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Hair arsenic levels and prevalence of arsenicosis in three Cambodian provinces

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ABSTRACT

Natural, inorganic arsenic contamination of groundwater threatens the health of more than 100 million people worldwide, including residents of the densely populated river deltas of South and Southeast Asia. Contaminated groundwater from tube wells in Cambodia was discovered in 2001 leading to the detection of the first cases of arsenicosis in 2006. The most affected area was the Kandal Province. The main objective of this study was to determine the prevalence of arsenicosis in Cambodia based on acceptable criteria, and to investigate the use of hair arsenic as a biomarker not only for arsenicosis-related signs but also for associated symptoms. A cross-sectional epidemiological study of 616 respondents from 3 purposely selected provinces within the Mekong River basin of Cambodia was conducted. The Kandal Province was chosen as a high arsenic-contaminated area, while the Kratie Province and Kampong Cham Province were chosen as moderate and low arsenic-contaminated areas, respectively. The most prevalent sign of arsenicosis was hypomelanosis with a prevalence of 14.5% among all respondents and 32.4% among respondents with a hair arsenic level of ≥1 μg/g. This was followed by hyperkeratosis, hyperpigmentation and mee's lines. Results also suggest a 1.0 μg/g hair arsenic level to be a practical cut off point for an indication of an arsenic contaminated individual. This hair arsenic level, together with the presence of one or more of the classical signs of arsenicosis, seems to be a practical criteria for a confirmed diagnosis. Based on these criteria, the overall prevalence of arsenicosis for all provinces was found to be 16.1%, with Kandal Province recording the highest prevalence of 35.5%. This prevalence is comparatively high when compared to that of other affected countries. The association between arsenicosis and the use of Chinese traditional medicine also needs further investigation.

1. Introduction

Natural, inorganic arsenic contamination of groundwater threatens the health of more than 100 million people worldwide, including residents of the densely populated river deltas of South and Southeast Asia. Arsenic can be found in geological formations associated with river deposit of young Holocene sediments rich in organic content. Reducing groundwater aquifer condition promotes decomposition of the organic carbon through anaerobic microbial respiration which mobilizes inorganic arsenic from host minerals, notably iron oxides (Winkel et al., 2008; Charlet and Polya, 2006). In Asia, high arsenic concentrations have been found in the aquifers of Bangladesh, West Bengal, Nepal, China, Cambodia and Vietnam (Winkel et al., 2008). The use of arsenic contaminated groundwater in Bangladesh has led to what has been described as the worst mass poisoning of a human population in history, where between 35 and 77 million are at risk and over 100,000 have developed skin lesions (Charlet and Polya, 2006; Smith et al., 2000).

The inorganic salts of arsenic are tasteless and odorless making its contamination in drinking water unsuspecting to the consumers. Soluble inorganic arsenic is acutely toxic, and ingestion of large doses leads to gastrointestinal symptoms, dysfunction of the cardiovascular and nervous systems, and eventually death. In survivors, bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and

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encephalopathy may be observed. Chronic exposure to arsenic in drinking-water is causally related to skin changes such as hyperkeratosis and pigmentation changes, as well as a severe form of peripheral vascular disease known as blackfoot disease (IPCS, 2001). Chronic exposure also leads to increased risks of cancers of the skin, lungs, bladder, kidney and liver. There are evidences that chronic exposure may also be related to hypertension, cerebrovascular disease, diabetes, reproductive effects, neurological effects and cancers at other sites (NRC, 2001; Tchounwou et al., 2003; Brinkel, et al., 2009; Smith, et al., 2000). Increased risks of lung and bladder cancers and of arsenic-associated skin lesions have been reported to be associated with ingestion of drinking-water at concentrations ≤50 μg/L, which is the present arsenic in drinking water guideline for countries like Bangladesh and Cambodia (IPCS, 2001). The World Health Organization however recommends a lower guideline level of 10 μg/L (WHO, 2008).

Limited information is known about the extent of arsenic contamination of groundwater and arsenicosis in Southeast Asia, especially on its potential health and economic burden. Using logistic regression models of geological and surface soil parameters, regions in Southeast Asia predicted to have groundwater arsenic levels in excess of 10 μg/L are the Red River Delta of Vietnam, Mekong Delta of Cambodia and Vietnam, Irrawaddy Delta of Myanmar, Chao Phraya Basin of Thailand and Palembang in Sumatra (Winkel et al., 2008). Surface water in many of these areas is normally contaminated by human and animal feces due to the lack of organized sewage treatment facility. Resorting to deep tube wells has exposed these populations to natural arsenic in groundwater.

The Mekong River has a large catchment area, draining weathered arsenic-bearing rocks in the Himalayan and Tibetan highlands (Polya et al., 2005). Only 34% of rural Cambodians have access to bacteriologically safe drinking water. This led the Cambodian Government and partner agencies to install tube wells which were later found to be arsenic contaminated in 1999 during a national drinking water quality assessment program (Sampson et al., 2008). However, a systematic review of the Cambodian groundwater quality which identified a more extensive arsenic contamination was only completed in 2001 (Feldman et al., 2007).

The first cases of suspected arsenicosis were discovered in 2003 which however were not clinically confirmed (Milton, 2003). The first confirmed cases of arsenicosis in Cambodia were discovered in August 2006 in Preak Russey Village in Kandal Province (Samnang, 2006 as reported in Sampson et al., 2008). Some 54% of the tube well water samples from the contaminated provinces of Kandal and Prey Veng have arsenic concentrations above 10 μg/L, with maximum concentrations as high as 900 and 1543 μg/L (Sthiannopkao et al., 2008, 2010). Based on these elevated arsenic concentrations, the lifetime excess cancer risk may be as high as 2 cancers per 1000 exposed population (Sthiannopkao et al., 2010). However, to this date, the prevalence of arsenicosis in the Cambodian population has never been determined. As arsenicosis was only recently discovered in 2006, coupled with the continued use of groundwater especially in rural Cambodia during the dry season, it would not be unreasonable to assume that more cases of arsenicosis and related cancers will appear in the population in years to come.

In Cambodia, where infant and under-5 mortality rates are 70 and 91 per 1000 live births, respectively, the problem or arsenicosis will not be an immediate health priority (WHO, 2009). Nevertheless, its cancer-causing potential is equally alarming which may exert a significant health and economic burden on the country in the future. Presently, no practical solution is yet in sight as the practical approach of providing centrally treated drinking water may not yet be economically feasible, except for the urban areas. The main objective of this study was to determine the prevalence of arsenicosis in Cambodia based on acceptable criteria, and to investigate the use of hair arsenic as a biomarker not only for arsenicosis-related signs but also for associated symptoms.

2. Materials and methods

2.1. Study area and sample

This study is a cross-sectional epidemiological study of 616 respondents from 3 purposely selected study areas or provinces within the Mekong River basin of Cambodia, to represent locations with varying levels of human arsenic exposure from drinking contaminated groundwater. One commune and 2 villages were purposely selected from each province. The Kandal Province was chosen as a high arsenic-contaminated area, whereby the Preak Russey and Lvea Toung villages within the Kampong Kong Commune were selected. The Kratie Province was chosen as a moderate arsenic-contaminated area, whereby the Preak Samrong I and II villages within the Khsarch Andaet Commune were selected. As a control or clean study area with low arsenic contamination, the Kampong Cham Province was chosen, whereby the Andoung Chros and Veal Sbov villages within the Ampil Commune were selected (Fig. 1).

The target was to sample 250 individuals from each province to give a total sample of 750 respondents. Due to reluctant participation from the people in the Kratie Province we only managed to sample 101 respondents from there, 284 respondents from the Kampong Cham Province and 231 respondents from the Kandal Province (Table 1). In each of the selected villages, quota sampling was used to reach the desired sample size. Only respondents who have lived in the area for 5 years or more were chosen as our study subjects. Since the number of samples fell short in the Kratie and Kandal provinces, the number of samples in the Kampong Cham Province was increased beyond 250. We finally achieved a total sample size of 616 respondents who participated in the questionnaire survey and physical examination. However out of these, only 453 respondents relented to having samples of their hair taken.

2.2. Health questionnaire, physical examination and biological samples

For each of the selected provinces, 2 local community nurses were appointed for the health questionnaire and physical examination study. They were trained by a qualified physician on how to conduct a proper health questionnaire interview and to carry out a physical examination of the respondents to screen for the signs of arsenicosis, namely hyperkeratosis, hypomelanosis, hyperpigmentation and mee's line. Khmer translation of the health questionnaire was given to the nurses to facilitate their questionnaire interview. They were also trained on how to properly collect hair samples from the respondents.

Hyperkeratosis was defined as diffused gritty thickening (hypertrophy) of the corneous (outer) layer of the skin, often with raised wart-like nodules several millimeters in size. Hyperpigmentation was defined as areas of skin darkening from increased pigmentation (melanin) either diffusely distributed in freckle-like appearance or in patches. Hypomelanosis was defined as areas of skin lightening, often characterized by “rain drop” pattern, diffused over the skin. Mee's lines was defined as horizontal bands of discoloration seen as transversed white lines across the nails of hands and feet due to arsenic poisoning. Blackfoot disease was defined as chronic severe damage to the peripheral arteries in the lower limbs, causing impaired vascular flow which progressively leads to gangrene and hence, the characteristic of a blackened appearance.

2.3. Hair arsenic

Respondents' hair samples were collected from the nape of their heads, as close as possible to the scalp, using a pair of stainless steel scissors. The hair samples were first washed sequentially with 18.2 MΩ MilliQ deionized water and acetone to remove any external contaminants. The hair samples were then digested with concentrated nitric acid (70% HNO_3). Arsenic concentrations in hair samples were

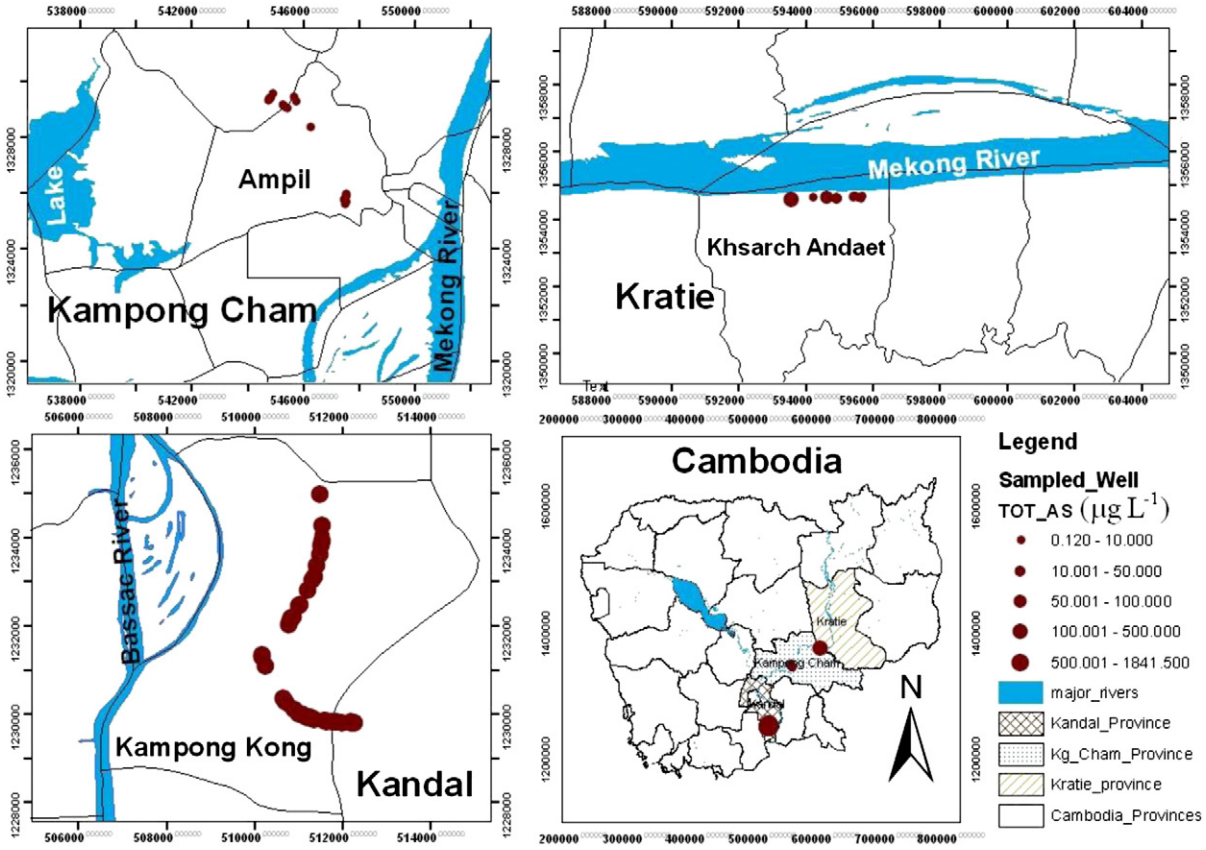

Fig. 1. Map of study provinces in Cambodia with groundwater arsenic levels from sampled well. Adapted from Phan et al. (2010).

then measured using inductively coupled plasma mass spectrometry (ICP-MS, model Agilent 7500ce) and validated using human hair certified reference material (GBW07601). The hair arsenic recovery rate was 94.8%. The details of this analytical method have been described in an earlier publication (Phan et al., 2010).

3. Results

Table 1 shows the gender of respondents by study province. There was no significant difference in the gender proportion between the 3 provinces. Table 2 compares the median age of respondents between the 3 provinces, whereby a significant difference was observed. The median age of respondents was highest in Kratie Province, followed by those from Kandal Province and Kampong Cham Province. Therefore, we can assume that respondents from the 3 provinces were comparable with respect to gender but different with respect to age.

Table 2 also compares groundwater arsenic from an earlier publication in the 3 provinces by Phan et al. (2010), as well as arsenic in hair of respondents from the same provinces in this present paper. Both papers are however reporting results from the same field work conducted in the 3 provinces from February to August 2009. Results indicate that groundwater arsenic and hair arsenic were consistently and significantly lowest in Kampong Cham Province, moderately high in Kratie Province, and highest in Kandal Province. This reaffirms our selection of Kandal Province as the most arsenic-contaminated study area, followed by Kratie Province as the moderate arsenic-contaminated study area and Kampong Cham Province as the control or clean study area.

Table 3 gives the smoking status and use of Chinese traditional medicine among the respondents by study province. There is no significant difference in the smoking status of respondents by study province. However, the use of Chinese traditional medicine is significantly more prevalent in Kandal Province when compared with the other 2 provinces.

Table 4 gives the prevalence of the classical signs of arsenicosis by the hair arsenic cut off point of 1 μg/g. The prevalence of all signs were significantly much higher among respondents with hair arsenic level of 1 μg/g or greater. When a hair arsenic cut off point of 0.5 μg/g was used, the prevalence rates for all signs of arsenicosis dropped consistently (Table 5). Therefore, a hair arsenic cut off point of 1 μg/g seems

Table 1
Gender of respondents by study provinces.

Sex	Study province						χ^2 (p value)
	Kampong Cham, n = 284 (clean)		Kratie, n = 101 (moderately contaminated)		Kandal, n = 231 (highly contaminated)		
	No.	%	No.	%	No.	%	
Male	126	44.37	44	43.56	102	44.16	0.990
Female	158	55.63	57	56.44	129	55.84	

Table 2
Age of respondents, total groundwater and hair arsenic levels by study provinces.

	Study province						Kruskal–Wallis[a] (p value)
	Kampong Cham (clean)		Kratie (moderately contaminated)		Kandal (highly contaminated)		
	n	Median	n	Median	n	Median	
Age	284	26.00	101	43.00	231	30.00	<0.001***
Total groundwater As (μg/L)[b]	18	1.22	12	1.30	46	822.63	
Hair As (μg/g)	173	0.090	77	0.240	203	4.810	<0.001***

*** Medians were significantly different at $p < 0.001$.
[a] Test for normality using the Kolmogorov–Smirnov test showed that data was not normally distributed, hence the non-parametric Kruskal–Wallis test was employed.
[b] Reported earlier by Phan et al. (2010).

Table 3
Smoking status and use of Chinese traditional medicine among respondents by study provinces.

	Study province						χ^2 (p value)
	Kampong Cham, n = 284 (clean)		Kratie, n = 101 (moderately contaminated)		Kandal, n = 231 (highly contaminated)		
	No.	%	No.	%	No.	%	
Smoking	32	11.3	9	8.9	28	12.1	0.694
Use of Chinese traditional medicine	15	5.3	2	2.0	54	23.4	<0.001***

*** Use of Chinese traditional medicine was significantly associated with study province at $p < 0.001$.

to be the logical choice to differentiate between the arsenic exposed and non-exposed individuals.

Table 6 presents the prevalence rate of the classical signs of arsenicosis by study provinces. The highest overall prevalence was for hypomelanosis (14.45%), followed by hyperkeratosis (9.09%), hyperpigmentation (8.28%), mee's lines (7.31%) and blackfoot disease (0.16%). For all the signs mentioned except for blackfoot disease, prevalence was highest for Kandal Province, followed by Kratie Province and Kampong Cham Province. The presence of one or more signs of arsenicosis on a study subject together with a hair arsenic level of 1 μg/g or greater was taken as a confirmed diagnosis for arsenicosis. Using these criteria, the overall prevalence of arsenicosis was 16.11%, with Kandal Province showing the highest prevalence of 35.47%.

Table 7 shows the prevalence rates of arsenicosis from several studies in other parts of the world, in comparison to this study. Of course, the diagnosis criteria vary from study to study. All studies were conducted relatively recent after the year 2000. The sample size varies from the smallest of 453 respondents in this study to the largest of 135,452 in China. By comparison, the prevalence of arsenicosis of 16.1% found in this study was one of the highest.

Table 8 presents the reported common symptoms of arsenicosis by the hair arsenic cut off point of 1 μg/g. Results clearly indicate that the prevalence of all symptoms were significantly higher for respondents having hair arsenic level of 1 μg/g or higher, except for the symptoms of hemoptysis and hallucinations. When the prevalence of these symptoms were compared across the 3 provinces, the prevalence were significantly highest for Kandal Province (Table 9). The bottom eleven symptoms in Tables 8 and 9 are related to the nervous system indicating the neurotoxicity potential of arsenic. Among the more pronounced symptoms were decreased concentration and forgetfulness, convulsion, weakness in upper and lower limbs, as well as tingling sensation, numbness and pain in extremities.

Table 10 shows the factors that may also have influence on the occurrence of arsenicosis. Those who are aged between 20 and 29 years old were 3.7 times at higher risk of getting arsenicosis compared to those under 20 years old. Those who used Chinese traditional medicine were 10.6 times at higher risk of getting arsenicosis compared to those who did not. All these were observed after controlling for gender and smoking.

4. Discussion

An attempt was made to compare the respondents of the 3 provinces. They were very comparable with respect to gender. However, respondents from Kratie Province were significantly older compared to those from Kandal Province and Kampong Cham Province. Age of course may affect the duration of exposure to arsenic in groundwater.

Table 4
Prevalence of signs of arsenicosis by hair arsenic level of 1 μg/g.

Signs of arsenicosis	Hair arsenic level				χ^2 (p value)
	<1 μg/g, n = 283		≥1 μg/g, n = 170		
	No.	Prev. (%)	No.	Prev. (%)	
Hyperkeratosis	14	4.94	32	18.82	<0.001***
Hypomelanosis	21	7.42	55	32.35	<0.001***
Hyperpigmentation	13	4.59	30	17.64	<0.001***
Mee's lines	14	4.94	19	11.17	0.014*

* Signs of arsenicosis were significantly associated with hair arsenic level at $p < 0.05$.
*** Signs of arsenicosis were significantly associated with hair arsenic level at $p < 0.001$.

Table 5
Prevalence of signs of arsenicosis by hair arsenic level of 0.5 μg/g.

Signs of arsenicosis	Hair arsenic level				χ^2 (p value)
	<0.5 μg/g, n = 253		≥0.5 μg/g, n = 200		
	No.	Prev. (%)	No.	Prev. (%)	
Hyperkeratosis	5	1.97	41	16.20	<0.001***
Hypomelanosis	13	5.13	63	24.90	<0.001***
Hyperpigmentation	7	2.76	36	14.22	<0.001***
Mee's lines	9	3.55	24	9.48	0.001**

** Signs of arsenicosis were significantly associated with hair arsenic level at $p < 0.01$.
*** Signs of arsenicosis were significantly associated with hair arsenic level at $p < 0.001$.

Table 6
Prevalence of signs of arsenicosis by study provinces.

Signs of arsenicosis	Study province								χ^2 (p value)
	Kampong Cham, n = 284[a]/173[b] (clean)		Kratie, n = 101[c]/77[d] (moderately contaminated)		Kandal, n = 231[e]/203[f] (highly contaminated)		All provinces, N = 616[g]/453[h]		
	No.	Prev. (%)	No.	Prev. (%)	No.	Prev. (%)	No.	Prev. (%)	
Hyperkeratosis	1	0.35[a]	4	3.96[c]	51	22.08[e]	56	9.09[g]	<0.001***
Hypomelanosis	0	0.00[a]	13	12.87[c]	76	32.90[e]	89	14.45[g]	<0.001***
Hyperpigmentation	0	0.00[a]	10	9.90[c]	41	17.75[e]	51	8.28[g]	<0.001***
Mee's lines	0	0.00[a]	16	15.84[c]	29	12.55[e]	45	7.31[g]	<0.001***
Blackfoot disease	0	0.00[a]	1	0.99[c]	0	0.00[e]	1	0.16[g]	0.078
At least 1 sign and hair As ≥ 1 μg/g	0	0.00[b]	1	1.30[d]	72	35.47[f]	73	16.11[h]	<0.001***

[a,b,c,d,e,f,g,h]Corresponding sample sizes for calculation of prevalence rates, as not all respondents with arsenicosis signs allowed their hairs to be sampled.
*** Signs of arsenicosis were significantly associated with study province at $p < 0.001$.

However, our resident selection criteria of choosing only respondents who have lived in their area for 5 years or more would help reduce the discrepancy in their duration of exposure.

Both hair and nails have been used as biomarkers for arsenic body burden (Samanta et al., 2004; Gault et al., 2008). They can be good indicators of toxic element exposures during the previous 2 to 18 months before sampling. The main concern of using hair and nail samples is the external contamination which must be removed before analysis (Samanta et al., 2004). Arsenic in hair may be deposited both from ingestion and external contamination from water, dust and cosmetics (Dart, 2004). The sequential washing of the hair samples with deionized water and acetone in this study as described by Phan et al. (2010), would have removed any external arsenic contamination. Normal hair arsenic for people living in a non-contaminated environment ranges from 0.08 to 0.25 μg/g. Hair arsenic level in excess of 1 μg/g normally indicates toxicity (Rahman et al., 2005; Caussy, 2005). Hair arsenic for patients with chronic poisoning ranges from 1 to 5 μg/g, but is often greater than 10 μg/g (Dart, 2004).

This study explores the practicality of using hair arsenic cut off point of 0.5 μg/g versus 1.0 μg/g by comparing the prevalence of signs of arsenicosis according to the 2 cut off points. Results suggest a 1.0 μg/g hair arsenic level to be a more practical cut off point for an indication of an arsenic contaminated individual. This supports the use of hair arsenic as an acceptable biomarker for arsenic toxicity.

The groundwater and hair arsenic levels found in this study clearly reaffirm Kandal Province as a highly arsenic-contaminated area. The median groundwater arsenic level of 822.63 μg/L is way above the World Health Organization's guideline of 10 μg/L (WHO, 2008) and the Cambodian guideline of 50 μg/L (Ministry of Rural Development Cambodia, 2010). The median hair arsenic level of 4.8 μg/g is also above the normal population range of 0.08 to 0.25 μg/g. This finding also seems to concur with that of another study by Gault et al. (2008) which found the hair arsenic level of 40 residents from Kandal Province to range from 0.10 to 7.95 μg/g. However, the hair arsenic level from this study is much higher than that found by Sthiannopkao et al. (2010) among 68 residents of Kandal Province with a median hair arsenic level of only 0.61 μg/g, even though the mean hair arsenic level was much higher at 3.2 μg/g. In the moderately contaminated Kratie Province, while the median groundwater arsenic level was low (1.3 μg/L), its median hair arsenic level (0.24 μg/g) is at the upper end of the normal range.

There is no international consensus on the criteria for the diagnosis and management of arsenicosis. Generally, skin manifestation is the primary condition leading a patient to seek medical care. However, it is also recognized that arsenicosis may present with or without skin manifestation. The World Health Organization defines arsenicosis as a chronic health condition arising from prolonged ingestion of arsenic above the safe dose for at least six months, usually manifested by characteristic skin lesions of melanosis and keratosis, occurring alone or in combination, with or without the involvement of internal organs. (Caussy, 2005). In this study, we looked for the presence of any of the classical signs of arsenicosis, namely melanosis (either as hyperpigmentation or hypomelanosis) hyperkeratosis, mee's lines on nails and blackfoot disease.

The most prevalent sign of arsenicosis was hypomelanosis with a prevalence of 14.5% among all respondents and 32.4% among respondents with a hair arsenic level of ≥1 μg/g. This was followed by hyperkeratosis, hyperpigmentation and mee's lines. Only 1 patient with blackfoot disease was identified. A peripheral vascular disease, blackfoot disease was mainly confined to the southwestern coast of Taiwan (Tseng et al., 2007). However, it is uncommon in mainland China, where even though 30,000 arsenicosis cases have been diagnosed, not a single case of blackfoot disease has been discovered (Sun, 2004).

The presence of one or more of these signs on a patient together with a hair arsenic level of 1 μg/g was taken as a confirmed diagnosis for the purpose of this study. Based on our proposed diagnosis criteria, the overall prevalence for all provinces was found to be 16.1%, with Kandal Province recording the highest prevalence of 35.5%. There are respondents with hair arsenic level ≥1 μg/g but without any sign of arsenicosis. This is expected as signs of arsenicosis are generally the manifestation of chronic exposure to arsenic. The use of hair arsenic as a biomarker for arsenic exposure should be promoted even if its practicality is limited in the less developed parts of the world. Nevertheless, hair samples can be easily obtained, transported

Table 7
Prevalence studies on arsenicosis from contaminated groundwater exposure.

Study location	Author	Year	Sample size	Prevalence (%)
Bangladesh	Hadi and Parveen (2004)	2000	1654	2.9
India (Rajapur, West Bengal)	Rahman et al. (2005)	2000–2003	825	18.1
China	Guangqian et al. (2007)	2001–2003	135,492	7.5
Nepal (Terai region)	Maharjan et al. (2006)	2001–2004	18,288	2.2
China	Sun (2004)	2002	45,570	9.4
Cambodia (Kandal Province)	Milton (2003)	2003	3905	1.4
Cambodia (Kandal Province)	Samnang (2006)	2006	Not stated	13.0
Cambodia	Jamal et. al.	2009	453	16.1

Table 8
Prevalence of symptoms of arsenicosis by hair arsenic level.

Symptoms of arsenicosis	Hair arsenic level				χ^2 (p value)
	< 1 μg/g, n = 283		≥ 1 μg/g, n = 170		
	No.	Prev. (%)	No.	Prev. (%)	
Frequent unexplained fever	113	39.93	134	78.82	<0.001***
Frequent headache	111	39.22	156	91.76	<0.001***
Garlic odor of breath	1	0.35	4	2.35	0.049*
Frequent breathing difficulty	17	6.00	37	21.76	<0.001***
Recurrent chesty cough	31	10.95	32	18.82	0.019*
hemoptysis	6	2.12	6	3.53	0.366
Loss of weight	15	5.30	50	29.41	<0.001***
Loss of appetite	12	4.24	41	24.12	<0.001***
Recurrent nausea and vomiting	18	6.36	68	40.00	<0.001***
Recurrent abdominal pain	47	16.61	115	67.65	<0.001***
Recurrent episodes of diarrhea	45	15.90	74	43.53	<0.001***
Decreased concentration and forgetfulness	27	9.54	66	38.82	<0.001***
Confusion or disorientation	7	2.47	15	8.82	0.002**
Slowed speech	1	0.35	6	3.53	0.008**
Slowed movement	3	1.06	10	5.88	0.003**
Hallucinations (delirium)	3	1.06	2	1.18	0.909
Convulsion	10	3.53	20	11.76	0.001***
Weakness in upper limbs	26	9.19	63	37.06	<0.001***
Weakness in lower limbs	18	6.36	35	20.59	<0.000***
Tingling sensation in extremities	27	9.54	46	27.06	<0.000***
Numbness in extremities	16	5.65	54	31.76	<0.000***
Pain in extremities	15	5.30	37	21.76	<0.000***

* Symptoms of arsenicosis were significantly associated with hair arsenic level at $p < 0.05$.
** Symptoms of arsenicosis were significantly associated with hair arsenic level at $p < 0.01$.
*** Symptoms of arsenicosis were significantly associated with hair arsenic level at $p < 0.001$.

and stored with little preparation and without preservation. It can also be sent by ordinary mail to distant places for analysis. This is in contrast to other biological samples like blood and urine.

When compared to other studies from Bangladesh, West Bengal, China, Nepal and Cambodia, the overall prevalence of arsenicosis found by this study among 453 studied subjects was relatively high. There might be a tendency to over sample arsenicosis patients in Kandal Province as they would voluntarily approach the nurses who were conducting the health survey to seek medical advice. Therefore, the prevalence of 35.5% for Kandal Province may be on the high side. However, there might also be a tendency to under sample arsenicosis patients in Kampong Cham Province and Kratie Province. This may be so as some arsenicosis patients may not present any sign. Therefore, the overall arsenicosis prevalence of 16.1% should be representative of the 3 provinces.

The overall arsenicosis prevalence of 16.1% in this study is much higher than the 1.4% recorded by Milton (2003) in an unpublished report, but closer to the 13% reported by Samnang (2006). Both previous surveys were conducted in Kandal Province where the arsenicosis prevalence recorded in this study was much higher at 35.5%. The surveys by Milton (2003) and Samnang (2006) were conducted 4 to 7 years after arsenic contaminated tube wells in Cambodia were discovered in 1991. Arsenicosis generally develops after 8 to 10 years of consumption of arsenic contaminated water. As this study was completed in 2009, the higher prevalence of arsenicosis recorded in this study may be due to the longer period of exposure. Moreover, this study focused on the most arsenic polluted villages in Kandal Province, namely Preak Russey and Lvea Toung.

When the prevalence of possible symptoms of arsenicosis were tabulated by hair arsenic level and by study province, certain symptoms like unexplained fever, frequent headache and recurrent abdominal pain stand out prominently. Neurological symptoms, even though not prominently high, are consistently more prevalent among the exposed respondents. Tsai et al. (2003) found poorer scores of neurobehavioral performance among school children exposed to arsenic in Taiwan. Two studies in an arsenic affected area of Bangladesh showed that exposure to arsenic in drinking water was associated with reduced children's intellectual function even after adjusting for socio-demographic confounders (Wasserman et al., 2004, 2007).

Table 9
Prevalence of symptoms of arsenicosis by study provinces.

Symptoms of arsenicosis	Study province								χ^2 (p value)
	Kampong Cham, n = 284 (clean)		Kratie, n = 101 (moderately contaminated)		Kandal, n = 231 (highly contaminated)		All provinces, N = 616		
	No.	Prev. (%)	No.	Prev. (%)	No.	Prev. (%)	No.	Prev. (%)	
Frequent unexplained fever	50	17.61	67	66.34	183	79.22	300	48.70	<0.001***
Frequent headache	28	9.86	80	79.21	213	92.21	321	52.11	<0.001***
Garlic odor of breath	0	0.00	0	0.00	7	3.03	7	1.14	0.003**
Frequent breathing difficulty	1	0.35	4	3.96	59	25.54	64	10.39	<0.001***
Recurrent chesty cough	14	4.93	9	8.91	53	22.94	76	12.34	<0.001***
hemoptysis	0	0.00	0	0.00	14	6.06	14	2.27	<0.001***
Loss of weight	6	2.11	0	0.00	71	30.74	77	12.50	<0.001***
Loss of appetite	1	0.35	0	0.00	61	26.41	62	10.06	<0.001***
Recurrent nausea and vomiting	0	0.00	1	0.99	97	41.99	98	15.91	<0.001***
Recurrent abdominal pain	2	0.70	20	19.80	163	70.56	185	30.03	<0.001***
Recurrent episodes of diarrhea	4	1.41	35	34.65	100	43.29	139	22.56	<0.001***
Decreased concentration and forgetfulness	2	0.70	13	12.87	96	41.56	111	18.02	<0.001***
Confusion or disorientation	3	1.06	0	0.00	23	9.96	26	4.22	<0.001***
Slowed speech	3	1.06	0	0.00	7	3.03	10	1.62	0.078
Slowed movement	2	0.70	0	0.00	16	6.93	18	2.92	<0.001***
Hallucinations (delirium)	1	0.35	1	0.99	4	1.73	6	0.97	0.285
Convulsion	3	1.06	1	0.99	31	13.42	35	5.68	<0.001***
Weakness in upper limbs	11	3.87	6	5.94	91	39.39	108	17.53	<0.001***
Weakness in lower limbs	9	3.17	3	2.97	52	22.51	64	10.39	<0.001***
Tingling sensation in extremities	0	0.00	11	10.89	73	31.60	84	13.64	<0.001***
Numbness in extremities	2	0.70	6	5.94	75	32.47	83	13.47	<0.001***
Pain in extremities	1	0.35	7	6.93	52	22.51	60	9.74	<0.001***

** Symptoms of arsenicosis were significantly associated with study province at $p < 0.01$.
*** Symptoms of arsenicosis were significantly associated with study province at $p < 0.001$.

Table 10
Multiple logistic regression to determine factors associated with diagnosed arsenicosis.[a]

Variable		Adjusted odds ratio (OR)	95% CI OR	χ^2 stat.	p-value
Gender	Female	1.11	0.56; 2.18	0.09	0.76[b]
	Male	1.00			
Age		–	–	15.05	0.01*
	>60	0.94	0.32; 2.76	0.01	0.91[c]
	50–59	0.88	0.30; 2.61	0.05	0.82[c]
	40–49	1.88	0.71; 4.94	1.63	0.20[c]
	30–39	2.19	0.88; 5.42	2.85	0.09[c]
	20–29	3.69	1.62; 8.42	9.64	0.002[c]**
	0–19	1.00			
Smoking	Yes	0.43	0.13; 1.39	2.29	0.13[b]
	No	1.00			
Use of Chinese traditional medicine	Yes	10.60	5.39; 20.82	50.02	<0.001[b]***
	No	1.00			

Adjusted odds ratio for gender and smoking.
[a] At least 1 sign and hair As ≥ 1 μg/g.
[b] Likelihood Ratio (LR) test.
[c] Wald test.
* Adjusted OR was significantly greater than 1 at $p < 0.05$.
** Adjusted OR was significantly greater than 1 at $p < 0.01$.
*** Adjusted OR was significantly greater than 1 at $p < 0.001$.

While controlling for gender and smoking as possible confounders of arsenicosis, age as well as the use of Chinese traditional medicine were both found to be associated with the occurrence of arsenicosis. The association with age is expected as it reflected the time of exposure to arsenic. The age group of 20 to 29 years was most associated with arsenicosis. Even though arsenic contamination of tube wells in Cambodia was first discovered in 2001, it is uncertain when contamination actually first occurred. If people 20 years and above are showing signs of arsenicosis, their exposures may have occurred in early or mid 1990's.

Chinese traditional medicine is a popular alternative medicine in Cambodia. The statistically significant association between arsenicosis and Chinese traditional medicine does not reveal whether the association is due to the fact that the use of Chinese traditional medicine increases one's exposure to arsenic, or that people with arsenicosis tend to seek treatment using this type of medications. According to Liu et al. (2008), arsenic in traditional medicine typically comes from deliberate addition for therapeutic purposes, mainly in the form of mineral arsenicals, including orpiment (As_2S_3), realgar (As_4S_4) and arsenolite (contains arsenic trioxide, As_2O_3).

5. Conclusion

This study as well as a number of previous studies have found Kandal Province to be one of the most arsenic polluted, if not the most polluted province in Cambodia. Hair arsenic with an exposure cut off point of 1.0 μg/g was found to be a reliable biomarker for arsenic exposure. It was then used with the presence of one of the traditional signs of arsenicosis as a proposed criteria for the diagnosis of arsenicosis. The overall prevalence of arsenicosis of 16.1% for Cambodia was comparatively high when compared to that of other affected countries. The association between arsenicosis and the use of Chinese traditional medicine also needs further investigation.

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