (n=120)	Percentage (%)
coronary atherosclerosis	66	55.0
myocardial fiber hypertrophy	43	35.8
fibrosis of LV myocardium	30	25.0
contraction band necrosis ventricular	24	20.0
myocardium	15	12.5
myocardial infarction	3	3.0
cardiomyopathy	3	3.0
dystrophic calcification aortic valve	2	1.7
myocarditis	2	1.7
dystrophic calcification mitral valve endocarditis	1	0.8

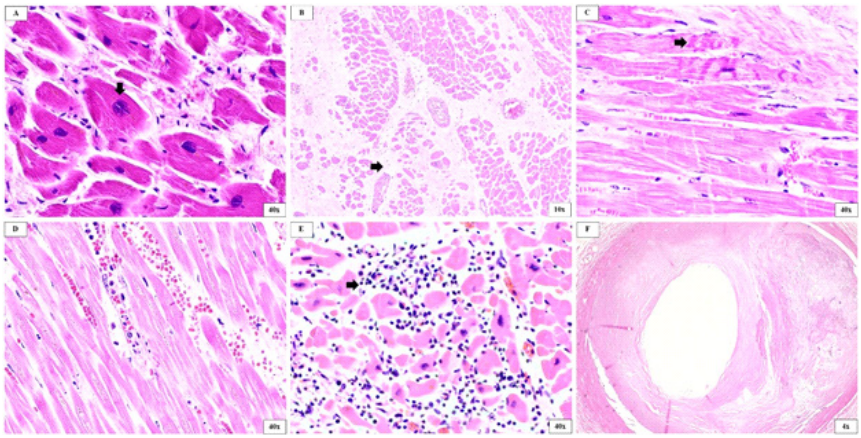

Figure 3: Heart pathology in methamphetamine-related postmortem case and stained with Hematoxylin and eosin. A)Myocardial fiber hypertrophy Arrow shown scattered enlarged nuclei in myocardial fiber (40x), B) Fibrosis of left ventricular myocardium showed by arrow (10x),C) Contraction band necrosis at ventricular myocardium showed by arrow (40x) D) Acute myocardial infarction. The myocardial fibershown coagulative necrosis and neutrophilic infiltration (40x), E) Myocarditis in methamphetamine-related postmortem case. Arrows shown interstitial lymphocytic infiltration. Hematoxylin and eosin (40x), F) Coronary atherosclerosis (4x).

Correlation of blood level of methamphetamine, amphetamine and Am/Meth ratio and pathological findings

The study found that only the levels of Meth and Am associated with myocarditis, cardiomyopathy and dystrophic calcification mitral valve. Akhgari presented that cardiovascular pathology was revealed about 68% and myocardial fiber hypertrophy, mild, moderate to severe atherosclerosis and focal degeneration/necrosis were found in Meth poisoning-related death[19]. For, Am/Meth ratio related with myocarditis and dystrophic calcification mitral valve. Correlation of blood level of Meth, Am and Am/Meth ratio and pathological findings are presented in Figure 3.In this research showed that A low dose level of Meth, Am, and the Am/Meth ratio is associated with a high rate of coronary atherosclerosis. The potential link between CYP2D6 genotype, Methamphetamine (Meth) use, and coronary atherosclerosis is a complex and ongoing area of research. Some studies suggest that poor metabolizers (individuals with two reduced-function alleles) have higher Meth blood levels, potentially increasing the risk of adverse effects[20].However, Meth-induced cardiotoxicity, it could well explain increasing reports of heart failure in Meth abusers[21].

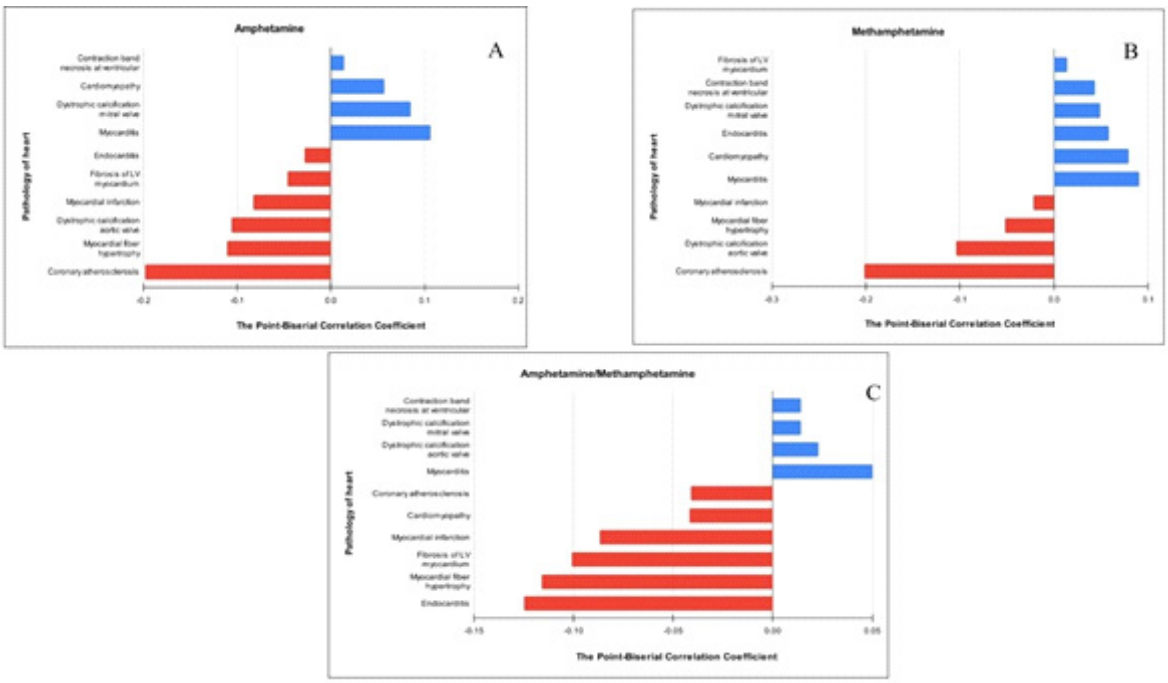

Figure 4: Correlation of blood level of methamphetamine, amphetamine and Am/Meth ratio and pathological findings by the point-biserial plot.

Discussion

Methamphetamine is a highly addictive central nervous system stimulant that can have severe and long-lasting effects on a person's physical and mental health. Meth poses a grave threat to both individual health and public safety, highlighting the importance of prevention, treatment, and support for those affected by addiction. Meth use can have profound effects on the cardiovascular system, ranging from acute complications such as

tachycardia and hypertension to chronic conditions like cardiomyopathy and increased risk of myocardial infarction.

For autopsy cases, the CYP2D6 activity showed low the ratio value and could be assumed as poor metabolizer[11]. CYP2D6 is an enzyme found in the liver that is responsible for metabolizing Meth[15]. People who have certain variations (polymorphisms) in the CYP2D6 gene metabolize Meth differently. Those with two reduced-function alleles (poor metabolizers) metabolize Meth more slowly, which can lead to higher levels of the drug in the bloodstream and increased risk of toxic effects[16]. Meth is a stimulant drug that can cause a variety of harmful effects, including addiction, psychosis, heart damage, and stroke. Heart wight of male and female who exposed to Meth were similar, however the heart wight in the Meth intoxication were higher than normal[17].

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The study found that only the levels of Meth and Am associated with myocarditis, cardiomyopathy and dystrophic calcification mitral valve. Akhgari presented that cardiovascular pathology was revealed about 68% and myocardial fiber hypertrophy, mild, moderate to severe atherosclerosis and focal degeneration/necrosis were found in Meth poisoning-related death[19].

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Conclusion

In this study, it was discovered that incidents of methamphetamine poisoning and fatalities linked to narcotics occur about 8 times more frequently in males than in females. it was noticed that only the levels of methamphetamine and amphetamine were connected to coronary artery disease. However, the Am/Meth ratio didn't show a correlation with this condition, suggesting that drugs and their by products might not be directly linked to the occurrence of CYP2D6 activity-related heart conditions. Thus, evaluating the levels of methamphetamine and amphetamine is critical for predicting the seriousness of heart-related pathological conditions.

Conflict of interest: No conflict of interest

Source of funding: There is no source of funding

Ethical clearance: This research has received approval from the the Research Ethics Committee, Faculty of Medicine, Chiang Mai University (Study code: FOR-2565-0069, Research ID: 0069).

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