Two Age-Related Accumulation Profiles of Toxic Metals

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Abstract: In order to investigate the body burden levels of toxic metals in Japanese, five toxic metal concentrations in scalp hair samples from 28,424 subjects from infant to elderly were determined with inductively coupled plasma mass spectrometry (ICP-MS). The geometric mean of hair mercury concentrations showed a high-significant age-correlated increase ($r = 0.341$, $p < 0.0001$) with a peak at the 6th decade of life and then decreased with further aging in both sexes. The mean mercury concentrations in male adults were significantly higher than those in female ($p < 0.001$), indicating the gender difference (male > female) in mercury accumulation. Arsenic also showed a similar accumulation profile with age-dependency and gender difference in adult subjects. In contrast, cadmium, lead and aluminium exhibited another type of accumulation profile: the highest burden level was observed in infants aged 0-3 years old for every element in both sexes. In addition, cadmium was found to have a character accumulating in aged females, with significant age-dependency ($r = 0.134$, $p < 0.0001$) and gender difference (female > male).

These findings suggest that toxic metals are classified into two families on the basis of their accumulation profiles, and that the three elements of mercury, arsenic and cadmium which accumulate age-dependently in adults, may play a role in aging process and higher burden with them may lead to acceleration of aging.

Keywords: Toxic metal accumulation, correlation with age, gender difference, mercury, arsenic, cadmium.

INTRODUCTION

Environmental pollution with toxic metals is one of the problems today. Hair is a unique tissue containing a special protein "hair-specific keratin" which is rich in cysteine capable of binding with toxic metals, and has a role of excreting them from the body. In fact, high toxic metal levels have been reported in the scalp hair samples from the subjects exposed to toxic metal pollutions, such as mercury, cadmium, arsenic or lead [1-4]. Thus, scalp hair is generally accepted as a biomarker specimen for estimating methylmercury exposure, and hair mineral analysis has been used in forensic medicine, in screening populations for toxic metal poisoning and in monitoring environmental pollutants [5-8]. In particular, Goulle *et al.* [7] have demonstrated that hair mineral analysis using a high specific and sensitive inductively coupled plasma mass spectrometry (ICP-MS) method is reliable and suitable for estimating chronic toxic metal pollution and mineral deficiency in human body. Thus, its clinical application has been tried for diagnosis of some diseases and symptoms [9-12].

The purpose of this study was to examine the burden levels of toxic metals in Japanese population. The data of representative five toxic metals including metalloid (mercury, arsenic, cadmium, lead and aluminium) in scalp hair samples from total 28,424 Japanese subjects aged 0-100 years were used, and their body burden levels were estimated.

This study demonstrates that there are two age-related accumulation profiles for toxic metals and that the three elements of mercury, arsenic and cadmium accumulate with age-dependency and their accumulation may play a role in aging process.

METHODS

Scalp Hair Samples and Toxic Metal Analysis

On the basis of informed consent, scalp hair samples from 28,424 (male: 9,612; female: 18,812) subjects aged 0-100 years were collected in the period from June 2005 to July 2007 (Table 1). Hair sampling was recommended to cut as close to the scalp of the occipital area as possible. Hair sample of 75 mg was weighed into 50ml plastic tube, and washed with acetone and then with 0.01% Triton solution, in accordance with the procedures recommended by the Hair Analysis Standardization Board [13]. The washed hair sample was mixed with 10 ml 6.25% tetra methyl ammonium hydroxide (TMAH, Tama Chemical, Kawasaki, Japan) and 50 µl 0.1% gold solution (SPEX Certi Prep, Metuchen, NJ, USA), and then dissolved at 75 °C with shaking for 2 hours. After cooling of the solution to room temperature, internal standard (Sc, Ga, and In) solution was added, and after adjusting its volume gravimetrically to 15.0 g, the obtained solution was used for mineral analysis. The toxic metal and metalloid concentrations were determined with inductively coupled plasma mass spectrometry (ICP-MS; collision-typed 7500c, Agilent Technologies, Santa Clara, CA, USA) by the internal standard method as previously reported [10-12,14] and are expressed as ng/g hair (ppb). The detection limit was 16, 3.6, 2.2, 3.4 and 47 ppb for mercury, arsenic, cadmium, lead and aluminium, respectively. The inter-daily variation

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Table 1. Tested Subjects (N = 28,424)

Age (year-old)	Female	Male
0-3	200	660
4-9	306	1,000
10-15	181	338
16-19	134	105
20-29	2,817	927
30-39	6,421	2,147
40-49	3,763	1,829
50-59	2,648	1,371
60-69	1,476	796
70-79	695	364
80-	171	75
Total	18,812	9,612

of analysis for mercury, arsenic, cadmium, lead and aluminium was calculated 9.4, 6.9, 15.2, 5.8 and 6.9 %, respectively.

This study has been approved by review of the ethical committee of La Belle Vie Inc. All of the data obtained are held securely in such a form as to ensure anonymity.

Statistical Analysis

Because scalp hair toxic metal concentrations are lognormal distributed, geometric rather than arithmetic means were used as representative of their hair concentrations. The relation between the toxic metal concentrations and age of the subjects was investigated by Pearson's correlation coefficient test. The statistically significant difference was determined using Welch's t-test.

RESULTS

Five toxic metal concentrations in the scalp hair specimens from 28,424 subjects were distributed in nearly lognormal profile. Fig. **1** for mercury exhibits a marked age-related accumulation profile with gender-difference (male > female) in adult subjects. The geometric mean of hair mercury concentrations in male adults showed a high-significant age-correlated increase ($r = 0.341$, $p < 0.0001$) from the lowest of around 2,000 ng/g (ppb) up to a peak of 5,700 ng/g at the 6th decade of life, and then decreased with further aging ($r = -0.170$, $p < 0.0001$) (Fig. **1**). A similar age-dependent accumulation profile was also observed in female ($r = 0.217$, $p < 0.0001$): the mean mercury concentrations increased from 1,700 to 3,600 ng/g, although these levels were significantly lower than those in male adults ($p < 0.001$).

A similar, age-correlated accumulation profile with gender difference was observed for arsenic (Fig. **2**). In male adults, the mean arsenic concentrations increased age-dependently from around 50 ng/g to over 90 ng/g at 70's ($r = 0.109$, $p < 0.0001$), while in female, the mean levels increased from around 20 ng/g to over 40 ng/g.

In contrast, cadmium, lead and aluminium showed another accumulation profile different from mercury and arsenic: the highest mean concentration was observed in the infant group aged 0-3 years for every element (Figs. **3-5**). In addition, for cadmium a significant age-correlated accumulation ($r = 0.134$, $p < 0.0001$) was observed in female adults with a peak at the 7th decade of life and its mean burden levels in aged women were significantly ($p < 0.0001$) higher than those in male subjects (Fig. 3).

The maximum accumulation level of toxic metals in children was observed in the individuals aged 0-3 years for every toxic element (Table **2**). In particular, the highest level of 1,972 ng/g for cadmium, which was detected in a 2-year old boy, was estimated about 280-fold higher than the reference mean level in adults. For lead and aluminium, almost 68-fold and 20-fold higher level was detected in a different 2-year old boy, respectively. Whereas, for mercury and arsenic, the highest level in children corresponded to only about 9- and 8-fold of the adult mean level, respectively.

DISCUSSION

In the present toxic metallomic study, we estimated the body burden levels of five toxic metals including metalloid, namely neurotoxic mercury, lead and aluminium, and carcinogenic cadmium and arsenic, for 28,424 Japanese subjects from infant to elderly.

On the basis of the accumulation profile in the scalp hair specimens, toxic metals were grouped into two families. The first family composing of mercury and arsenic showed a characteristic profile of a marked age-dependency and gender difference (male > female) in adult subjects (Fig. **1** and **2**). The second family including cadmium, lead and aluminium showed another characteristic profile exhibiting the highest accumulation in infants aged 0-3 years for both sexes (Figs. **3-5**), although only for cadmium a considerable age-correlated accumulation profile with inverse gender-difference (female > male) was observed.

On the mechanism of the gender difference for mercury and arsenic (male > female), it is reported that some sex hormonal control may be involved in human mercury uptake and elimination, because a marked sex difference in the tissue uptake and elimination was observed in methyl-mercury treated animals [15]. In addition, the lower level in female may be due to the low amounts of dietary fish consumption and high incidence of artificial hair waving among women [6,14,16].

The mechanism of the inverse gender difference for cadmium (female > male), may relate to its ability to form a high-affinity complex with estrogen receptor and exhibit potent estrogen-like activities [17]. Furthermore, the highest cadmium accumulation in the aged-female group at the 7th decade of life (Fig. 3) explains the reason why many patients suffered from Itai-itai disease were senile women [18,19].

This study demonstrated that mercury exhibits the highest significant correlation to aging, with the regression coefficient of $r = 0.341$ in male and $r = 0.217$ in female

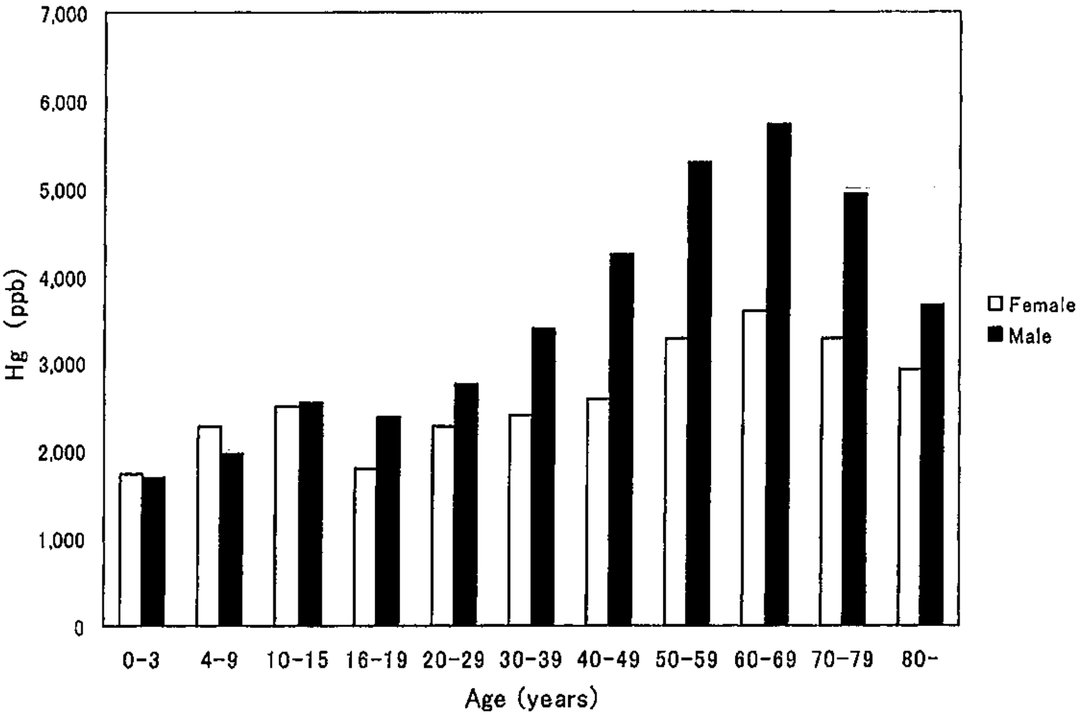

Fig. (1). Mean hair mercury concentrations in Japanese subjects (N = 28,424).
Each column represents the geometric mean of hair mercury concentrations (ng/g hair) for female and male subjects aged 0-3, 4-9, 10-15, 16-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80 years over.

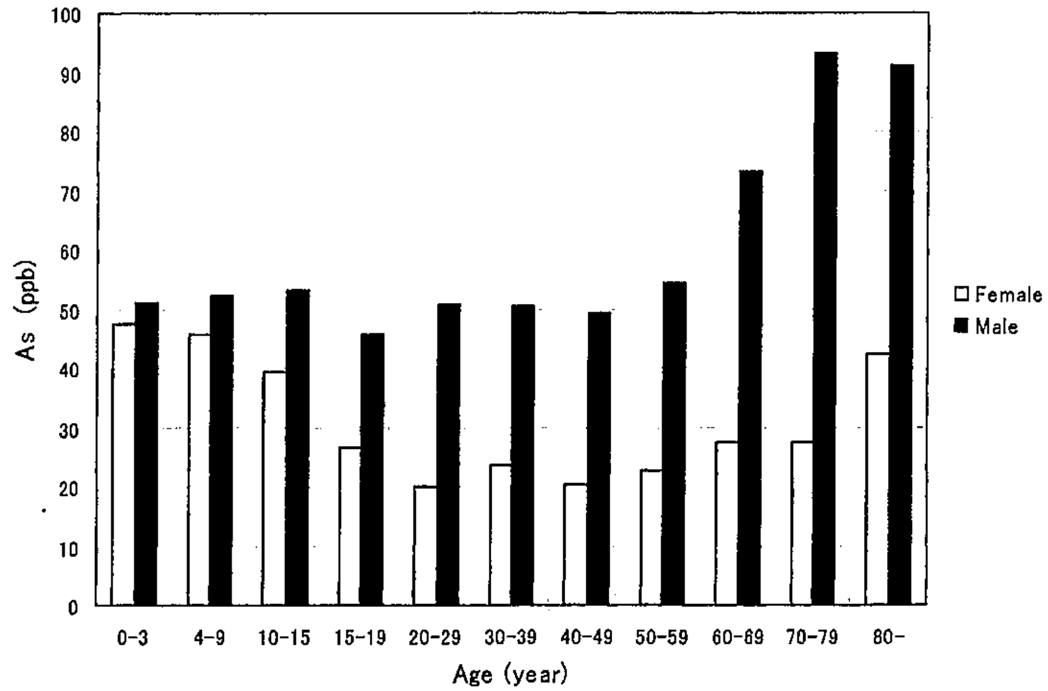

Fig. (2). Mean hair arsenic concentrations in Japanese subjects (N = 28,424).
Each column represents the geometric mean of hair arsenic concentrations (ng/g hair) for female and male subjects aged 0-3, 4-9, 10-15, 16-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80 years over.

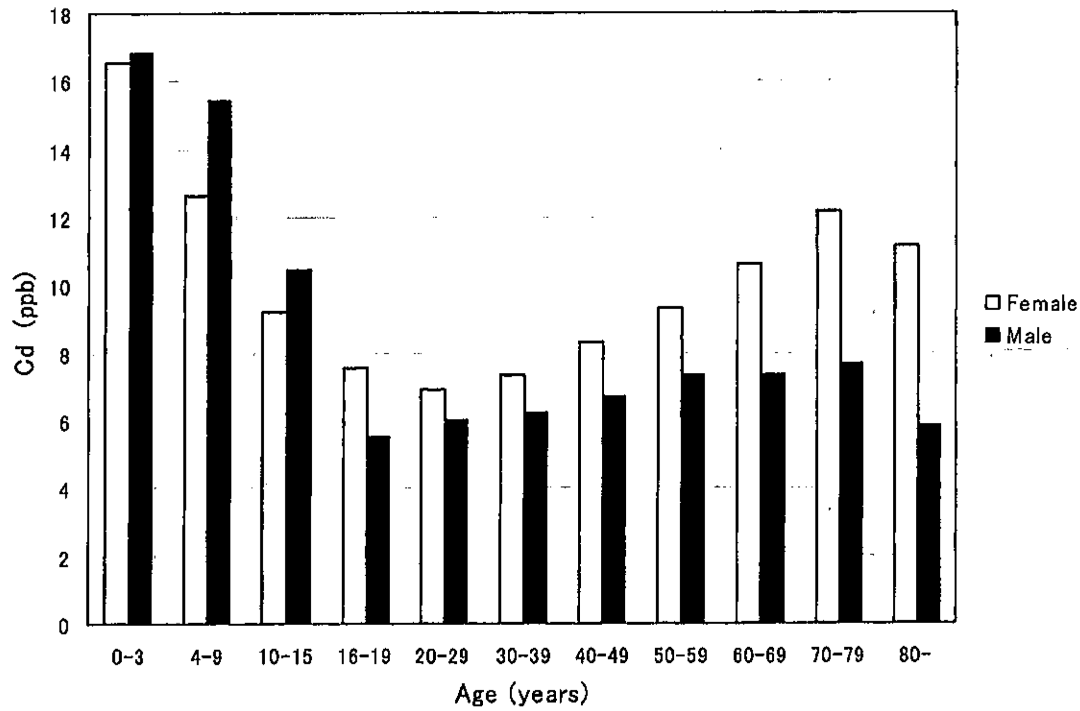

Fig. (3). Mean hair cadmium concentrations in Japanese subjects (N = 28,424).
Each column represents the geometric mean of hair cadmium concentrations (ng/g hair) for female and male subjects aged 0-3, 4-9, 10-15, 16-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80 years over.

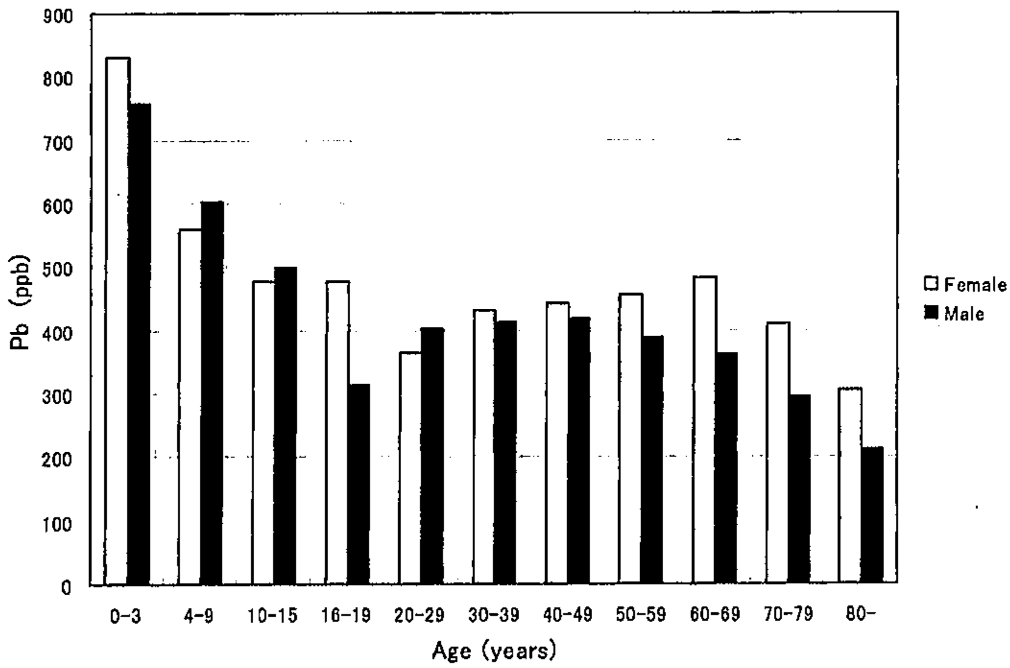

Fig. (4). Mean hair lead concentrations in Japanese subjects (N = 28,424).
Each column represents the geometric mean of hair lead concentrations (ng/g hair) for female and male subjects aged 0-3, 4-9, 10-15, 16-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80 years over.

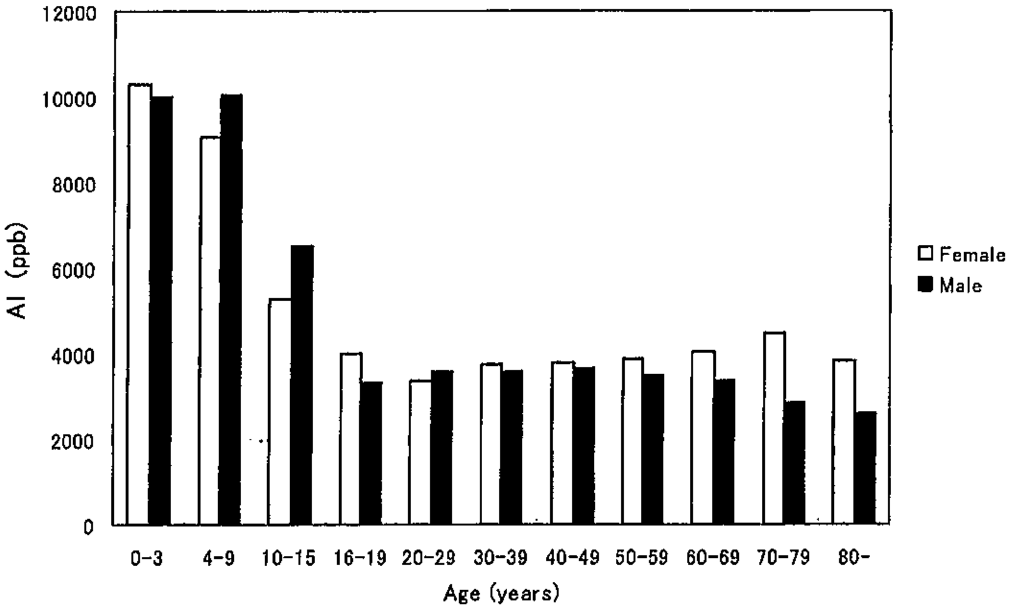

Fig. (5). Mean hair aluminium concentrations in Japanese subjects (N = 28,424).
Each column represents the geometric mean of hair aluminium concentrations (ng/g hair) for female and male subjects aged 0-3, 4-9, 10-15, 16-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80 years over.

Table 2. Maximum Accumulation Levels of Toxic Metals in Infants

	Mercury	Arsenic	Cadmium	Lead	Aluminum
Female					
Geomean in Adults	2,685	30.0	8.9	461	4,044
in Infants	1,725	47.6	16.6	829	10,318
Maximum Level	19,210	566.4	1,541.0	12,130	62,770
(Age)	(3-y)	(1-y)	(2-y)	(1-y)	(1-y)
Male					
Geomean in Adults	3,894	51.0	7.0	434	3,758
in Infants	1,685	51.1	16.8	758	10,009
Maximum Level	36,280	394.1	1,972.0	29,480	74,310
(Age)	(2-y)	(1-y)	(2-y)	(2-y)	(2-y)
					ng/g (ppb)

($p < 0.0001$). This finding suggests that mercury accumulation plays a considerable role in physiological senescence, particularly in Japanese, because mercury is well known to not only inhibit various enzyme reactions and metabolic processes but also enhance lipid per-oxidation, progression of atherosclerosis and the risk of myocardial infarction and/or stroke [20-22]. We also reported that hair mercury concentration in male Japanese is significantly correlated to their body mass index [11], which is well accepted to be a risk factor associated with overall mortality. Probably, this correlation of mercury burden with body mass index may explain the reason why the peak of mercury accumulation appears at the 6th decade of life (Fig. 1), corresponding to that little senile persons with obesity are alive.

Table 2 demonstrates the probability of toxic metal pollution in infants: all of the highest accumulation levels detected in children were observed in infant individuals aged 0-

3 years. In particular, the maximum burden level for cadmium (1,972 ng/g) in a 2-year old boy corresponded to about 280-fold higher than the adult reference level. For lead and aluminium, the highest level of 29,480 ng/g and 74,310 ng/g corresponded to almost 68-fold and 20-fold of each reference level, respectively. Even for mercury and arsenic, a high level of 36,280 ng/g and 394 ng/g detected in the infant aged 1- and 2-year old, respectively, corresponded to about 9-fold and 8-fold of the adult reference level. We have to consider that the mercury burden level of 36,280 ng/g is highly over the threshold of 14,000 ng/g (14 ppm) that is the upper limit recommended by FAO/WHO Meeting (JECFA2003).

The high accumulation of toxic metals in infants may be explained by their higher intestinal absorption and less effective renal excretion in newborns [23]. Freundlich *et al.* [24] reported that in two infants with severe kidney failure, the absorption and retention of aluminium from a cow's milk-based formula resulted in clinical toxicity. Thus, newborns and infants are likely at increased risk of absorbing high amount of toxic metals and retaining them in their bodies.

Another origin of these toxic metal accumulations in infants is presumed to be due to the cigarette smoking by their parents, in particular by the mothers [25-27]. Maternal cigarette smoking is reported to be associated with higher cadmium and lead and lower zinc concentrations in their neonates [28]. During pregnancy and lactation, these toxic metals accumulated in maternal bone tissues seem to be co-transferred with calcium to foetal and newborn bodies through increased bone-resorption [29,30].

In the present study on 5 toxic metal burdens, the three elements of mercury, arsenic and cadmium were found to show age-dependent accumulation profile, suggesting that these toxic elements may play a role in aging process and that higher intake of them may accelerate aging. Therefore, the dietary intake of some essential minerals competitive against toxic metals, such as zinc, calcium, magnesium and selenium, may be useful for detoxification and controlling aging [31]. Further understanding of molecular and cellular mechanisms of aging could not only improve medical care of the elderly but also hold out some hope in finding feasible solutions to slow down the aging process.

CONCLUSION

This study demonstrates that toxic metals are classified into two families on the basis of their accumulation profiles in scalp hair specimen. The first family consisting of mercury and arsenic exhibits a characteristic accumulation profile with a marked age-dependency and gender-difference (male > female) in adults. The second family consisting of cadmium, lead and aluminium exhibits a common accumulation profile with the highest burden level in infants, although cadmium has also another character accumulating in aged females, with gender difference (female > male) and age-dependency. These findings suggest that mercury, arsenic and cadmium may be associated with aging, and that higher burden with these toxic metals may lead to acceleration of aging in human.

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