Legal Medicine 17 (2015) 451–458

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

An objective approach using three indexes for determining fatal hypothermia due to cold exposure; statistical analysis of oxyhemoglobin saturation data

Daisuke Yajima [a,b], Masaru Asari [a], Katsuhiro Okuda [a], Chikatoshi Maseda [a], Hiromi Yamada [a], Chisato Ichimaru [a], Kazuo Matsubara [c], Hiroshi Shiono [a], Hirotaro Iwase [b,d], Yosuke Makino [b,d], Keiko Shimizu [a,*]

[a] Department of Legal Medicine, Asahikawa Medical University, 2-1-1-1 Midorigaoka-Higashi, Asahikawa 078-8510, Japan
[b] Department of Legal Medicine, Graduate School of Medicine, Chiba University, Inohana 1-8-1, Chuo-ku, Chiba City 260-8670, Japan
[c] Department of Pharmacy, Kyoto University Hospital, Sakyo-ku, Kyoto 606-8507, Japan
[d] Department of Forensic Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:
Received 26 March 2015
Received in revised form 28 September 2015
Accepted 30 September 2015
Available online 8 October 2015

Keywords:
Hypothermia
Oxyhemoglobin saturation
Oximeter
Difference in color

ABSTRACT

Analysis of oxyhemoglobin (O_2-Hb) saturation levels in the left and right heart blood is useful in the assessment of exposure to cold surroundings before death. We quantified conventional subjective visual evaluation of O_2-Hb saturation levels and developed useful diagnostic criteria for fatal hypothermia: O_2-Hb saturation in the left heart blood (L-O_2Hb) was $\geqslant$36%, the O_2-Hb saturation gap between the left and right heart blood (L–R gap) was $\geqslant$13%, and the O_2-Hb saturation ratio of the left to right heart blood (L/R ratio) was $\geqslant$1.8. When we used L-O_2Hb of $\geqslant$36% as a basic criterion and applied a further criterion of an L–R gap of $\geqslant$13% or an L/R ratio of $\geqslant$1.8, these criteria registered a sensitivity level of $\geqslant$86% and specificity level of $\geqslant$93% for the diagnosis of fatal hypothermia. This method can be useful for determining fatal hypothermia in connection with conventional autopsy findings, as well as histological and biochemical markers.

1. Introduction

There is no specific sign or symptom when we examine cases of fatal hypothermia due to cold exposure. Although, the environment surrounding the victim may be all-important generally, comprehensive investigation is needed for postmortem diagnosis of death from cold exposure, including complete autopsy, histology, toxicology and biochemistry, excluding other causes of death. The diagnostic criteria include the evidence for antemortem cold exposure and fatal outcome due to hypothermia, as well as exclusion of other contributors or fatal insults [1–6]. Especially, a color difference between the left and right heart blood (CDL/RHB) is a common sign characteristic of fatal hypothermia due to cold exposure, for which the incidence is reported as around 95% in Japan [7,8]. The mechanism is deduced to be as follows: first, a decrease in body temperature lowers oxygen consumption in the body, which results in maintaining an antemortem composition of arterial and venous blood after death. Second, the hypothermic effect enhances binding between hemoglobin (Hb) and oxygen (O_2) to suppress oxygen release; thus left heart blood circulating the lungs with cold air contains more oxygen than right heart blood [8,9].

Some studies have attempted to analyze the oxyhemoglobin (O_2-Hb) saturation level using an oximeter in hypothermia cases because visual color discrimination is subjective [10–14]. Previous studies suggested the bilateral heart blood oxyhemoglobin level is useful as an indicator of death involving exposure to cold air; however, its diagnostic validity has not been statistically established due to the small sample size. The present study analyzed postmortem oximetric data in 77 serial autopsy cases of fatal hypothermia to establish quantitative diagnostic criteria. We have devised three indexes for determining hypothermia; the O_2-Hb saturation in left heart blood (L-O_2Hb), the O_2-Hb saturation gap between left and right heart blood (L–R gap) and the O_2-Hb saturation ratio between the left and right heart blood (L/R ratio) [10]. We introduce useful diagnostic criteria for fatal hypothermia using the three indexes in this study.

* Corresponding author.
E-mail address: okeichan@asahikawa-med.ac.jp (K. Shimizu).

http://dx.doi.org/10.1016/j.legalmed.2015.09.005
1344-6223/

2. Materials and methods

2.1. Subjects and objective

We appropriated 77 fatal hypothermia (cold exposure) cases and 143 control cases wherein full autopsies had been performed within 7 days after death between January 2002 and April 2015 in our institution. Hypothermia cases ($n = 77$) were diagnosed based on the following three criteria: (1) previous exposure to cold environment; (2-i) both CDL/RHB and gastric erosions (Wischnewski-Flecke) were present, or (2-ii) only either CDL/RHB or gastric erosions were present, and where at least one of the following conventional autopsy findings was observed: bright red lividity, frost erythema, compact arrangement of myocardial fibers, or urine ketones; and (3) exclusion of trauma, suffocation, poisoning, and disease (based on histological, biochemical, and toxicological examinations).

We selected control cases not exposed to cold conditions after close investigation of police reports about death situations: 71 cases due to disease and 72 due to extrinsic causes.

We introduced four cases whereby although the victims had clearly been exposed to cold conditions prior to death, causes of death other than hypothermia were found.

We also introduced eight cases whereby bright red lividity had developed after death as a result of refrigerated storage to assess whether refrigeration influences O_2-Hb saturation in heart blood.

2.2. Methods

2.2.1. Sampling of heart bloods and determination of left vs right heart blood color

Before removing the heart during autopsy, the left and right heart blood samples were respectively taken from the left atrium or pulmonary vein and the right atrium or inferior vena cava using syringes. We compared the color of two syringes containing blood from the left and right heart. If color difference was unclear, we checked marbling of blood color in the pericardium at resection of the heart.

2.2.2. Estimation of postmortem time and exposure to a cold environment

Postmortem times were estimated by forensic pathologists based on autopsy findings and circumstances surrounding the victims at the time they were found. Days were converted to hours and the medians were used as the postmortem times if they were originally expressed as periods of time.

We confirmed the evidence of exposure to a cold environment by information of ambient temperature from police records or meteorological bureau.

2.2.3. Analysis of O_2-Hb saturation in the heart blood by oximeter

Each blood sample (85 μL) was applied to an analysis device (ABL800 FLEX CO-OX OMS; Radiometer, Brønshøj, Denmark) for measuring O_2-Hb during an autopsy or as soon as possible after an autopsy. Each sample was analyzed twice and the mean value was used in the present study.

2.2.4. Analysis of alcohols and ketones, and toxicological examinations

Alcohols were analyzed by gas chromatography (GC). Ketones were analyzed by GC after testing with urine test paper (UROLABSTIX® SIEMENS health care Japan, Tokyo). The test paper detected acetoacetic acid, while GC detected acetone. When acetoacetic acid or acetone is detected, ketones are positively identified [15].

We used Triage® or liquid chromatography tandem mass spectrometry for toxicological analysis.

2.2.5. Statistical analysis

Three indexes were calculated: (1) the O_2-Hb saturation value in left heart blood (L-O_2Hb); (2) L–R gap; and (3) L/R ratio. Values were statistically verified using the Student *t*-test. We used Excel Statistics (add-in software, Social Survey Research Information Co. Ltd., Tokyo, Japan) and Origin (Lightstone Corp., Tokyo, Japan) as statistical analysis software. Differences where *P* values of less than 0.05 were considered significant.

2.3. Ethical issue

The present study was approved by the ethics committee of our University (No. 14117).

3. Results

Results of examinations, autopsy findings and other information in hypothermia cases are shown in Table 1.

3.1. Comparing data between hypothermia and control cases

The mean values of O_2-Hb saturation in the hypothermia cases and controls registered 69.9 ± 19.4% and 19.1 ± 16.4% in the left and 20.6 ± 11.8% and 16.3 ± 11.6% in the right heart blood, respectively. L–R gaps and L/R ratios were 49.3 ± 19.5% and 4.6 ± 2.9 in hypothermia, while those of control cases were 2.9 ± 13.6% and 1.4 ± 1.3, respectively (Table 2). Values in the hypothermia cases were significantly different from those in controls ($P < 0.05$).

3.2. Cause of death and data analysis on control cases

The breakdown according to causes of death in controls (Table 3) indicated that 71 deaths were attributable to diseases while 72 cases were attributable to extrinsic causes; and the mean values of O_2-Hb saturations were 21.6 ± 18.7% and 16.7 ± 13.5% in the left and 15.8 ± 10.9% and 16.8 ± 12.4% in the right heart blood, respectively. The L–R gaps and L/R ratios registered 5.8 ± 15.8% and 0 ± 10.3% in the diseased cases and 1.6 ± 1.3 and 1.2 ± 1.2 in the extrinsic cases, respectively. Values in the diseased cases ($n = 71$) were not significantly different from those in the extrinsic cases ($n = 72$) except for the L–R gaps ($P = 0.05$).

3.3. Setting cut-off values for the three indexes as diagnostic criteria for hypothermia

Using the three indexes, we analyzed the receiver-operator characteristics (ROC) curves of L-O_2Hb, L–R gap, and L/R ratio (Fig. 1). Areas under the ROC curves of L-O_2Hb, L–R gap, and L/R ratio were 0.962, 0.971, and 0.920, respectively, and they exhibited highly discriminatory levels between the two samples. ROC analyses of L-O_2Hb, L–R gap, and L/R ratio suggested that the cut-off values discriminating between fatal hypothermia cases and controls were 36.8%, 13.8%, and 1.8, respectively. Based on these values, we designated the cut-off values of L-O_2Hb, L–R gap, and L/R ratio as 36%, 13%, and 1.8, respectively (Table 4).

3.4. Evaluation of cut-off values to discriminate between hypothermia and control cases

Using scatter plots with L-O_2Hb expressed in the abscissa, and L–R gap or L/R ratio in the ordinate, we estimated the number of hypothermia cases and controls using the cut-off values. In the scatter plot of L-O_2Hb against L–R gap, 72 of the 77 hypothermia cases were diagnosed correctly (94% sensitivity), and 133 of the 143 control cases were appropriately discriminated from the