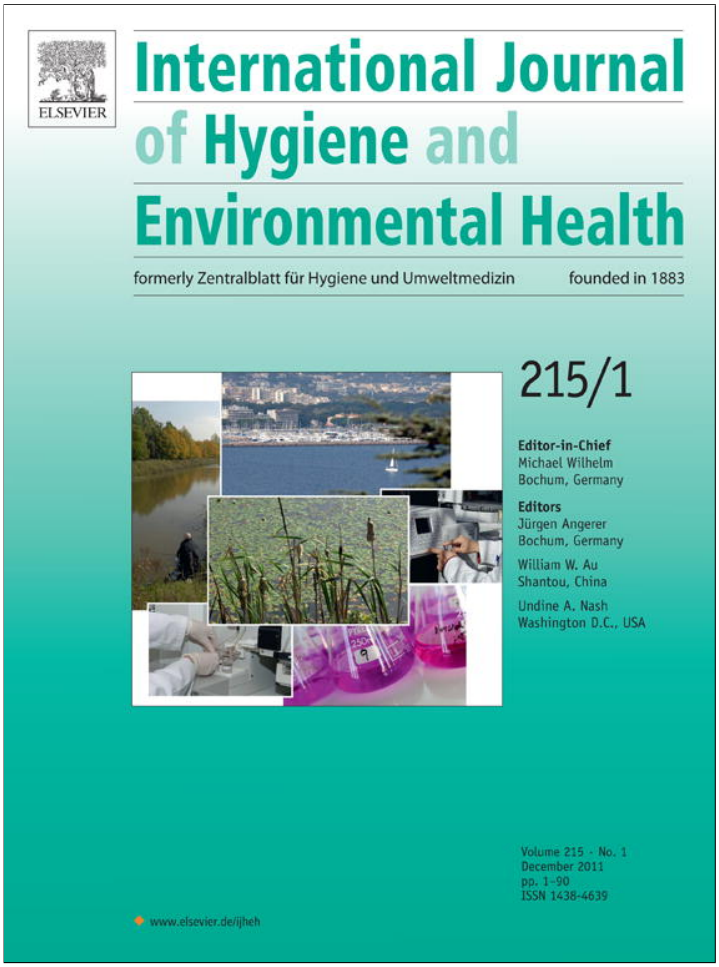

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Surveillance on chronic arsenic exposure in the Mekong River basin of Cambodia using different biomarkers

Kongkea Phan[a,c], Suthipong Sthiannopkao[b,*], Kyoung-Woong Kim[a,**]

[a] School of Environmental Science and Engineering, Gwangju Institute of Science and Technology, Gwangju 500-712, Republic of Korea
[b] Department of Environmental and Occupational Health, National Cheng Kung University, Tainan City, Taiwan
[c] Resource Development International-Cambodia, Kean Svay, Kandal, P.O. Box 494, Phnom Penh, Cambodia

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ABSTRACT

Thousands of Cambodia populations are currently at high risks of both toxic and carcinogenic effects through drinking arsenic-rich groundwater. In order to determine and assess the use of arsenic contents in different biological samples as biomarkers of chronic arsenic exposure from drinking arsenic-rich groundwater in Cambodia, individual scalp hair, fingernail and toenail were collected from three different provinces in the Mekong River basin of Cambodia. After washing and acid-digestion, digestate was analyzed for total arsenic by an inductively coupled plasma mass spectrometry. Chemical analysis of the acid-digested hair revealed that among 270 hair samples cut from Kandal, 78.1% had arsenic content in scalp hair (As_h) greater than the typical As_h (1.00 $\mu g\,g^{-1}$), indicating possible arsenic toxicity. Concurrently, 1.2% and 0.6% were found elevated in Kratie ($n = 84$) and Kampong Cham ($n = 173$), respectively. Similarly, the upper end of the ranges for arsenic contents in fingernail (As_{fn}) and toenail (As_{tn}) clipped from Kandal (fingernail $n = 241$; toenail $n = 187$) were higher than the normal arsenic content in nail (0.43–1.08 $\mu g\,g^{-1}$), however, none was observed elevated in both Kratie (fingernail $n = 76$, toenail $n = 42$) and Kampong Cham (fingernail $n = 83$; toenail $n = 52$). Significant positive intercorrelations between groundwater arsenic concentration (As_w), average daily dose (ADD) of arsenic, As_h, As_{fn} and As_{tn} suggest that As_h, As_{fn} and As_{tn} can be used as biomarkers of chronic arsenic exposure from drinking arsenic-rich groundwater, in which As_h is more favorable than As_{fn} and As_{tn} due to the ease of sample processing and analytical measurements, respectively.

Introduction

Chronic exposure to naturally occurring arsenic-rich groundwater has become a major public health concern in Cambodia and elsewhere. Our recent cross-sectional health risk assessment of inorganic arsenic intake of Cambodia residents living the Mekong River basin through groundwater drinking pathway using USEPA model revealed that thousands of Cambodia populations were at high risks of both toxic and carcinogenic effects. Approximately, 99% and 14% of the residents of the Kandal and Kratie province study areas were at risk of arsenic toxicity, respectively. Moreover, cancer risk index was found in average of 5 in 1000 exposure in the Kandal province study area (Phan et al., 2010). In order to better understand a relationship between arsenic exposure and its adverse health impacts, dose–response effects might be characterized by the study of biomarkers of arsenic exposure (Abernathy et al., 2003). Biomarker (biological marker) was defined as a substance, physiological characteristic, genes and so on that indicates or may indicate the presence of disease, a physiological abnormality or a physiological condition (Youngson, 2007). Since a small fraction of arsenic was generally excreted in the feces, urine has been studied for most of kinetic and metabolic processes; however, the kinetic of arsenic varied depending on the chemical form of arsenic and animal species (Brown, 2008). For instance, urine could be used to determine arsenical metabolism; in consequence, it was found that groundwater arsenic concentration was positively associated with urinary arsenicals (Calderon et al., 1999). Similarly, blood arsenic concentrations have been reported to be positively correlated with groundwater arsenic concentrations (Hall et al., 2006).

Because arsenical compounds were quickly and extensively removed from blood through kidney within 2–3 h and lasted in urine for 3–4 days, blood or urinary arsenic was considered to

Abbreviations: As_h, arsenic content in scalp hair; As_{fn}, arsenic content in fingernail; As_{tn}, arsenic content in toenail; As_w, groundwater arsenic concentration; ADD, average daily dose.

* Corresponding author. Tel.: +886 6 235 3535; fax: +886 6 236 1245.
** Corresponding author. Tel.: +82 62 715 2821; fax: +82 62 715 2434.
E-mail addresses: suthisuthi@gmail.com (S. Sthiannopkao), kwkim@gist.ac.kr (K.-W. Kim).

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indicate on the recent exposure (Hall et al., 2006; Adair et al., 2006; Mandal et al., 2003). Although urine collection was not invasive, the requirement to freeze the samples during storage might be problematic for the extended period of field sampling in the rural parts of the developing worlds (Gault et al., 2008). Similarly, blood collection presented the same problem of storage plus invasive nature of sampling procedure. Therefore, these challenges were significant drawbacks to use urine and blood as biomarkers of arsenic exposure (Gault et al., 2008).

Recently, there has been increasingly use of scalp hair and nail as biomarkers of arsenic exposure because of the ease of sample collection, storage and transportation. In addition, arsenic had affinity to bind with abundant sulfhydryl groups in keratin of hair and nail tissues (Hall et al., 2006; Mandal et al., 2003), which led to higher arsenic accumulation. Nail and scalp hair matrices were isolated from metabolism pathways of the body while they were formed and grown; therefore, collecting of these tissues was considered to monitor the past exposure of arsenic (Gault et al., 2008). For example, scalp hair and nail were used to analyze for arsenic and other elements of arsenic victims (Samanta et al., 2004; Mandal et al., 2003; Adair et al., 2006; Gault et al., 2008). Numerous studies showed that arsenic contents in scalp hair and nail were positively correlated with groundwater arsenic concentrations (Gault et al., 2008; Kubota et al., 2006; Mandal et al., 2003). Among keratin-rich tissues, hair contained soft and flexible keratin with 10–14% of cysteine while nail contained harder and more brittle keratin of up to 22% of cysteine (Mandal et al., 2003). Nail might have more advantages over scalp hair; this was due to the less presence of external contaminants and less variable growth rates; typically, scalp hair grew 6–36 mm per month while nail grew 0.9–1.5 mm per month (Slotnick and Nriagu, 2006). Therefore, scalp hair cutting might reflect a time window of 2–5-month exposure and nail clipping might reflect 6–18-month exposure (Samanta et al., 2004; Slotnick and Nriagu, 2006). However, in order to sound application of nail as biomarker of arsenic exposure, some drawbacks should be addressed; those drawbacks included standardization of washing and collecting procedures, investigation of potential modifiers of exposure–biomarker relationship, investigation of biomarker–disease relationship and further exploration of temporal variability and exposure time windows (Slotnick and Nriagu, 2006).

To date, notice that none of standardized procedures for nail washing and digestion has been officially approved and recognized by any international scientific organizations/institutions (Slotnick and Nriagu, 2006). Nevertheless, some studies have optimized the washing procedures, digestion methods and analytical instrumentation in order to assess fingernails and toenails as biomarkers (Gault et al., 2008; Button et al., 2009; Adair et al., 2006). Therefore, the objectives of this present study were to (i) determine the arsenic contents in all scalp hair, fingernail and toenail tissues, which were gathered from inhabitants living in the Mekong River basin of Cambodia, using inductively coupled plasma mass spectrometry, (ii) compare the individual arsenic accumulations in all of three biological samples among the study populations and (iii) assess the use of arsenic contents in scalp hair (As_h), fingernail (As_{fn}) and toenail (As_{tn}) as biomarkers of chronic arsenic exposure from drinking arsenic-rich groundwater.

Materials and methods

Field sampling

The design of the present project was a cross-sectional study. After our research proposal was approved by the National Ethics Committee for Health Research (Reference No. 131NECHR, 12/12/2008) under the Ministry of Health of the Kingdom of Cambodia and informed consent was obtained, sampling was conducted in two villages of each of three purposely selected provinces with different anticipated level of arsenic contaminations in the Mekong River basin of Cambodia. Kandal province (Preak Russsey and Lvea Toung villages, Kampong Kong commune, Koh Thom district) was selected as a highly contaminated area where Kratie province (Preak Samrong I & II villages, Khsarch Andaet commune, Chhloung district) and Kampong Cham province (Andoung Chros & Veal Sbov villages, Ampil commune, Kampong Siem district) were chosen as the moderately and mildly contaminated areas, respectively. In fact, samples were simply collected based upon the accessibility to the tube well, the willingness of inhabitants to provide their biological samples and inhabitant claims of living in the village at least five years. Hair samples were collected from the nape of the head as near as possible to the scalp of several members of the volunteered family in the Kandal ($n = 270$), Kratie ($n = 84$) and Kampong Cham ($n = 173$) province study areas, who claimed to routinely use a tube well, using stainless steel scissors. Concurrently, fingernail and toenail samples were gathered from all digits of same individuals, who lived in Kandal (fingernail $n = 241$; toenail $n = 187$), Kratie (fingernail $n = 76$; toenail $n = 42$) and Kampong Cham (fingernail $n = 83$; toenail $n = 52$), using stainless steel nail clippers. The collected scalp hair, fingernail and toenail samples were separately kept in three different labeled plastic ziplock bags and stored in darkness until analyses. In addition, groundwater and individual demographic information were collected from each of the visited families to calculate the individual average daily dose of arsenic. Individual ages, genders, ingestion rates and exposure duration were collected using a structured questionnaire. Body weight was measured using a bathroom scale that was calibrated zeroed before each measurement. Groundwaters were simply sampled using the acid-cleaned polyethylene bottles, acidified (with 70% HNO_3) to pH less than 2, kept in a cooler at field and transferred to a refrigerator where they were stored at 4 °C until analyses.

Sample preparation and analyses

Hair was cut into small pieces (3 mm) and washed with a recommended method described by Ryabukhin (1978). However, fingernail and toenail were alternatively washed with 1.5 M HNO_3, deionized water, acetone, and deionized water. Fingernail and toenail were first sonicated twice with 5 mL of 1.5 M HNO_3 for 10 min, and then rinsed with 10 mL of deionized water. Finally, 10-min sonication with 5 mL of acetone, followed by twice rinse with 10 mL of deionized water were performed. In order to determine the elemental contaminant residues in the washing procedure, the initial and final washing solutions were collected and instantly analyzed for arsenic and other elemental concentrations (Table 1). Washed hair, fingernail and toenail were dried at 60 °C overnight before digestion. Acid-digestion was performed using a method described by Phan et al. (2010). Chemical measurements of all groundwater and digestate were employed by an inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500ce).

Average daily dose of arsenic

Individual average daily dose (ADD) of arsenic was calculated using a model from USEPA (Integrated Risk Information System (IRIS): arsenic, inorganic, CASRN 7440-38-2, 1998) as follows:

$$\mathrm{ADD} = \frac{\mathrm{As_w} \times \mathrm{IR} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{AT} \times \mathrm{BW}}$$

where ADD is the average daily dose of arsenic from the oral ingestion ($mg\,kg^{-1}\,d^{-1}$); As_w is the groundwater arsenic concentration ($mg\,L^{-1}$); IR is the ingestion rate ($L\,d^{-1}$); EF is the exposure

Table 1
Mean, median, SD, min and max concentrations in $\mu g\,L^{-1}$ of the elemental contaminant residues in nail washing solutions ($n = 5$).

Elements	DL	Initial washing solution					Final washing solution				
		Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max
Ag	0.009	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Al	0.610	736.36	589.80	474.29	263.50	1370.00	NA	NA	NA	NA	NA
As	0.050	6.72	7.24	5.71	1.33	15.28	NA	NA	NA	NA	NA
B	0.370	4.66	4.19	1.62	3.10	6.64	NA	NA	NA	NA	NA
Ba	0.197	159.50	122.20	151.14	26.15	377.10	NA	NA	NA	NA	NA
Cd	0.008	0.62	0.63	0.30	0.30	0.96	NA	NA	NA	NA	NA
Co	0.009	1.06	0.57	0.91	0.24	2.43	NA	NA	NA	NA	NA
Cr	0.013	1.74	1.87	0.63	0.86	2.36	NA	NA	NA	NA	NA
Cu	0.008	80.95	81.58	46.21	19.28	147.20	NA	NA	NA	NA	NA
Ga	0.016	46.92	35.90	44.84	7.91	113.10	NA	NA	NA	NA	NA
Mn	0.059	369.84	215.20	341.38	75.79	878.20	0.28	0.25	0.17	0.11	0.51
Mo	0.060	0.15	0.12	0.07	0.10	0.23	NA	NA	NA	NA	NA
Ni	0.022	26.33	20.65	18.37	3.93	51.40	NA	NA	NA	NA	NA
Pb	0.037	83.78	97.34	42.53	12.59	124.90	NA	NA	NA	NA	NA
Rb	0.098	15.12	13.74	9.14	6.55	30.23	NA	NA	NA	NA	NA
Se	0.000	0.06	0.06	0.06	0.01	0.12	NA	NA	NA	NA	NA
Tl	0.013	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
U	0.003	0.43	0.30	0.52	0.02	1.11	NA	NA	NA	NA	NA

NA: not applicable for calculation; this was because the concentrations of the elemental contaminant residues were below the limit of detection; DL: detection limit; SD: standard deviation; Min: minimum; Max: maximum.

Table 2
Summary of body weight (BW), age, ingestion rate (IR) and exposure duration (ED) in each of the study areas.

Statistics	Kandal				Kratie				Kampong Cham			
	BW	Age	IR	ED	BW	Age	IR	ED	BW	Age	IR	ED
Gender (F/M)	169/128				56/33				114/70			
Mean	42.9	31.6	1.6	7.9	46.8	38.1	1.5	10.0	44.5	30.6	1.8	4.9
Median	45.5	28.0	1.5	8.0	49.0	42.0	1.5	11.0	48.0	26.0	2.0	5.0
SD	14.3	19.5	0.6	4.2	14.0	22.5	0.4	2.6	17.0	20.7	0.7	3.0
Min	7.0	3.0	0.5	1.0	14.0	6.0	1.0	4.0	11.0	2.0	0.5	1.0
Max	89.0	84.0	4.0	19.0	80.0	83.0	2.5	13.0	80.0	85.0	4.0	12.0

BW: body weight (kg); age (y); IR: ingestion rate ($L\,d^{-1}$); ED: exposure duration (y); SD: standard deviation; F: female; M: male; Min: minimum; Max: maximum.

frequency ($d\,y^{-1}$); ED is the exposure duration (y); AT is the average time/life expectancy (d) and BW is the body weight (kg). The survey results used as input data for calculating ADD are presented in Table 2. The field surveys also revealed that the residents in Kandal ($n = 297$) and Kratie ($n = 89$) province study areas have consumed groundwater for 9 months ($EF = 270\,d\,y^{-1}$) per year and with rainwater and surface river floodwaters for the remainder of the year. However, Kampong Cham residents have used groundwater for the whole year ($EF = 365\,d\,y^{-1}$). Life expectancy of female and male Cambodian people is 65 years (23,725 days) and 59 years (21,535 days), respectively (Phan et al., 2010).

Data quality control and statistical analyses

Digestions of the replicated hair, fingernail and toenail samples were conducted to verify the validity of acid digestion method. A human hair standard reference material (GBW07601) was also treated in the same manner as the sample to check the accuracy of this digestion method (Table 3). Two-related-samples test was analyzed to assess the reliability of repeated measurements, which null postulated "there was no significant difference between arsenic concentrations in each replicated subsample". In addition, simple regression was used to investigate how well the arsenic content analyzed in a subsample could predict that in the reanalysis (Fig. 1).

Statistical analyses of the obtained data were employed by SPSS for windows (Version 13.0). Kruskal–Wallis test was applied to assess the differences in region and age group of arsenic contents in scalp hair (As_h), fingernail (As_{fn}) and toenail (As_{tn}). In addition, Mann–Whitney's *U* test was applied to verify the differences in gender between male and female whereas Wilcoxon Signed Ranks test was performed to certify the individual differences in As_h, As_{fn} and As_{tn}. The strength of intercorrelations between As_w, ADD, As_h, As_{fn} and As_{tn} was measured by the Spearman's rho correlation coefficient (r_s). Significance was considered in circumstance where $p < 0.05$.

Table 3
Recovery rate from acid digestion (Human Hair CRMs GBW07601).

Elements	Certified value ($\mu g\,g^{-1}$)	Recovery rate (%) (70% HNO_3)
As	0.28	94.80
Ba	17	87.29
Cd	0.11	108.55
Cu	10.6	101.00
Mn	6.3	93.91

A human hair standard reference material was treated in the same manner as samples.

Results

Arsenic content in scalp hair

The results of ICP-MS analyses of acid-digested hair samples revealed that arsenic content in scalp hair (As_h) of the residents in the Kandal province study area ($n = 270$) ranged from 0.27 to 57.21 $\mu g\,g^{-1}$ with mean and median of 6.40 $\mu g\,g^{-1}$ and 4.03 $\mu g\,g^{-1}$, respectively. 78.1% of this group had As_h greater than the typical As_h (1.00 $\mu g\,g^{-1}$), indicating possible arsenic toxicity. Concurrently, As_h of the Kratie residents ($n = 84$) ranged from 0.05 to 1.42 $\mu g\,g^{-1}$

Table 4
Summary of groundwater arsenic concentration (As_w), average daily dose (ADD) of arsenic, arsenic content in scalp hair (As_h), fingernail (As_{fn}) and toenail (As_{tn}) in each of the study areas.

Statistics	Kandal					Kratie					Kampong Cham				
	As_w	ADD	As_h	As_{fn}	As_{tn}	As_w	ADD	As_h	As_{fn}	As_{tn}	As_w	ADD	As_h	As_{fn}	As_{tn}
N[a]	46	297	270	241	187	12	89	84	76	42	18	184	173	83	52
Mean	846.14	3.50	6.40	2.54	2.34	22.22	0.0996	0.29	0.26	0.44	1.28	0.0053	0.12	0.13	0.15
Median	822.63	2.99	4.03	1.23	1.05	1.30	0.0188	0.24	0.23	0.41	1.22	0.0044	0.09	0.12	0.15
SD	298.11	2.46	8.01	3.77	3.47	43.89	0.1637	0.21	0.13	0.15	0.58	0.0044	0.10	0.06	0.06
Min	247.08	0.19	0.27	0.24	0.32	0.12	0.0004	0.05	0.07	0.10	0.12	0.0003	0.01	0.03	0.03
Max	1841.5	10.75	57.21	28.47	21.89	140.60	0.6261	1.42	0.73	0.76	2.37	0.0221	1.01	0.28	0.28

[a] The number of groundwater sample is fewer than the number of scalp hair, fingernail and toenail due to the use of a tube well more than one household. The difference in sample size among scalp hair, fingernail and toenail is because some individuals could not provide all of three biological samples at the time of sampling. As_w ($\mu g\,L^{-1}$); ADD (10^{-3} mg As kg^{-1} d^{-1}); As_h ($\mu g\,g^{-1}$); As_{fn} ($\mu g\,g^{-1}$); As_{tn} ($\mu g\,g^{-1}$); SD: standard deviation; Min: minimum; Max: maximum.

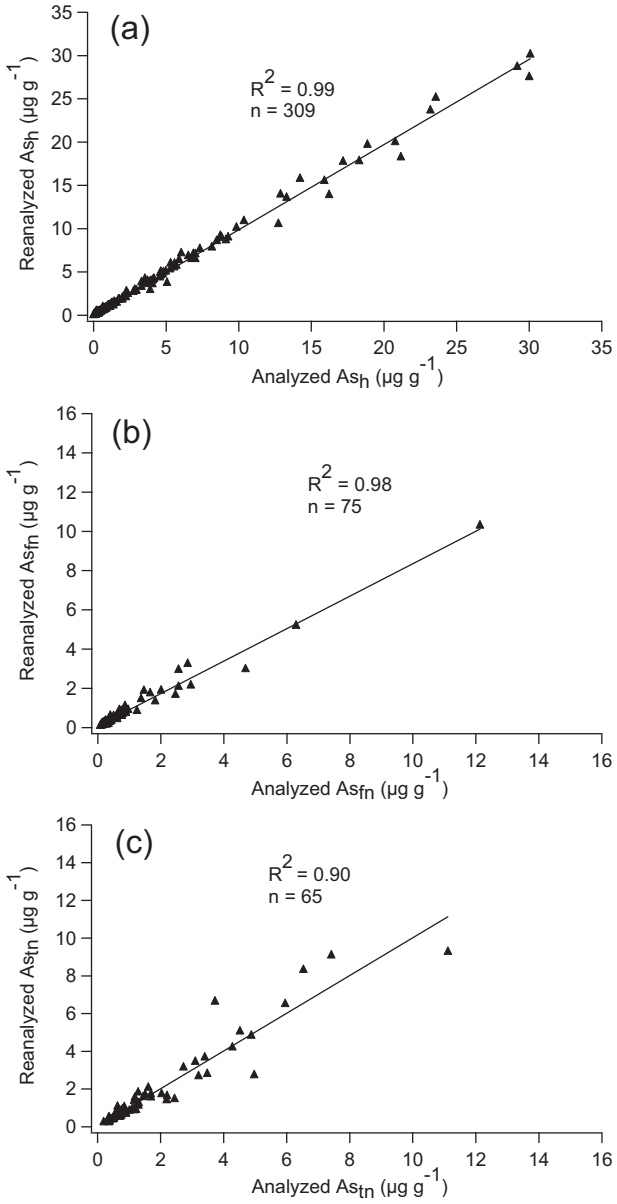

Fig. 1. Simple regression analysis for arsenic contents in (a) scalp hair (As_h), (b) fingernail (As_{fn}) and (c) toenail (As_{tn}).

with mean of 0.29 $\mu g\,g^{-1}$ and median of 0.24 $\mu g\,g^{-1}$ whereas As_h of the residents in the Kampong Cham province study area ($n = 173$) ranged from 0.01 to 1.01 $\mu g\,g^{-1}$ with mean of 0.12 $\mu g\,g^{-1}$ and median of 0.09 $\mu g\,g^{-1}$ (Table 4). The upper end of the ranges for the last two groups were higher than the typical As_h; 1.2% and 0.6% of the residents in Kratie and Kampong Cham province study areas, respectively, indicating possible arsenic toxicity. Plotting the As_h against As_w (Fig. 2a) illustrated a significant positive correlation (r_s (525) = 0.75, $p < 0.0001$). The As_h was also positively associated with ADD (r_s (525) = 0.74, $p < 0.0001$) (Fig. 3a).

Arsenic content in nails

Analyses of acid-digested nail samples clipped from the resident in the Kandal province study area revealed that the arsenic content in fingernail (As_{fn}) ranged from 0.24 to 28.47 $\mu g\,g^{-1}$ with mean of 2.54 $\mu g\,g^{-1}$ and median of 1.23 $\mu g\,g^{-1}$ whereas arsenic content in toenail (As_{tn}) ranged from 0.32 to 21.89 $\mu g\,g^{-1}$ with mean and median of 2.34 $\mu g\,g^{-1}$ and 1.05 $\mu g\,g^{-1}$, respectively (Table 4). The upper ends of the ranges for As_{fn} and As_{tn} exceeded the normal arsenic content in nail, 0.43–1.08 $\mu g\,g^{-1}$ (Karim, 2000). However, none was observed elevated in the Kratie and Kampong Cham province study areas. Statistical analyses depicted that there were significant positive correlations between As_w with As_{fn} (r_s (398) = 0.72, $p < 0.0001$, Fig. 2b) and As_{tn} (r_s (279) = 0.61, $p < 0.0001$, Fig. 2c). Similarly, significant positive correlations between ADD with As_{fn} (r_s (398) = 0.71, $p < 0.0001$, Fig. 3b) and As_{tn} (r_s (279) = 0.59, $p < 0.0001$, Fig. 3c) were also observed. Moreover, plotting the As_h against the As_{fn} (Fig. 4a) and As_{tn} (Fig. 4b) revealed significantly positive correlations r_s (355) = 0.87, $p < 0.0001$ and r_s (251) = 0.83, $p < 0.0001$, respectively. Concurrently, significant positive correlation between As_{fn} and As_{tn} (r_s (267) = 0.93, $p < 0.0001$) was observed in Fig. 4c.

Comparison of arsenic contents in biological samples

Pairwise comparison revealed that the individual mean of As_h was significantly higher than that of As_{fn} (Wilcoxon Signed Ranks test, $Z = -7.24$, $n = 357$, $p < 0.0001$) and As_{tn} (Wilcoxon Signed Ranks test, $Z = -5.84$, $n = 253$, $p < 0.0001$). However, the individual mean of As_{fn} was not significantly different from that of As_{tn} (Wilcoxon Signed Ranks test, $Z = -1.25$, $n = 269$, $p = 0.212 > 0.05$). Further analyses revealed that there were significant regional differences in As_h among the three study areas (Kruskal–Wallis test, $p < 0.0001$). However, there were not significant differences in gender (Mann–Whitney's U test, $p = 0.45 > 0.05$) and age group (Kruskal–Wallis test, $p = 0.92 > 0.05$) of As_h (Fig. 5). Although there are significant regional differences in As_{fn} (Kruskal–Wallis test, $p < 0.0001$) and As_{tn} (Kruskal–Wallis test, $p < 0.0001$), no significant differences in gender of As_{fn} (Mann–Whitney's U test, $p = 0.22 > 0.05$) and As_{tn} (Mann–Whitney's U test, $p = 0.47 > 0.05$) and age group of As_{fn} (Kruskal–Wallis test, $p = 0.13 > 0.05$) and As_{tn} (Kruskal–Wallis test, $p = 0.42 > 0.05$) were observed (Fig. 5).

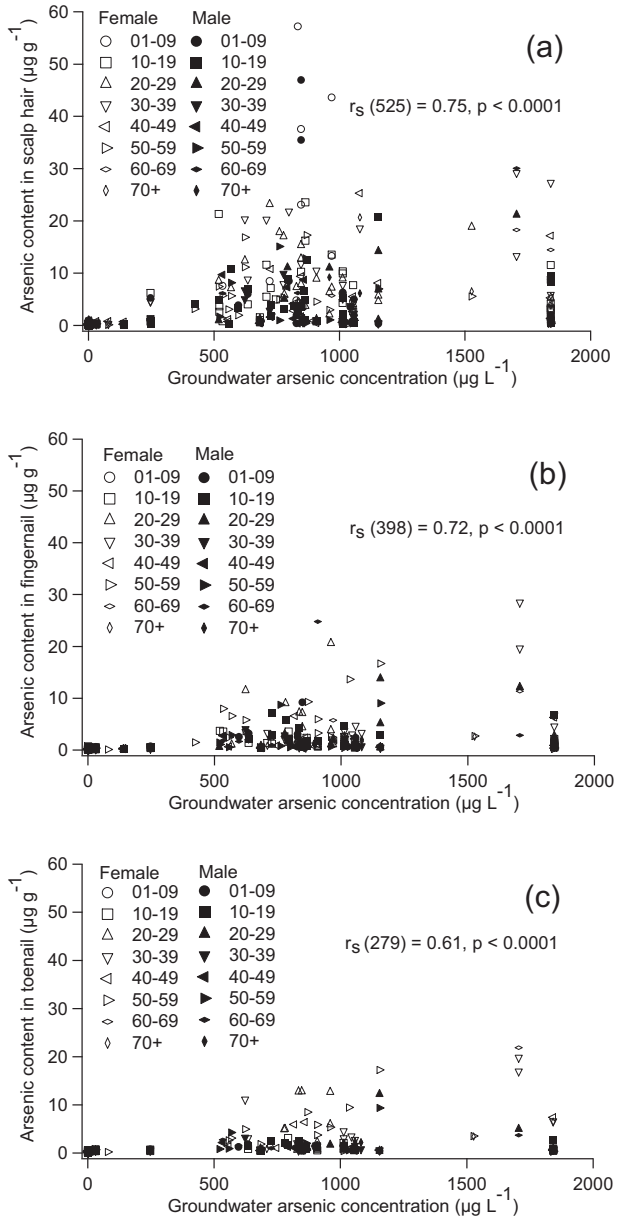

Fig. 2. Association between groundwater arsenic concentrations (As_w) with arsenic contents in (a) scalp hair (As_h), (b) fingernail (As_{fn}) and (c) toenail (As_{tn}).

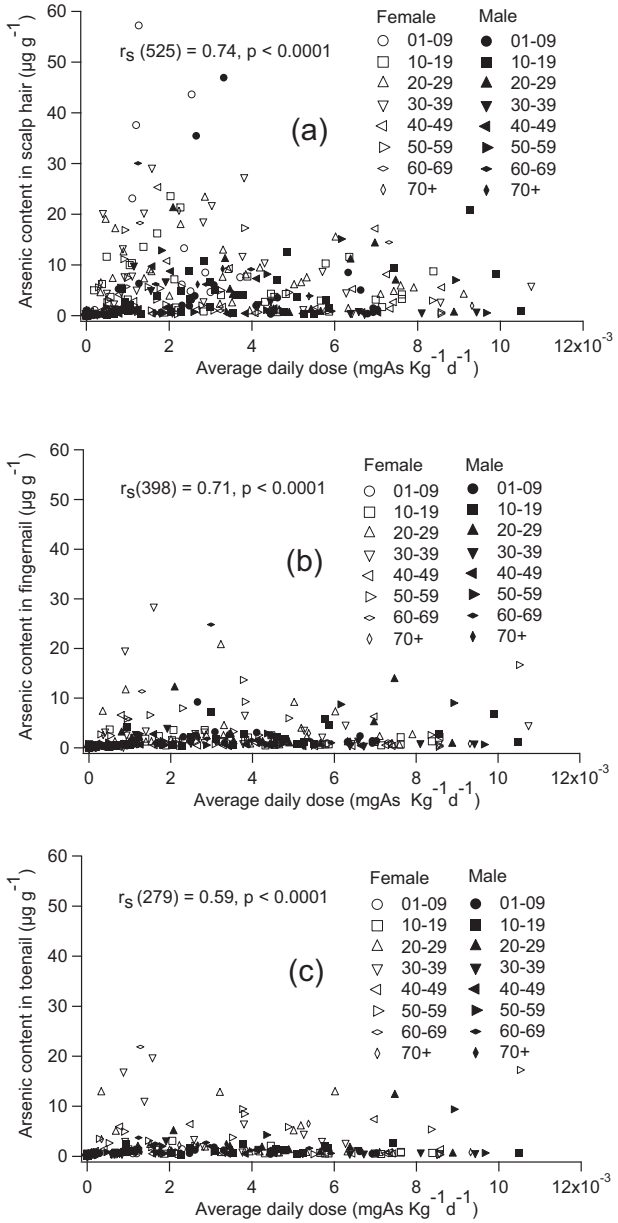

Fig. 3. Association between average daily dose (ADD) of arsenic with arsenic contents in (a) scalp hair (As_h), (b) fingernail (As_{fn}) and (c) toenail (As_{tn}).

Discussion

Analyses of acid-digested hair samples presented that the mean of As_h in Kandal was significant higher than that in Kratie and Kampong Cham province study areas, respectively. However, the As_h of Cambodia residents could be found in the literatures. For instance, Gault et al. (2008) reported that the As_h of Kandal residents ranged from 0.10 to 7.95 $\mu g\,g^{-1}$ ($n = 40$, mean 1.41 $\mu g\,g^{-1}$, median = 0.54 $\mu g\,g^{-1}$); Sthiannopkao et al. (2010) presented that the As_h collected from six villages of Kandal province ranged from 0.06 to 30 $\mu g\,g^{-1}$ ($n = 58$) with mean of 3.2 $\mu g\,g^{-1}$ and median of 0.6 $\mu g\,g^{-1}$; Sampson et al. (2008) released that the As_h in Preak Russey village, where arsenicosis patients were discovered, in Kandal province was in a range from 2.1 to 13.94 $\mu g\,g^{-1}$ ($n = 36$) with geometric mean of 5.64 $\mu g\,g^{-1}$ while Mazumder et al. (2009) subsequently reported that the As_h of the same Preak Russey residents, Kandal province, ranged from 0.92 to 25.60 $\mu g\,g^{-1}$ ($n = 93$). The present study does not only reveal the elevated As_h of the Preak Russey residents, but also in the neighbored village, Lvea Toung village in Kampong Kong commune, Koh Thom district, Kandal province. These findings suggest that the residents in the Kandal province study area are currently at high risk of arsenic toxicity. Concurrently, Mazumder et al. (2009) found that approximate 70 cases ($n = 97$) of Preak Russey residents showed evidences of arsenical skin lesions with either pigmentation or keratosis. The largest number of cases belonged to age group of 31–45 years old. In addition, 37% of children belonging to the age group of less then 16 years old had skin lesion of arsenicosis. The youngest child having the evidence of keratosis and pigmentation was 8 years old although the feature of redness and mild thickening of the palms were found on children with age of 4–5 years old (Mazumder et al., 2009). This finding corresponds to that of our present study that As_h are found highest in children belonging to the age group of 1–9 years old followed by adults belonging to the age group of 30–39 years old (Figs. 2a, 3a, and 5). Similarly, Kubota et al. (2006) reported a mean value of As_h of the Kratie residents was 1.77 ± 2.94 $\mu g\,g^{-1}$

Table 5
Comparison of arsenic contents in various biological samples in $\mu g\,g^{-1}$ in different countries.

Country	As_h	As_{fn}	As_{tn}	Mean	Median	Range	References
Bangladesh	74	–	–	–	–	1.10–19.84	Karim (2000)
	–	74	–	–	–	1.30–33.98	Karim (2000)
India	44	–	–	3.43	2.29	0.17–14.40	Samanta et al. (2004)
	–	33	–	7.24[a]	4.73	0.74–36.63	Samanta et al. (2004)
	47	–	–	4.5	–	0.70–16.20	Mandal et al. (2003)
	–	47	–	7.32	–	2.14–40.30	Mandal et al. (2003)
Vietnam	59	–	–	–	–	0.088–2.77	Agusa et al. (2006)
	43	–	–	–	–	0.12–1.09	Nguyen et al. (2009)
England	–	–	8	5.41[a]	–	0.858–25.98	Button et al. (2009)
United States	–	–	94[b]	–	–	0.087–16.30	Adair et al. (2006)
Cambodia							
Kandal	40	–	–	1.41	0.54	0.10–7.95	Gault et al. (2008)
Kandal	–	70	–	1.9	1.3	0.20–6.50	Gault et al. (2008)
Kandal	58	–	–	3.2	0.6	0.06–30.00	Sthiannopkao et al. (2010)
Kandal	36	–	–	5.64[a]	–	2.10–13.94	Sampson et al. (2008)
Kandal	93	–	–	–	–	0.92–25.60	Mazumder et al. (2009)
Kandal	–	93	–	–	–	1.06–69.48	Mazumder et al. (2009)
Kratie	–	–	–	1.77	–	–	Kubota et al. (2006)
Kandal	270	–	–	6.4	4.03	0.27–57.21	Present study
	–	241	–	2.54	1.23	0.24–28.47	
	–	–	187	2.34	1.05	0.32–21.89	
Kratie	84	–	–	0.29	0.24	0.05–1.42	
	–	76	–	0.26	0.23	0.07–0.73	
	–	–	42	0.44	0.41	0.10–0.76	
Kampong Cham	173	–	–	0.12	0.09	0.01–1.01	
	–	83	–	0.13	0.12	0.03–0.28	
	–	–	52	0.15	0.15	0.03–0.28	

As_h: arsenic content in scalp hair; As_{fn}: arsenic content in fingernail; As_{tn}: arsenic content in toenail.
[a] Geometric mean.
[b] Number of detectable samples.

(mean $\pm\,\sigma$) with 42.6% of hair samples exceeded the level of possible indication of the arsenic toxicity ($1.00\,\mu g\,g^{-1}$) whereas 1.2% was observed elevated in the present study. In addition, Gault et al. (2008) reported that As_{fn} collected from Kandal province study area ranged from 0.2 to $6.50\,\mu g\,g^{-1}$ ($n = 70$, median = $1.30\,\mu g\,g^{-1}$ and mean = $1.90\,\mu g\,g^{-1}$) while Mazumder et al. (2009) subsequently presented that the arsenic content in nail of the Preak Russey residents ranged from 1.06 to $69.48\,\mu g\,g^{-1}$ ($n = 93$). However, our results showed that the As_{fn} and As_{tn} were consistent with As_h when we performed a pairwise comparison of the individual As_h with As_{fn} and As_{tn}. The data of As_{fn} and As_{tn} were not available for the elevated 1.2% and 0.6% (shown by scalp hair's results) in the Kratie and Kampong Cham province study areas, respectively. These shortages were because some individuals could not provide all of their three scalp hair, fingernail and toenail samples at the time of sampling. In fact, the elevated As_h, As_{fn}, As_{tn} and their variations have been also well-documented elsewhere (Table 5). A comparison revealed that the range of As_h in Bangladesh and India were close to that observed in Kandal province study area where the case of Vietnam was comparable to those in Kratie and Kampong Cham provinces in our present study. Similarly, the range of As_{fn} in India and Bangladesh and the range of As_{tn} in the United States and England were close to those observed in the Kandal province in the present study.

The accuracy of a new biomarker of exposure could be verified with correlations between the validated and proposed biomarkers; however, a biomarker of exposure was also examined by sample collection, processing, and sensitivity of analysis if the validated biomarker was not available (Adair et al., 2006). In the present study, we defined a biomarker of arsenic exposure based on the sampling and washing procedures and reliability of the repeated measurements. Therefore, deposition and/or absorption of exogenous materials such as dirt and other particulates on the surface of scalp hair, fingernail and toenail were considered as influence in determining the exposure–biomarker relationship for biomarker validation. However, the degree of influence was variable depending on the contaminants of interest and behavior of study subjects, likely due to individual hygiene, time spent outdoor that was difficult to determine (Slotnick and Nriagu, 2006). Numerous studies have demonstrated that exogenous materials and particulates could be removed from the study subjects using dilute acid or week solvents (Button et al., 2009; Agahian et al., 1990; Chen et al., 1999). Therefore, fingernail and toenail in the present study were subjected to sonication with dilute acid and acetone and rinsed with water. Chemical measurements of the final washing solutions of nails (Table 1) revealed that most of the elemental contaminant residues were not detectable, suggesting that influence of environmental exposures were effectively removed from the surface of nail tissues. Human hair standard reference material (GBW07601) was treated in the same manner as samples to verify the validity of the acid-digestion method. In fact, the recovery rates of acid-digestion of human hair standard reference material were in good agreement with the certified values (Table 3). In addition, two replications of sample were digested and analyzed to access the reliability of repeated measurements. As a consequence, there was not statistically significant difference in arsenic content in each kind of replicated subsamples (Wilcoxon Signed Ranks test, $p > 0.05$). Similarly, simple regression revealed that arsenic content in the analysis could well predict that in the reanalysis ($R^2 > 0.90$), suggesting that data could be reproduced (Fig. 1).

Concurrently, we also investigated the exposure–biomarker relationship using correlations between groundwater arsenic concentration (As_w) and average daily dose (ADD) of arsenic with arsenic contents in scalp hair (As_h), fingernail (As_{fn}) and toenail (As_{tn}) (Table 6). As a consequence, statistically significant positive association between As_h with As_w and ADD and significant regional difference in the As_h undoubtedly suggested that the arsenic accumulations in scalp hair of Cambodia residents were mainly through a groundwater drinking pathway. Similarly, statistically significant positive correlations between As_w and ADD with As_{fn} and As_{tn} were also found. These significant positive correlations undoubtedly indicated that inhabitants who drank groundwater

Table 6
Intercorrelation, mean, standard deviation for average daily dose, gender, age group, groundwater arsenic concentration, arsenic contents in scalp hair, fingernail and toenail.

Variables	As_{fn}	As_{tn}	ADD	As_w	Gender	Age group	Mean	SD
As_h	0.870**	0.827**	0.743**	0.752**	0.033	0.009	3.36	6.52
As_{fn}	–	0.931**	0.710**	0.716**	0.061	−0.088	1.61	3.14
As_{tn}	–	–	0.589**	0.606**	−0.043	0.062	1.65	2.99
ADD	–	–	–	0.919**	0.077	−0.02	0.0018	0.0025
As_w	–	–	–	–	0.007	0.014	507.98	564.76
Gender	–	–	–	–	–	−0.125**	0.41	0.49
Age group	–	–	–	–	–	–	2.78	2.06

As_h: arsenic content in scalp hair; As_{fn}: arsenic content in fingernail; As_{tn}: arsenic content in toenail; As_w: groundwater arsenic concentration; ADD: average daily dose of arsenic; SD: standard deviation, female: "0" and male: "1".
** $p < 0.01$.

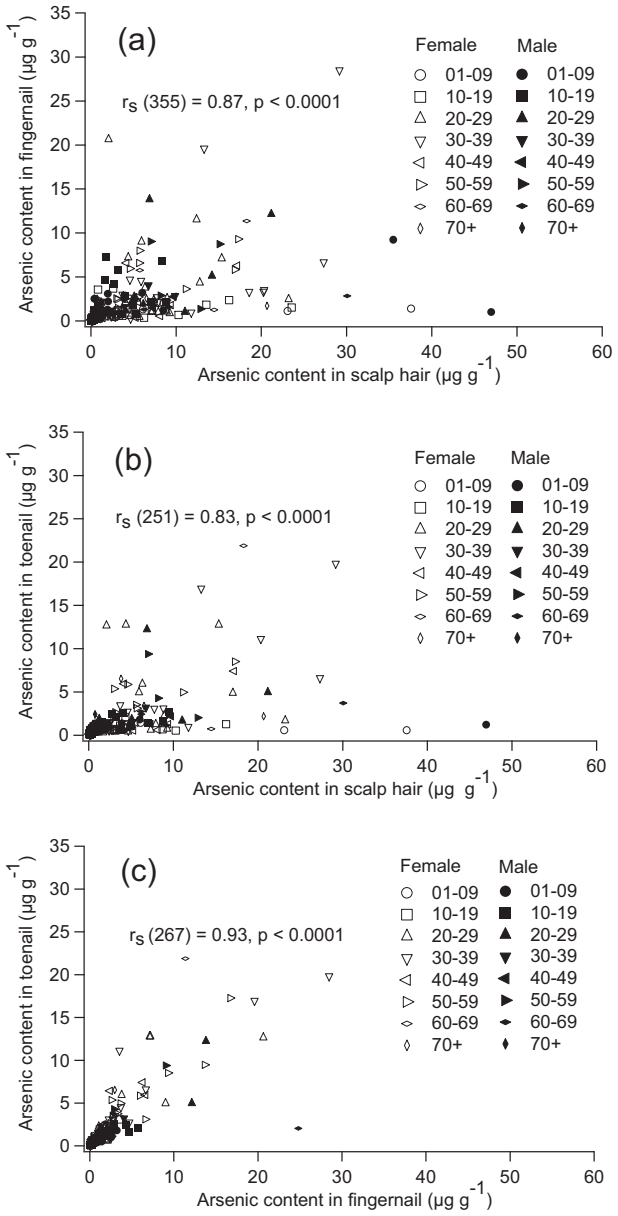

Fig. 4. Bivariate plots among biological samples (a) arsenic content in scalp hair (As_h) with arsenic content in fingernail (As_{fn}), (b) arsenic content in toenail (As_{tn}) with arsenic content in scalp hair (As_h) and (c) arsenic content in fingernail (As_{fn}) and arsenic content in toenail (As_{tn}).

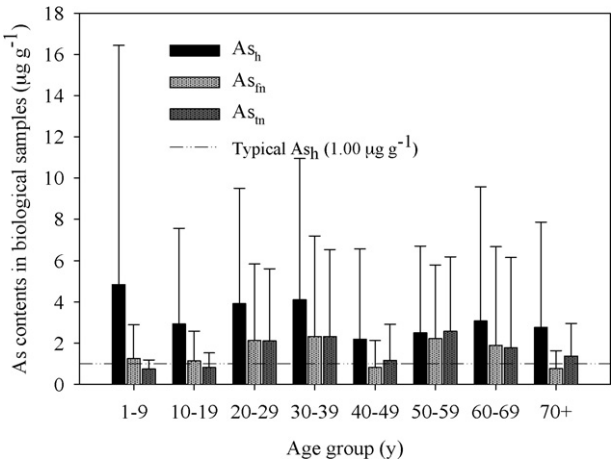

Fig. 5. Distribution of arsenic contents in scalp hair (As_h), fingernail (As_{fn}) and toenail (As_{tn}) among all age groups.

containing higher arsenic concentrations tended to have higher arsenic accumulations in their scalp hair, fingernail and toenail. However, the relatively stronger correlations of As_w and ADD with As_h comparing to As_{fn} and As_{tn} (Table 6) suggest that arsenic appears to accumulate more in individual scalp hair than in individual fingernail and toenail. The higher accumulation rate of arsenic in scalp hair has led to the finding of individual mean of As_h which was significantly higher than individual mean of As_{fn} and As_{tn}. Significant positive intercorrelations between As_w, ADD, As_h,As_{fn} and As_{tn} suggested that either As_h or As_{fn} or As_{tn} could be used as biomarkers of arsenic exposure. However, these biomarkers reflect the different time windows of exposure. As_h may indicate the past exposure of 2–5 months prior to hair cutting (Samanta et al., 2004); As_{fn} is more likely to reflect the past exposure of 6–12 months while As_{tn} may depict the past exposure as long as 12–18 months before clipping (Slotnick and Nriagu, 2006). In addition, the correlations of As_w and ADD with As_h are better than those with As_{fn} and As_{tn} (Table 6), suggesting that As_h is more favorable biomarker than As_{fn} and As_{tn}, respectively. Similarly, the preferred biomarker can be defined by reliability of the repeated measurement. In fact, the measurements of As_h (Fig. 1) are more precise than those of As_{fn} and As_{tn}, respectively.

Conclusions

Analytical results demonstrate that As_h, As_{fn} and As_{tn} are highly elevated in the Kandal province study area. Statistically significant difference in region of As_h As_{fn} and As_{tn} coupled with significant positive correlations between As_w and ADD with As_h, As_{fn} and As_{tn} apparently indicate that deleterious arsenic concentrations in groundwater of the Mekong River basin is the main sources of chronic arsenic toxicity in Cambodia. Concurrently, statistically sig-

nificant positive intercorrelations between As_w, ADD, As_h, As_{fn} and As_{tn} are suggestive that As_h, As_{fn} and As_{tn} can be used as biomarkers of chronic arsenic exposure, in which As_h is more favorable than As_{fn} and As_{tn} due to the ease of sample processing and analytical measurements, respectively. In fact, arsenicosis appears to be continued as a major public health concern since most rural Cambodian people are relying on the arsenic-rich shallow wells. Theses are due to the lack of safe water supplies, long dry season (November–May) which has been leading to failure of rainwater catchments, access of some households to inexpensive and easily drilling borehole tubing into shallow aquifer and the lack of regular monitoring system of well water qualities in each community. In addition, some international authorities and/or NGOs may not be fully aware of where and what kind of safe water sources are appropriate for their target working communities because the available databases of arsenic high risk areas in rural Cambodia are very limited and less accessible. Failures to communicate between international and local authorities and NGOs in accessing those available databases has been leading to continuous installation of hand pump wells in the communities known of high risk of arsenic exposure, which increase the prevalence of arsenicosis. However, our present study reveals that the use of As_h, As_{fn} and As_{tn} as biomarkers can provide useful information of the early warning stage of chronic arsenic exposure while arsenicosis symptoms are generally assumed to develop after a decade of consuming arsenic-rich groundwater. Nevertheless, analyses of arsenic content in biological samples are not feasibly applicable in the world's rural parts as developing as Cambodia; therefore, development of simpler diagnostic tools to detect and monitor arsenicosis should be encouragingly taken into consideration.

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Conflict of interest statement

They have no conflict of interests to declare.

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