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【Original article】

Application of electrolyte analysis of pleural effusion to diagnosis of drowning

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Abstract

We measured concentrations of sodium (Na), chloride (Cl), calcium (Ca), and magnesium (Mg) in pleural effusion from forensic autopsy cases to examine whether they were useful for a diagnosis of drowning. We analyzed a total of 51 cases (15 seawater drowning, 10 freshwater drowning, and 26 non-drowning), and determined the following reference values. If the concentration of Na or Cl is under 65 mEq/l, a diagnosis of freshwater drowning can be made. If the concentration of Na is higher than 175 mEq/l, or that of Cl is higher than 155 mEq/l, or that of Ca is higher than 16 mg/dl, or that of Mg is higher than 15 mg/dl, a diagnosis of seawater drowning can be made. We recommend that pleural effusion from the left and the right thoracic cavities should be collected and analyzed separately because large differences may be observed between each side in the case of drowning. If one side corresponds to the reference value for seawater or freshwater drowning and the other side does not, a diagnosis of drowning can still be made according to the obtained value.

Keywords:

Drowning

Pleural effusion

Electrolytes

Seawater

Freshwater

1. Introduction

Drowning is diagnosed mainly from macroscopic findings, such as froth in the air passage, or drowned lungs etc. if the postmortem interval is within a few days and decomposition is not observed [1,2]. If the postmortem interval exceeds this period, the above mentioned findings are no longer observed. Instead, extensive pleural effusion is often observed in the thoracic cavity, and this finding is applied to the diagnosis of drowning since victims of drowning are assumed to have inhaled a large amount of water and the water exudes into the thoracic cavity due to postmortem changes [3-9].

On the other hand, extensive pleural effusion is also observed in the thoracic cavity if cardiac or pulmonary disease existed before death, or severe pulmonary edema developed in the agonal period regardless of the cause of death and fluid exuded into the thoracic cavities due to postmortem changes. It is therefore difficult to diagnose drowning from extensive pleural effusion alone. Moreover, it is also often very difficult to distinguish between drowning in freshwater (freshwater drowning) and drowning in the sea (seawater drowning) just from the extent and appearance of pleural effusion.

If drowning is suspected during the forensic autopsies, parts of the lungs, spleen, liver, and kidneys etc. are collected and dissolved in strong acids or enzymes to detect diatoms contained in the water inhaled when drowning. The diatom species are then identified using a light microscope, and the site of drowning is estimated from the ecology of the diatom [10-22]. However, if the victim drowned in water containing few diatoms, it is difficult to determine whether the drowning took place in fresh or seawater.

Recently, Usamoto et al. reported that it was valuable for the diagnosis of seawater drowning to measure concentrations of sodium (Na) and chloride (Cl) in pleural effusion [23,24]. They analyzed the means of the electrolytes concentrations of the left and right pleural effusions. However, a large difference can be observed in electrolyte concentration between the two

cavities because a large difference is sometimes observed in the volume of pleural effusion between the two cavities. Moreover, calcium (Ca) and magnesium (Mg), besides Na and Cl, are abundant in seawater. Therefore, we collected pleural effusions from the left and the right thoracic cavities separately, and analyzed concentrations of the electrolytes (Na, Cl, Ca, and Mg), respectively for the cavities.

2. Materials and methods

We selected cadavers with over 10 ml of pleural effusion in the left and right thoracic cavities, respectively, that underwent forensic autopsy between April 2008 and May 2011. Cadavers with thoracic injuries, thoracic hemorrhage or severe decomposition, as well as those where the cause of death could not be determined were excluded from the analysis.

In total, there were 51 cases (27 males and 24 females): 15 seawater drowning, 10 freshwater drowning and 26 non-drowning (Tables 1-3).

Drowning was diagnosed after confirming several findings, such as froth in the air passage, drowned lungs, a large amount of fluid in the thoracic cavity, detection of diatoms in lung, spleen and heart blood specimens, as well as the absence of findings indicative of other causes of death. None of the cadavers presented a finding of cardiac or lung diseases which could induce antemortem pleural effusion formation. All drowning cases were accidental or suicidal, and place of drowning was in freshwater or seawater as revealed by police investigation and autopsy findings. There was no drowning in a brackish water area.

The cause of death of Nos. 30, 36, 38, and 39 was undetermined due to decomposition. The highly probable causes of death based on police investigation and autopsy findings are shown in Table 3. Postmortem interval was determined from cadaver findings and police investigations.

The pleural effusion was collected from the thoracic cavity with a catheter and a syringe after partial thoracotomy cutting from ribs at the lower level up to the second rib to avoid damaging subclavian veins and contaminating the pleural effusion with the blood.

All samples were stored at -30°C within two hours after collection. Electrolyte concentrations were measured at the Hokkaido laboratory of SRL Corporation (Sapporo, Japan). Na and Cl concentration were measured by an ion-selective electrode method. Those of Ca and Mg were measured by the arsenazo III method and xylidyl blue method, respectively.

All statistical analyses were performed with the use of JMP version 8, Japanese Edition (SAS Institute, INC., Cary, NC, USA). Differences among the aforementioned three groups were analyzed by the Tukey-Kramer method. Linear regression analysis was carried out to determine the relationships between electrolyte concentrations and postmortem interval in the three groups. A p-value of 0.05 or less was considered statistically significant.

3. Results

Electrolyte concentrations, the volume of pleural effusion, and postmortem interval in the seawater and freshwater drowning groups are shown in tables 1 and 2. Table 3 shows similar data as well as the cause of death in the non-drowning group. Table 4 shows the mean and standard deviation (SD) of the postmortem interval and age in each group. A significant difference was observed in the postmortem interval between the seawater drowning and the non-drowning groups.

Table 5 shows the mean and SD of electrolyte concentrations in each group. Significant differences were observed in the concentrations of Na, Cl, Ca, and Mg in the pleural effusion between the seawater drowning group and the

other two groups. Significant differences were also observed in the Na concentration between the freshwater drowning and the non-drowning group. A significant difference was observed in the volume of pleural effusion between the seawater drowning and the non-drowning groups.

Table 6 shows the number of the cases classified by Na and Cl concentrations. Table 7 shows the number of the cases classified by Ca and Mg concentrations.

Differences in Na concentration between the left and right side (obtained by subtracting the right-side value from the left-side value.) were $-78 \sim +86$ mEq/l in the seawater drowning group, $-19 \sim +31$ mEq/l in the freshwater drowning group and $-16 \sim +12$ mEq/l in the non-drowning group. For differences in Cl concentration, they were $-116 \sim +94$ mEq/l, $-12 \sim +21$ mEq/l and $-6 \sim +11$ mEq/l, respectively. The number of cases which had a difference of more than 20% of the lower value (either left or right side) between the left and the right side was 4 in the seawater drowning (Nos.5, 6, 7, and 12) and 2 in the freshwater drowning (Nos.19 and 25). The rest had a difference of less than 20% of the lower value (either left or right).

Na, Cl, and Ca concentrations in the pleural effusion did not correlate with the postmortem interval in any of the groups. Mg concentration did not correlate with the postmortem interval in the seawater drowning and non-drowning group. The concentration of Mg in the left and right pleural effusion correlated with the postmortem interval in the freshwater drowning group (Left: $Y_{Mg}=0.0611X_{day}+3.5335$, adjusted R -square=0.4552, $P=0.0096$, Right: $Y_{Mg}=0.0572X_{day}+3.8978$, adjusted R -square=0.4177, $P=0.0138$).

4. Discussion

Electrolyte concentrations in pleural effusion in the seawater drowning group tended to be higher, while those in the freshwater group tended to be

lower than those in the non-drowning group (Table 5). However, in some of the seawater or freshwater drowning cases, concentrations were not markedly different from those in the non-drowning cases (Nos.8, 17). Pleural effusion in drowning cases is most likely a mixture of inhaled fresh or seawater, intracellular fluid, and extracellular fluid exuded by postmortem change. Namely, electrolyte concentrations in the pleural effusion do not necessarily reflect those of inhaled fresh or seawater only. The degree of cell decomposition, the volume of inhaled water in drowning, and the volume of fluid exuded from the lungs by postmortem change are thought to affect concentrations of electrolytes in pleural effusion. Therefore, it is important to compare values with those from a control, namely non-drowning group to determine reference values for a diagnosis of seawater or freshwater drowning.

Usumoto et al. reported that concentrations of potassium (K) and total protein (TP) tended to increase in correlation with postmortem interval and that they were not useful for a diagnosis of drowning [24]. They also reported that seawater drowning could be diagnosed if concentrations of Na and Cl were higher than 170 mEq/l and 125mEq/l respectively, because concentrations in 7 non-drowning cases were below such values [24].

In our non-drowning group, the highest concentrations of Na and Cl were 171 mEq/l and 150 mEq/l, respectively (Table 3, No.46). Therefore, if the concentration of Na is higher than 175 mEq/l, or the concentration of Cl is higher than 155 mEq/l, this is a strong indication of seawater drowning.

In this study, cadaver (No.46) suffered from cardiac failure caused by ischemic heart disease. He went into cardiac arrest while in hospital, and died from hypoxic encephalopathy 8 days later. We have not been able to determine the reason why electrolyte concentrations were comparatively high in this case compared to other non-drowning cases. Literature on this topic is rare in clinical medicine or forensic pathology and it remains to be investigated what diseases and medical treatments influence the

concentrations of electrolytes in pleural effusion.

In the 20 non-drowning cases with the exception of the aforementioned case, concentrations of Na and Cl were under 145 mEq/l and 115 mEq/l, respectively (Table 3 and Table 5). Those in 6 of the 7 non-drowning cases reported by Usumoto et al.[24] were also under 145 mEq/l and 115 mEq/l, respectively. Based on such data, if the Na concentration is higher than 145 mEq/l, or that of Cl is higher than 115 mEq/l, a diagnosis of seawater drowning is likely.

Concentrations of Na and Cl were higher than 66 mEq/l in all non-drowning cases, and lower than 65 mEq/l only in the freshwater drowning. Therefore, if the concentration of Na or Cl is under 65 mEq/l, we can make a diagnosis of freshwater drowning. These reference values we propose are supported by the data reported by Usumoto et al [24].

In the non-drowning and freshwater groups, concentrations of Ca were under 16.0 mg/dl (Table 7). If the Ca concentration is higher than 16.0 mg/dl, a likely diagnosis of seawater drowning can be made. In the non-drowning and the freshwater drowning groups, concentrations of Mg were under 15.0 mg/dl (Table 7). Therefore if the Mg concentration is higher than 15.0 mg/dl, a diagnosis of seawater drowning can be made. We should note that Ca concentrations were under 16.0 mg/dl in some seawater drowning cases whereas the concentrations of Mg were higher than 15.0 mg/dl in all seawater cases (Table 7) because the Mg concentration has about three times as high as the Ca concentration in the seawater. We therefore suggest that the measurement of Mg is more valuable than that of Ca for a diagnosis of seawater drowning. On the other hand, we cannot distinguish freshwater drowning from non-drowning by measurements of Mg and Ca alone because significant differences were not observed between the groups.

The difference in Na and Cl concentrations between the left and right pleural effusion were less than 20 % of the lower values (concentration of either the left or right) in all non-drowning cases and most of drowning cases.

In contrast, we observed differences of more than 20% in 4 seawater drowning cases (Nos.5, 6, 7, and 12) and 2 freshwater drowning cases (Nos. 19 and 25). From these, if the difference is more than 20% of lower value, we can make a diagnosis of drowning.

In the cases of Nos.6, 7, and 12, the concentration of Na or Cl on one side corresponded to our reference value, and that of the other side did not. If one side value corresponds to the reference value for seawater or freshwater drowning, we can also make the diagnosis in such a case, even though values on the other side do not. In such cases, the means of the electrolytes concentrations in the left and right pleural effusion do not necessarily correspond to any of the reference values of drowning, which could make it impossible to make a diagnosis although in most cases we can make a diagnosis from the means. Therefore, it is necessary to measure electrolyte concentrations in left and right pleural effusion separately and analyze whether the values correspond to any of the reference values.

In cases Nos.5, 7, 12, and 25, it was speculated that the left lung contained more seawater or freshwater than the right lung, and in the cases of Nos.6 and 19, the right lung contained more seawater or freshwater than the left lung before pleural effusion developed due to postmortem changes. We speculate that the disproportionate amount of inhaled water in each lung was caused from the following two reasons. If someone inhaled more water into one lung than the other during drowning, a disproportionate amount would result. We did not know the position of cadavers (Nos. 5, 6, and 7) because they were found floating at sea. Nos.12 and 25 were found in the left lateral position, No. 19 was found in the right lateral position. Considering these, we speculate that inhaled water moves to the lung located in the lower position after death, as by the same mechanism that causes postmortem lividity, and the lung located in the lower position would inevitably contain more sea or freshwater than the other lung.

However, in some of the drowning cases (Nos.1, 2, 11, 18), little difference

was found in electrolyte concentrations between the left and the right side while the difference in the volume of pleural effusion was more than 100ml (Tables 1 and 2). Therefore, we cannot explain this inconsistency at this point and further study is necessary.

Usumoto et al. reported that concentrations of Na and Cl did not correlate with postmortem interval [24]. Our data supports this result. In freshwater drowning, concentrations of Mg correlate with postmortem interval, but they do not affect the diagnosis because the rate of increase is mild. Therefore, the reference values we propose are reliable, although we recognize that the mean of postmortem interval in the non-drowning group was shorter than those in the other two groups. At present, the references for the relationship between electrolyte concentrations in pleural effusion and postmortem interval are few and the number of cases in our study is not enough to determine the influence of postmortem change completely, thus further investigation is needed.

In conclusion, an analysis of concentrations of Na, Cl, Ca and Mg in pleural effusion is useful for the diagnosis of freshwater or seawater drowning. The left and right pleural effusion should be collected and analyzed separately because in drowning cases a large difference is often observed between the pleural effusion taken from each side.

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Table 1. Concentrations of electrolytes of pleural effusion in seawater drowning group.

No.		Na(mEq/l)	Cl(mEq/l)	Ca(mg/dl)	Mg(mg/dl)	Volume(ml)	PMI(days)	Age	Sex
1	L	232	303	2.8	23.9	500	10	21	M
	R	215	290	5.3	23.8	810			
2	L	240	252	20.2	54.0	70	4	58	F
	R	216	225	18.8	51.0	330			
3	L	219	250	17.7	49.0	150	10	57	F
	R	224	255	17.1	51.0	200			
4	L	369	404	31.9	81.0	270	19	69	M
	R	354	396	37.2	84.6	300			
5	L	315	342	26.6	80.0	600	6	61	F
	R	229	248	19.3	54.6	500			
6	L	110	118	8.0	26.6	650	45	74	M
	R	188	234	10.0	35.0	750			
7	L	161	214	24.1	34.0	400	60	54	M
	R	126	175	11.9	30.1	250			
8	L	160	139	13.1	20.0	50	1	59	M
	R	156	137	13.3	21.6	100			
9	L	165	277	8.2	31.9	30	30	58	M
	R	148	250	5.8	20.5	120			
10	L	293	313	24.3	67.0	100	2	43	M
	R	286	308	24.1	56.0	170			
11	L	224	268	7.6	38.5	870	30	34	M
	R	225	265	11.0	42.9	1100			
12	L	191	203	16.3	38.5	470	3.5	67	F
	R	148	160	14.8	29.1	370			
13	L	199	209	17.4	46.5	330	1	38	F
	R	186	191	16.8	45.0	250			
14	L	250	275	21.9	54.0	90	2	84	F
	R	252	278	23.0	56.0	60			
15	L	269	312	24.5	67.0	700	11	74	M
	R	249	290	23.1	65.0	700			

L: left, R: right, PMI: postmortem interval, M: male, F: female.

Table 2. Concentrations of electrolytes of pleural effusion in freshwater drowning group.

No.		Na(mEq/l)	Cl(mEq/l)	Ca(mg/dl)	Mg(mg/dl)	Volume(ml)	PMI(days)	Age	Sex
16	L	61	88	0.1	1.3	20	5	65	M
	R	61	84	0.7	3.2	50			
17	L	108	84	8.3	4.9	75	2.5	26	M
	R	106	82	8.5	5.1	50			
18	L	42	50	7.3	5.6	700	23	60	M
	R	46	51	8.2	6.5	920			
19	L	82	77	3.1	5.4	120	5	67	M
	R	51	56	1.8	3.5	400			
20	L	55	73	1.9	4.7	300	6	61	M
	R	65	74	1.3	4.5	350			
21	L	72	64	5.4	2.8	70	1.5	59	M
	R	76	64	5.8	2.9	170			
22	L	64	89	2.9	7.1	390	60	34	F
	R	63	79	2.9	6.7	320			
23	L	68	60	7.8	5.0	150	12	65	F
	R	67	60	8.4	5.1	170			
24	L	52	46	4.2	3.0	240	1.5	28	F
	R	55	48	4.7	3.1	240			
25	L	53	42	4.2	4.1	150	1	71	M
	R	72	54	5.1	4.6	50			

L: left, R: right, PMI: postmortem interval, M: male, F: female.

Table 3. Concentrations of electrolytes of pleural effusion in non-drowning group.

No.		Na (mEq/l)	Cl (mEq/l)	Ca (mg/dl)	Mg (mg/dl)	Volume (ml)	Cause of death	PMI (days)	Age	Sex
26	L	80	81	7.0	8.9	200	CO poisoning	1.5	28	M
	R	96	86	7.6	7.6	300				
27	L	139	103	8.8	3.8	450	Death due to burn	1.5	83	F
	R	140	102	9.2	4.1	300				
28	L	138	104	6.6	3.4	20	Interstitial pneumonia	0.5	62	M
	R	138	101	6.9	3.7	20				
29	L	141	110	9.7	2.9	100	Hemorrhagic shock	0.5	70	F
	R	142	111	8.6	2.4	300				
30	L	98	105	1.2	2.8	230	Unknown	30	78	F
	R	96	94	1.4	3.4	20	(Natural death)			
31	L	109	95	13.5	5.9	20	Brain contusion	1.5	81	M
	R	112	95	12.8	5.6	20				
32	L	80	82	8.4	9.8	100	Cardiac tamponade	7	59	F
	R	71	87	12.6	12.8	120	(Dissection of the aorta)			
33	L	111	89	9.2	4.6	60	Ischemic heart disease	2	59	M
	R	109	87	9.1	5.2	10				
34	L	136	89	8.6	5.0	10	Asphyxia	0.5	15	F
	R	143	94	8.9	4.5	10				
35	L	86	76	5.7	8.4	20	Hepatic failure	1	44	M
	R	86	74	6.2	8.9	20				
36	L	70	73	7.7	10.7	70	Unknown	7	32	F
	R	70	74	10.1	13.9	180	(Poisoning)			
37	L	104	79	10.8	8.7	50	Asphyxia	2.5	28	F
	R	115	84	11.5	7.5	70				
38	L	117	100	9.7	4.5	350	Unkown	9	60	M
	R	106	91	10.2	7.2	90	(Natural death)			
39	L	68	77	15.3	12.6	300	Unknown	13	37	F
	R	77	75	11.8	13.5	60	(Poisoning)			
40	L	134	109	11.3	5.2	400	Brain contusion	1	26	F
	R	140	115	10.6	4.8	700				
41	L	110	84	5.9	3.4	100	Brain infarction	1.5	67	M
	R	110	84	6.4	3.9	100				
42	L	127	96	13.0	5.5	50	Asphyxia	1.5	65	F
	R	115	90	11.9	6.0	20				
43	L	125	93	11.4	3.8	20	Asphyxia	1	16	F
	R	129	94	11.9	4.3	20				
44	L	109	86	9.3	5.7	20	Hemorrhagic shock	1.5	75	F
	R	115	90	8.9	6.4	10				
45	L	112	91	8.0	4.4	300	Sepsis	1.5	67	M
	R	105	86	8.7	5.3	20				
46	L	167	145	7.2	3.9	100	Ischemic heart disease	1	79	M
	R	171	150	6.4	3.3	260				
47	L	136	90	12.0	4.2	70	Cardiac tamponade	1	71	F
	R	133	85	10.2	4.0	20	(Cardiac infarction)			
48	L	139	109	8.3	2.9	150	Cor pulmonale	0.5	79	M
	R	140	109	7.3	2.2	950	(Pulmonary emphysema)			
49	L	75	89	6.4	9.4	20	Hyperthermia	2	60	M
	R	75	80	7.1	11.2	10				
50	L	118	95	11.5	5.7	20	Pulmonary thrombosis	1	40	F
	R	115	92	10.8	5.7	40				
51	L	79	72	8.6	9.0	30	Asphyxia	6	27	F
	R	90	76	10.0	8.2	70				

L: left, R: right, PMI: postmortem interval, M: male, F: female.

Table4. Summary of the examined cases

	Seawater drowning	Freshwater drowning	Non-drownig
<i>n</i>	15	10	26
Sex			
Male	9	7	11
Female	6	3	15
PMI (days)			
Min	1	1	0.5
Max	60	60	30
Mean $\pm$ SD	15.6 $\pm$ 18.0*	11.8 $\pm$ 18.2	3.7 $\pm$ 6.2
Age (years)			
Min	21	26	15
Max	84	71	83
Mean $\pm$ SD	56.7 $\pm$ 16.8	54.0 $\pm$ 17.0	54.2 $\pm$ 21.8

SD: standard deviation, PMI: postmortem interval.

Significantly different from non-drowning. * $P < 0.05$.

Table5. Concentrations of electrolytes in pleural effusion in the three groups.

Left	Na (mEq/l)	Cl (mEq/l)	Ca (mg/dl)	Mg (mg/dl)	Volume(ml)
Seawater	226.5±67.2	258.6±74.8	17.6±8.3	47.5±19.6	352.0±271.6
Freshwater	65.7±18.7*	67.3±17.5*	4.5±2.7*	4.4±1.7*	221.5±202.6
Non-drowning	111.8±26.3*,†	93.2±15.5*	9.0±2.9*	6.0±2.8*	125.4±131.7*

Right	Na (mEq/l)	Cl (mEq/l)	Ca (mg/dl)	Mg (mg/dl)	Volume(ml)
Seawater	213.5±59.3	246.8±65.3	16.8±8.2	44.4±18.0	400.7±306.9
Freshwater	66.2±16.7*	65.2±13.5*	4.7±3.0*	4.5±1.4*	272.0±261.3
Non-drowning	113.0±26.0*,†	92.5±16.0*	9.1±2.5*	6.4±3.3*	143.8±225.9*

Values are means ±SD. Significantly different from seawater. **P* < 0.05.
Significantly different from freshwater. †*P* < 0.05.

Table 6. Numbers of the cases classified by the concentrations of Na and Cl.

Left						Left					
Na (mEq/l)	-65	66-109	110-145	146-174	175-	Cl (mEq/l)	-65	66-90	91-115	116-154	155-
Seawater	0	0	1	3	11	Seawater	0	0	0	2	13
Freshwater	6	4	0	0	0	Freshwater	5	5	0	0	0
Non-drowning	0	11	14	1	0	Non-drowning	0	13	12	1	0

Right						Right					
Na (mEq/l)	-65	66-109	110-145	146-174	175-	Cl (mEq/l)	-65	66-90	91-115	116-154	155-
Seawater	0	0	1	3	11	Seawater	0	0	0	1	14
Freshwater	6	4	0	0	0	Freshwater	6	4	0	0	0
Non-drowning	0	11	14	1	0	Non-drowning	0	14	11	1	0

Table 7. Numbers of the cases classified by the concentrations of Ca and Mg.

Left				Left			
Ca (mg/dl)	0-9.9	10.0-15.9	16.0-	Mg (mg/dl)	0-9.9	10.0-14.9	15.0-
Seawater	4	1	10	Seawater	0	0	15
Freshwater	10	0	0	Freshwater	10	0	0
Non-drowning	18	8	0	Non-drowning	24	2	0

Right				Right			
Ca (mg/dl)	0-9.9	10.0-15.9	16.0-	Mg (mg/dl)	0-9.9	10.0-14.9	15.0-
Seawater	2	5	8	Seawater	0	0	15
Freshwater	10	0	0	Freshwater	10	0	0
Non-drowning	14	12	0	Non-drowning	22	4	0