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ORIGINAL ARTICLE

Arsenic-induced toxicity and carcinogenicity: a two-wave cross-sectional study in arsenicosis individuals in West Bengal, India

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In the state of West Bengal in India, over 26 million individuals are exposed to arsenic via drinking water. Dermatological, non-dermatological disorders and cancers are associated with arsenic toxicity. Of late, there has been a decrease in the arsenic concentration in drinking water owing to governmental efforts, raising the possibility of remediation. A cross-sectional study was conducted, where 189 arsenicosis and 171 unexposed individuals were recruited at two time points, (2005–06 and 2010–11) with concomitant decrease in the level of arsenic exposure via drinking water in the arsenicosis group in 2010–11. Parameters studied included dermatological, non-dermatological health status and cytogenetic damage. Decrease of arsenic exposure (190.1 μg/l to 37.94 μg/l) resulted in significant decline in the number of individuals having dermatological disorders ($P<0.01$) and in the severity of each dermatological outcome ($P<0.0001$). Micronucleus formation in urothelial cells and lymphocytes decreased significantly ($P<0.001$). However, there was a significant ($P<0.001$) rise in the incidence of each of the non-dermatological diseases, that is, peripheral neuropathy, conjunctivitis and respiratory distress over the period. Thirteen (6.87%) of the initially recruited arsenicosis individuals died of cancer, in this period. Remediation by arsenic-safe drinking water can reduce dermatological manifestations and cytogenetic insult; but is unable to counter the non-dermatological symptoms.

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INTRODUCTION

Chronic arsenic toxicity is a pandemic concern, affecting more than 150 million individuals in 70 countries that includes Bangladesh, Japan, Mexico, Argentina, USA and so on.[1–3] In the state of West Bengal in India, more than 26 million individuals are affected by drinking arsenic-contaminated ground water, which is well above the current maximum permissible level of 10 μg/l, stipulated by the World Health Organization.[4] Characteristic skin lesions and skin pigmentation patterns are recognized as the hallmarks of chronic arsenicosis. Several investigations over the years have associated various forms of skin pigmentation such as raindrop hypo-pigmentation as well as hyper-pigmentation and palmo-palmer hyper-keratosis with chronic arsenic toxicity.[5] However, these skin lesions often appear after a latency period of 6 months up to 10 years or more from the initial exposure and the dictum of arsenic susceptibility in humans is often determined by individual genetic variability (NRC[6]). Hence, a reliable biomarker for both early screening and chronic exposure are the cytogenetic damage indicators such as micronucleus (MN).[7–11] Apart from the arsenical skin lesions, arsenic shows its toxic effects by leading to the development of chronic lung diseases that include chronic bronchitis, chronic obstructive pulmonary disease, liver diseases such as non-cirrhotic portal fibrosis and other diseases such as poly-neuropathy, peripheral vascular disease, hypertension and ischaemic heart disease.[5,10,12] Several studies have also found association of persistent conjunctivitis with prolonged arsenic consumption.[10] In addition, investigations have demonstrated association between arsenic ingestion with anaemia, solid oedema of the legs, liver fibrosis, gangrene of the toes (Blackfoot disease) and neuropathy.[13] Cancers of skin, lung, liver, bladder, kidney and prostate have also been shown to be associated with chronic arsenic consumption.[14–18] The time for development of symptoms and degree of complexity as well as severity depends on the length of exposure as also on the concentration of arsenic ingested, health, nutritional status as also the genetic composition of the individuals exposed.[19]

At present, providing arsenic-free drinking water is considered to be the best remedial measure to combat arsenic toxicity, as medications for this purpose, are yet to be developed. Consequently, to cope up with this massive onslaught of arsenic exposure and resulting mass toxicity, the authorities in India have begun adopting large-scale mitigation procedures to combat this deadly menace. As a result, in several areas, the high arsenic-contaminated water used for drinking purposes have been replaced by safer options, bringing down considerably the arsenic load from drinking water sources. We had observed a very high

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incidence of cytogenetic damage as measured by micronuclei formation in both lymphocytes and urothelial cells and also from dermatological symptoms and three major non-dermatological health effects, that is, peripheral neuropathy, respiratory disorders and eye problems. As, in the region of our study population, the mean arsenic content in the drinking water was nearly five times less in 2010–11 compared with 2005–06; our objective was to analyse whether consumption of lower load of arsenic through drinking water ameliorates the cytogenetic damage, dermatological and non-dermatological health condition, which when previously observed in 2005–06, was grim. In 2010–11, 37.94 μg/l of arsenic through drinking water was consumed by our study population. Although this mean is higher than the WHO recommendation,[20] our objective is to find out the ameliorative effects due to lowering of the arsenic load in drinking water, in a population having a history of chronic arsenic exposure.

Study Design

Individuals having a history of arsenic exposure through drinking water are referred as exposed population; whereas individuals with no history of arsenic exposure above the threshold (10 μg/l) are termed as unexposed, control population. The exposed study participants were recruited from three highly exposed villages of Murshidabad district[21] and it was also in accordance to the areas, based on the database (2002–2006) of ground water arsenic contamination obtained from the online registry (http://www.wbphed.gov.in) of West Bengal Public Health Engineering Department (WBPHED), Government of West Bengal. The unexposed participants were recruited from East Midnapore, one of the unaffected districts of West Bengal, India. The sample collection strategy is elaborated in Figure 1.

Initially, trained non-physician volunteers were sent to the affected villages to screen individuals with probable arsenic-induced skin lesions.[17,22] The initial screening identified 2012 individuals. A thorough screening was conducted by expert physicians and subsequent samples were collected after a written consent and its analysis finally considered 189 arsenic-exposed individuals with skin lesions, for the study in 2005–06. The screening procedure for the exposed individuals with skin lesions is illustrated in Figure 1. From the unexposed population, 171 individuals were recruited and clinical and demographic data were recorded in a comparable manner with respect to the socio-economic status of the study participants from the two populations.

The same study population was again monitored in a similar manner in 2010–11 eliciting all the demographic and clinical data for a second time for each study participant. However, at this point in time, in the exposed population, 13 individuals had already died owing to cancer and hence, the final exposed population consisted of 176 only for whom the data were available at both the time points. The unexposed population consisted of 171 individuals initially, of whom 1 died. Hence, 170 of the initially recruited 171 individuals were followed up. This study was conducted in accord with the Helsinki II Declaration and was approved by the institutional ethics committee.

METHODS

Chemicals and Instruments

RPMI-1640 media was purchased from Himedia (India), Cytochalasin B, Giemsa stain powder were purchased from Sigma chemical company (St. Louis, MO, USA). Potassium chloride, trisodium citrate and sodium chloride were purchased from Qualigens Fine Chemicals (India). Glacial acetic acid, methanol, hydrochloric acid, sodium borohydrate, nitric acid were purchased from E. Merck (India). A PerkinElmer model Analyst 700 was used for atomic absorption spectrometry.

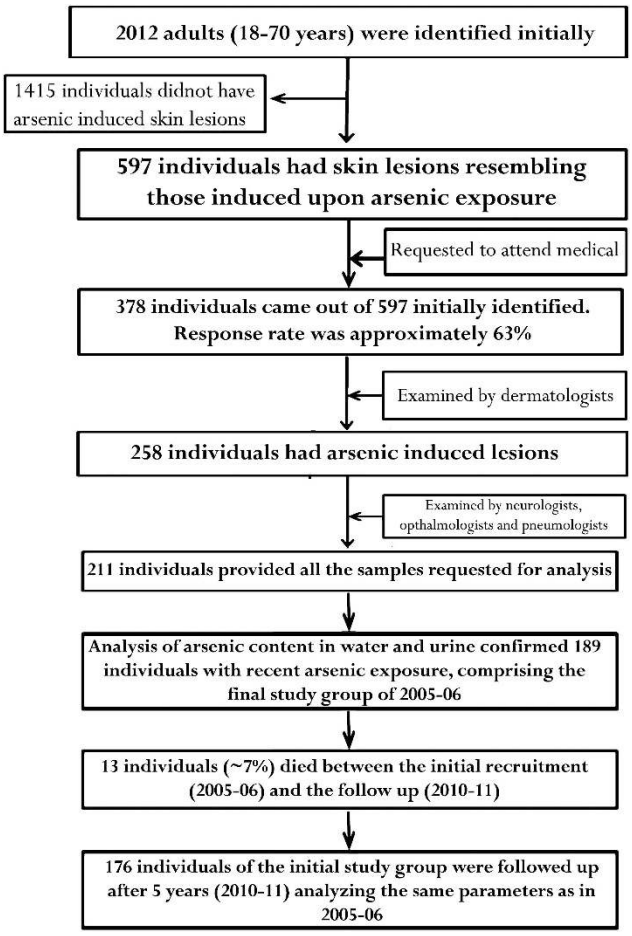


Figure 1. Sampling strategy of arsenicosis individuals in 2005–06 and followed up in 2010–11.

Identification of Eye Problems

The eye problems checked by the ophthalmologist in the field include eye irritation, redness and watering that mostly lead to conjunctivitis in the individuals consuming high concentrations of arsenic, which was also observed previously.[10] Cases having a medical history of conjunctivitis due to bacterial infections, virus or allergens were not considered in the study.

Identification of Neurological Symptoms

Symptoms that were considered to arsenic-induced neuronal problems were muscle cramps, numbness, weakness, pain as well as paraesthesias in stocking and glove distributions. The various types of clinical manifestations were recorded by the neurologist team including the power and deep tendon reflexes, calf tenderness, pressure and pain as detailed previously.[10] The participants identified clinically to have neuropathy were brought to the National Neuroscience Centre, Kolkata for confirmatory electrophysiological studies such as nerve conduction velocity test (NCV) and electromyography (EMG).

Identification of Respiratory Diseases

Chronic ingestion of arsenic results in pulmonary problems such as persistent coughs, thoracic sounds, throat irritation and loss of breath, hoarseness of the voice due to arsenic-induced laryngitis are some of the common symptoms observed in arsenic-exposed individuals.[10] For an unbiased analysis, subjects with an individual or family history of bronchitis, seasonal cough and asthma, which could be attributed to other aetiological factors, were not considered.

Table 1. Demographic characteristics of the study population at two different time points (year 2005–06 and 2010–11).

Parameters	2005–06		2010–11	
	Unexposed	Exposed	Unexposed	Exposed
Participants	171	189	170	176
Age in years (mean ± SD)	34.76 ± 9.52	34.62 ± 12.93	39.05 ± 9.08	38.65 ± 13.09
Gender (N*(%)*)				
Male	88 (51.46)	94 (49.74)	87 (51.17)	85 (48.29)
Female	83 (48.54)	95 (50.26)	83 (48.82)	91 (51.71)
Tobacco consumption (N*(%)*)				
Male				
Smoking (bidi/cigarette)	40 (45.45)	35 (37.23)	41 (47.13)	32 (37.65)
Chewing tobacco (pan)	17 (19.31)	12 (12.76)	21 (24.14)	13 (15.29)
Both	6 (6.82)	10 (10.64)	7 (8.04)	8 (9.41)
Female				
Smoking (bidi/cigarette)	6 (7.23)	4 (4.21)	8 (9.64)	4 (4.39)
Chewing tobacco (pan)	28 (33.73)	25 (26.31)	30 (36.14)	27 (29.67)
Both	0	2 (2.10)	1 (1.20)	2 (2.19)
Arsenic content (mean ± SD)				
Drinking water (μg/l)*,**	4.13 ± 3.18	190.1 ± 110.53	3.7 ± 3.0	37.94 ± 27.08
Urine (μg/l)*,**	27.62 ± 21.74	274.87 ± 149.83	24.8 ± 13.9	121.9 ± 94.93
Nail (μg/g)*,**	0.46 ± 0.24	3.24 ± 1.86	0.44 ± 0.23	0.99 ± 0.53
Hair (μg/g)*,**	0.29 ± 0.18	1.90 ± 1.29	0.37 ± 0.21	0.82 ± 0.46

*P-value < 0.001 unpaired two-tailed t-test between exposed and unexposed of same time period (year 2005–06 or 2010–11).
**P-value < 0.0001 unpaired two-tailed t-test between datasets of same groups (exposed *vs* exposed and unexposed *vs* unexposed) at two time points (year 2005–06 and 2010–11).

Sample Collection and Exposure Analysis

Nitric acid (1 ml/l) was added as preservative to the 100 ml of drinking water, collected from each of the study participants. Mid-stream first morning urine void (about 100 ml) was also collected from each of the participants for recent arsenic exposure estimation. The samples were brought in ice and stored in $-20\,^{\circ}C$ till the analysis was carried out. For determination of the amount of arsenic exposure before the sample collection time point, nail (~500 mg) and hair (~400 mg) were collected. Arsenic estimation was conducted using a PerkinElmer Spectrophotometer, Model Analyst 700 in our laboratory, at Indian Institute of Chemical Biology, Kolkata. The quality control and details of the procedure are as described previously.[10]

Urothelial MN Analysis

The urothelial MN assay was carried out following previously described protocol.[23] Briefly, urothelial cells were recovered after centrifuging the urine samples at 1000 r.p.m. for 10 min. The density of the cells spun down was analysed by phase contrast microscope and adjusted accordingly, bringing the density to 1.5–2.0×10^6 cells. The pellet suspension was fixed using Carnoy's fixative on glass slide and stained with Giemsa and observed under the microscope. A total of 2000 cells per slide were scanned to obtain the micronuclei count index per 1000 cells, as described earlier.[23] Two slides per sample was scored by trained individuals, blind to the exposure status of the samples.

Lymphocyte MN Analysis

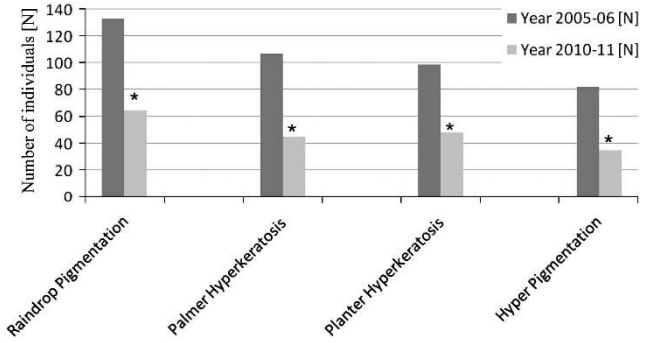
Venous blood samples (5 ml) were obtained from the study participants and whole-blood lymphocyte culture was carried in RPMI-1640 (Himedia). At least 2000 binucleated cells, following the standard specifications,[23] were scored for each coded slides and the MN frequency was expressed as per 1000 cells. Two trained individuals scored each slide, blind to the exposure status of the samples.

Statistical Analyses

For the two-wave longitudinal study, unpaired two-tailed t-test, was performed to compare the parameters between exposed and unexposed categories of a particular time point. Again, paired t-test was performed to compare the parameters within exposed as well as within the unexposed individuals at two time points (initial data set at 2005–06 and follow-up data set at 2010–11). Analysis of effect of age on the development of non-dermatological health disorders was concluded using standardized morbidity ratio (SMR). SMR helps in normalizing a study outcome (especially death) taken at two time points, taking age or sex into consideration. Mathematically, the ratio is obtained between "observed number of individuals" by "expected number of individuals" in the taken study population, within a particular age- or sex- group. If the ratio is >1.0, then there is an increase of occurrence of the outcome and *vice versa*. SMR is one of the best tool that gives an analytical view of the change in outcome, in a varying subset of population based on the age- or sex-specific parameter. The statistical significance was calculated based on the guidelines provided by New Mexico's Indicator- Based Information System (NM-IBIS).[24] The SMR analysis for non-dermatological health effects was conducted of the same individuals of 2005–06 who were alive during the follow-up study in 2010–11. Risk evaluation was done by calculating point estimates, that is, odds ratio (OR) and 95% confidence intervals (CI). Alteration in the health status such as conjunctival irritation, melanosis and keratosis, respiratory problems and peripheral neuropathy in follow-up study (2010–11) was compared with the initial study (2005–06) was analysed using Chi-Square test in GraphPad InStat (San Diego, USA) software.

RESULTS

The demographic characteristics at both the time points are summarized in Table 1. Both the exposed and unexposed groups were well matched for gender and age, showing no significant



* *p value* <0.01, Chi-squared test

Figure 2. Distribution of various arsenic-induced skin lesions in the individuals exposed to arsenic at two different study periods.

difference ($P > 0.05$). The number of individuals using tobacco in form of smoking (*bidis* and cigarettes) and chewing (*pan* and *pan masala*), did not have any significant variation among the study populations at any time point ($P > 0.05$). The arsenic content in the drinking water, urine, nail and hair were significantly higher ($P < 0.0001$) in the exposed individuals when compared with the unexposed individuals at both the time points. The arsenic content in water, urine, nail and hair in unexposed group were similar at both the time points, although there were a significant decrease in all of those exposure parameters in the exposed groups in 2010–11, compared with that in 2005–06.

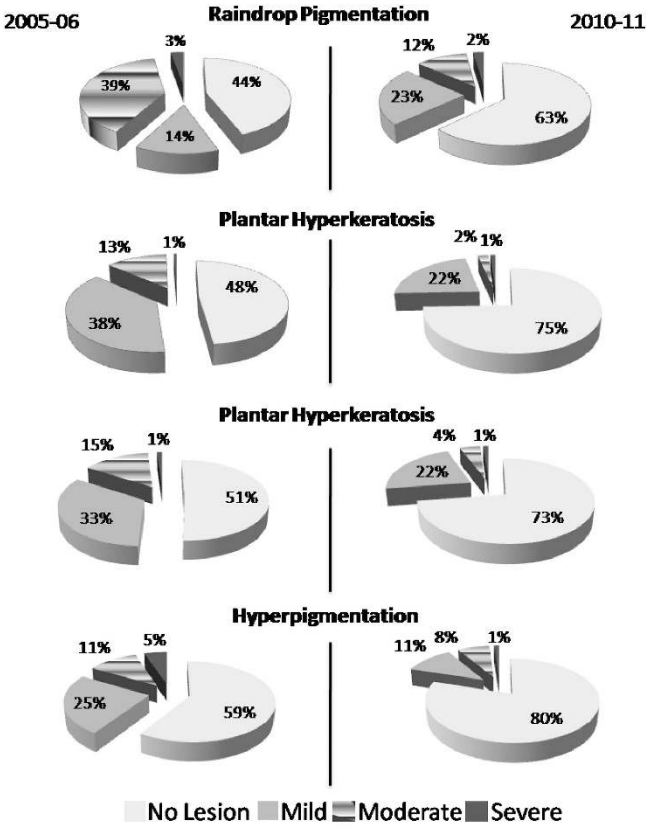
Figure 2 illustrates the distribution of the various types of skin lesion in the exposed population at the two different time points. The result reveals that, in 2010–11, there was a significant ($P < 0.01$) decline in the occurrence of each kind of dermatological lesion studied, compared with 2005–06. The percentage comparison between the follow-up cohorts in 2010–11with the parent population in 2005–06 revealed a 38.7% decline in the raindrop pigmentation, 35.2% and 28.9% decline in the palmer and plantar hyperkeratosis, respectively, while 26.9% decline in the hyperpigmentation outcome in 2010–11 compared with 2005–06.

In addition, there was a significant decrease in the severity of all skin lesion types studied as is evident from the steady increase in the number of individuals in the low-severity groups ($P < 0.0001$) in 2010–11 compared with 2005–06 as depicted in Figure 3.

Table 2 elaborates the effect of decrease in arsenic exposure towards cytogenetic damage as measured by MN count of urothelial cells and the lymphocytes. There is a significant decrease ($P < 0.001$) in MN count in exposed individuals in 2010–11 when compared with 2005–06 in both the end points measured. Nevertheless, there was no significant alteration in the basal level of genetic damage in unexposed individuals over the period of time. The amount of cytogenetic damage as indicated by the MN frequency, induced by chronic arsenic exposure, was significantly higher than in case of unexposed individuals at both time points.

This study documents the death among arsenicosis individuals. The number of deaths among the initially recruited study cases was 13, with an average age of 44 years, which accounts to a death percentage of 6.87% and mostly due to skin cancer (Table 3). In comparison, only one individual among the unexposed died due to natural cause, in this period of study.

Table 4 reflects the general depreciation in the non-dermatological health outcomes in arsenic-exposed individuals. The SMR for the three non-dermatological health disorders (peripheral neuropathy, conjunctival irritation and respiratory disorders) studied, was statistically significant. The SMR (95% CI) for peripheral neuropathy was calculated to be 1.77 (1.36, 2.19), conjunctival irritation was 1.47 (1.17, 1.77) and respiratory ailment was 1.53 (1.13, 1.92). The unexposed individuals were also



p value < *0.01,* Chi Squared Test.

Figure 3. Change in the severity of skin lesions in arsenic-exposed individuals over this study period due to decrease in the arsenic exposure.

Table 2. Change in micronucleus (MN) frequency in urothelial cells and lymphocytes of individuals from arsenic-exposed and -unexposed areas over the period of study.

Study group (*N*)	Year 2005–06 (MN/1000 cells) mean ± SD	Year 2010–11 (MN/1000 cells) mean ± SD
Urothelial		
Exposed (176)	5.97 ± 1.52*	1.95 ± 1.10**,***
Unexposed (170)	1.45 ± 0.51	1.42 ± 0.57†
Lymphocyte		
Exposed (159)	11.11 ± 2.5*	3.55 ± 1.48**,***
Unexposed (156)	2.39 ± 0.81	2.06 ± 0.81†

**P*-value < 0.0001; unpaired *t*-test between exposed and unexposed individuals in the year 2005–06.
***P*-value < 0.0001; paired *t*-test between exposed individuals between two time points (year 2005–06 and 2010–11).
****P*-value < 0.0001; unpaired *t*-test between exposed and unexposed individuals in the year 2010–11.
†*P*-value > 0.5; paired *t*-test between unexposed individuals between two time points (year 2005–06 and 2010–11).

subjected to the SMR calculation in the similar manner and it showed insignificant alteration in between 2005–06 and 2010–11. For the above three mentioned non-dermatological ailments, the unexposed controls had an SMR (95% CI) of 0.85 (0.59, 1.12), 1.00 (0.78, 1.22) and 1.00 (0.72, 1.28), respectively.

Table 3. Details of the cancer-induced death in arsenic-exposed individuals in between the two study periods.

Cancer type	Number of individuals		Arsenic content of all cancer deaths considered in the study (mean frequency ± SD)				Micronucleus[a] (mean frequency ± SD) $N = 13$ (MN/1000 cells)	
	Male	Female	Water (μg/l)	Urine (μg/l)	Nail (μg/g)	Hair (μg/g)	Urothelial	Lymphocyte
Skin cancer	5	3	198.35 ± 126.3	308.74 ± 115.67	3.97 ± 1.67	2.08 ± 1.52	6.07 ± 2.37	10.27 ± 3.57
Lung cancer	3	0						
Liver cancer	0	1						
Bone cancer	1	0						
Mean age at death (years)	4.25 ± 8.54						44.26	44.27

[a]Data were recorded in the year 2005–06 for the individuals who died from cancer death during the study period.

Table 4. Age-adjusted rate of non-dermatological health effects for the exposed individuals in 2010–11 compared with 2005–06.

Age-specific (in years)	Individuals				Expected in 2010–11[a]	Standardized morbidity rate (observed/expected in 2010–11)	SMR (95% CI)
	2005–06		2010–11				
	Total	Observed	Total	Observed			
Peripheral neuropathy							
<20	25	3	11	3	1	3.00	1.77 (1.36, 2.19)
20–29	43	13	34	14	10	1.40	
30–39	51	9	52	17	9	1.89	
40–49	48	14	49	25	14	1.78	
50–59	13	2	18	7	3	2.33	
60	9	2	12	5	3	1.67	
Conjunctival irritations							
<20	25	10	11	6	4	1.50	1.47 (1.17, 1.77)
20–29	43	14	34	18	11	1.64	
30–39	51	18	52	23	18	1.28	
40–49	48	23	49	32	23	1.39	
50–59	13	3	18	3	4	2.25	
60	9	3	12	6	4	1.50	
Respiratory problems							
<20	25	5	11	2	2	1.00	1.53 (1.13, 1.92)
20–29	43	7	34	16	6	2.67	
30–39	51	8	52	13	8	1.62	
40–49	48	12	49	14	12	1.67	
50–59	13	4	18	7	6	1.67	
60	9	3	12	6	4	1.50	

[a]Indicates the number of individuals in 2010–11 who theoretically should have the disease outcome similar to the trend observed in the age-specific group in 2005–06. It is obtained by

$$\text{SMR} = \frac{\text{Observed number of cases (2005}-\text{2006)}}{\text{total individuals in the agespecific subgroup (2005}-\text{2006)}} \times \text{total individuals in the age}-\text{specific group (2010}-\text{2011)}$$

Table 5. Risk of the development of non-dermatological health effects at two time points.

Non-dermatological health outcomes	Year	OR	95% CI
Peripheral neuropathy	2005–06	9.08	3.48, 23.72
	2010–11	18.48	7.75, 44.06
Conjunctivitis	2005–06	11.15	4.91, 25.32
	2010–11	20.51	9.84, 42.72
Respiratory disorders	2005–06	6.07	2.47, 14.95
	2010–11	11.45	5.04, 25.97

Abbreviations: CI, Confidence Interval; OR, Odds Ratio.

True to the expectation, within the exposed group, there was a significantly higher risk of the development of non-dermatological health effects when compared with the unexposed individuals. It was found that there was a significant rise in non-dermatological outcomes in 2010–11 compared with 2005–06 (Table 5).

DISCUSSION

Chronic exposure to arsenic induces several dermatological and non-dermatological clinical manifestations including cancers of the skin and internal organs such as lung, liver, bladder and kidney.[5] In the present study, the demographic characteristics of exposed and unexposed individuals, such as age, gender and

tobacco consumption were well matched (Table 1) and hence, the outcome of our study truly portrays arsenic manifestations and subsequent decrease thereof.

Biomarker, in the form of MN induction is long considered to be one of the most versatile tools to determine the degree of genetic toxicity and particularly in case of arsenic toxicity.[11] In the present study, we have found that, with decrease in arsenic exposure there was a concomitant statistically significant decline in the MN count, when compared over the period of time. As evident from Table 2, there was a 3-fold decrease of the MN frequency in urothelial cells, whereas a 2.5-fold decrease in lymphocytes, in 2010–11 when compared with the results of 2005–06. Thus, it can be inferred that removal of arsenic exposure can lead to the reversal of the induction of at least some types of genetic damage at the cellular level. The genesis of MN is thought to be incurred via DNA backbone damage caused probably by generation of certain reactive oxygen species (ROS), as a result of arsenic metabolism;[25,26] which lowered due to consumption of lesser concentration of arsenic.

In addition, we find corroborating evidence of decrease in arsenic exposure leading to the alleviation of arsenic-induced dermatological disorders. Our data show a significant decrease in the number of exposed individuals exhibiting each type of arsenic-specific dermatological symptoms (Figure 2). There was also a significant decrease in the severity levels of each of these arsenic-induced dermatological symptoms (Figure 3). This might be a very significant observation, keeping in mind that these individuals have started using arsenic-free water for drinking purposes only for a short duration, compared with the time period for which they were continually exposed to this toxicant. Hence, it would only be logical to expect that with further decrease in the drinking water arsenic concentration to below 10 μg/l, and with longer duration of use of potable water, the incidence as well as the severity of these arsenic-induced skin lesions might be further reduced drastically, along with the amelioration of the genotoxic insult to a basal level.

Anomalously though, in spite of consumption of lesser amount of arsenic; we found that the non-dermatological health effects showed an increased risk of development with time. The SMR (95% CI) indicates the increased susceptibility of the exposed individuals towards the non-dermatological health outcomes, namely, peripheral neuropathy (1.77 (1.36, 2.19)), conjunctival irritation (1.47 (1.17, 1.77)) and respiratory disorders (1.53 (1.13, 1.92)), in an age-specific manner. However, there was no appreciable change in the incidence of these non-dermatological disorders in unexposed population at the two time points. From Table 4, we find that for each age group, when reconsidered within the scope of SMR, the exposed group showed a higher number of cases for respective non-dermatological health outcomes when compared with the expected outcomes. But, this was not so for the unexposed controls. From the age-adjusted SMR analysis (Table 4), we can interpret that arsenic exposure itself has a predominant role for adverse non-dermatological health outcomes.

In addition, we observed that 13 (6.87%) of the initially recruited 189 exposed individuals had died before the follow-up sampling in 2010–11. Interestingly, these individuals who died of cancer had an average age of 44.25 ± 8.54 years at death, which is far below the normal life expectancy of Indian population, which is around 63.4 years (SRS Database, Registrar General of India, 2003).[27] Data reflects that the maximum individuals succumbed with skin cancer, which is only natural considering skin is the primary target organ for arsenic toxicity. On the contrary, there was only one death (0.58%) in the unexposed group, resulting from cardiac arrest and the age at death was 65 years. As per the epidemiological survey in 2005–06, it can be stated that all the 13 individuals who have died owing to different types of cancers had severe skin lesions of several types as well as significant cytogenetic damage as measured by MN assay from the two cell types. According to WHO criteria, all the 13 individuals were arsenicosis patients with a very high arsenic exposure status as reflected by the arsenic content in their water, urine, nail and hair samples (Table 3). All these evidence strongly suggest that the individuals who died within this period of time were mainly owing to arsenicosis. MN formation is not only a classical biomarker for a population exposed to arsenic but also a potent tool for monitoring susceptibility of arsenic-induced dermatological cancers.[11]

Hence, life expectancy in humans decreases owing to arsenic exposure, which has also been reported earlier.[28] This severe health effects are due to the fact that this particular study population were exposed to very high concentration of arsenic through drinking water for more than 10 years. Life expectancy is a very potent tool to monitor the health of a population.[29] The data, although on a small scale, does imply that the life span is shortened for exposed individuals. Thus, it seems that once the arsenic-induced physiological damage crosses a certain threshold, even removal of exposure may not always be enough to prevent eventual casualty.

The underlying molecular basis of arsenic-induced clinical manifestations is not well understood. Literature suggests the pronounced effect of arsenic upon the lipoic acid metabolism, inhibiting pyruvate dehydrogenase enzyme.[30] This in turn slows the production of energy units (i.e adenosine tri-phosphate, ATP) within the mitochondria. A study conducted by our group[31] has shown the influence of arsenic on mitochondrial instability, leading to cell death. As mitochondrial dysfunction, under stress have been associated with neurotoxicity,[32] we can propose the route of development of certain types of peripheral neuropathy that are associated with mitochondrial instability[33] in arsenic-exposed population. In lung cancer, arsenic has been associated with activation of caspase cascade along with mitochondrial dysfunction.[34] Hence, the frequency of respiratory tract irritations, shortness of breath, hoarseness of voice and so on,[10] related to arsenic-exposed individuals may be due to these cellular wasting. Redness of the eye, frequent watering and conjunctival irritations are commonly found in the arsenic-exposed individuals.[10] *In vitro* studies on laboratory mice have shown development of optic disorders such as anophthalmia,[35] that can cause such recurrent conjunctival irritations. The population studied here were exposed to high concentration of arsenic in their drinking water for >10 years. In our study period, although there was a significant reduction of arsenic in water but still it was much higher than the permissible limit recommended by WHO (10 μg/l; 1996). So, it may appear that over the period of time of exposure, the non-dermatological health effects increased, as the arsenic exposure was not reduced to international standard. This may be the reason of having more incidence of peripheral neuropathy and other non-dermatological health effects observed in 2010–11 compared with 2005–06.

Risk assessment is an effective tool to monitor the risk management due to any environmental toxicant and determine the maximum permissible limit for their interaction with humans.[36] Drinking arsenic-free water is a widely practised method to curb the development of arsenicosis. But, the results from this particular cross-sectional study shows that considerable decrease of arsenic content in the drinking water corresponds to a decrease in the severity of arsenic-specific dermatological symptoms, as also cytogenetic damage significantly. However, this reduction of arsenic in drinking water failed to reduce the non-dermatological health effects. Therefore, our study demonstrates the acute necessity for the development of curative measures in conjunction to reduction of arsenic in drinking water as an ideal mitigation strategy for chronic arsenic toxicity.

ABBREVIATIONS

ATP, Adenosine tri-phosphate; CI, Confidence interval; EMG, Electromyography; MN, Micronucleus; NCV, Nerve conduction velocity; NM-IBIS, New Mexico's Indicator-based Information

System; OR, Odds ratio; ROS, Reactive oxygen species; SEARO, South East Asia Regional Office; SMR, Standardized morbidity ratio; WBPHED, West Bengal Public Health Engineering Department; WHO, World Health Organization

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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