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Occurrence of arsenicosis in a rural village of Cambodia

D. N. GUHA MAZUMDER[1], K. K. MAJUMDAR[2], S. C. SANTRA[3], HERO KOL[4] and CHAN VICHETH[5]

[1]*DNGM Research Foundation, Kolkata , India*
[2]*Community Medicine, Calcutta National Medical Collage, Kolkata, India*
[3]*Environmental Science Department, University of Kalyani, India*
[4]*Ministry of Health, Cambodia*
[5]*National Clinic for Dermatology & STD Unit, Phnom Penh, Cambodia*

Ninety-seven subjects belonging to 40 families in a village in Cambodia were examined in a health camp where all the cases with skin disease assembled. These people had evidences of chronic arsenic exposure from reports of testing of water samples and of hair and/or nail studied. Seventy cases were diagnosed to be suffering from arsenicosis (Clinically and laboratory confirmed according to WHO criteria) as all these cases had evidences of pigmentation and/or keratosis characteristic of arsenicosis and history of exposure of arsenic contaminated water and/or elevated level of arsenic in hair and/or in nail. Highest number of cases belonged to age group of 31 to 45 yrs, both the sexes are more or less affected equally. Evidence of both pigmentation and keratosis were found in 60 cases (85.7%) while only pigmentation and only keratosis was found in 6 (8.5%) and 4 (5.7%) cases respectively. It was interesting to find 37.04% of children below the age of 16 years had skin lesions of arsenicosis. The youngest child having definite evidence of keratosis and pigmentation was aged 8 years, though two children aged 4 and 5 yrs had feature of redness and mild thickening of the palms. The minimum and maximum arsenic values detected in the nails were 1.06 and 69.48 mg/Kg respectively and the minimum and maximum arsenic values in hair were 0.92 and 25.6 mg/Kg respectively. No correlation was observed between arsenic concentration in drinking water and arsenic level in nail and hair. This is the first report of clinical and laboratory confirmed cases of arsenicosis in Cambodia.

Keywords: Arsenic, arsenicosis, Cambodia, hair, nail, children, pigmentation, keratosis.

Introduction

Many aquifers in various parts of the world are contaminated with arsenic (As) at concentration above 0.05 mg/L. Of these the most noteworthy occurrences are in large areas of India, Bangladesh, Taiwan and Northern China. Other countries having reports of significant arsenic contamination of ground water are Hungary, Mexico, USA, Chile and Argentina. Other south Asian countries from where reports of contamination of ground water due to arsenic are available are Lao PDR (People Democratic Republic), Cambodia, Myanmar, and Vietnam.[1]

Arsenic is a normal component of the human body and readily absorbed from the gastrointestinal tract. The absorption rate varies from 40–100%.[2] Skin abnormalities such as pigmentation and keratosis have long been known to be hallmark signs of chronic arsenic exposure. Although limited epidemiological data exist, the other clinical manifestations resulting from ingestion of arsenic-contaminated drinking water include weakness, conjunctival congestion, hepatomegaly, portal hypertension, respiratory system effects, polyneuropathy, solid oedema of limbs, peripheral vascular disease and malignant neoplasm. [3–6] Pigmentation changes and keratotic lesions are the most common health effect found in populations exposed to arsenic-contaminated drinking water. Pigmentation and keratosis caused by arsenic are quite distinctive.

The pigmentation of chronic arsenic toxicity commonly appears as a finely freckled, "raindrop" pattern that is particularly pronounced on the trunk and extremities distributed bilaterally symmetrically. The raindrop appearance results from the presence of numerous rounded hyperpigmented macules widely dispersed in the body. Pigmentation might also involve mucous membranes such as undersurface of tongue or buccal mucosa. Other patterns include diffuse hyperpigmentation, localised patchy pigmentation, and leucomelanosis, in which the hypopigmented macules take on a spotty white appearance. Leucomelanosis appears to occur in an arsenicosis patient following stoppage of drinking arsenic contaminated water for some duration.[4] Arsenical keratosis appears as diffuse thickening involving palms and soles, alone or in combination with nodules usually symmetrically distributed. The nodular forms are

Address correspondence to Dr. D. N. Guha Mazumder, Director, DNGM Research Foundation, 37C, Block-B, New Alipore, Kolkata – 700 053, India; E-mail: guhamazumder@yahoo.com
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Table 1. Water test data of wells of Prek Reusey village.

0.005 mg/L = 5 open wells	0.3 mg/L = 16 wells
0.1 mg/L = 2 wells	0.4 mg/L = 17 wells
0.2 mg/L = 3 wells	0.5 mg/L = 32 wells

encountered most frequently on the thenar and lateral borders of palms, on roots or lateral surfaces of fingers and soles, heels and toes of feet. Such small nodules may coalesce to form large verrucous lesions. The nodular form may also occur in the dorsum of the hands and feet and other parts of the body. In severe cases, cracks and fissures may be seen in the soles.

Keratosis is further subdivided into mild, moderate and severe. The form mild appears as slight thickening or minute papules (less than 2 mm) of palms and soles, often associated with a grit-like texture, which may be primarily detectable by palpation. Moderate forms are multiple raised keratosis (2–5 mm) while severe forms are large discrete or confluent keratotic elevations (>5 mm) on palms and soles, with nodular, wart-like or horny appearance.[4,6] Skin lesions pose an important public health problem because advanced forms of keratosis are painful, and the consequent disfigurement can lead to social isolation in the villages. In contrast to cancer, which takes decades to develop, these skin lesions are generally observed 6–9 years after exposure commences.[6]

In Cambodia, up to December 2005, 15673 wells of 7 provinces were tested for arsenic by field test kits. The results showed that 2924 wells (18.5%) of the total wells tested proved to have arsenic concentrations above the national limit of 0.05 mg/l. However no confirmed cases of arsenicosis were reported from that country till date. The arsenic contamination of groundwater does not occur evenly in Cambodia. The most highly affected province is Kandal (Fig. 1). Forty seven percent of the wells tested in this province have arsenic level > 0.05 mg/l. The village chief of Prek Reusey village, Kampong Kong Commune, Koh Than district of Kandal province (Fig. 2) reported that during the last 2–3 years, 3 young people died who had skin problems.[7] Ministry of Rural Development (MRD) Govt. of Cambodia, carried out arsenic testing with Field Test Kits in water samples taken from 75 tube wells in Prek Reusey village and these included community wells, private wells, and wells belonging to school and Pagoda. Results of testing of 75 tube wells by MRD are given in Table 1.[7]

A community-based study was conducted in the Perk Russey village in the month of October 2006 to ascertain the occurrence of arsenicosis cases in Cambodia.

Materials and methods

Clinical study and biological sample collection of suspected arsenicosis cases were done at Prek Reusey Village, Kampong Kong Commune, Koh Than district of Kandal province of Cambodia. In Kampong Commune there are 11 villages having 2,387 households covered by one health center. The Prek Reusey village is located 4 km from Kampong. Kong Health Center (Fig. 2). It has 383 households and most villagers who have skin problems consume water from tube wells all year round.

After extensive Information Education and Communication (I.E.C) campaign in the Perk Russey village, a health camp was organized near a primary school by the village head. Suspected cases of arsenicosis gathered at the health camp. After taking consent from the head of each family (the form was written in Cambodian language), all information collected was recorded in a proforma. Information was collected regarding location of source and duration of current and past water intake. Demographic features like age, sex and occupation of participants were also recorded. A clinical examination including history taking was done on each member of the families who attended the health camp and the specific symptoms were noted. Detail dermatological examination by an expert was carried out in each case in daylight to ascertain presence of arsenical skin lesions e.g., pigmentation and keratosis. Subjects having other skin disease simulating arsenicosis were excluded to make a diagnosis of clinically confirmed case of arsenicosis. Diagnosis of clinically confirmed case of arsenicosis was made according to the criteria of WHO.[6]

Hair samples were collected from each subject by cutting from the base and kept in plastic bag. Nail samples were collected by clipping finger and toenails and kept in a vial. All the samples were kept at room temperature till they were brought to Kolkata. These were then stored at 4°C till testing.[8]

Next day, water samples were collected from tube wells used by the participants of previous day's study by visiting the household of each family. Both the current water source and the previous water sources were collected. It was found that many of the families were taking water from common tube wells. Water samples were collected in plastic containers with a small quantity of residual hydrochloric acid to preserve the oxidation state. The samples were kept at room temperature for a week till it was brought to Kolkata. Subsequently these were stored at –20°C till testing.

Arsenic levels in the water were analyzed by AAS (Atomic Absorption Spectrophotometer) with Hydride generation system. (Perkin Elmer A Aanalyst 400). Arsenic level in hair and nail was estimated after proper preparation and digestion by AAS with Hydride generation system.[8]

Results

Ninety-seven subjects belonging to 40 families of Prek Reusey village were examined. All the villagers were farmers. These people had evidences of chronic arsenic exposure from reports of testing of water samples (collected

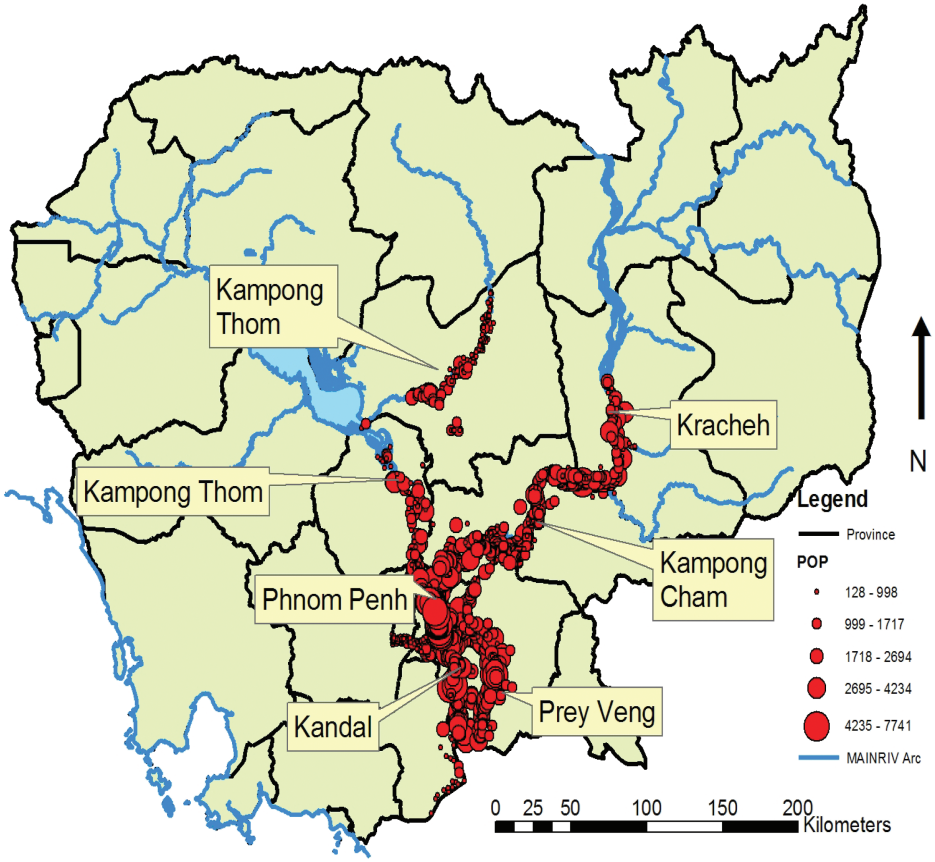

Fig. 1. Geographic distribution of arsenic affected region of Cambodia.

from 57 water sources used by all the participants) and of hair and/or nail studied in 93 subjects. Of these, 70 cases had evidences of arsenical skin lesion. These 70 cases were diagnosed to be suffering from arsenicosis (Clinically and laboratory confirmed, according to WHO criteria, WHO [6])as all these cases had evidences of pigmentation and/or keratosis characteristic of arsenicosis and had history of exposure of arsenic contaminated water and/or elevated level of arsenic in hair and/or in nail. The age distribution in relation to skin lesion is given in Table 2. The highest number of cases belonged to the age group of 31 to 45 yrs. It was interesting to find that about 37% of children belonging the age group of less than 16 years had skin lesions of arsenicosis. The youngest child having definite evidence of keratosis and pigmentation was 8 years old (Fig. 3), though two children aged 4 and 5 years had feature of redness and mild thickening of the palms. These children also looked ill. Both sexes are more or less affected equally (Female 40/56, 71.43%, male 30/41, 73.17%). Evidence of both pigmentation and keratosis were found in 60 cases while only pigmentation and only keratosis was found in 6 and 4 cases each respectively.

Table 2. Arsenical skin lesion in relation to age.

AGE	*n*	*Skin lesion*	*%*
<16 Year	27	10	37.04
16–30 Yr	25	19	76
31–45 Yr	29	27	93.1
46–60 Yr	16	14	87.5
All	97	70	72.16

Both pigmentation and keratosis were nearly equally distributed among the participants examined (Table 3). It could be seen from Table 4 that both spotty pigmentation and leuco-melanosis were more or less equally distributed among both sexes in all age groups. Distribution of diffuse and nodular keratosis (severe form of disease) in relation to age and sex is given in Table 5. Nodular form of keratosis was observed in about 25% cases while diffuse form of keratosis was found in about 52% participants examined. Both the types were equally distributed in the two sexes.

In addition to arsenical skin lesions other skin diseases found in these people are:Urtecaria-2, Fungal infection-2 and Dermatitis-5. Symptoms of systemic diseases found in the study population with and without arsenical skin lesions are given in Table 6. Significant increased incidences of weakness and body pain were found in people who had

Table 3. Age and sex distribution of participants in relation to pigmentation and keratosis.

	Pigmentation						*Keratosis*					
Age	*Male (n = 41)*	*%*	*Female (n = 56)*	*%*	*Total (n = 97)*	*%*	*Male (n = 41)*	*%*	*Female (n = 56)*	*%*	*Total (n = 97)*	*%*
<16 yrs	6	14.63	3	5.36	9	9.28	7	17.07	2	3.57	9	9.28
16–30 Yr	7	17.07	12	21.43	19	19.59	7	17.07	10	17.86	17	17.53
31–45 Yr	8	19.51	16	28.57	24	24.74	10	24.39	15	26.79	25	25.77
46–60 Yr	6	14.63	8	14.29	14	14.43	5	12.20	8	14.29	13	13.40
All	27	65.85	39	69.64	66	68.04	29	70.73	35	62.50	64	65.98

arsenical skin disease. Out of 97 subjects examined in the health camp one case was found to be suffering from suspected skin cancer. He had an ulcerated tumor-like lesion in the sole of one foot having evidence of keratosis (Fig. 4).

Samples of water could be collected from present and past water sources from 57 sources from where all the participants were drinking water. Very high level of arsenic contamination was found in the wells of Prek Reusey village from where the people came (see Table 7). Highest amount of arsenic exposure (1.286 mg/L) was found to occur in 29 subjects studied. Duration of arsenic exposure varied from 5–10 years. Arsenic level in the newly dug wells from where most of the people were currently drinking water was found to be safe (arsenic level-0.008mg/L). Arsenic level tested in water taken from Barak River, which was flowing by the side of the village, was found to be below detection limit.

Arsenic level in nail or hair of 93 participants showed above normal value suggesting that nearly all the participants who attended the health camp in the Prek Reusey village had chronic exposure to arsenic. The minimum and maximum arsenic values detected in the nails were 1.06 and 69.48 mg/Kg respectively, the distribution of the values being given in Table 8. It will be seen that 69% of individuals had arsenic value varying from 1.5 to 20 mg/kg. The minimum and maximum arsenic values in hair were 0.92 and 25.6 mg/Kg, respectively. It could be seen that 80% of cases had hair arsenic value ranging between 1.0 and 10 mg/kg (Table 8). Arsenic value in hair and nail was found to be within normal limit in one case each, but the person who had normal arsenic value in nail had high arsenic value in hair and the person who had normal arsenic value in hair had high arsenic value in nail. Arsenic values in the nail and hair were correlated with arsenic exposure data through water taken by the participants. It could be seen that there was no correlation between arsenic values in these biomarkers with arsenic exposure data.

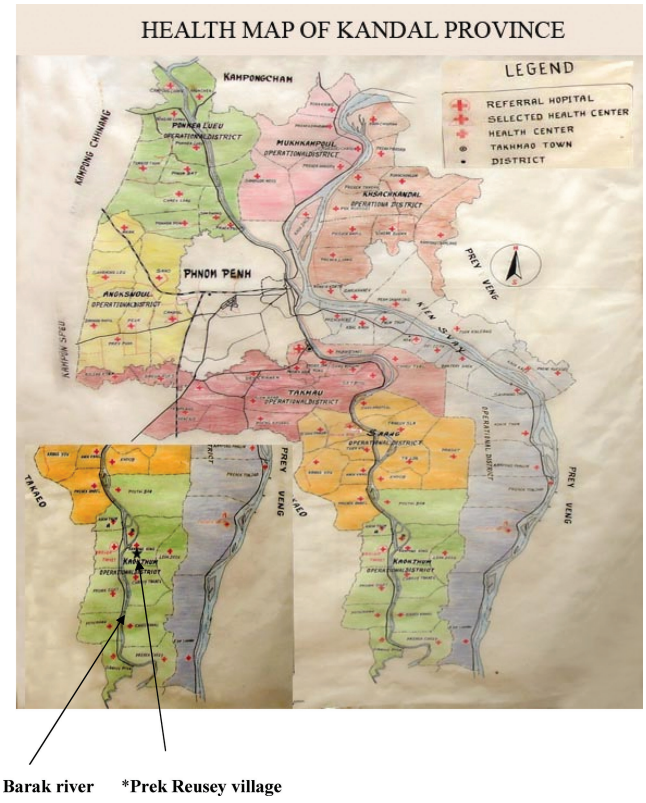

Fig. 2. Kandal province.

Discussion

This appears to be the first report of clinical survey on occurrence of arsenicosis cases in Cambodia. However,

Fig. 3. A child aged 8 years showing arsenicosis.

Table 4. Age and sex distribution of participants in relation to spotty pigmentation and Leuco melanosis.

	Spotty pigmentation						*Leuco melanosis*					
Age	*Male (n = 41)*	*%*	*Female (n = 56)*	*%*	*Total (n = 97)*	*%*	*Male (n = 41)*	*%*	*Female (n = 56)*	*%*	*Total (n = 97)*	*%*
<16 yrs	6	14.63	3	5.36	9	9.28	3	7.32	2	3.57	5	5.15
16–30 Yr	7	17.07	11	19.64	18	18.56	7	17.07	11	19.64	18	18.56
31–45 Yr	4	9.76	14	25.00	18	18.56	6	14.63	12	21.43	18	18.56
46–60 Yr	6	14.63	7	12.50	13	13.40	3	7.32	7	12.50	10	10.31
All	23	56.10	35	62.50	58	59.79	19	46.34	32	57.14	51	52.58

arsenic in ground water has been reported earlier in the Lower Mekong region.[9] Pigmentation and keratosis are the hallmark signs of arsenicosis and are the only criteria used by WHO[6] for case diagnosis of clinically confirmed cases of arsenicosis. In the present study large number (72%) of people showed evidences of arsenical skin lesion (Pigmentation and/or keratosis) among the residents of Prek Reusey village, Koh Than district of Kandal province of Cambodia. This observation of large number of cases in study subjects may be due to selective attendance of suspected cases of people residing in the village to the health camp for their examination. However most of the people (86%) examined were drinking water with very high (>0.5 mg/L) level of arsenic in their drinking water source and among them 56.1% had a exposure level more than 1 mg/Kg. In India Arsenic-enriched Holocene ground water is reported to be present. [10,11]

Earlier, population survey carried out in a district of West Bengal, India[12] showed prevalence of arsenicosis to be 6.73% among the participants studied. The survey was carried out on 7683 participants (4093 female and 3590 male) with current water sources ranging up to 3.4 mg/L. However less than 20% of participants consumed water with arsenic level >0.5 mg/L.[12] In West Bengal water from 132,262 government installed hand pumps in eight districts has been tested and overall 25.5% of samples were found to contain arsenic > 0.5mg/L and 57.9% having concentrations >0.1mg/L.[13] Studies by the National Arsenic Mitigation Information centre (NAMIC) in Bangladesh showed a prevalence of arsenic above 0.05mg/L in app 20% tube wells.[14] Another cross-sectional study was conduced in Bangladesh, where occurrence of arsenic in groundwater due to hydrogeological conditions were reported,[15] 1,481 subjects ≥30 years of age were interviewed and examined, and a total of 430 subjects were found to have skin lesions. Arsenic water concentrations ranged from 0.01 to 2.04 mg/L and the crude overall prevalence rate for skin lesions was 29%[16] Increased incidence of skin manifestations among males was reported from Bangladesh even in the same families having exposure through drinking the same arsenic-contaminated water.[17] As in Cambodia various studies in Bangladesh also indicated that majority of the patients belongto low income group.[18] Studies conducted in other countries have also investigated the prevalence of pigmentation and keratosis in region with elevated arsenic levels in drinking water; however, the studies either lacked individual exposure data or had small numbers. It was interesting to note that although majority (85.7%) of participants in Cambodia showed both pigmentation and keratosis, a small number of cases were found to have only pigmentation (8.5%) and only keratosis (5.7%).

Fig. 4. A case of suspected skin cancer with keratosis of sole.

There was not much difference in the incidence of pigmentation and keratosis among males and females in Cambodia. This is in contrast to the observation of sex distribution of arsenicosis cases in Indo-Bangladesh subcontinent where males are more affected. In an epidemiological study in West Bengal 127 out of 4093 (3.1%) females had pigmentation while in males it was found in 234 out of 3590 (6.5%) subjects.[12] However in the Alashan area of Inner Mongolia, China the incidences of skin lesion in males and females were found to be similar (36.4 % and37.8 % respectively) when studied in a population of 1176.[19] Thus there appears to be a racial variation in regard to susceptibility to arsenicosis among male and female gender. Children below 16 years of age were found to show evidences of arsenical skin lesions in significant number (10 out of 27, 37.04%) in Cambodia. In Inner Mongolia, China, from where various adverse health effects of arsenicosis have been reported, in one study it was found that the incidence of arsenicosis in children below 19 years was found to be 12.2% when studied in a population of 728 subjects of the same age group.[19,20] On the other hand, in Bangladesh, out of 4877 children below 11 years, 298 children (6.11%) had evidence

Table 5. Age and sex distribution of participants in relation to defuse and nodular keratosis.

	Defuse keratosis						*Nodular keratosis*					
Age	*Male (n = 41)*	*%*	*Female* (n = 56)	*%*	*Total (n = 97)*	*%*	*Male (n = 41)*	*%*	*Female (n = 56)*	*%*	*Total (n = 97)*	*%*
<16 yrs	4	9.76	2	3.57	6	6.19	4	9.76		0.00	4	4.12
16–30 Yr	6	14.63	9	16.07	15	15.46	2	4.88	3	5.36	5	5.15
31–45 Yr	8	19.51	12	21.43	20	20.62	3	7.32	6	10.71	9	9.28
46–60 Yr	4	9.76	6	10.71	10	10.31	2	4.88	5	8.93	7	7.22
All	22	53.66	29	51.79	51	52.58	11	26.83	14	25.00	25	25.77

of arsenical skin lesion.[21] In West Bengal, India only 114 out of 6695 (3.7%) children below 11 years had evidences of arsenicosis.[22] In another study in West Bengal out of 2748 subjects below 19 years of age 66 (2.4%) had evidences of arsenical skin lesions.[12] However, before any definite conclusion in regard to age and sex distribution of arsenicosis cases are made, an epidemiological study involving large number of people need to be carried out in Cambodia.

Although limited epidemiological data exist, the reported non dermatological clinical manifestations resulting from ingestion of arsenic-contaminated drinking water include weakness, conjunctival congestion, hepatomegaly, portal hypertension, respiratory system effects, polyneuropathy, solid oedema of limbs, anaemia, burning sensation of eyes, liver fibrosis, chronic lung disease, gangrene of toes, neuropathy, and skin cancer and other malignant neoplasm[4,5,22,23] and various models like LQM were developed to find the associations between these diseases and arsenic.[24]. However in the present study, increase in the incidence of symptoms of weakness (14%) and body pain (21.4%) were only observed in a significant number of patients in Prek Russy village. Previous report showed that occurrence of weakness increase significantly with increase in arsenic exposure through water in those with skin lesions.[25] The reason why people exposed to high arsenic levels reported feeling weak is not clear. While arsenic can cause peripheral neuropathy, it is not known to cause central nervous system effects that could explain general feelings of weakness. None of our cases had any evidence of peripheral vascular disease as has been reported in people in Taiwan and Chile.[25,26]

The minimum and maximum arsenic value detected in the nails in our study was 1.06 and 69.48 mg/Kg, respectively. However it will be seen that 69% of individuals had arsenic value varying from 1.5 to 20 mg/Kg . Studies in six districts of West Bengal, India[8] showed max level of arsenic in nails to be 52 mg/kg. In the present study the minimum and maximum arsenic value in hair was 0.92 and 25.6 mg/Kg, respectively. However 80% of participants had hair arsenic value ranging between 1.0 and 10 mg/kg. Maximum level of arsenic in hair in West Bengal, was found to be 31 mg/Kg.[8] In Nova Scotia, Canada, arsenic level in hair was studied in 110 people who were drinking water having maximum arsenic value of 1.4 mg/Kg. Highest arsenic value in hair was found to be 55 mg/Kg.[27]

In our study in Cambodia we did not find any correlation between arsenic level in water consumed by the participants and arsenic level in hair and nail. Several other workers also observed poor correlation of arsenic level in drinking water and arsenic level in hair and nail. [27–29] Arsenic level in nails and hair may be increased as a result of surface contamination.[4] It is claimed that the source of arsenic on the outer surface of hair is both ingestion and external contamination, and that the two sources cannot be differentiated.[27,28] Hence observation of very high level of arsenic in nail and hair among Cambodian people,

Table 6. Symptoms of systemic disease found in arsenic-exposed population.

	Arsenical skin lesion Present n = 70		*Arsenical skin lesion absent n = 27*	
Symptoms	*n*	*%*	*n*	*%*
Cough, dyspnoea	9	12.8	4	14.8
Body pain	15	21.4	0	0
Dyspepsia	7	10	2	7.4
Headache	12	17.1	4	14.8
Vertigo	7	10	2	7.4
Weakness	10	14.3	0	0

Table 7. Arsenic level in water tested from different water sources.

Sl. No.	*Arsenic level in water (mg/L)*	*No. of water water sources*
1.	1.286	29
2.	1.026	3
3.	0.896	9
4.	0.752	1
5.	0.600	6
6.	0.526	1
7.	0.300	1
8.	0.296	2
10.	0.148	5
	Total	57

Table 8. Arsenic level (mg/kg) in hair and nail.

Hair		*Nail*	
Arsenic level (mg/Kg)	*No. (%)*	*Arsenic level (mg/Kg)*	*No. (%)*
≤ 1	1 (1.08)	≤ 1.5	1 (1.08)
>1 to ≤5	53 (56.99)	>1.5 to ≤10	21 (22.58)
>5 to ≤10	22 (23.66)	>10 to ≤20	43 (46.24)
>10 to ≤15	8 (8.60)	>20 to ≤30	16 (17.20)
>15 to ≤20	4 (4.30)	>30 to ≤40	7 (7.53)
>20	5 (5.38)	>40	5 (5.38)
Total :	93 (100.00)	Total :	93 (100.00)

reported here, may not be a true indicator of arsenic exposure through drinking water.

Limitation of this study is that the study population has been drawn from organizing health camp where suspected cases have gathered by previous publicity campaign by the village chief. As all the population of the village was not examined, the true prevalence of disease could not be ascertained. However water sources of all the participants have been collected by home visit subsequent to the health camp. Thus arsenic exposure data could be used for exposure assessment of the cases and clinical and laboratory confirmation of diagnosis of arsenicosis in participants studied.

Conclusion

Ninety-seven subjects belonging to 40 families in a village in Cambodia were examined in a health camp where all the cases with skin disease assembled. These people had evidences of chronic arsenic exposure from reports of testing of water samples and of hair and/or nail studied. Seventy cases were diagnosed to be suffering from arsenicosis (clinically and laboratory confirmed according to WHO criteria, WHO[6]) as all these cases had evidences of pigmentation and/or keratosis characteristic of arsenicosis history of exposure of arsenic contaminated water and/or elevated level of arsenic in hair and/or in nail. Highest number of cases belonged to age group of 31 to 45 yrs; both the sexes are more or less affected equally.

Evidence of both pigmentation and keratosis were found in 60 cases (85.7%) while only pigmentation and only keratosis was found in 6 (8.5%) and 4 (5.7%) cases respectively. It was interesting to find 37.04% of children below the age of 16 years had skin lesions of arsenicosis. The youngest child having definite evidence of keratosis and pigmentation was aged 8 years. The minimum and maximum arsenic values detected in the nails were 1.06 and 69.48 mg/Kg respectively and the minimum and maximum arsenic values in hair were 0.92 and 25.6 mg/Kg respectively. No correlation was observed between arsenic concentration in drinking water and arsenic level in nail and hair. This is the first report of clinical and laboratory confirmed cases of arsenicosis in Cambodia.

A supply of safe arsenic free potable water is the available preventive measure and while the surface water is not potable without treatment and filtration and a major portion of the groundwater is also not potable due to presence of many toxic species so development of a low cost filter is the only way to solve the present drinking water crisis.[30]

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