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REVIEW ARTICLE

Chronic arsenic toxicity: Studies in West Bengal, India

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Abstract Chronic arsenic toxicity (arsenicosis) as a result of drinking arsenic-contaminated groundwater is a major environmental health hazard throughout the world, including India. A lot of research on health effects, including genotoxic effect of chronic arsenic toxicity in humans, have been carried out in West Bengal during the last 2 decades. A review of literature including information available from West Bengal has been made to characterize the problem. Scientific journals, monographs, and proceedings of conferences with regard to human health effects, including genotoxicity, of chronic arsenic toxicity have been reviewed. Pigmentation and keratosis are the specific skin diseases characteristic of chronic arsenic toxicity. However, in West Bengal, it was found to produce various systemic manifestations, such as chronic lung disease, characterized by chronic bronchitis, chronic obstructive and/or restrictive pulmonary disease, and bronchiectasis; liver diseases, such as non cirrhotic portal fibrosis; polyneuropathy; peripheral vascular disease; hypertension; nonpitting edema of feet/hands; conjunctival congestion; weakness; and anemia. High concentrations of arsenic, greater than or equal to 200 μg/L, during pregnancy were found to be associated with a sixfold increased risk for stillbirth. Cancers of skin, lung, and urinary bladder are the important cancers associated with this toxicity. Of the various genotoxic effects of arsenic in humans, chromosomal aberration and increased frequency of micronuclei in different cell types have been found to be significant. Various probable mechanisms have been incriminated to cause DNA damage because of chronic arsenic toxicity. The results of the study in West Bengal suggest that deficiency in DNA repair capacity, perturbation of methylation of promoter region of p53 and p16 genes, and genomic methylation alteration may be involved in arsenic-induced disease manifestation in humans. P53 polymorphism has been found to be associated with increased occurrence of arsenic-induced keratosis. Of the various genes involved in the regulation of arsenic metabolism, single-nucleotide polymorphisms of purine nucleoside phosphorylase, in one study, showed increased occurrence of arsenicosis.

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Introduction

Reports of arsenic contamination in water are available from more than 30 countries in the world. However, the major regions affected are in the river basins of the Ganga, Brahmaputra, and Meghna in India and Bangladesh and those in China. It is suspected that about 6 million people in West Bengal (Fig. 1), India, are exposed to arsenic-contaminated groundwater [1]. The occurrence of a large number of cases of arsenic-induced skin lesions was

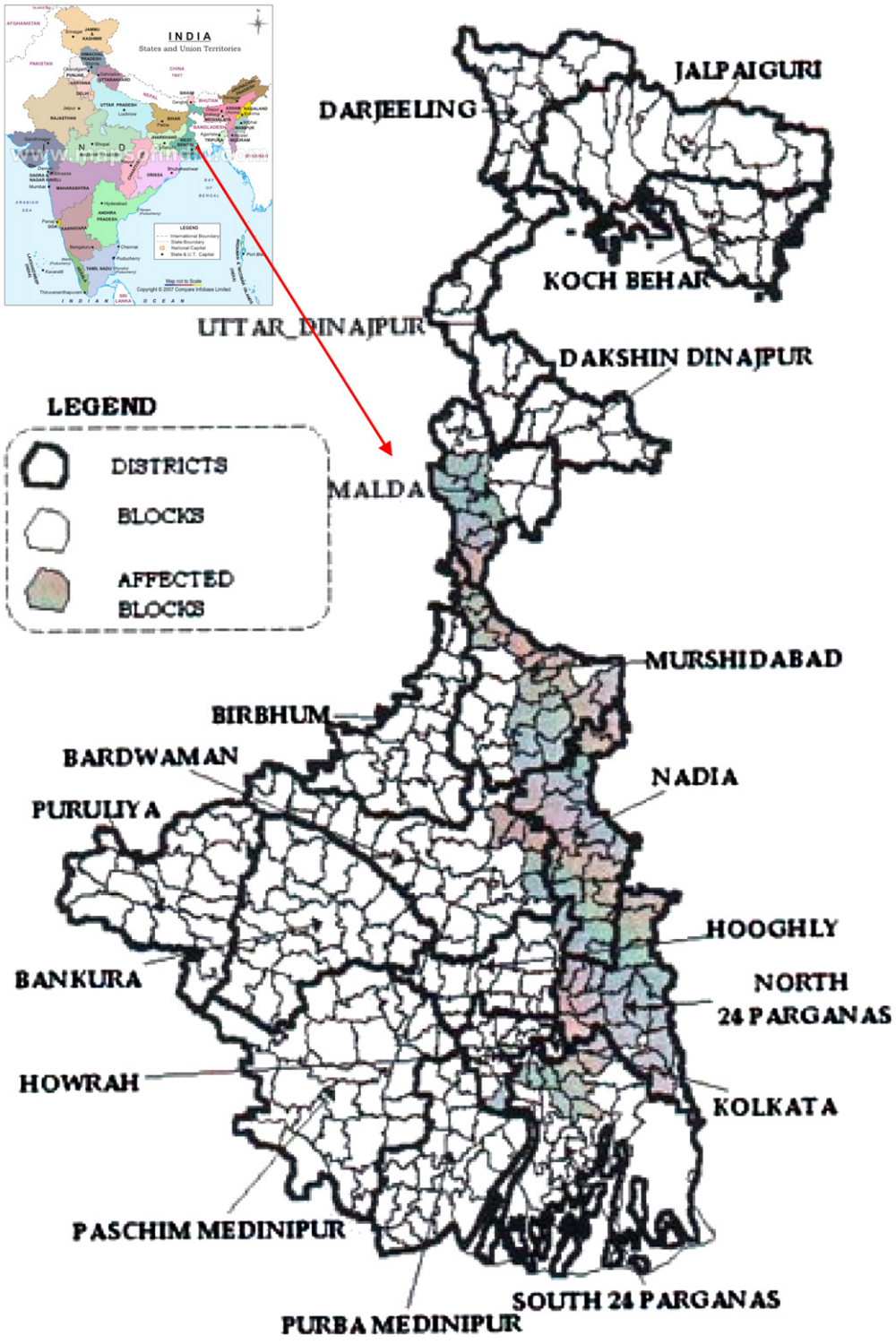

Figure 1. Map of West Bengal.

reported from Kolkata, West Bengal, in 1984 [2]. Since then, incidences of chronic arsenic toxicity have been reported in most of the states adjoining the upper, middle, and lower Ganga and Brahmaputra plains. Arsenic contamination has been found in the states of Bihar, Uttar Pradesh, Jharkhand, Assam, Chhattisgarh, and Andhra Pradesh [3,4]. As a large amount of data have been generated from research on clinical health effect and genotoxicity in man as a result of drinking arsenic-contaminated groundwater in West Bengal, India, this article has focused attention to present data on results of research in these areas in two sections and has incorporated a review of relevant literature from studies in other countries. Because of space constraints, the literature survey could not be exhaustive.

Human health effect (arsenicosis)

The term arsenicosis to designate human health effect of chronic arsenic toxicity was first coined by our group [5] and later used by the World Health Organization [6] to imply a chronic disease caused by prolonged exposure of arsenic in humans. Previously, the condition was described as arseniasis, arsenism, arsenicism, and others. Most of the reports of chronic arsenic exposure in man focus attention on skin manifestations because of their diagnostic specificity. However, data derived from population-based studies; clinical case series; and reports relating to intake of inorganic arsenic through drinking water, medications, or occupational and environmental exposure, show that chronic arsenic exposure adversely affects multiorgan system of human body.

Skin manifestations

Pigmentation and keratosis are the specific skin diseases characteristic of chronic arsenic toxicity. To ascertain the prevalence of keratosis and pigmentation in relation to arsenic exposure, the first population-based survey was carried out on 7,683 participants (4,093 females and 3,590 males) in West Bengal with participants arsenic-exposure data [7]. The arsenic content of current water source of the participants ranged up to 3,400 μg/L; however, more than 80% of the participants consumed water with arsenic level less than 50 μg/L. The age-adjusted prevalence of keratosis and pigmentation was strongly related to water arsenic levels, rising from 0 and 0.3 per 100, respectively, in the lowest exposure level (<50 μg/L), to 8.3 and 11.5 per 100, respectively, for females drinking water containing greater than 800 μg/L arsenic, and increasing from 0.2 and 0.4 per 100, respectively, in the lowest exposure category, to 10.7 and 22.7 per 100, respectively, for males in the highest exposure level (>800 μg/L). Calculation by dose per kilogram body weight showed that men had roughly two to three times the prevalence of both keratosis and pigmentation compared with women apparently ingesting the same dose of arsenic from drinking water. Participants who were less than 80% of the standard body weight for their age and sex had a 1.6-fold increase in the prevalence of keratosis, suggesting that malnutrition may play some role in increasing susceptibility. However, the survey examined only the participants' primary current drinking-water source. Results of a nested case–control study using detailed lifetime (at least 20 years) exposure assessment showing low dose of arsenic exposure (<50 μg/L) among the aforementioned study population were further available. The exposure assessment incorporated arsenic concentration data from current and past water sources used in households and work sites. The lowest peak arsenic level ingested by a confirmed case was 115 μg/L. Strong dose–response gradients with both peak and average arsenic water concentrations were also observed [8]. In another cross-sectional study, conducted in Bangladesh, 430 out of 1,481 participants aged 30 years or older and drinking arsenic-contaminated water were found to have arsenical skin lesions. Arsenic water concentrations ranged from 10 μg/L to 2,040 μg/L, and the crude overall prevalence rate for skin lesions was 29%. This study also showed a higher prevalence rate of arsenical skin lesions in males than in females with clear dose–response relationship [9].

Systemic manifestations

Chronic arsenic toxicity produces various systemic manifestations over and above skin lesions. It needs to be noted that although systemic manifestations occur mostly in association with arsenical skin lesions, symptoms do occur in significantly higher number of cases in arsenic-exposed people even in the absence of arsenical skin disease compared with arsenic-unexposed people [10].

Respiratory disease

Initial report of nonmalignant lung disease was available from a study of 180 residents of Antofagasta, Chile, exposed to drinking water containing arsenic (800 μg/L). About 38% of the 144 participants with abnormal skin pigmentation complained of chronic cough, compared with 3.1% of 36 participants with normal skin [11]. Symptoms of chronic lung disease were present in 89 (57%) out of 156 cases of chronic arsenic toxicity caused by drinking arsenic-contaminated water in West Bengal (Table 1) [12]. Lung function tests carried out on 17 patients showed features of restrictive lung disease in nine (53%) and combined obstructive and restrictive lung disease in seven (41%) cases.

To ascertain the relationship of chronic arsenic exposure with occurrence of lung disease, an analysis of data of cross-sectional epidemiological survey of nonsmokers (6,864 participants) was carried out in West Bengal. The study participants included those who had arsenic-associated skin lesions and who were also highly exposed at the time of the survey (arsenic water concentration ≥ 50 μg/L). Participants with normal skin and low arsenic water concentration (<50 μg/L) were used as the reference group. In participants with skin lesions, the age-adjusted prevalence odds ratio (OR) estimates for cough, crepitations, and shortness of breath for females were 7.8, 9.6, and 23.2, respectively, and for males, 5, 6.9, and 3.7, respectively [13]. In an epidemiological study carried out on 218 nonsmokers (94 exposed to arsenic, 136–1,000 μg/L, and 124 unexposed cases) in Bangladesh, the crude

Table 1 Symptoms and signs of 167 arsenicosis patients during hospital admission in West Bengal, India

	n	%
Symptoms		
Weakness	110	70.5
Headache	32	20.5
Burning of the eyes	69	44.2
Nausea	17	10.9
Pain in the abdomen	60	38.4
Epigastric	39	25.0
Paraumbilical	21	13.4
Diarrhea	51	32.6
Cough	89	57.0
With expectoration	53	33.9
Without expectoration	36	23.1
Hemoptysis	8	5.1
Dyspnea	37	23.7
Paresthesia	74	47.4
Signs		
Pigmentation	156	100.0
Keratosis	96	61.5
Anemia	74	47.4
Hepatomegaly	120	76.9
Splenomegaly	49	31.4
Ascites	5	3.0
Pedal edema	18	11.5
Sign of lung disease	45	28.8
Sign of polyneuropathy	21	13.4

Adapted from Ref. [12].

prevalence ratios for chronic bronchitis were found to be 1.6 [95% confidence interval (CI): 0.8—3.1] and 10.3 (95% CI: 2.4—43.1) for males and females, respectively [14].

During 1998—2000, relationship between lung function and exposure to arsenic in drinking water was ascertained in West Bengal among a cohort of 287 participants selected among study population who were exposed to low dose of arsenic (up to 500 μg/L) [15]. The average forced expiratory volume in 1 second, adjusted for age, height, and smoking, was reduced by 256.2 mL (95% CI = 113.9—398.4, $p < 0.001$), and the average adjusted forced vital capacity reduced by 287.8 mL (95% CI = 134.9—440.8, $p < 0.001$) in men with skin lesions compared with those without skin lesions. An increase of 100 μg/L arsenic in drinking water was associated with a decrease in forced expiratory volume in 1 second of 45 mL (95% CI = 6.2—83.9, $p = 0.02$) and a decrease in forced vital capacity of 41.4 mL (95% CI = 0.7—83.5, $p = 0.05$) in men. Women showed little evidence of lung function alteration. Thus, over and above respiratory symptoms, consumption of arsenic-contaminated water in man was found to be associated with reduced pulmonary function.

In a hospital-based study carried out on 29 cases of chronic arsenic toxicity with nonmalignant lung disease in Kolkata, West Bengal [16], obstructive lung disease was diagnosed in 17 (58.6%), interstitial lung disease in nine (31.2%), and bronchiectasis in three (10%) cases. To ascertain the incidence of bronchiectasis in the population, 108 participants with arsenic-caused skin lesions and 150 participants without skin lesions were studied in an arsenic-endemic population in West Bengal [17,18]. The median highest level of arsenic in drinking water was 330 μg/L [standard deviation (SD) = ±881 μg/L] in participants with skin lesions compared with 28 μg/L (SD = ±147 μg/L) in those without such lesions. Thirty-eight study participants who reported at least 2 years of chronic cough underwent high-resolution computed tomography. The mean bronchiectasis severity score was 3.4 (SD = ±3.6) in the 27 participants with skin lesions and 0.9 (SD = ±1.6) in the 11 participants without these lesions (controls). In participants who reported chronic cough, high-resolution computed tomography evidence of bronchiectasis was found in 18 (67%) cases with skin lesion and in three (27%) controls. Adjusted OR was found to be 10.1 (95% CI = 2.7—37.1). This study showed that drinking high-arsenic-contaminated water was associated with increased incidence of bronchiectasis in man. Many other investigators also reported chronic respiratory disease in the form of chronic cough or chronic bronchitis because of prolonged drinking of arsenic-contaminated water [19—21].

Gastrointestinal disease

Dyspepsia was observed in 60 out of 156 (38.4%) cases of chronic arsenic toxicity studied in West Bengal (Table 1) [12]. However, in an epidemiological study carried out in the affected population, there was no difference in the incidence of pain in abdomen among people drinking arsenic-contaminated water and the control population (27.84% *vs.* 31.81%) [22]. Gastroenteritis was reported in a study of 1,447 cases of chronic arsenicosis caused by drinking arsenic-contaminated water with a concentration greater than 50 μg/L in the Inner Mongolian Autonomous Region of China [20]. Many investigators variously reported symptoms, such as nausea, diarrhea, anorexia, and abdominal pain in cases of chronic arsenic toxicity [5,11,23—25].

Liver disease

Many workers have reported, earlier, cases of liver damage after treatment of patients with arsenic as Fowler's solution [26—28]. All these patients developed features of portal hypertension with signs of liver fibrosis. Typical cutaneous signs of long-term arsenic exposure were also observed in some of the patients. There have also been case reports of liver cirrhosis after medication with inorganic arsenic compounds [29,30].

Hepatomegaly was found in 62 out of 67 members of families who drank arsenic-contaminated water (200—2,000 μg/L) in West Bengal, whereas it was found in only 6 out of 96 people who drank safe water in the same area. Thirteen of those arsenic-exposed patients who had hepatomegaly were further investigated in a hospital. All showed various degrees of portal zone expansion and fibrosis on liver histology. Four out of five patients who had splenomegaly showed evidence of increased intrasplenic pressure (30-cm to 36-cm saline), suggesting portal hypertension. Splenoportography done in those cases showed evidence of intrahepatic portal vein obstruction. Although

routine liver function tests were normal in all those 13 cases investigated, the bromsulphthalein retention test, done in three patients, was found to be abnormal. The arsenic level in liver tissue (estimated by neutron activation analysis) was found to be elevated in 10 out of 13 cases (As levels—cases: 500—6,000 μg/L/kg; controls: 100 ± 40 μg/L/kg) [5]. In a subsequent report from the same hospital, hepatomegaly was found in 190 out of 248 cases of chronic arsenicosis investigated. Evidence of portal fibrosis on liver histology was found in 63 out of 69 cases of hepatomegaly who were biopsied. Liver function tests carried out in 93 such patients showed evidence of elevation of alanine transaminase, aspartate transaminase, and alkine phosphatase, in 25.8%, 6.3%, and 29% of cases, respectively. Serum globulin was found to be high (>3.5 g/dL) in 19 (20.7%) cases [31].

In an epidemiological study carried out in West Bengal, the incidence of hepatomegaly was found to have a linear relationship with increasing exposure of arsenic in drinking water in both sexes ($p < 0.001$) [22]. Liver enlargement was also reported after drinking of arsenic-contaminated water by other workers [19—21,32]. All these studies show that prolonged drinking of arsenic-contaminated water is associated with hepatomegaly, the predominant lesion being hepatic fibrosis.

Cardiovascular disease

Blackfoot disease (BFD), a form of peripheral vascular disease, has been reported to be one of the important complications of chronic arsenic toxicity in Taiwan. The prevalence of BFD has been reported to be 8.9 per 1,000 among 40,421 inhabitants studied in Taiwan [33]. Comparable peripheral vascular disorders with varying degrees of severity, including Raynaud's syndrome and acrocyanosis, have also been reported among people drinking arsenic-contaminated water by others [11,12,19,20,30,33]. The incidence of peripheral vascular disease was found to be low in West Bengal. Out of 246 arsenicosis patients who attended a referral hospital in Kolkata, three cases (1.2%) of peripheral vascular disease were found [22]. However, out of 4,865 cases surveyed in a village population of West Bengal, one case (0.02%) of gangrene was detected [32]. Similarly, low incidence has been found in Chile and Bangladesh, whereas there is no report from Mexico and Argentina [34].

An epidemiological study reported an increased prevalence of hypertension among residents in the endemic area of BFD and a dose—response relationship between ingested inorganic arsenic and prevalence of hypertension [35]. An association of cumulative arsenic exposure in drinking water was also found with increased risk of hypertension in a study of 1,595 people in Bangladesh [36]. Increased prevalence of hypertension was also reported in 6.2% patients affected with arsenic-induced skin lesions (144) compared with none without skin lesion in Antofagasta, Chile [11]. Increased incidence of hypertension was found in a study of 108 arsenicosis cases compared with 100 arsenic-unexposed participants in a rural population in West Bengal (Guha Mazumder, unpublished data). Mortality rates from ischemic heart disease with endemic arsenicosis (from 1973 through 1986) were correlated with the arsenic level in drinking water among residents of 60 villages in Taiwan [37].

Diseases of nervous system

There are many reports of occurrence of peripheral neuropathy because of chronic exposure of arsenic through drinking water [20,21,24,32,38,39]. Peripheral neuritis characterized by paresthesia (tingling, numbness, limb weakness, and others) was present in 74 (47.4%) out of 156 patients of chronic arsenicosis as a result of drinking arsenic-contaminated water (500—14,200 μg/L) in West Bengal, India (Table 1). Objective evaluation of neuronal involvement, done in 29 patients, showed abnormal electromyography (EMG) in 10 (30.8%) and altered nerve conduction velocity and EMG in 11 (38%) cases [12,40]. In another electrophysiological study carried out on 88 patients of arsenicosis in West Bengal, sensory neuropathy was found in 24 (27.3%), motor neuropathy in 13 (14.7%), and abnormal EMG in 5 (5.7%) cases [41]. Abnormal EMG findings, suggestive mostly of sensory neuropathy, was reported in 10 out of 32 participants exposed to drinking arsenic-contaminated well water (range, 60—1,400 μg/L) in Canada [42].

There are several reports of increased incidence of cerebrovascular disease in patients suffering from chronic arsenicosis [20,38,43]. However, no study data are available from West Bengal regarding the occurrence of cerebrovascular disease.

Peripheral neuritis, sleep disturbances, weakness, and cognitive and memory impairment have been reported in residents of Byan College Station, Texas, exposed to arsenic from air and water from arsenic trioxide used to produce defoliants from an Atochem plant (TX,USA) [39]. Headache has been reported to occur in people drinking arsenic-contaminated water in Mexico [24] and in West Bengal (Table 1) [12]. Irritability, lack of concentration, depression, sleep disorders, headache, and vertigo were reported in arsenicosis people showing features of neuropathy in West Bengal [41].

Hematological effects

Hematological abnormalities have been reported in acute and chronic arsenic poisoning [44]. A characteristic pattern of anemia, leucopenia, and thrombocytopenia was found in 55 participants exposed to arsenic in drinking water in Niigata Prefecture in Japan for approximately 5 years, half of the patients having arsenical skin lesions [44]. In one study in West Bengal, anemia was reported in all the 13 people exposed to arsenic-contaminated groundwater (200—2,000 μg/L) [5]. Further study in West Bengal on 156 people exposed to arsenic-contaminated water (50—14,200 μg/L) showed incidence of anemia in 47.4% of cases (Table 1) [12]. However, no association of anemia was found in people drinking well water (mean, 220 μg/L) in Alaska [45] and in two towns of Utah (arsenic exposure, 180 μg/L and 270 μg/L) [46].

Diabetes

A dose—response relationship between cumulative arsenic exposure and prevalence of diabetes mellitus was observed in Taiwan after a study on 891 persons living in arsenic-endemic areas [47]. From Bangladesh, significantly

increased prevalence of diabetes mellitus as a result of drinking arsenic-contaminated water was also reported among individuals with keratosis compared with those who did not have such lesions [48]. However, no study on the incidence of diabetes mellitus in arsenicosis has been carried out in West Bengal.

Pregnancy outcome

Limited information on pregnancy outcome and infant mortality in relation to arsenic levels in drinking water is available in literature as few studies included participants assessment of arsenic concentrations in all water sources used during each pregnancy. A retrospective study of pregnancy outcomes and infant mortality was conducted in West Bengal, India, among 202 married women selected from a source population of 7,683 between the years 2001 and 2003. Reproductive histories were ascertained by structured interviews. Arsenic exposure during each pregnancy was assessed based on all water sources used, involving measurements from 409 wells. Odds ratios for spontaneous abortions, stillbirth, and neonatal and infant mortality were estimated with logistic regressions based on the method of generalized estimating equations. High concentration of arsenic, greater than or equal to 200 μg/L, during pregnancy, was associated with a sixfold increased risk of stillbirth after adjusting for potential confounders (OR = 6.25, 95% CI = 1.59–24.6, $p = 0.009$). Arsenic-related skin lesions were found in 12 women who had a substantially increased risk of stillbirth (OR = 13.1, 95% CI = 3.17–54.0, $p = 0.002$). The OR for neonatal death was 2.03 (95% CI = 0.57–7.24). No association was found between arsenic exposure and spontaneous abortion (OR = 0.90, 95% CI = 0.36–2.26) or overall infant mortality (OR = 1.18, 95% CI = 0.38–3.64). This study adds to the limited evidence that exposure to high concentrations of arsenic during pregnancy increases the risk of stillbirth. However, there was no indication of increased rates of spontaneous abortion and overall infant mortality [49].

In an ecological study carried out in Chile, stillbirth (rate ratio = 1.7; 95% CI = 1.5–1.9) and neonatal and post-neonatal infant mortality rates were found to be increased in the high-arsenic-exposure city of Antofagasta as compared with the low-exposure-city Valparaiso [50]. A study conducted in Bangladesh showed an increased risk of stillbirth in women with current arsenic levels greater than or equal to 100 μg/L, although the risk estimates were smaller (OR = 2.5; 95% CI = 1.5–5.9). The authors further reported increased effects on spontaneous abortions (OR = 2.5, 95% CI = 1.5–4.4) [51]. However, no information was available on arsenic exposure during pregnancy, and high exposure levels of 200 μg/L and more were not considered separately in this study. One earlier cross-sectional study from Bangladesh compared the rates of spontaneous abortions, stillbirths, and preterm deliveries among 96 women in one village who were exposed to greater than or equal to 100 μg/L arsenic with the rates in 96 women in another village who were exposed to less than 20 μg/L arsenic and showed two to three times higher rates among exposed women [52]. Both the Bangladesh studies reported a relationship with the overall duration of women's exposure without taking into account exposure during the actual time period of pregnancy. In a recent publication from Bangladesh, pregnancy outcome and infant death were ascertained by health surveillance program on 29,134 pregnancies with a history of drinking arsenic-contaminated water with arsenic level greater than 50 μg/L. Significant increased risk of fetal loss (relative risk = 1.14, 95% CI = 1.04–1.25) and infant death (relative risk = 1.17, 95% CI = 1.04–1.32) was observed in this study. There was a significant dose response of arsenic exposure to risk of infant death ($p = 0.02$) [53].

Other effects

High incidence of weakness and fatigue have been reported in chronically arsenic-exposed people after drinking arsenic-contaminated water in West Bengal and in many other countries [5,12,22,23,32,39,54]. Conjunctival congestion and nonpitting edema of the legs (Fig. 2) and hands have also been reported in patients of chronic arsenic toxicity in West Bengal and Bangladesh [12,21,25,55].

Arsenicosis and cancer

The evidence of carcinogenicity in humans from exposure to arsenic is based on epidemiological studies of cancer in relation to arsenic in drinking water. The working group of International Agency for Research on Cancer [3] evaluated data from ecological, cohort, and case–control studies from many countries and observed that arsenic was

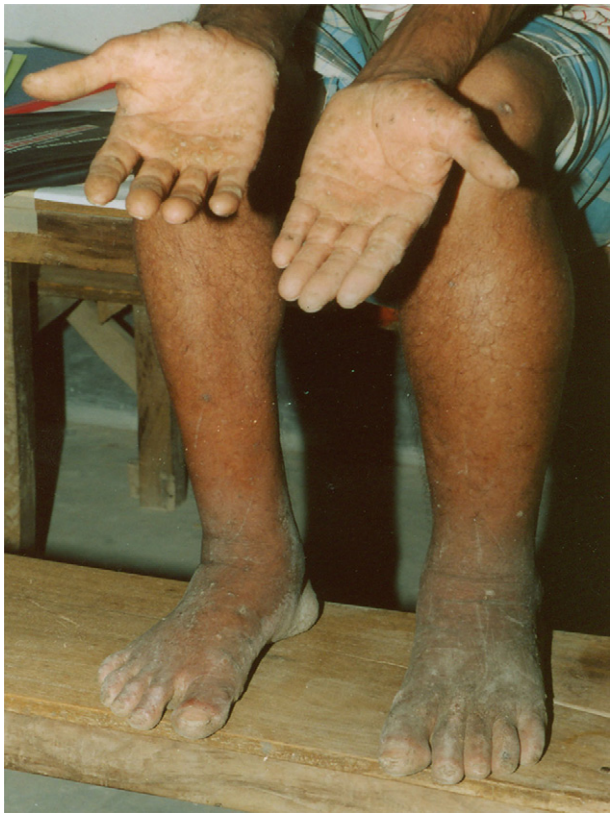

Figure 2. Nonpitting edema of legs with keratosis in a patient of arsenicosis.

potentially responsible for skin, urinary bladder, and lung cancers as result of chronic exposure to arsenic. In West Bengal, 212 (4.35%) cases of skin cancer and 38 (0.78%) cases of internal cancers were detected among 4,865 cases of arsenicosis studied in arsenic-affected villages [56].

Genotoxicity

Several studies have investigated the genotoxic effects of arsenic after long-term exposure in drinking water. Increased incidence of chromosomal aberration and frequency of micronuclei in buccal and urothelial cells was observed by many workers after drinking of arsenic-contaminated water [3,57,58]. In a study in West Bengal, the frequencies of micronuclei in oral mucosal cells, urothelial cells, and peripheral lymphocytes were found to be significantly high in 163 participants exposed to high level of arsenic in drinking water (214.72 ± 9.02 μg/L) compared with 154 unexposed control participants with little arsenic in drinking water (9.2 ± 0.31 μg/L). The analyzed data revealed that micronuclei frequencies in the exposed group were significantly elevated to 5.33-fold over unexposed levels for lymphocytes, 4.63-fold for oral mucosal cells, and 4.71-fold for urothelial cells (increases in micronuclei frequencies significant at $p < 0.01$). The results indicate that chronic ingestion of arsenic in drinking water in exposed participants is linked to the enhanced incidence of micronuclei in all the cell types, a slightly higher level of micronuclei being observed in lymphocytes compared with oral mucosal and urothelial cells [59]. Over and above micronuclei study, further cytogenetic studies included the study of chromosomal aberration on 422 (244 symptomatic and 178 asymptomatic) participants. Higher level of cytogenetic damage was found in symptomatic participants compared with asymptomatic participants, and asymptomatic participants had significantly higher genotoxicity than unexposed participants [60].

DNA damage

Various probable mechanisms have been incriminated to cause DNA damage because of chronic arsenic toxicity. Arsenic appears to have little ability, if any, to induce point mutation [44,61]. Altered DNA repair, oxidative stress, cell transformation, altered cell proliferation, altered cell signaling, altered steroid receptor binding, and altered gene expression and amplification are many of the mechanisms postulated to be involved in causing DNA damage because of chronic arsenic exposure [3]. A study in Taiwan showed that p53 gene mutation rate in arsenic-related skin cancers from the BFD-endemic area of Taiwan was high, and that the mutation types were different from those in UV-induced skin cancers [62].

Altered DNA repair

To ascertain whether keratosis, a premalignant condition associated with arsenicosis, is involved in the alteration in DNA repair capacity, 30 individuals with keratosis exposed to chronic arsenic-contaminated water and 30 unexposed controls were studied in West Bengal by comet, chromosomal aberration (CA), and challenge assays. DNA damage and CA was significantly higher in the arsenic-exposed participants compared with those in unexposed participants ($p < 0.001$). Within the exposed group, there was no significant difference of DNA damage ($p > 0.05$), but CA was significantly higher in the exposed group with keratosis than in that without exposure ($p < 0.01$). Challenge assay showed that, on induction of DNA damage, the repair capacity in the exposed group with keratosis was significantly less (<0.001) than that of participants without skin lesion, although the basal level of DNA damage was similar in both. The results of the study suggested that deficiency in DNA repair capacity may be involved in arsenic-induced carcinogenesis [63].

Altered DNA methylation

It has been hypothesized that alteration of DNA methylation by arsenic may play a role in the development of cancer. This mechanism has been proposed because the S-adenosyl methionine (SAM)/methyltransferase pathway for biotransformation of arsenic overlaps with the DNA methylation pathway, in which donation of methyl groups from SAM to cytosine produces 5-methylcytosine in DNA. DNA methylation is now known to be one of the main epigenetic mechanisms, which perpetuates the repression of genes in vertebrates and is intimately associated with development. Hypermethylation and consequent silencing of tumor suppressor and repair genes have been implicated as an early step in many cancers, such as those of lung, colorectum, breast, and others. Hypomethylation, on the other hand, provides growth advantage. Earlier experimental studies indicated that the carcinogenicity of arsenic may be mediated by alteration in the methylation status of DNA either by hypermethylation or hypomethylation [64,65].

To ascertain whether perturbation of methylation plays a role in carcinogenesis in humans, the degree of methylation of p53 and p16 genes in DNA obtained from blood samples of people chronically exposed to arsenic and skin cancer patients was studied in West Bengal. Methylation-specific restriction endonuclease digestion followed by polymerase chain reaction (PCR) of gene p53 and bisulfite treatment followed by methylation-sensitive PCR of gene p16 have been carried out to analyze the methylation status of the samples studied. Significant DNA hypermethylation of the promoter region of p53 gene was observed in the DNA of arsenic-exposed people compared with that of the control participants. This hypermethylation showed a dose–response relationship. Furthermore, hypermethylation of p53 gene isolated from peripheral blood was also observed in arsenic-induced skin cancer patients compared with that in individuals having skin cancer unrelated to arsenic, though not at a significant level. However, a small subgroup of cases showed hypomethylation with high arsenic exposure. Significant hypermethylation of gene p16 was also observed in cases exposed to high level of arsenic [65]. Further DNA was isolated from arsenic- and non-arsenic-induced skin cancer tissues, and p53 promoter methylation was studied. The degree of methylation in p53 promoter region of DNA of arsenic-induced cancer biopsy tissues showed significantly higher

hypermethylation compared with the biopsy tissues obtained from non-arsenic-induced skin cancer ($p < 0.05$) [66].

The changes in methylation status of whole genome because of arsenic exposure have been studied in West Bengal by monitoring the variation of methyl acceptance capacity of DNA extracted from peripheral blood of persons exposed to various doses of arsenic using the SssI methylase assay. To monitor the level of whole-genome methylation in persons exposed to different levels of arsenic through drinking water, DNA was extracted from peripheral blood mononuclear cells of 64 persons. Uptake of methyl group from ^{3}H-labeled *S*-adenosyl methionine after incubation of DNA with SssI methylase was measured. Results showed statistically significant ($p = 0.0004$) decrease in uptake of ^{3}H methyl group in the persons exposed to 250–500 μg/L arsenic, indicating genomic hypermethylation. This significance is lost in Group D that was exposed to greater than 500 μg/L of arsenic in drinking water [67]. To explain the results, it is noted that inorganic arsenic induces cytosine methyltransferase in tissue culture system [68], and array analysis of tissue from mice exposed to different doses of arsenic also showed up the regulation of DNA methyltransferase 3a gene [69]. This can cause the initial hypermethylation of DNA after arsenic exposure. However, as arsenic load increases, its detoxification through enzymatic methylation causes depletion of the methyl donor SAM that has a vital role in the detoxification process, leading to inefficient arsenic excretion, and DNA hypomethylation. DNA methyltransferase uses the same methyl donor SAM, and maintenance methylation is disrupted when the level of SAM in the body drops. In the last group with arsenic dose greater than 500 μg/L, there is probably too much methionine requirement for excretion of the large amount of arsenic ingested, and the extent of hypermethylation is no longer statistically significant because of the reduced SAM level. This probably signifies the beginning of the process of hypomethylation. It might be mentioned that, in an earlier work too, a small number of samples of the group having much higher dose of arsenic exposure showed hypomethylation, the level of significance increasing from 0.02 to 0.001 as the arsenic dose increased [70].

Genetic polymorphism

Arsenic-induced keratosis is considered as a precancerous state of *in situ* carcinoma of skin. Several reports have suggested the role of p53 polymorphisms as a potential marker for the risk assessment of different types of cancers. This prompted to study the association of three p53 polymorphisms with arsenic-induced keratosis in a population exposed to arsenic through drinking water. A total of 366 unrelated participants (177 participants with arsenic-induced keratosis and 189 participants with no arsenical skin lesions) were recruited from North 24 Parganas, Nadia, and Murshidabad districts of West Bengal for study of genotype distribution of three p53 polymorphisms [16-bp duplication at intron 3, codon 72 Arg/Pro, and G>A at intron 6 (nt 13,494)] by PCR-restriction fragment length polymorphism. The arginine homozygous genotype at codon 72 and homozygous genotype of no duplication polymorphism at intron 3 were overrepresented in the participants with keratosis compared with participants with no skin lesion (OR = 2.086, 95% CI = 1.318–3.299; and OR = 2.086, 95% CI = 1.257–3.457, respectively). This study indicated that participants carrying the arginine homozygous genotype of codon 72 and/or no duplication homozygous genotype at intron 3 are at risk for the development of arsenic-induced keratosis [71].

Studies from 115 study participants from Taiwan showed that genetic polymorphisms of *GSTM1* and *GSTT1* were significantly associated with arsenic methylation. Participants having the null genotype of *GSTM1* had an increased percentage of inorganic arsenic in urine, whereas those with null genotype of *GSTT1* had elevated percentage of DMA in urine [72].

A further study on 170 participants in Argentina showed that women with null genotype of *GSTM1* excreted a significantly higher proportion of arsenic as monomethylarsonic acid than women with active genotype [73]. However, incidence in *GSTM1* null gene frequencies studied in 244 skin symptomatic participants and 178 asymptomatic participants exposed to arsenic in West Bengal, significantly higher *GSTM1* null gene frequencies were found in the asymptomatic group. No difference in allelic variants in *GSTT1* and *GSTP1* was observed between these two groups [60]. Studies from 600 cases and controls (each) in Bangladesh also did not find any significant interaction for *GSTM1* for primary methylation ratio. The investigators observed significant interaction on the multiplicative scale between the *GSTT1* wild type and secondary methylation ratio [74].

To find out any probable association between arsenicosis and the exonic single-nucleotide polymorphisms of genes involved in the regulation of arsenic metabolism, purine nucleoside phosphorylase (*PNP*), arsenic(+3) methyltransferase, and glutathione *S*-transferase omega 1 and omega 2 were studied in arsenic-exposed population in West Bengal. Among the four candidate genes, distribution of three exonic polymorphisms, His 20His, Gly 51Ser, and Pro 57Pro of *PNP* was found to be associated with arsenicosis. Genotypes with minor alleles were significantly overrepresented in the case group: OR = 1.69 (95% CI = 1.08–2.66) for His 20His; OR = 1.66 (95% CI = 1.04–2.64) for Gly 51Ser; and OR = 1.67 (95% CI = 1.05–2.66) for Pro 57Pro.The results indicate that three *PNP* variants render participants susceptible toward developing arsenic-induced skin lesions [75].

Discussion and conclusion

Chronic arsenic toxicity (arsenicosis) as a result of drinking arsenic-contaminated groundwater is a major environmental health hazard throughout the world, including India. A lot of new information is emerging from extensive research on health effects of chronic arsenic toxicity in humans during the last two decades. Pigmentation and keratosis are the specific skin lesions characteristic of chronic arsenic toxicity. Of the various systemic manifestations, chronic lung disease, peripheral neuropathy, and chronic liver disease appeared to be the major causes of morbidity reported by most of the investigators from different parts of the world [76]. A systematic review of the epidemiological evidence on arsenic exposure and

cardiovascular disease showed that methodological limitations restricted the interpretation of the moderate to strong association between high arsenic exposure and cardiovascular outcome in Taiwan. In other populations and in occupational setting, the evidence was inconclusive [77]. There is consensus in all the studies on pregnancy outcome with the finding that stillbirth occurs in significantly higher number of cases in pregnant women with chronic arsenic exposure. There is sufficient epidemiological evidence to incriminate arsenic as an important cause of cancers of skin, lungs, and urinary bladder [3].

Arsenic causing genotoxicity as a result of drinking arsenic-contaminated water has been extensively studied in West Bengal. Chromosomal aberration and increased frequency of micronuclei in different cell types have been found to be significant. Further studies are needed to ascertain whether these could be used as biomarker of chronic arsenic toxicity. Various probable mechanisms have been incriminated to cause DNA damage because of chronic arsenic toxicity and have been correlated with disease manifestations. However, further studies are needed to establish specific genotoxic effects of arsenic in causing cancer. There appears to be no consensus with the various findings of genetic polymorphism and disease manifestation.

The key approach for addressing the arsenic problem is to provide scientifically correct information to the people at risk and develop comprehensive water-quality surveillance system. The most important step is to arrange for identification of arsenic-contaminated tube wells and make the people aware of not drinking arsenic-contaminated water. Management of health effect because of arsenic toxicity requires an integrated approach of collaboration of health personnel of Ministry of Health and engineering personnel of Public Health Engineering Department of the government. For its mitigation in a developing country, such as India, assistances may also be required from nongovernmental organizations and international agencies, for example, World Health Organization, United Nations Children's Fund, and World Bank. Research on epidemiology, including determination of disease burden in the population, confounding factors for variability in disease manifestation, standardization of proper disease management protocol, and arsenic in food chain and human health are some of the issues that require further attention.

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