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Blood Levels of Acrolein, Polyamine Oxidases, and Several Other Markers in Cases of Bathtub Death

Sari MATSUMOTO, Shojiro TAKASU, and Kimiharu IWADATE

Department of Forensic Medicine, The Jikei University School of Medicine

ABSTRACT

The Japanese have the habit of taking Japanese-style baths in bathtubs. Bathtub deaths are among the most common types of unexpected death in elderly Japanese persons. Bathtub death has been hypothesized to have several causes, but the cause remains unclear. The widely accepted theory is that the main cause of drowning in the bathtub is a syncopal attack associated with a transient ischemic attack. In patients with brain infarction, serum levels of protein-conjugated acrolein, polyamine oxidases, interleukin 6, and C-reactive protein are reportedly elevated. Therefore, to assess the hypothesis that these markers are elevated in patients who drown in a bathtub, in the present study we measured serum levels in 10 cases of bathtub death and 10 cases of non-bathtub death. We found that serum levels of these markers did not differ significantly between cases of bathtub and non-bathtub death. On the basis of this result, we suspect that the brain ischemic attack is not involved in the mechanism of bathtub death and that marker levels undergo postmortem changes. Therefore, future studies should include a larger number of cases and examine the changes in markers according to the time since death. The present study suggests that these markers cannot be used now to diagnosis cases of bathtub death. (Jikeikai Med J 2018 ; 65 : 1-5)

Key words : bathtub deaths, acrolein, polyamine oxidase, brain ischemic attack, autopsy

INTRODUCTION

The Japanese have the habit of taking Japanese-style baths in bathtubs, which they take hot water in the bathtub, get in, and warm their body. However, bathtub deaths are among the most common types of unexpected death among elderly Japanese persons. The annual number of bathtub deaths in Japan is more than 10,000, which is 30 times as many as the number of drown people in each Western countries. Many studies of bathtub deaths in Japan have revealed its epidemiology. Bathtub deaths occur most frequently in winter, and their number is negatively correlated with the mean atmospheric temperature[1]. A risk factor for bathtub death is a water temperature of more than 42°C[2], and a contributing factor is a difference in temperature between the bathroom and the bathtub.

Despite these epidemiologic findings, the pathogenesis of bathtub deaths remains unclear. The direct cause of many such deaths is thought to be a person drowning from a loss of consciousness while soaking in a bathtub ; however, the underlying cause of the loss of consciousness is controversial. Although many persons who have died in bathtubs have undergone forensic autopsy, the cause of unconsciousness has rarely been found to be brain hemor-

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柗本　紗里，髙須翔志郎，岩楯　公晴
Mailing address : Sari MATSUMOTO, Department of Forensic Medicine, The Jikei University School of Medicine, 3-25-8 Nishi-shimbashi, Minato-ku, Tokyo, Japan
E-mail : kineduka@jikei.ac.jp

rhage, subarachnoid hemorrhage, myocardial ischemia, or other pathological lesions. Most other cases have shown only macroscopic findings of drowning and no obvious indication of a syncopal attack[3-6]. Many cases have shown mild cerebral/coronary arteriosclerosis or mild cardiomegaly, suggesting the possibility of an intrinsic syncopal attack, which is difficult to diagnose morphologically, and other possible conditions are a transient ischemic attack (TIA), acute myocardial infarction, and arrhythmia are possible conditions[4]. In particular, TIA in concurrence with arrhythmia is considered to be a principal differential diagnosis for unconsciousness in a bathtub.

Plasma levels of protein-conjugated acrolein (PC-Acro) and polyamine oxidases, such as spermine oxidase (SMO) and acetylpolyamine oxidase (AcPAO), have been reported to increase in patients who have had or are at high risk for brain infarction[7-9]. Polyamines are present in cells and are necessary for normal cell growth[10]. When cells have been damaged, acrolein and H_2O_2 are produced by polyamine oxidases from polyamines, especially spermine[7]. Spermine is divided by SMO into spermidine and 3-aminopropanal or is acetylated by spermidine/sperumine N1-acetyltransferase and divided by AcPAO into spermidine and 3-aminopropanal. Acrolein is then produced after NH_3 breaks away from 3-aminopropanal[11]. As a result, when cells are damaged, serum levels of acrolein and polyamine oxidase increase.

Much as PC-Acro is elevated in patients with metabolic or psychiatric diseases, also elevated are C-reactive protein (CRP) and interleukin (IL) 6, which are markers for the inflammation by cellular disorders[12,13]. Therefore, these markers might be useful for estimating brain damage.

We have hypothesized that serum levels of PC-Acro and polyamine oxidases are increased if TIA or acute brain ischemia causes unconsciousness, which results in bathtub death. Based on this hypothesis, the present study aimed to compare serum levels of PC-Acro, polyamine oxidases, and other markers between cases of bathtub death and cases of non-bathtub death with the short process of dying.

MATERIALS and METHODS

The study included 10 cases of bathtub death and 10 cases of death due to other causes as negative control cases, in which cardiac blood had not clotted. These cases underwent forensic autopsy from January 2008 through May 2012 at The Jikei University School of Medicine.

In our department, we routinely obtain blood samples from the right side of the heart at the time of forensic autopsy and preserve the serum at −20°C. Serum levels of PC-Acro, SMO, AcPAO, IL-6, and CRP were measured by the Amine Pharma Research Institute Co., Ltd., Chiba, Japan.

Data are presented as median ± interquartile deviation. The cases were compared by means of the Wilcoxon rank sum test with the statistical software program Stata (StataCorp LLC, College Station, TX, USA).

This study was approved by the ethics committee of The Jikei University School of Medicine for Biomedical Research (receipt number : 24-108(6874)).

RESULTS

Between cases of bathtub death and cases of non-bathtub death, we identified no significant differences of any of the markers (Tables 1 and 2). Levels of PC-Acro, AcPAO, and IL-6 tended to be higher and levels of SMO and CRP tended to be lower in cases of bathtub death than in cases of non-bathtub death (Fig. 1) ; however, the differences were not statistically significant.

DISCUSSION

Of the markers measured in the present study, all have been found in previous studies to be higher in the people who have had or are at high risk for brain infarction brain infarction than in healthy subjects[7,9]. Although we assessed the makers with a focus on brain ischemic attack as a possible cause of bathtub death, we found no significant difference in the levels of these markers between cases of bathtub death and non-bathtub death. These results might be explained by several factors. First, brain ischemic attack might not be involved in the mechanism of bathtub death. Second, the time from brain ischemic attack to death might be too short to increase the marker levels. Third, the marker levels might show postmortem changes. Fourth, the number of cases might be too small.

The serum levels of PC-Acro in the present cases of bathtub death and non-bathtub death were 2 to 3 times as high as those previously reported in living, healthy subjects[12,13]. Additionally, the levels of AcPAO were 1.5 to 6

Table 1. Patient data and the value of makers in cases of bathtub and non-bathtub death

Bathtub deaths	Age (years)	Cause	PC-Acro (nmol/m)	SMO (nmol/m)	AcPAO (nmol/m)	IL-6 (pg/mL)	CRP (mg/dL)
1	75	drowning	99.4	2.00	6.57	89.0	0.06
2	80	drowning	84.5	1.16	9.47	21,637.7	0.04
3	76	drowning	125.5	0.00	2.81	8.7	0.06
4	76	drowning	88.8	0.00	1.07	33.0	0.55
5	66	drowning	93.3	4.99	9.16	54,072.9	0.07
6	79	drowning	87.0	0.37	9.43	7.9	0.30
7	70	drowning	82.8	1.07	3.31	10,072.1	0.04
8	59	drowning	103.8	1.68	9.96	19.6	0.00
9	70	drowning	165.3	9.72	3.27	4,588.2	0.03
10	81	drowning	93.7	1.87	9.01	154.3	4.14

Non-bathtub deaths	Age (years)	Cause	PC-Acro (nmol/m)	SMO (nmol/m)	AcPAO (nmol/m)	IL-6 (pg/mL)	CRP (mg/dL)
1	37	hanging	90.5	5.52	3.34	213.5	0.04
2	62	acute myocardial infarction	112.0	5.94	11.83	250.4	8.75
3	74	acute myocardial infarction	36.9	0.32	4.34	88.0	5.64
4	64	hanging	93.7	0.62	5.35	2.6	0.04
5	56	acute myocardial infarction	97.6	1.47	2.87	17.2	0.27
6	57	aorta dissection	112.7	0.00	7.66	126.8	0.17
7	65	aorta dissection	80.9	1.66	3.28	183.5	0.09
8	78	acute myocardial infarction	63.9	2.13	1.01	129.7	0.23
9	52	ischemic heart failure	95.8	3.13	5.74	132.7	0.12
10	65	ischemic heart failure	94.4	3.44	6.02	14.5	1.05

PC-Acro, protein-conjugated acrolein ; SMO, spermine oxidase ; AcPAO, acetylpolyamine oxidase ; IL-6, interleukin 6 ; CRP, C-reactive protein

Table 2. Median, interquartile deviation, and p value of the Willcoxon rank sum test of bathtub and non-bathtub deaths

	PC-Acro (nmol/mL)	SMO (nmol/mL)	AcPAO (nmol/mL)	IL-6 (pg/mL)	CRP (mg/dL)
Bathtub deaths	93.5 ± 16.8	1.42 ± 1.65	7.79 ± 6.16	121.65 ± 10,052.5	0.06 ± 2.96
Non-bathtub deaths	94.05 ± 16.7	1.895 ± 2.82	4.845 ± 2.74	128.25 ± 166.3	0.2 ± 0.96
p value	0.623	0.545	0.756	0.68	0.129

PC-Acro, protein-conjugated acrolein ; SMO, spermine oxidase ; AcPAO, acetylpolyamine oxidase ; IL-6, interleukin 6 ; CRP, C-reactive protein

times as high and the levels of IL-6 were 100 times as high as those in healthy subjects. These phenomena might contribute to the effects of the moment of death or postmortem changes. The process of dying occurs before death, but the time it occurs is not clearly defined. As a patient dies, many functions (such as example cardiac, respiratory, and central nervous function) deteriorate, and the bodies are placed under a great deal of stress and respond to dying in various

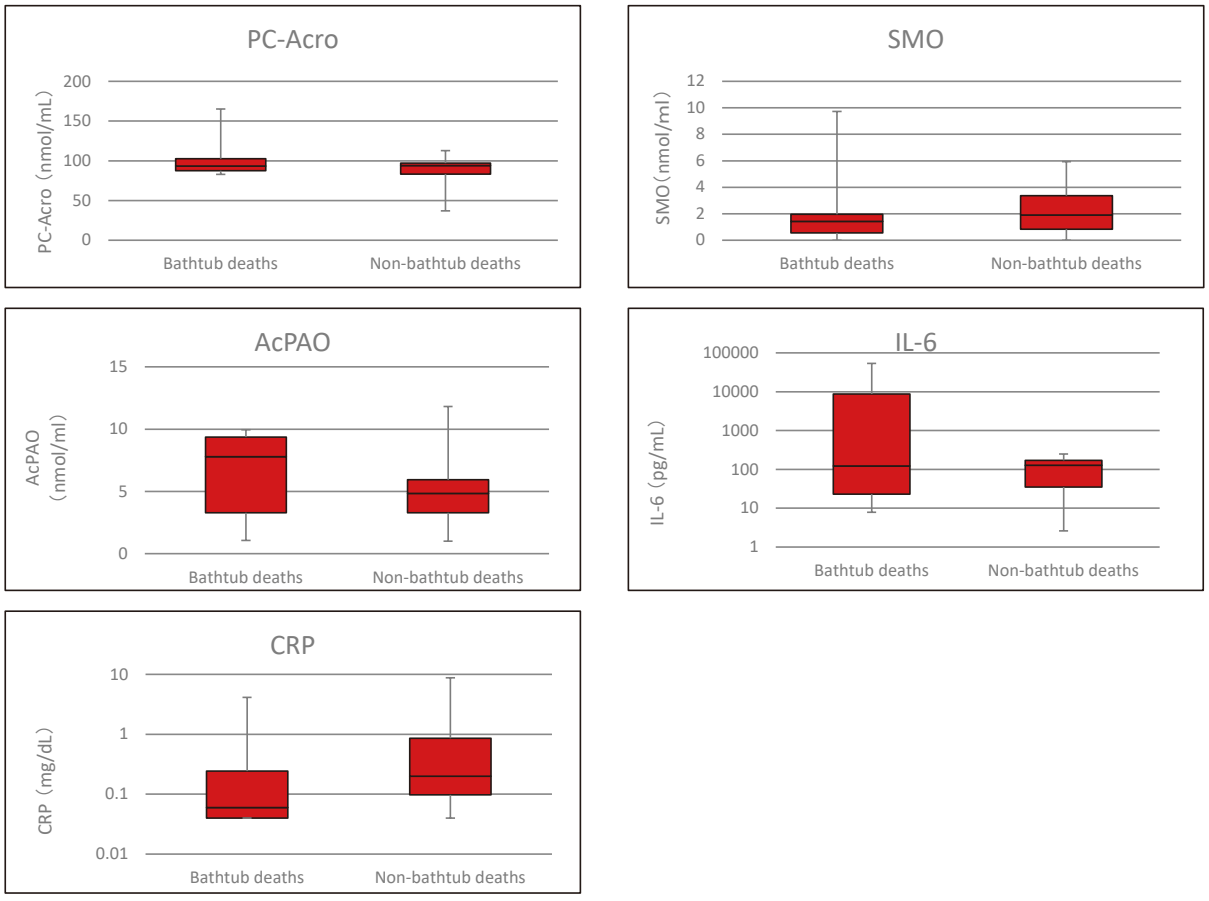

Fig. 1. The graphs of each marker comparing bathtub deaths and non-bathtub deaths. PC-Acro, protein-conjugated acrolein ; SMO, spermine oxidase ; AcPAO, acetylpolyamine oxidase ; IL-6, interleukin 6 ; CRP, C-reactive protein

ways. Some substances increase in the serum owing to ischemic cellular damage, some substances, used for activities during life, decrease in the serum. The differences of levels of PC-Acro, AcPAO, and IL-6 in the deceased subjects of the present study from those in healthy subjects might contribute to the decrease in cardiac output and the hypoxic conditions of the brain as death occurs, following the same path as brain infarction. Another possibility is that the serum levels of the markers increased because of leakage from cells by cytolysis owing to postmortem changes. On the other hand, the serum levels of SMO were one-third to one-quarter lesser than those in healthy subjects of the previous studies[12,13]. This marker might be expended while a person dies or might be deteriorate after death.

In the present study, serum levels of SMO and CRP were decreased, but not to a significant degree, in cases of bathtub death, which were suspected to involve brain ischemia, when compared with cases of non-bathtub death. In contrast, live patients with brain ischemia show an increase in each marker[7,9]. In cases of bathtub deaths, the temperature of the hot water in the bathtub around the body might be greater than the atmospheric temperature ; therefore, there is a high tendency of accelerated postmortem changes. If SMO is deteriorated owing to postmortem changes, its decomposition in bathtub deaths might be greater than that in non-bathtub deaths, and serum SMO levels might be lower when forensic autopsy is performed.

Serum levels of IL-6 in 4 of the present cases of bathtub death were extremely high. In general, IL-6 is the cytokine that increases in cases of inflammatory disease[14], and serum levels are reportedly increased in autopsy cases with trauma[15-17]. Of 4 cases of the present study with increased IL-6 levels, 2 cases had acute injuries of the head and 1 case had an old head injury. The remaining case showed no injury associated with death but showed interstitial pneumonia and chronic hepatitis, which is a chronic inflammato-

ry disease.

To assess any of the possibilities mentioned earlier, for example the healthy history, the postmortem changes or the influences by the moment of death or by hot water, the number of cases needs to be increased and the change in the markers according to time since death needs to be examined. If serum levels of the markers are affected by postmortem changes, studies should examine whether urine or vitreous fluid, which are considered to not be greatly affected by postmortem changes, can be used as alternative samples.

Moreover, we had tried to measure the serum levels of markers in other patients who had died of brain infarction, non-bathtub deaths. However, the serum could not be obtained from most patients because the blood in the right side of the heart had largely clotted, owing to the long process of dying due to brain infarction. Because coagulation factors in plasma are activated, fibrin is generated from fibrinogen, and the blood in the heart coagulate when the process of dying is prolonged[18].

Because the cause of sudden bathtub deaths remains unclear, further studies should be performed of brain ischemic attack and other mechanisms.

In the present study, we found no significant differences of serum markers, which are elevated in cases of brain infarction, between cases of bathtub deaths and non-bathtub deaths.

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