

Comparative Study of Blood Metal Levels in Post-Mortem and Living Population Samples from Dammam, KSA

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Abstract

Post-mortem testing of drugs and toxic agents is becoming an increasingly indispensable part of the forensic pathology routine that has recently made considerable progress. However, little attention has been given to the post-mortem blood and tissue metal levels compared to reference values of living population. Therefore, this study was designed to determine the post-mortem blood levels of arsenic (As), selenium (Se), silver (Ag), cadmium (Cd), antimony (Sb), mercury (Hg), thallium (Tl) and lead (Pb) and to compare their levels with the measured reference values from a living population sample. Moreover, the relationship between the post-mortem blood metal levels and the post-mortem interval (PMI) was investigated. Two hundreds and twelve post-mortem femoral blood samples were collected from autopsied cases at the Forensic Medicine centre in Dammam Saudi Arabia. In addition, a living population sample of 200 subjects was recruited from the same region. The blood metal levels were measured by inductively coupled plasma mass spectrometry (ICP-MS). The calculated means for blood As, Se, Ag, Cd, Sb, Hg, Tl and Pb were 2.7, 10.16, 3.38, 12.06, 1.61, 5.49, 1.33, and 13.5 µg/dl respectively. Blood Cd, Hg and Pb levels were significantly higher in the post-mortem samples compared to the living population with p values 0.001, 0.001 and 0.02 respectively. Moreover, there were statistically significant differences among post-mortem blood Cd and Pb levels with regard to post-mortem interval (PMI) which could be attributed to post-mortem redistribution (PMR).

Keywords: Post-mortem redistribution; Metals and inductive coupled plasma

Introduction

Post-mortem assaying of drugs and toxic agents has been there for decades and the accurate interpretation of post-mortem testing results plays a crucial role in the diagnosis of the cause of death [1-3]. The correct interpretation of post-mortem drug analysis could provide vital evidences in the successful prosecution, conviction and subsequent imprisonment of the guilty person, for instance the well-known case of Dr Harold Shipman. Also, the right conclusion about post-mortem levels of xenobiotic is indispensable to avoid executing innocent persons [4-6]. Noteworthy, the databases of necropharmacology have recently grown considerably [7]. For example, Prouty and Anderson listed the concentrations of 69 drugs from different organs and blood vessels from hundreds of autopsied cases died under various conditions [8]. Moreover, Druid and Holmgren reported the femoral concentrations of 83 drugs from thousands of medicolegal autopsies in Swedish laboratories [9]. Furthermore, Schulz et al. compiled a reference table of almost 1000 drugs and xenobiotics from various sources [10].

These comprehensive databases of post-mortem xenobiotic concentrations are considered valuable sources of information that resulted in much better understanding of the mechanisms and predictions of xenobiotics that alter their concentrations after death. Although the database of post-mortem drug concentrations is ever-growing, the available information in the literature about the post-mortem metal levels and the possibility of their redistribution after death is scarce.

The metals are a unique class of toxins as they cannot be broken down to non-toxic forms by the biological system. The heavy metals affect multiple organ systems and target biochemical processes and cellular organelles. The heavy metal toxicity usually involves

an interaction between free metal ion form and the target site. They become toxic because the human body cannot metabolise, thus they accumulate overtime in the soft tissues, heart, lungs and other body organs until they reach toxic levels. Blood and urinary levels only reflect the amount of element circulating at the time of sampling, which may not relate to the degree of exposure [11,12].

Interestingly, the detection of very high post-mortem blood Cd level in a single case raised the suspicion of homicide, however further criminal investigation excluded such possibility. Environmental contamination of the biologic specimens and normal post-mortem redistribution of cadmium between tissues were the reasons behind such false allegation [13]. Such cases highlight how the interpretation of the analytical results obtained from autopsy samples represents a real challenge to scientists. Also, they demonstrate the current paucity of information regarding post-mortem metal levels and the possibility of their redistribution after death as an area that need further extensive research.

Thus, in this study we measured the post-mortem whole blood levels of As, Se, Ag, Cd, Sb, Hg, Tl and Pb in autopsied cases from

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Dammam Forensic Medicine centre in Saudi Arabia and compared the data with the estimated blood levels of the same metals from a living population sample in the same region. Then, we investigated whether the post-mortem interval could have an impact on the measured levels of these metals after death.

Materials and Methods

Data source and collection

According to Saudi Arabia regulations, all suspected unnatural deaths (including cases with suspected poisoning, unknown cause of death, unknown identity, or sudden death cases) must be reported to the police by the physician issuing the death certificate. At the institute of Dammam Forensic Medicine, autopsy is always performed in such cases upon request from the police. Peripheral blood, urine, vitreous, gastric content, liver and kidney tissue samples are collected and submitted to the Forensic Chemistry department at Dammam Poison Control Center "DPCC" where all the samples are routinely screened for xenobiotics including metal levels by inductively coupled plasma mass spectrometry (ICP-MS).

This cross-sectional study was conducted in the period between January 2010 and December 2013. During this period, 212 subjects were selected and their official medical examiner records were scrutinized. The circumstances surrounding death, premorbid clinical and demographic information (age, sex, smoking history, nationality, occupation) and co-morbid medical condition were all recorded in order to assess the relationship between post-mortem blood metal levels and the post-mortem interval (period between estimated time of death and time of sample collection). A 10-15 ml sample of peripheral blood-being the most reliable specimen for toxicological testing-was withdrawn from the femoral vein during external examination. Due to lack of equivalent referent population for comparison, a 200 living population sample that undergo pre-employment toxicology screening were enrolled as a control. All the control subjects consented to participate in the study and their demographic data were recorded. Venous blood samples were withdrawn from the antecubital vein with all the necessary precautions to avoid any source of sample contamination.

Analytical methods

Forensic pathologists instructed technicians on sampling techniques and provided all the necessary equipment. The samples were stored at 4°C until transport to the Forensic Chemistry department at DPCC, where the levels of the whole blood As, Se, Ag, Cd, Sb, Hg, Ti and Pb were measured by ICP-MS. Precisely, 1 ml of each blood sample was transferred to a Teflon digestion tube (120 ml) and mixed up with 7 ml of the acid mixture Nitric/Hydrofluoric/Hydrochloric acids (HNO₃/HF/HCl, 4.5: 2: 0.5) [14]. The tube was sealed and the sample was digested inside a microwave oven (Milestone ETHOS 1600). After digestion, the tubes left to cool down to ambient temperature, Then, the tube was opened and the inside of the lid was rinsed with distilled and de-ionized water (DDW) and the mixture heated on a hot plate (120°C) for 30 minutes to drive off the residual HF and HCl. The resulting digest was filtered in a polypropylene flask using 1% HNO₃ and made up to 50 ml volume with DDW. For ICP-MS measurement the clear digest obtained were diluted 10 times using DDW.

Samples and standard reference materials (SRM) were prepared in a batch of six, including a blank (HNO₃/HF/HCl) digest [10,15]. The eight different metals (As, Se, Ag, Cd, Sb, Hg, Ti and Pb) in all the prepared solutions were quantified subsequently by ICP-MS.

Statistical analysis

Statistical analysis was carried out using SPSS (statistical package for social science) program version 22 (SPSS Inc., Chicago, Illinois, USA) on an IBM compatible computer. Descriptive results were expressed as mean and standard deviation (SD). The student t-test was used to compare the means of blood metal levels between post-mortem samples and the living population control. The post-mortem samples were further subdivided into three groups (Group 1: Absence of putrefaction "no evidence of external or internal signs of putrefaction", Group 2: Early degree of putrefaction "evidence of early external or internal signs of putrefaction" and Group 3: "evidence of advanced external or internal signs of putrefaction" Advanced degree of putrefaction) based on the post-mortem interval. The Levene's test was used to test for variance between subgroups of the post-mortem samples and the p values were considered statistically significant if they were equal or less than ≤ 0.05 .

Results

This study was conducted on 212 death cases from Dammam Forensic Medicine center over a period of 4 years (between January 2010 and December 2013). They were 191 males (91.1%) and 21 females (8.9%) whose ages ranged between 7 and 80 years with a mean of 37.7 year, age distribution is shown.

As regards the nationality, 24.5% of the studied cases were Saudi (n=52) whereas, the remaining 75.5% were non-Saudi (n=160). With reference to the occupation of the studied cases, both farmers (n=30) and mechanical workers (n=30) were equally represented (14.2% each). Also, the majority (86.3%) of the studied cases were non-smokers (n=183). The three most prominent causes of death were hanging (n=71; 33.4%), sudden natural death (n=39; 18.4%) and cut throat (n=23; 10.8%). The other causes of death were falling from height, stab wound, drowning, drug overdose and electrocution whereas in 11.5% of cases (n=24) the causes of death were undetermined, as shown in Table 1. With reference to the place of death, it was indoors in 44.3% of the studied cases (n=94) whereas in 35.45% of cases (n=75), death was outdoors. Also, 14.20% died in medical facility and 6.10% died from drowning. In more than half of the studied cases putrefaction did not start (n=135, 63.7%), whereas, 55 cases were in a state of early putrefaction (25.9%) and the remaining 22 cases were in a state of advanced putrefaction (10.4%), as shown in Table 1.

As regards the two hundreds living population samples, they were all males. More than half of them were Non-Saudi (n=116; 58%) whereas, the other 42% were Saudi (n=84). The majority of them were non-smoker (n=176; 88%), as shown in Table 2.

As regards the measured blood metal levels in this study, as shown in Table 3; firstly the mean post-mortem blood Cd, Hg and Pb levels were statistically significant higher (with mean \pm SD of 12.06 ± 4.62 , 5.49 ± 3.25 and 13.50 ± 3.39 $\mu\text{g/dl}$ respectively) than their calculated means in the living population samples (with mean \pm SD of 0.10 ± 0.07 , 1.75 ± 1.12 and 7.01 ± 2.39).

Secondly, the mean post-mortem blood Se and Sb levels were statistically significant lower (with mean \pm SD of 10.1 ± 6.03 and 1.61 ± 1.92 $\mu\text{g/dl}$ respectively) than their calculated means in the living population samples (with mean \pm SD of 18.95 ± 8.38 and 2.82 ± 2.03 $\mu\text{g/dl}$ respectively) p values: 0.001. However, there were not statistically significant differences of the blood As and Ti levels between the post-mortem and the living population samples.

Levene's test revealed a significant increase in post-mortem blood

Metal	Group 1 (n=135)		Group 2 (n=55)		Group 3 (n=22)		Levene's test p Value
	Mean	± SD	Mean	± SD	Mean	± SD	
As	2.65	3.3	3.14	5.29	2.14	1.75	Gp 1 and 2: P=0.34 Gp 1 and 3: P=0.38 Gp 2 and 3: P=0.35
Se	10.54	6.21	10.61	5.47	10.34	4.07	Gp 1 and 2: P=0.23 Gp 1 and 3: P=0.44 Gp 2 and 3: P=0.23
Ag	3.54	1.14	3.48	1.12	3.42	0.87	Gp 1 and 2: P=0.77 Gp 1 and 3: P=0.64 Gp 2 and 3: P=0.23
Cd	12.27	3.75	9.39	3.47	24	3.39	Gp 1 and 2: P=0.10 Gp 1 and 3: P=0.04* Gp 2 and 3: P=0.03*
Sb	1.6	1.94	1.81	1.94	1.55	1.38	Gp 1 and 2: P=0.26 Gp 1 and 3: P=0.28 Gp 2 and 3: P=0.26
Hg	5.89	3.24	5.1	2.06	5.28	1.79	Gp 1 and 2: P=0.78 Gp 1 and 3: P=0.28 Gp 2 and 3: P=0.78
Ti	1.25	1.24	1.41	1.19	2.15	5.24	Gp 1 and 2: P=0.50 Gp 1 and 3: P=0.30 Gp 2 and 3: P=0.40
Pb	12.66	2.77	13.87	2.44	24.55	7.88	Gp 1 and 2: P=0.10 Gp 1 and 3: P=0.01* Gp 2 and 3: P=0.02*

Group 1: No putrefaction; Group 2: Early putrefaction; Group 2: Advanced putrefaction

*Significant p value<0.05

Table 1: Blood metal levels at variable post-mortem intervals.

Post-mortem Cases			
Total number of cases: 212			
Year distribution		Nationality	
2010:00:00	6	Saudi	52 (24.5%)
2011:00:00	42	Non-Saudi	160 (75.5%)
2012:00:00	79	Gender Distribution	
2013:00:00	85	Male	191 (90.1%)
-	-	Female	21 (9.9%)
Age Groups		Occupation	
Gp1: <10 years	01 (0.5%)	Mechanical- worker	30 (14.2%)
Gp2: 10-19 years	04 (1.9%)	Farmer	30 (14.2%)
Gp3: 20-29 years	66 (31.1%)	Un-detected	152 (71.6%)
Gp4: 30-39 years	56 (26.4%)	-	-
Gp5: 40-49 years	44 (20.8%)	-	-
Gp6: 50-59 years	35 (16.5%)	-	-
Gp7: 60-69 years	04 (1.9%)	Smoking Habit	
Gp8:>70 years	02 (0.9%)	Non-Smoker	183 (86.3%)
Mean ± SD (Min.-Max.) µg/dl (37.7 years) (7-80 years)		Smoker	29 (13.7%)
Place of Death		Postmortem Changes	
Indoor	94 (44.3%)	Absence of putrefaction	135 (63.7%)
Outdoor	75 (35.4%)	Early degree of putrefaction	55 (25.9%)
In medical facility	30 (14.2%)	Advanced degree of putrefaction	22 (10.4%)
Under water	13 (6.1%)		
Cause of Death			
Hanging	71 (33.4%)	Drowning	10 (4.7%)
Sudden Death	39 (18.4%)	Poisoning and drug overdose	09 (4.2%)
Cutthroat	23 (10.8%)	Electra-caution	08 (3.8%)
Falling from a height	14 (6.0%)	Undetermined	24 (11.5%)
Stab wound	14 (6.0%)		

Table 2: Demographic data of the studied population (age, gender, nationality and smoking habit).

Metal	Post-mortem		Living		t-test		LOD*
	(n=212)		(n=200)		t	p	
	Mean	± SD	Mean	± SD			
As	2.7	1.73	2.37	1.47	1.11	0.29	<LOD-35.80
Se	10.16	6.03	18.95	8.38	12.27	0.001*	<LOD-54.00
Ag	3.38	1.29	3.78	7.07	0.8	0.43	<LOD-10.00
Cd	12.06	4.62	0.1	0.07	6.16	0.001*	<LOD-295.50
Sb	1.61	1.92	2.82	2.03	13.23	0.001*	<LOD-9.10
Hg	5.49	3.25	1.75	1.12	5.01	0.001*	<LOD-24.40
Ti	1.33	1.98	1.07	0.94	1.67	0.09	<LOD-24.00
Pb	13.5	3.39	7.01	2.39	2.42	0.02*	<LOD-214.00

*Level of Detection
**Significant p value<0.05

Table 3: Comparison between blood metal levels of both the post-mortem and living population samples.

Cd levels with advancing putrefaction. The post-mortem blood Cd levels were significantly higher in the group with advanced putrefaction (with mean ± SD of 24.00 ± 2.39) in comparison with the other two groups: absence of putrefaction (with mean ± SD of 12.27 ± 3.75) and early putrefaction (with mean ± SD of 9.39 ± 30.47), with p values: 0.04 and 0.03 respectively, as shown in Table 3. Moreover, the post-mortem blood Pb levels were significantly higher in the group with advanced putrefaction (with mean ± SD of 24.55 ± 7.88) in comparison with the other two groups: absence of putrefaction (with mean ± SD of 12.66 ± 2.77) and early putrefaction (with mean ± SD of 13.87 ± 2.44), with p values: 0.01 and 0.02 respectively, as shown in Table 3. However, there were no statistically significant differences between the post-mortem blood levels of As, Se, Ag, Sb, Hg and Ti among the three groups, as shown in Table 3.

Discussion

Post-mortem toxicological analysis has become as important as histopathology for the diagnosis of the cause of death. However, Post-mortem changes, such as post-mortem diffusion and redistribution, make the interpretation of xenobiotic concentrations measured in post-mortem samples difficult or impossible. Moreover, post-mortem xenobiotic concentrations unpredictably change with the site and time of sampling, as a result of the phenomenon of post-mortem redistribution [16-20]. The phenomenon of post-mortem redistribution was coined by Pounder and Jones [21]. Since then, the studies investigating drugs redistribution after death are ever-growing; however, very little studies discussed the post-mortem redistribution possibility of heavy metals and whether a true relationship between lethal events and the post-mortem tissues and blood metal levels does exist, because of the lack of such comprehensive studies, tabulations of ‘fatal concentrations’ could be misleading and the post-mortem measurements must be interpreted with extreme caution [21].

In the present study, the post-mortem blood Cd level was significantly higher than its level in the living population sample, as shown in Table 2. Moreover, the post-mortem blood Cd levels were significantly higher in the group with advanced putrefaction compared to the other two groups (absence of putrefaction and early putrefaction with p values: 0.04 and 0.03 respectively, as shown in Table 3. In some cases, the post-mortem blood Cd reached levels incompatible with life. In such cases, the medical examiner reports were re-examined to trace back any evidence of pre-mortem clinical toxicities. In addition, all the precautions were undertaken during sample collection to avoid any source of blood contamination. Therefore, the suspicion of cadmium toxicity was excluded and the only plausible explanation for such thousand-fold elevation of post-mortem Cd level is post-mortem

redistribution from the surrounding tissue and organs. In agreement with our finding, a previous study measured Cd, Zn, Cu, and Fe in blood and post-mortem tissue (both fresh and formalin fixed) samples. This study revealed that the post-mortem blood Cd level was hundredfold higher than its pre-mortem level and did not indicate ante-mortem exposure to Cd. This elevated post-mortem Cd level was attributed to the very rapid elution of Cd from body organs [22].

Also, our results were consistent with Schier et al., 2010 who reported the same finding [13]. It is well documented that most drugs and toxic agents undergo post-mortem redistribution, particularly drugs in cardiac blood is affected by post-mortem redistribution from surrounding tissue (e.g., lung, gastrointestinal tract, and myocardium) [23,24]. Moreover, drugs excreted in the liver may be redistributed via the hepatic veins to inferior vena cava, right cardiac chamber, and pulmonary vessels [19]. The same principle apply to metals; Cd accumulates in very high concentrations in various body tissues (especially liver and kidney) during life [23,25,26]. However, inequitable distribution of red blood cells due to clotting or hypostasis cannot explain increased post-mortem whole blood Cd level [7]. Thus, post-mortem redistribution is the likely explanation for the significant increase of post-mortem blood Cd in cases with advanced putrefaction compared to those with no/early putrefaction.

Noteworthy, we found that blood Hg and Pb levels were statistically significant higher in the post-mortem cases compared with the living population samples, with p values of 0.001, as shown in Table 2. Moreover, the post-mortem blood Pb levels were significantly higher in the group with advanced putrefaction compared to the other two group (absence of putrefaction and early putrefaction), with p values: 0.01 and 0.02 respectively, as shown in Table 3. This elevation of post-mortem blood Pb levels could be attributed to the aforementioned phenomenon of post-mortem redistribution. However, there were no statistically significant differences between the post-mortem blood levels of As, Se, Ag, Sb, Hg and Ti among the three groups.

Interestingly, we found that blood Se and Sb levels were statistically significant lower in the post-mortem cases compared with the living population samples, with p values of 0.001, as shown in Table 2. However, there were not statistically significant differences of the blood As and Ti levels between the post-mortem and the living population samples, as shown in Table 2. The finding of low post-mortem blood Se and Sb levels might be attributed to back diffusion or redistribution of these two metals from blood to the surrounding tissues.

The authors used living population samples as control for comparison in this study due to the lack of pre-mortem blood samples and absence of established reference ranges for the studied population in the literature. Although, it is not optimal, our results of the living population samples could be used as reference ranges for comparison in future studies in the region.

Conclusion

In the present study, we showed that post-mortem blood Cd and Pb levels were significantly higher than the measured values in the living population samples and their levels increased with post-mortem interval. These variations in post-mortem levels are most probably due to post-mortem diffusion and redistribution. The researchers also found that post-mortem blood levels of both Se and Sb were significantly different from the measured reference range of the living population sample studied. Indeed, experimental research is needed to further investigate the post-mortem redistribution of metals for accurate interpretation of post-mortem analysis results.

Conflict of Interests

The authors declare that they have no competing interests.

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