Changes in thallium distribution in the scalp hair after an intoxication incident

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Highlights

- Hair samples store information concerning the exposure period to thallium.
- The time course of thallium distribution changes in the hair were evaluated.
- Variation among individuals was seen in the decrease of thallium in hair.

Introduction

Thallium has been using as an industrial material in the manufacture of optical lenses, semiconductors, scintillation counters, low-temperature thermometers, green-colored fireworks and chemical catalysts [1]. Thallium intoxication typically shows symptoms of gastrointestinal disturbances, peripheral and central nervous system disorders, and hair loss [2–5]. Its presence in blood, urine or hair are essential for confirmation of exposure [6,7]. Industrial usage of thallium has decreased over recent decades because of its severe toxicity [2]. However, because of its colorless, odorless and tasteless nature, thallium is used for criminal purposes [8-10], which has attracted social attention in Japan [11]. For the assistance of prompt investigation of future thallium poisonings, it is, therefore, desirable to collect for much biological sample information about thallium exposure. In cases of criminal thallium poisoning, forensic investigation is required to identify the amount and time of thallium exposure [12]. Usually, blood and urine thallium levels are respectively used as biomarkers for identifying intoxication [13] and predicting the long-term outcome [1]. Because of variance among individuals and the long half-life of thallium (2–15 days) [14], blood or urine concentrations one month after exposure may be used to qualitatively confirm exposure, but do not correlate quantitatively with exposure amount. Moreover, it is difficult to estimate the exposure period using blood or urine.

Hair has the unique potential to reveal retrospective information. Thallium is incorporated into the growing hair from blood plasma, and incorporated thallium remains stable for long periods of time.

Moreover, because human hair is known to grow at an average rate of approximately 1 cm/month [15], several studies suggest that thallium levels in hair segments which differ in proximity to the scalp can, under certain conditions, be used as a retrospective calendar of thallium intoxication during time periods preceding sample collection [16-18]. However, in criminal cases, it is possible that the hair cannot be sampled at an appropriate time for such retrospective analysis. Moreover, it is known that drug distribution along a hair sample may vary from many causes [19], including hair growth rate and cycle, and incorporation from sweat or sebum. Although several studies have attempted to clarify how thallium is distributed in hair after thallium poisoning [6,7], none have evaluated the time course of changing thallium distribution.

In the study presented here, we investigated changes in the distribution of thallium in scalp hair at different time points after poisoning, to assess the utility of hair for retrospective analysis of poisoning.

Material and Methods

Subject and sample collection

Four male and one female workers at a company in Kanagawa Prefecture, Japan, were poisoned with thallium. Table 1 shows characteristics of these five thallium intoxication cases. According to the police, thallium sulfate had been added to a bottle of tea that they consumed. The thallium concentration in the tea was estimated at approximately 400 mg/l; five victims drank one or two cups (300–700 ml) of

the tea. All cases developed severe pain with paresthesia in the lower extremities, beginning 2 or 3 days after exposure. Two males (cases 3 and 4) and one female (case 5) showed characteristic loss of hair from 10 days after the exposure. The remaining two males (cases 1 and 2) did not show this symptom. Because one victim complained that they were poisoned by thallium, the employer considered that police investigation was necessary, which began on day 26. The police science laboratory found thallium in blood and spot urine samples collected on day 29.

For further forensic analyses, the police brought blood and urine samples to our department. They also gave us scalp hair samples collected from each case as evidential material. The hair samples were collected from the upper back of the head by cutting at exactly at the scalp surface, on day 79 or 80 (2.6 months after exposure, the first sample collection), and on day 125, 126, 127 or 134 (4.2–4.5 months after exposure, the second sample collection). Since the police collected hair at the time of interview about the incident, hair samples were in different lengths. Case 5 provided hair samples only once, refusing the later collection. The Ethical Committee of Juntendo University review board decided that this study did not require ethical approval as it was carried out by a commissioned service.

Measurement of thallium by inductively coupled plasma mass spectrometry (ICP-MS)

Thallium concentrations in scalp hair, blood and urine samples were determined by ICP-MS after

microwave digestion, using a previously reported method [20-22]. For each case, 28 scalp hair samples from the first sample collection were segmented every 3 mm from the scalp surface to 30 mm, and the remaining portions were cut every 10 mm to 60 mm. Similarly, 28 scalp hair samples from the second sample collection were segmented every 10 mm from the scalp surface to the tip, until there was no hair residue. Each bundle of segmented hair was placed into a perfluoroalkoxy alkane–Teflon (PFA) vial (GL Sciences Inc., Tokyo, Japan). Samples of blood or urine (100 μl) were also placed in PFA vials.

The hair, blood and urine samples were then digested with 0.4 ml of concentrated nitric acid (Ultrapure Grade, Tama Chemicals Co., Kawasaki, Japan) and 0.2 ml hydrogen peroxide (Ultrapure Grade, Tama Chemicals Co.) in a microwave oven (MLS-1200 MEGA, Milestone S.R.L., Bergamo, Italy) in the following five steps: power was set at 250, 0, 250, 400 and 600 W for 5, 1, 5, 5 and 5 min, respectively. The volume of the digested sample was then adjusted to 1.0 ml with ultrapure water. The fixed-volume solution was diluted 100 times with 0.5% nitric acid and 20 ng/ml yttrium as an internal standard. Thallium concentrations in the diluted solution were determined using an inductively coupled plasma mass spectrometer (Elan DRC-II, PerkinElmer, Waltham, MA, USA) at a mass-to-charge ratio (*m/z*) of 205. The ICP-MS conditions were optimized using a 1 ng/ml tuning solution containing thallium in 0.5% nitric acid. Quantification was performed by the internal standard method using an *m/z* of 89 for yttrium. Thallium measurements about hair were repeated three times and blood and urine were repeated twice. The average value was used for subsequent analysis.

Whereas thallium concentrations were shown the result per weight in other studies [16, 17], in the present study, the amount of thallium was expressed as the data per length of hair like Yoshinaga's study [18]. This was because of decrease in the hair density after thallium intoxication (Appendix A), which might have led to the difficulty in an estimation of exposure period from the distribution of hair thallium concentration. For reference, the results using the data per weight of hair are given in Appendix B.

Quality control and quality assurance

Suitable certified reference materials for the assessment of chemical analyses of thallium in scalp hair, blood and urine are not commercially available. For internal quality assurance of the thallium determination, we analyzed the quality control materials, Seronorm Trace Elements Whole Blood Control Level-2 and Level-3 (SERO, Billingstad, Norway) [21] with target thallium value of 5.2 and 10.2 ng/ml, respectively. Our mean concentrations from day-to-day (n = 20) for these control materials were 5.3 and 9.9 ng/ml, respectively, which is in good agreement with the target values. The relative standard deviations of the mean concentrations ranged from 5.8–8.4%. The limit of detection (LOD) and the limit of quantification (LOQ) were the concentrations equivalent to the signal of thallium, which was respectively equal to 3 and 10 times the standard deviation of 10 repeated measurements of the blank signal at m/z = 205. For the quantities of thallium in hair per mm length, values of the LOD and LOQ

were 0.02 and 0.07 pg/mm hair, respectively.

Results

The thallium distributions of the first and second sample collections are shown in Figure 1A and B, respectively. We found a considerable amount of thallium in all of the first-sample hair segments for all five cases. In each case, the maximum amount of thallium was found within the range of 21–30 mm from the scalp surface. The highest amount of thallium was 7.26±0.10 pg/mm in the 27–30 mm segment of case 5. In the second sample collection, the maximum amount of thallium was observed in the 30–40 mm segment in case 1 (2.04±0.03 pg/mm), case 2 (0.60±0.06 pg/mm) and case 3 (1.13±0.06 pg/mm). In case 4, the maximum amount of thallium was found in the 40–50 mm segment (1.42±0.05 pg/mm). As compared with the first sample collection, the maximum thallium amount in the second sample collection showed decreases of −39.1%, −80.8%, −82.2% and −68.4% for cases 1, 2, 3 and 4, respectively.

Table 2 shows whole blood and urine thallium concentrations in the five cases analyzed in our laboratory. More than 30 ng/ml of thallium was found in the blood evidence material collected on day 29, one month after the incident. Urinary thallium concentrations were higher than 200 ng/ml on day 29 in all five cases, and were reduced by 20–50% eight days later.

Discussion

Thallium in scalp hair was evaluated twice in each of four cases, at an interval of approximately 2 months. The thallium distribution changed during this time period, and showed an asymmetrical bell-shape. In addition, the widths of the peaks expanded with the passage of time. In hair analyses of drug abuse cases, the spread of the distribution largely depends on variation in the elongation rate of individual hairs [19]. Hayashi et al. [23] showed that the growth rates of single hairs vary by more than 50%. It is likely that the characteristic distribution of thallium in the present study was also caused principally by variation in hair growth rates, because we used several hairs for the segmentation analysis. Our results indicated that, to estimate the thallium exposure period from its hair distribution, a single hair was not sufficient and several hairs were required. According to Hayashi's results [23], 30–50 hairs would be suitable for estimating the distribution of thallium because this number would be sufficient to sample the growth rate distribution. We also observed that some thallium was present not only on the scalp surface side of the peak, but also on the hair tip side. Although the details were not clear, the presence of thallium at the tip of the hairs was likely to arise from redistribution in sweat or sebum.

A detailed investigation by LeBeau et al. [15] showed that scalp hair grows at a mean rate of 1.06 cm/month, and a length of 0.8 cm is left under the scalp surface after the collection of hair specimens. By applying their model, we used the distribution of thallium in hair to estimate the thallium exposure period. The maximum-thallium segments of the first sample collection (2.6 months after exposure) were

distributed 21–30 mm from the scalp, indicating that the thallium exposure period was estimated to be 2.2–3.1 months before collection. Similarly, as maximum-thallium peaks were 30–40 or 40–50 mm from the scalp in the second sample collection (4.2–4.5 months after exposure), the thallium exposure period was estimated at 3.1–5.0 months before collection. Thus, the thallium exposure date estimated from both hair sample collections matched the actual exposure date.

The maximum amount of thallium in hair tended to be associated with the subjects' estimated thallium dose and subjective symptoms. Cases 4 and 5 reported greater consumption of thallium-containing tea than the other cases, agreeing with their testimony and subjective symptoms. In case of criminal exposure of thallium, sometimes it is difficult to collect blood or urine at suitable period for retrospective analysis. The hair is, therefore, still valuable evidence material. Our results support that using hair as biological samples is useful for the criminal investigation.

Variation among individuals in the decrease of thallium in hair between 2.6 and 4.2–4.5 months after exposure suggested individual differences in thallium loss from the hair. Maurice et al. [16] reported thallium concentrations in unwashed and washed hair samples from a thallium-poisoned person and found no significant effect of washing. They suggested that hair thallium derived from the body fluid appears to be tightly bound and is not leached out during the washing procedure. In contrast to their observation, thallium in the hair decreased from 2.6 to 4.2–4.5 months after exposure in the present study. Although it is difficult to unconditionally compare with washing before measurement and routine

washing, it was thought that this discrepancy is due to differences in washing conditions between the two studies. Maurice et al. [16] used International Atomic Energy Agency washing procedures (the hair samples were soaked in water [1 min], acetone [1 min], and water [1 min]). They only performed washing once, whereas the hair of our subjects was washed repeatedly in daily life. Although the decrease in thallium was small, the repetition of washing resulted in the leaching out of thallium from the hair.

The present study, however, has some flaws: Due to a scarce number of victims, any statistical analysis was not performed. Second, information about the actual exposure amount and symptoms were obtained only from police questioning; no objective data were available. We hope that the present study could contribute to a better understanding of identifying the date of the crime, even if the information is not sufficient.

Conclusion

We found that determination of thallium amounts in hair samples divided into consecutive segments provides valuable information about exposure period even if a considerable time passes after exposure. Moreover, the hair thallium distribution partly helps an information about the exposure amount in addition to blood and urinary thallium concentration. In that case, it is necessary to pay sufficient attention to individual differences in thallium decrease from hair.

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Table 1. Characteristics of five thallium intoxication cases.

	Case				
	1	2	3	4	5
Sex	Male	Male	Male	Male	Female
Age group	50–59	50–59	30–39	20–29	30–39
Intake of tea containing thallium (cups)	1	1	1	2	2
Estimated thallium intake (mg)	120–140	120–140	120–140	240–280	240–280
Signs and symptoms					
Lower limb pain	+	+	+	+	+
Mee's line/deformed nail	+			+	+
Alopecia			+	+	+
Abdominal pain	+	+	+		
Chest tightness		+		+	+

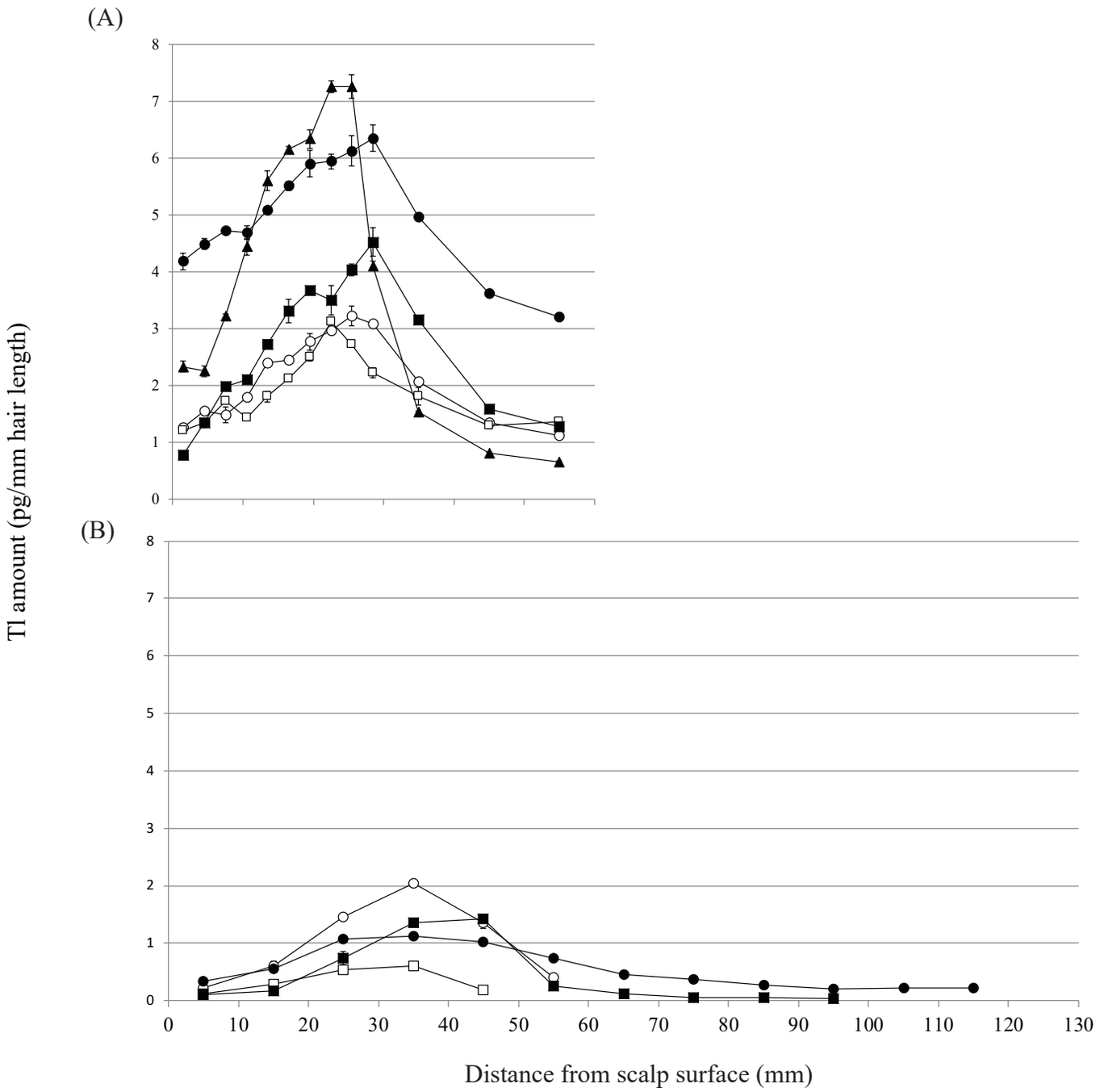
Table 2. Concentration of thallium in whole blood (29 days after exposure) and urine (29 and 37 days after exposure); mean (ng/ml).

Case	Whole blood	Urine	
		29 days	37 days
1	69.91	357.18	218.15
2	39.39	643.09	179.14
3	97.21	319.14	267.96
4	116.77	634.71	431.31
5	92.07	245.50	176.74

Figure legends

Figure 1. Thallium amounts in segmented hair samples from five cases. Samples were collected at 2.6 months (A) and 4.2–4.5 months (B) after the incident, from case 1 (open circles), 2 (open squares), 3 (closed circles), 4 (closed squares) and 5 (closed triangles). Data are mean ± standard deviation of three independent measurements. Where the standard deviation is small, the error bar is not visible.

Fig.


(A)
(B)
Tl amount (pg/mm hair length)
Distance from scalp surface (mm)
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
70
80
90
100
110
120
130

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Role of the Funding Source

The commercial sponsor had no role in study design, data collection, analysis and interpretation, or writing of the report.

Declaration of Interest

The authors declare that they have no competing interests.

Appendix A

Mean hair density of each fraction 2.6 (A) and 4.2-4.5 (B) months after thallium exposure (μg/mm).

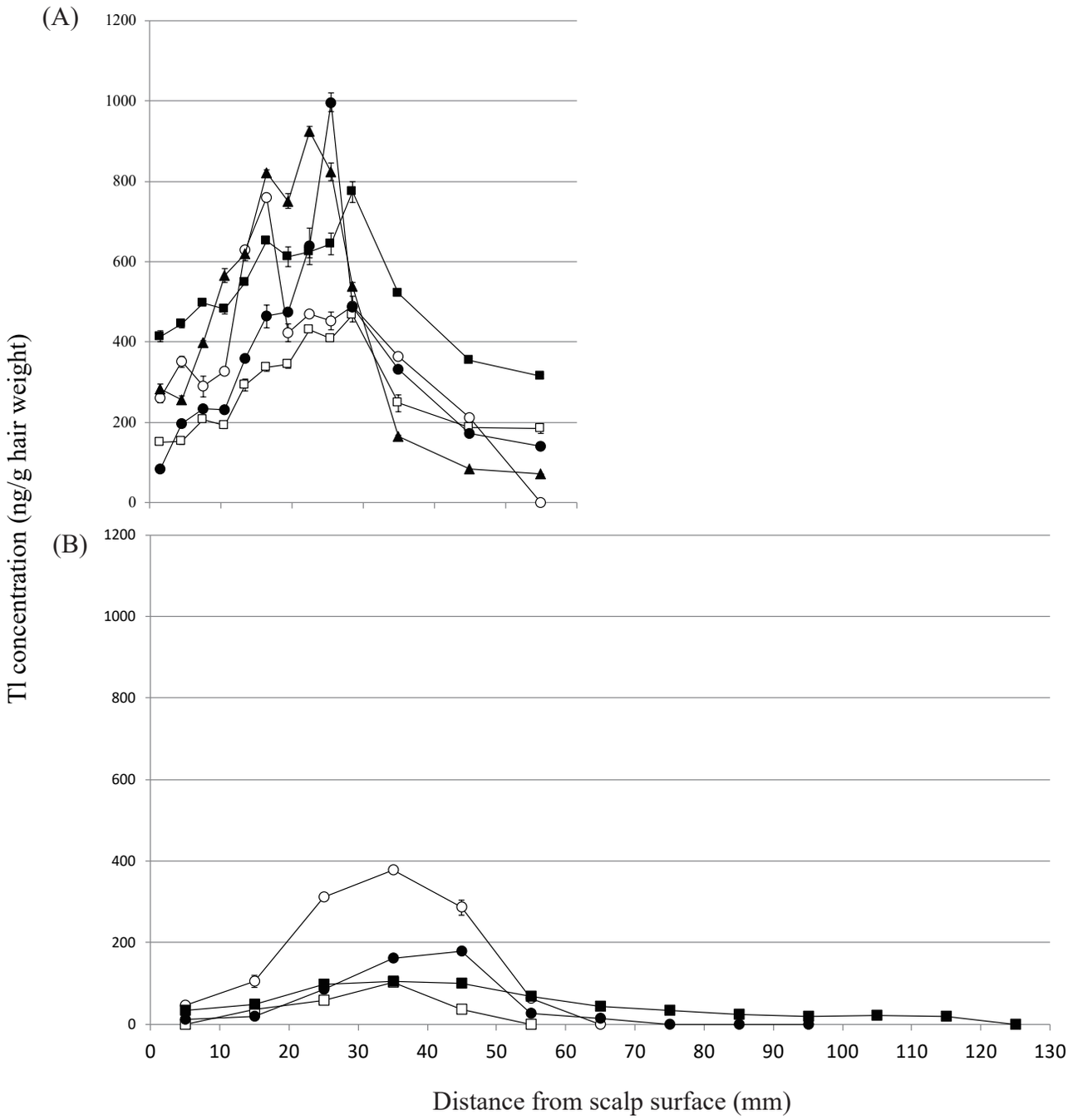
(A)

hair fraction	Case				
(mm-mm)	1	2	3	4	5
0-3	4.81	8.10	10.12	9.29	8.21
3-6	4.40	8.77	10.12	6.90	8.81
6-9	5.12	8.33	9.52	8.45	8.10
9-12	5.48	7.50	9.76	9.17	7.86
12-15	3.81	6.19	9.29	7.62	9.05
15-18	3.21	6.31	8.45	7.14	7.50
18-21	6.55	7.26	9.64	7.74	8.45
21-24	6.31	7.26	9.52	5.48	7.86
24-27	7.14	6.67	9.52	4.05	8.81
27-30	6.31	4.76	8.21	9.29	7.62
30-40	5.68	7.32	9.50	9.50	9.21
40-50	6.39	6.88	10.21	9.21	9.68
50-60	6.40	7.33	10.19	9.00	9.25

(B)

hair fraction	Case				
(mm-mm)	1	2	3	4	5
0-10	4.79	7.82	10.54	9.00	-
10-20	5.79	8.11	11.25	9.29	-
20-30	4.68	9.40	10.96	8.61	-
30-40	5.37	5.86	10.82	8.36	-
40-50	4.72	5.05	10.18	8.00	-
50-60	6.50	5.11	10.68	9.21	-
60-70	4.75	-	10.68	8.56	-
70-80	-	-	10.96	8.43	-
80-90	-	-	11.18	8.44	-
90-100	-	-	10.32	9.14	-
100-110	-	-	11.13	-	-
110-120	-	-	11.18	-	-
120-130	-	-	-	-	-

Appendix B



Thallium concentrations in segmented hair samples from five cases. Samples were collected at 2.6 months (A) and 4.2–4.5 months (B) after the incident, from case 1 (open circles), 2 (open squares), 3 (closed circles), 4 (closed squares) and 5 (closed triangles). Data are mean ± standard deviation of three independent measurements. Where the standard deviation is small, the error bar is not visible.