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Carboxyhemoglobin Determination in The Sternum Red Marrow at Lethal Carbon Monoxide Poisoning

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Abstract

It is difficult to determine the cause of death of some corpses found dead in fire whether they are incinerated, dismembered, bloodless or putrefied corpses. This study evoked a new investigatory method to detect the carboxyhaemoglobin (CoHb) in sternum bone marrow of corpses found dead in fire and compare that with blood level of CoHb of the same cases. It may be impossible to sample liquid blood in incinerated, exsanguinated or dismembered corpses, so this test is considered a confirmatory test for detecting CoHb. This study was carried out on 17 corpses found dead in fire, the sternum bone marrow and blood was investigated for CoHb by spectrophotometery. It was found that there is a correlation between CoHb level in blood and bone marrow, the absence of CoHb in blood is also negative in marrow. When blood carboxyhemoglobin concentration was 49% to 71% its marrow concentration was 27% to 81%. but marrow carboxyhemoglobin content was 1-15% lower and 6-47% higher than its blood samples. Only 4 of 17 cases (23.5%) of marrow CoHb did not differ from its blood concentration in more than 10% (except those cases of negative CoHb in the blood).No significant difference between blood and marrow COHb concentrations was found. It was proved that the degree of corpse incineration had no effect on the difference in blood and marrow COHb (Kruskal–Wallis test; p = 0.78). These results suggest that further studies on COHb detection in marrow can extend the forensic diagnostic methods in examinations of incinerated, dismembered, bloodless or putrefied corpses.

Keywords: carboxyhemoglobin, carbon monoxide poisoning, forensic medicine

INTRODUCTION:

Examination of fatal carbon monoxide poisoning remains an actual problem of Forensic Medicine. In the Republic of Belarus carbon monoxide (CO) poisoning, including combined intoxication with CO and ethyl alcohol, is about 15-20 % of all poisonings, and about 5% of all violent deaths.Quantitative detection of carboxyhemoglobin (COHb) in corpse blood has so far been used in forensic practice for the diagnosis of fatal CO poisoning. It is evident that blood COHb level may be affected by a number of factors: individual human body characteristics, diseases, injuries, alcohol intoxication, possibility of post-mortem penetration of CO and formation of carboxyhemoglobin in blood and organs of corpse, and the effect of heating [1–3].

There are conflicting data in literature regarding the effect of heating on blood carboxyhemoglobin.

Several authors [4, 5] using practical and experimental data revealed a direct correlation between CoHb and degree of body area damaged by flame. It is proved that CoHb decreases until its complete disappearance in blood tissue when exposed to heating, particularly an open flame [4]. Ageeva N.M. et al. [5] found that heat causes CoHb breakdown. They found that blood heating at 50-70°C with exposure of 10 to 30 minutes results in carboxyhemoglobin decrease by 3-60%, while heating at 80-100°C showed CoHb drop down to its minimum level, or it can't be detected at all.

According to Yamamoto K. et al. [3], blood HbCO decrease in corpse after heating results from hemoglobin CO elimination and its partial volatilization.

At the same time, J. Markiewicz found a CoHb increase in 17 blood samples after heating to 65°C , in 7 cases CoHb increase was 0.6-2%, in 11 cases CoHb was 9.7- 49.5%.

According to Y. Seto et al. [1] and Dobroriz A.M. [2] heating does not result in blood COHb decrease. Ageeva N.M. et al. [5] ascertained, that heating causes COHb breakdown, so COHb content in blood of incinerated corpses may be low.

It is proved that the first sign of poisoning occurs if blood CoHb is greater than 30%. When blood CoHb exceeds 60% death is usual.

It is also known that the combustion of modern building materials can produce not only CO but other combustion products (primarily hydrogen cyanide and acrylonitrile).

It may be impossible to sample liquid blood in incinerated, exsanguinated or dismembered corpses. This has aroused the interest to CoHb detection in dry blood, internal organs, and carboxymyoglobin in muscle. Thus, Babakhanyan R.V. and Busova N.S. [6] showed the possibility of quantitative spectrophotometric CoHb detection in spots of dried blood. CoHb in dry blood spots was 28-66 %, while CoHb in the liquid blood was 46 to 87%. Ageeva N.M. et al. [13] analyzed CoHb in the internal organs and muscles. CoHb concentration in fatal poisonings was 16-75.8%. Babakhanyan R.V. et al. [7] attempted to study the possibility of quantitative COHb detection in marrow. They found that marrow COHb concentration was 10-67% while in the parallel blood samples was 29-88%.

AIM OF WORK:

The aim of our study is to evaluate carboxyhemoglobin content in sternum marrow of the corpses in cases of carbon monoxide poisoning.

MATERIALS AND METHODS:

Blood and marrow sampling was performed in 17 corpses found in fire (marrow was taken from sternum with bone spoon).

Blood CoHb was determined by spectrophotometric method of Dobroriz A.M. et al. approved for use in the State Committee of forensic examinations [8]. To detect marrow CoHb chloroform was added to marrow sample (3 g) in a volume ratio of 1:3. The mixture was shaken for 2 min, then centrifuged 10 min at 3000 rpm, chloroform phase was collected, then sediment was mixed with 3 ml of chloroform, shacked for 2 min and centrifuged 10 min at 3000 rpm. Marrow remainder was topped with distilled water to volume of 25 ml. CoHb was extracted in dark during 1 hour with periodic stirring with glass rod. The solution was filtered. Ammonia was added to a final concentration of 0.1%, and then sample was examined according to approved methods of detecting CoHb in blood [8]. Resulting solution was filtered, poured in cuvette of spectrophotometer CARY-50 (Varian Medical Systems, Inc., USA) and was examined for its absorbance with 1 cm solution layer [wavelength range 600-510 nm, scan mode «Baseline correction-Medium» (A0 solution)]. Then 3.5 mg of sodium dithionite was mixed with 5 ml of this solution, the absorbance of this solution examined as described above (solution A). 10 ml of blood was saturated with carbon monoxide for 10 minutes, mixed with 3.5 mg of sodium dithionite and saturated with carbon monoxide for 5 min. Absorbance examination of this solution is described above (solution B). Concentration of carboxyhemoglobin was calculated by the formula:

% HbCO = (Sol A531 nm - Sol A583 nm) / (Sol B531 nm - Sol B583 nm) × 100%

In each case incineration degree was visually evaluated. Light incineration was assumed as soft tissue charring and severe incineration was assumed as bone defect, thoracic and abdominal wall defect, exposing internal organs.

Correlation between blood and marrow CO was estimated using Spearman test. Correlation between corpse incineration degree and difference in blood and marrow CoHb was assessed using the Kruskal-Wallis test.

RESULTS AND DISCUSSION

Red marrow CoHb concentrations and corresponding blood CoHb concentrations are shown in Table 1.

Table1: Marrow and blood CoHb concentrations

№ OF SAMPLE	CARBOXYGEMOGLOBIN CONCENTRATION (%)		INCINERATION DEGREE
	Blood	Sternum marrow	
1	52	44	No
2	0	0	light
3	49	72	light
4	0	0	No
5	57	64	No
6	67	81	severe
7	0	0	No
8	56	53	light
9	55	55	No
10	59	46	light
11	68	27	severe
12	65	75	severe
13	52	45	light
14	68	39	light
15	60	41	No
16	51	54	severe
17	71	49	No

In 3 cases CoHb was not detected in blood. Marrow of these corpses also showed no CoHb (Table 1.observations 2, 4, 7).

Correlation between sternum marrow CoHb and blood CoHb was not found (Spearman's criterion, p = 0.39).

In 14 other cases blood CoHb was 49 % to 71 %. Marrow CoHb in these cases ranged from 27% to 81%.These 14 cases were sorted as follows:

In one case blood and marrow CoHb concentrations were equal (55%, no incineration).

In five cases marrow CoHb was higher than concentration in blood by 6-47 %. In this subgroup, 1 corpse was not incinerated, 1case was lightly incinerated and severe incineration was found in 3 cases .

In 8 cases marrow CoHb was lower than concentration in blood by 1-15 %. In this subgroup, severe incineration was observed in only one case.

Thus, in 13 cases sternum marrow CoHb differed from blood CoHb by 1-47%, and in 11 cases the difference was greater than 10%.

It should be noted that our findings are not consistent with the results of Babakhanyan R.V. et al. [1]. He showed, that marrow carboxyhemoglobin was 15-30% lower than that one in corresponding liquid blood samples.

Assessment of the impact of corpse incineration degree on the difference in blood and marrow CoHb using the Kruskal-Wallis test revealed no statistically significant differences between three groups (no incineration, light and severe incineration) (p = 0.78).

Conclusion

The results of this study showed that the absence of carboxyhemoglobin in blood is accompanied as well with an absence of CoHb in sternum marrow. As such lack of marrow carboxyhemoglobin in incinerated corpses (either anemic or dismembered), when blood sampling is impossible, suggests ruling out of carbon monoxide poisoning as a cause of death.

Statistically significant correlation between blood and marrow carboxyhemoglobin was not found. However, when blood carboxyhemoglobin was higher than 49%, its concentration in sternum marrow was not lower than 27%. So carboxyhemoglobin in marrow can assume carbon monoxide poisoning, but in order to implement this technique in practice of medico-legal examination it is necessary to extend the study and evaluate its diagnostic value (repeatability, sensitivity and specificity) for the diagnosis of carbon monoxide poisoning.

There is no significant effect of corpse incineration degree on the difference between blood and marrow CoHb concentration.

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