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OPIS PRZYPADKU
CASE REPORT

Post mortem ethanol detection in corpses after embalming – preliminary study

Pośmiertna detekcja etanolu w zwłokach poddanych balsamacji – badania wstępne

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ABSTRACT

Nowadays, embalming has become more popular as it is often performed due to sanitary, hygienic, or psychological reasons. In special circumstances, like transporting a corpse from abroad, embalming is an administrative requirement. Embalming is performed using chemical substances that inhibit enzymes, bacteriological activity, and therefore cease decomposition processes. The study shows the toxicological analyses of sampled materials collected during post mortem examinations performed on six corpses subjected to the embalming process. It also presents the concentration of used preservatives in the tissues and body fluids. Analysis performed using gas chromatography with a flame ionization detector (GC-FID) revealed that in five cases, the deceased were intoxicated with ethanol during the time of death, and liquid chromatography-mass spectrometry (LC-MS) excluded the presence of psychoactive substances in all six cases. The results showed that in cases of toxicological analyses of embalmed corpses, it is crucial to secure isolated body fluids, such as cerebrospinal fluid, vitreous humor, and a wide range of reference material.

KEY WORDS

ethanol, embalming, post mortem analysis

STRESZCZENIE

Balsamacja zwłok staje się coraz bardziej popularna ze względów sanitarnych, higienicznych lub psychologicznych. W szczególnych okolicznościach, jak transport zwłok z zagranicy, balsamowanie jest wymogiem administracyjnym. Balsamacja odbywa się za pomocą substancji chemicznych, które hamują aktywność enzymatyczną i bakteriologiczną, wstrzymując tym samym procesy rozkładu. Prezentowana praca przedstawia analizy toksykologiczne materiałów pobranych podczas sekcji sześciu zwłok poddanych procesom balsamowania oraz wyniki pomiarów stężenia użytych kon-

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serwantów w tkankach i płynach ustrojowych badanych zwłok. Analiza przeprowadzona za pomocą chromatografii gazowej z detektorem płomieniowo-jonizacyjnym (*gas chromatography with a flame ionization detector* – GC-FID) udowodniła obecność etanolu w pięciu przypadkach. Chromatografia cieczowa połączona ze spektrometrią mas (*liquid chromatography-mass spectrometry* – LC-MS) wykluczyła obecność substancji psychoaktywnych we wszystkich sześciu przypadkach. Wyniki wykazały, że w przypadku analiz toksykologicznych zabalsamowanych zwłok kluczowe znaczenie ma zabezpieczenie izolowanych płynów ustrojowych, takich jak płyn mózgowo-rdzeniowy, płyn gałki ocznej, oraz wielu materiałów referencyjnych.

SŁOWA KLUCZOWE

etanol, balsamacja, analiza pośmiertna

INTRODUCTION

The available literature indicates that mummification is gaining popularity. In ancient times, mummification was performed on corpses using natural substances, i.e., liquid resin to fill up the skull cavity, palm wine, myrrh, cinnamon, essential oils, natron (natural soda, sodium carbonate with sodium chloride, sodium sulfate, and other impurities) and resin. The visceral organs were removed and preserved separately [1]. The ears, nose, and mouth were covered with beeswax exhibiting antibacterial properties. The newest research on canopic jars, which were used for visceral organ storage in ancient Egypt, showed the presence of longiborneol, abietadiene acid derivatives, guaiacol, aniseed constituents, salicylic acid, chamazulene, and jacobine [2]. During the Middle Ages and Renaissance, corpses were embalmed using substances like mercury, myrtle, mint, frankincense, lime, and, possibly, cinnamon and copper [3]. In the 18th century, embalming involved, among others, myrtle, honey, and lime [4].

Nowadays, natural substances have been replaced with specialized chemicals. Removing visceral organs became unnecessary as the specialized chemicals preserve the veins and arteries, clean the intestines and the stomach, and preserve the liver. The skin's natural color and elasticity and even the dehydrated eyeballs can be brought back to the natural state. The chemicals used for embalming deactivate enzymes and microbes, which stops the decomposition process. The most frequently used methods are formalin, Thiel's, and saturated salt solution methods. Formalin is still used ubiquitously due to its low cost and wide availability [5]. Embalming is widespread due to the sanitary and epidemiological requirements of transporting corpses across borders [6]. Embalmed donor corpses are used for teaching anatomy [7], practicing surgical skills [8] or anesthetic procedures [9]. The skin of formalin embalmed corpses is almost bacteriologically sterile and free from active viruses [10].

For autopsy technicians and forensic pathologists, embalming is a protective measure against infection during the post mortem autopsy [11] of corpses with positive quantitative polymerase chain reaction (qCR) test results of embalmed tissues [12]. The tissue collected from embalmed corpses allows immunohistological assays and PCR tests to be conducted for virus detection [13]. The early inactivation of microbes substantially reduces the production of endogenic ethanol, which is relevant for the analysis of forensic toxicology reports. Cadavers embalmed using formalin did not show any microbial growth up to eight months after embalming [14].

Nowadays, the most frequently used chemicals in arterial solutions comprise preservatives (18–35% formaldehyde for burial purposes; glutaraldehyde, phenol, formalin (40% formaldehyde) for preserving anatomical specimens), germicides (like thymol, quaternary ammonium compounds, glutaraldehyde), buffers (citrates, EDTA disodium salt, borax, sodium phosphate), modifying agents (buffers, humectants, inorganic salts) influencing and controlling the action of preservative agents, humectants used to hydrate tissue (like glycerol, sorbitol, glycol, lanolin), perfuming agents (benzaldehyde, oil of cloves, methyl salicylate), surfactants reducing the molecular cohesion of liquid and enabling the penetration of small arteries and veins (like sulfonates), dyes (eosin, Ponceau Red, erythrosine and amaranth), and anticoagulants reducing blood viscosity (like sodium citrate, sodium oxalate, EDTA disodium salt) [15,16].

The research on drug stability in formalin-fixed tissues is limited [17]. Only a few xenobiotics have been analyzed to date in human embalmed corpses: midazolam and oxycodone [18], ethchlorvynol, and phenobarbital [19], morphine [6], and acetone and acetoacetate [20]. The presented study shows the results of toxicological analyses of six cadavers subjected to embalming processes for the purpose of transporting them abroad. The study aimed to analyze a wide range of samples of biological material collected from cadavers subjected to the embalming process and to indicate the biological material that is the least susceptible to penetration of the embalming agent. It also underlines the need to standardize the procedures and to provide samples of the embalming agents for comparative analyses.

CASE REPORTS

Case 1

A thirty-seven-year-old Caucasian male died on a Thai island in a motorcycle accident due to not complying with speed limits leading to losing control over the vehicle and hitting a metal guard rail. The post mortem

autopsy carried out in a local hospital established the cause of death as traumatic shock due to excessive blood loss and pleural hematoma resulting from the accident.

The corpse was embalmed for transportation purposes. The post mortem examination carried out after transporting the corpse to Poland revealed a stitched incision on the inner surface of the right arm, below the armpit, which was consistent with the condition after introducing the preservative substance into the vascular system (Figure 1). Similar incisions were revealed just below both inguinal pits on the front surface of both thighs. Additionally, the examination revealed the following trauma: a half-length fracture of the left humerus, fracture of the left clavicle, and fractures of the 1st and the 3rd ribs on the left mid-clavicular line, complete detachment of the aorta (slightly below the arch), with massive hemorrhage into the pleural cavities, multiple ruptures of the spleen parenchyma and intestinal mesentery, extensive subcutaneous and back muscle hemorrhages in the lumbar region. The extensive trauma might have resulted from the motorcycle accident. The toxicological analysis was done 17 days after the death and 14 days after embalming.

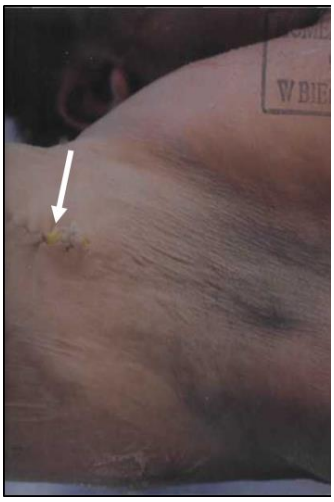

Fig. 1. Stitched incision after introducing preservative substance into vascular system located on inner surface of right arm, just below armpit.
Ryc. 1. Zszyte nacięcie po wprowadzeniu substancji konserwującej do łożyska naczyniowego zwłok, zlokalizowane tuż poniżej dołu pachowego po wewnętrznej stronie prawego ramienia.

Case 2

A twenty-eight-year-old Caucasian male died after falling from a height at his workplace in Azerbaijan. The post mortem examination carried out in Azerbaijan revealed trauma in the lungs and abdominal cavity, multiple fractures of the ribs, and fractures of the II and III vertebrae, and indicated them, and the following hemorrhage, as a direct cause of death.

The post mortem examination performed after transporting the corpse to Poland revealed: 1) signs of the initial post mortem examination and fabrics soaked in, most probably, formalin located in the chest, 2) the following trauma: bruised head wound in the occipital area, intensive hemorrhages with intramuscular effusions around the shoulders, scapulae, and in the spinal column, fractures of the spinous processes of the C7, Th1-Th3, and Th7-Th12, fracture of the spine with a rupture of the spinal canal at the Th12-L-1 level, fracture of the left ribs from V to X in the paraspinal line, and from VII to X in the scapular line, as well as fractures in the mid-clavicular line of the 1st and the 2nd right rib. The revealed trauma might have been caused by the fall from the height to the hard surface. The toxicological analysis was conducted 14 days after the death and 10 days after embalming.

Case 3

A sixty-five-year-old Caucasian male was found dead in a hotel pool in Egypt. The embalming was done 11 days after the death. The corpse was soaked in formalin, which could penetrate the veins and the soft tissues of the entire body. Formalin was also injected into the brain. The corpse was wrapped in a gauze soaked with formalin and sprayed with Dettol, naphthalene, and necessary disinfectants. The post mortem examination carried out in Poland revealed a poorly saturated bruise at the right elbow and signs of acute pulmonary emphysema, cerebral edema and congestion, and the presence of single subpleural hemorrhages. The post mortem examination results indicated that the cause of death might have been suffocation resulting from drowning. The autopsy and material collection for the analyses were performed 17 days after the death and 6 days after embalming.

Case 4

A twenty-five-year-old Caucasian male died in a traffic accident after losing control of a quad bike and a collision with a passenger car on a winding road in Greece. The autopsy showed that the direct cause of death was an extensive intracranial hemorrhage. The embalming involved administering formalin into the body cavities. The autopsy and material collection for analyses were performed 186 days after the death and 163 days after embalming.

Case 5

The body of a twenty-four-year-old Caucasian female, with a trouser leg tied around the neck, was revealed in the victim's apartment bathroom in England. The trousers were hung on the shower fixture. For transport purposes, the consolidation of the body consisted of partially filling the body cavities with formalin. The forensic post mortem examination established that the cause of death was violent strangulation in the hanging mechanism. The toxicological analysis was done 21 days after the death and 18 days after embalming.

Case 6

The hanging corpse of a twenty-seven-year-old Caucasian male was discovered in a hotel room in Egypt. Formalin was introduced into the venous system and body cavities before air transport to the homeland. The body was wrapped in bandages soaked with formalin and then placed in a wooden coffin with an internal zinc coating and the presence of mothballs. The

autopsy stated that the cause of death was asphyxiation by hanging. Toxicological analyses were performed 139 days after death and 133 days after embalming (Table I).

Table I. Descriptive variables of examined group of cadavers
Tabela I. Charakterystyki opisowe zabalsamowanych zwłok poddanych analizom

Case no.	Gender	Age [years]	Weight [kg]	Height [m]	BMI	PMI [days]	Time elapsed from embalming to autopsy [days]
1	male	37	79	1.72	26.7036	17	14
2	male	28	95	2.01	23.5143	14	10
3	male	65	95	1.73	31.7417	17	6
4	male	25	95	1.86	27.4598	186	163
5	female	24	50	1.69	17.5064	21	18
6	male	27	82	1.72	27.7177	139	133

BMI – body mass index, PMI – post mortem interval (time elapsed from death to autopsy)

MATERIALS AND METHODS

Detailed characteristics of the group of corpses subjected to the embalming process are presented in Table I. The samples of various organs were collected for toxicological analyses during post mortem examination performed after transporting the corpses to Poland. In one case, a sample of the fabric saturated with the embalming solution was additionally collected. To our knowledge, no preservatives containing ethanol were used for embalming. The initial toxicological analysis of the samples was carried out by liquid chromatography-mass spectrometry (LC-MS) on a Thermo Scientific TSQ Quantum Access Max mass spectrometer (Thermo Fisher Scientific, Pittsburgh, PA, USA). The analysis of volatile substances, including ethanol and methanol, was performed using gas chromatography with a flame ionization detector (GC-FID) by means of a Focus GC gas chromatograph equipped with a Triplus autosampler, FID detector (Thermo Fisher Scientific Inc., Milan, Italy), and an Rtx® – BAC2 column (Restek Corp., Bellefonte, PA, USA). The collected samples were also analyzed for the presence of formaldehyde using the chromotropic acid spectrophotometric method described by Fagnani et al. [21].

RESULTS

The screening for drugs and psychoactive substances carried out by LC-MS showed that the collected samples were negative for psychoactive substances. In Cases 1 and 3, 4, 5, 6, the analyses showed ethanol in the blood and in other analyzed tissues (Table II). In Case 2, the analyses showed no presence of ethanol in the blood. In the remaining cases, the ethanol concentration in the blood ranged from 0.6 mg/mL in Case 4 to 2.74 mg/mL in Case 3. The presence of ethanol in the blood coexisted with its presence in other analyzed tissues.

The detected concentrations of ethanol in the blood and other tissues indicated that the respective victims were intoxicated at the time of death. In Case 1, the highest ethanol concentration was detected in the urine and the hematoma, while the lowest concentration was detected in the liver and kidney. In Case 3, the highest ethanol concentration was recorded in the urine, bile, and vitreous humor, and the lowest in the liver and kidney. In both cases, the ethanol concentration in the urine was higher than in the blood, which indicated that the substance was undergoing the elimination phase. In Case 4 and 5, the highest ethanol concentration was detected in the blood, and the lowest in the vitreous humor, which suggests that the victims died shortly after alcohol consumption or during the initial phase of alcohol absorption. In Case 6, the highest ethanol concentration was detected in the urine.

In all six cases, the samples showed the presence of methanol and formaldehyde (Table II). In Case 1, the highest formaldehyde concentration was noted in the urine, liver, and blood samples, while the lowest was noted in the cerebrospinal fluid and the hematoma. In Case 2, the highest formaldehyde concentration was noted in the fabric filling the cadaver chest, treated as the reference sample, and in the blood, liver, and muscle samples. The lowest formaldehyde concentration was recorded in the vitreous humor – the concentration was 0.06 mg/ml. In Case 3, the highest formaldehyde concentration was detected in the urine and the liver, while the lowest was noted in the brain, blood, and vitreous humor. In Case 1, methanol and formaldehyde were absent in the cerebrospinal fluid, while in Case 2 and 3, their concentrations in the vitreous humor were the lowest, suggesting that the penetration of the preservatives into these compartments is hindered. In Case 4 and 6 the highest formaldehyde concertation was detected the kidney, while in Case 5 it was in the liver.

Table II. Ethanol (EtOH), methanol (MetOH), and formaldehyde concentrations in different tissue samples and materials collected from embalmed corpses
Tabela II. Stężenie etanolu (EtOH), metanolu (MetOH) i formaldehydu w tkankach i materiałach pobranych ze zwłok poddanych balsamacji

Case no.	Analyzed substance	Blood [mg/mL]	Hematoma [mg/mL]	Urine [mg/mL]	Vitreous humor [mg/mL]	Bile [mg/mL]	Muscle [mg/g]	Liver [mg/g]	Kidney [mg/g]	Brain [mg/g]	Cerebrospinal fluid [mg/mL]	Fabric* [mg/mL]
1	EtOH	1.65	1.97	2.00	–	–	–	1.10	1.52	1.6	1.61	–
	MetOH	4.8	0.23	6.65	–	–	–	6.50	4.77	0.38	0.16	–
	Formaldehyde	2.54	0.22	5.60	–	–	–	4.46	3.48	0.46	0.00	–
2	EtOH	0.00	0.00	0.00	0.00	–	0.00	0.00	0.00	0	–	0
	MetOH	0.29	0.18	0.25	0.03	–	0.40	0.32	0.24	0.15	–	0.88
	Formaldehyde	0.51	0.86	0.16	0.06	–	0.33	0.40	0.25	0.27	–	2.7
3	EtOH	2.74	–	3.09	2.87	3.04	2.51	2.28	2.02	2.29	–	–
	MetOH	0.07	–	0.39	0.14	0.28	0.04	0.18	0.09	0.01	–	–
	Formaldehyde	0.04	–	0.46	0.10	0.16	0.08	0.20	0.08	0.02	–	–
4	EtOH	0.6	–	–	0.00	–	0.03	0.34	0.4	–	–	–
	MetOH	1.06	–	–	0.15	–	0.56	0.82	0.81	–	–	–
	Formaldehyde	1.61	–	–	0.35	–	2.60	3.69	4.65	–	–	–
5	EtOH	2.45	–	–	1.03	–	–	1.47	1.13	–	–	–
	MetOH	0.018	–	–	0.034	–	–	0.009	0.011	–	–	–
	Formaldehyde	0.93	–	–	0.58	–	–	1.1	0.7	–	–	–
6	EtOH	1.1	–	1.58	1.51	–	–	0.54	1.01	0.64	–	–
	MetOH	1.07	–	2.98	0.25	–	–	0.53	0.38	0.01	–	–
	Formaldehyde	0.62	–	0.78	0.21	–	–	0.42	0.28	0.02	–	–

* In Case 2, fabric soaked in with preservative substances was intentionally left in cadaver's chest during initial autopsy performed by local forensic authorities in country of death; "–" no analyses were performed.

DISCUSSION

The embalming process entails removing the blood from the body through the veins and replacing it with formaldehyde-based chemicals by means of the arteries. The embalming of human bodies can pose significant difficulties for forensic toxicologists in the context of interpreting the results. Only a few studies have analyzed the concentrations of volatile substances in corpses subjected to the embalming process or preserved in formalin. Takayasu et al. showed that preserving the material sampled during the autopsy in 10% unbuffered formalin for 14 days resulted in a 16––92% decrease in the concentrations of volatile substances, with ethanol showing the slowest decrease and diethyl ether, chloroform, and toluene exhibiting a faster decrease in the concentration [22].

The cases we present confirmed that embalming with formalin did not affect the identification or detection of ethanol. The simultaneous quantitative analysis of methanol and formaldehyde in various samples showed that the components of the preservatives inefficiently penetrate the isolated body fluids, like the vitreous humor and cerebrospinal fluid. It suggests that these body fluids are less prone to mistakes when interpreting the results of toxicological analyses, and therefore might be valuable diagnostic materials in forensic toxicology.

Case 2 shows that embalming averts the formation of endogenous alcohol. None of the analyzed tissues showed the presence of ethanol. Leaving the fabric soaked in with formalin in the cadaver chest and sampling it for toxicological analyses requires special acknowledgments, as it could be used as a reference material. The fabric sample analyses showed that the embalming process, in this case, was done using formalin stabilized with methanol with no trace of ethanol.

In one of the sporadic cases, ethanol was detected only in the vitreous humor – the reason being the cleaning of the eyeball with ethanol during the embalming process [23,24]. Therefore, it seems to be very important to deliver the sample of the preservative so that it can be treated as a control material and to analyze samples of various tissues from the source material. Unfortunately, in the presented study, only in one case (Case 2) was such a reference material available, which, according to the protocol, was soaked in formalin and left to fill in the body cavities.

Methanol is used as a formalin stabilizing agent in most commercially available embalming fluids [15]. The analyses showed methanol and formaldehyde in the samples of all the presented cases, which was related to the performed embalming process. The range of detected methanol and formaldehyde concentrations may result from using different embalming techniques (transfusion, soaking, immersion) and using different non-standardized preservatives.

The ethanol concentration ratio between the blood and urine of the deceased with the confirmed ethanol intoxication was 0.83, 0.89, and 0.70, respectively for Case 1, Case 3, and Case 6. Our earlier study on unembalmed cadavers (n = 39) showed that the average blood/urine ethanol concentration ratio was 0.85 ± 0.24 [25]. The comparative analysis showed that the average blood/urine ethanol concentration ratio for unembalmed cadavers [25] does not differ from the values obtained for presented Case 1 and Case 3 ($p = 0.669$ and $p = 0.286$, respectively) but is higher than in presented Case 6 ($p < 0.001$), which most probably results from the longer time interval between the time of death and the time of the toxicological analyses.

CONCLUSIONS

The toxicological analysis showed that almost all the victims were intoxicated with ethanol at the time of death, the Case 2 victim was sober at the time of death, and none of the victims were under the influence of psychoactive substances. The results also showed that the best materials for toxicological analyses are isolated body fluids resistant to the penetration of preservatives. It seems that the preservatives used for the embalming process did not influence the ethanol concentrations in the blood and urine, which suggests that it is possible to evaluate the intoxication status of cadavers subjected to the embalming process.

DECLARATIONS

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Ethics approval

No consent was needed to conduct research on the cadavers, as in each case, a forensic post mortem examination and toxicological analyses were ordered by the Prosecutor's Office.

Declaration of competing interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material

All the data generated in this study are presented in the manuscript.

Code availability

The code is available on request from the corresponding author.

Author's contribution

Study design – M. Tomsia
Methodology – J. Nowicka
Statistical analysis – E. Chełmecka
Manuscript preparation – M. Tomsia
Literature research – M. Tomsia
Final approval of the version to be published – M. Tomsia, J. Nowicka, E. Chełmecka

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