Int. J. Cancer: **123,** 283–287 (2008)

DNA repair deficiency leads to susceptibility to develop arsenic-induced premalignant skin lesions

Mayukh Banerjee[1], Nilendu Sarma[2], Rupanwita Biswas[3], Jyoeeta Roy[1], Anita Mukherjee[3] and Ashok K. Giri[1]*

[1]*Molecular and Human Genetics Division, Indian Institute of Chemical Biology, Kolkata, India*
[2]*Department of Dermatology, Nil Ratan Sircar Medical College and Hospital, Kolkata 700 014, India*
[3]*Centre of Advanced Study, Department of Botany, University of Calcutta, Kolkata, India*

In West Bengal, India, although more than 6 million people are exposed to arsenic through drinking water, only 15–20% showed arsenic-induced skin lesions, including premalignant hyperkeratosis. This indicates toward some factors that confer susceptibility to arsenic-induced carcinogenicity. In this work, we wanted to explore whether differences in DNA repair capacity could impart arsenic-induced carcinogenicity, through Comet assay, chromosomal aberration (CA) assay and challenge assay. Sixty arsenic exposed (30 individuals with arsenic-induced premalignant hyperkeratosis and 30 without skin lesion, but drinking similar arsenic contaminated water) and 30 arsenic unexposed individuals were recruited as study participants. Alkaline comet assay, and challenge assay were carried out in whole blood and CA study in lymphocytes to find out the DNA damage and DNA repair capacity in both hyperkeratotic and without skin lesion individuals. DNA damage as well as CA were found to be significantly higher in the arsenic-exposed individuals compared to unexposed individuals ($p < 0.001$). Within the exposed group, there was no significant difference as far as the level of DNA damage is concerned ($p > 0.05$), but CA was significantly higher in exposed individuals with hyperkeratosis than exposed individuals without hyperkeratosis ($p < 0.01$). Challenge assay showed that upon induction of DNA damage, the repair capacity in the exposed individuals with premalignant hyperkeratosis is significantly less ($p < 0.001$) than that of individuals without skin lesion, although the basal level of DNA damage was similar in both. Thus, the deficiency in DNA repair capacities in the hyperkeratotic individuals emerges as a prime contender for arsenic carcinogenicity.

Key words: arsenic susceptibility; premalignant hyperkeratosis; challenge assay; DNA damage; DNA repair

In West Bengal, India, ground water of 9 districts is contaminated with arsenic, concentrations ranging from 52–1055 μg/l, much higher than the current permissible maximum contamination level.[1,2] More than 6 million people are exposed to arsenic in these districts. This is considered as the greatest arsenic calamity in the world. Long-term exposure to arsenic-contaminated drinking water causes a wide array of adverse health effects, principally, characteristic dermatological symptoms like raindrop pigmentation, palmo-plantar hyperkeratosis and hypo and hyperpigmentation and might ultimately lead to cancer of skin, lung and/or bladder.[3] Hyperkeratosis of skin is considered as a precursor of arsenic-induced skin cancer,[4,5] as skin cancers often appear at the sites of existing hyperkeratosis.[6] A strong relationship between arsenic levels in water with the prevalence of hyperkeratosis in the exposed individuals has been reported earlier.[7] Additionally, chronic arsenic exposure might also cause vascular diseases, conjunctivitis in the eyes, neuropathy, lung diseases and nonmelanocytic cancer of skin and different internal organs.[7] Anomalously enough, of the huge number of people exposed to arsenic-contaminated drinking water, only 15–20% exhibit arsenic-specific skin lesions.[8] This fact indicates that some factors must be present that confer susceptibility to certain persons to arsenic toxicity and carcinogenicity.

What makes a person susceptible to arsenic carcinogenicity has been a matter of much speculation and remains poorly understood until today. One of the most potent ways by which arsenic exerts its toxic effect is the induction of DNA damage *via* the production of reactive oxygen species (ROS).[9] Literature shows that arsenic can cause DNA damage *in vivo*[10] as well as *in vitro*.[11] This is well supported by cytogenetic studies, which show that arsenic exposure leads to increased incidence of micronuclei as well as chromosomal aberrations (CAs) in peripheral blood lymphocytes.[12,13] Our group has long been involved in deciphering the probable factors that might lead to arsenic susceptibility.[8,14,15] It has been shown that in many cases, DNA repair pathway genes are implicated[14,15] in the development of premalignant hyperkeratoses. Also, it is well known that arsenic and its metabolites can depress DNA repair mechanism.[16] These observations led us to hypothesize that, if the DNA repair capacity is suboptimal in some individuals, then they might continually keep on accumulating DNA damage brought about by arsenic, and its metabolites. Ultimately, in the long run, these individuals might exhibit premalignant hyperkeratosis, which are considered to be the hallmarks of arsenic toxicity. As a result, these individuals run a high risk of development of skin cancers at later stages. Hence, status of DNA repair capacity might be a key player in susceptibility to develop arsenic-induced precancerous skin lesions. Consequently, in the present work, we have aimed to find out the DNA damage status of each of the 2 exposed groups (individuals with hyperkeratosis and individuals without arsenic-induced skin lesions), and also the unexposed group by comet assay and CA assay. To clarify further whether or not DNA repair capacity plays any role, we have checked the DNA repair capacity in the 2 exposed groups (with hyperkeratosis and without skin lesions) by means of challenge assay.

Material and methods

Study area and selection of subjects

Murshidabad, a severely arsenic-affected district was selected as our study site and the criteria for the selection of study subjects were described previously.[8,12] Physicians examined the study participants for detection of various types of arsenic-related skin lesions. Both individuals with and without skin lesions, drinking water, were selected as study participants. We recruited 60 exposed individuals; among which 30 had arsenic-induced premalignant hyperkeratosis and 30 without any skin lesion. Hyperkeratotic individuals were chosen irrespective of the presence of other arsenic-induced skin lesions. However, none of these subjects had skin cancer. All the study participants had been exposed to arsenic

Abbreviations: BER, base excision repair; CA, chromosomal aberration; DPC, DNA-protein crosslink; DSBs, double strand breaks; EDTA, ethylene diamine tetra acetic acid; FI-HG-AAS, flow injection-hydride generation-atomic absorption spectrometry; NER, nucelotide excision repair; OTM, olive tail moment; ROS, reactive oxygen species.

Grant sponsor: Council of Scientific and Industrial Research (CSIR), Govt. of India; Grant number: CMM-0003.

***Correspondence to:** Molecular and Human genetics Division, Indian Institute of Chemical Biology, 4 Raja S. C. Mullick Road, Jadavpur, Kolkata 700 032, India. Fax: +91-33-2473-5197 or 91-33-2472-3967.
E-mail: akgiri15@yahoo.com or akgiri@iicb.res.in.

Received 5 October 2007; Accepted after revision 18 January 2008
DOI 10.1002/ijc.23478
Published online 3 April 2008 in Wiley InterScience (www.interscience.wiley.com).

containing drinking water for a period of 10 years or more. Thirty unexposed subjects with no history of arsenic contamination in their drinking water and age group between 15 and 70 years were selected from the East Midnapur district. Water and other biological samples such as blood, urine, nail and hair were collected from all the participants. The selected subjects provided informed consent to participate and fulfilled the inclusion criteria.[12] Our study was conducted in accord with the Helsinki II declaration and approved by our institutional ethics committee.

Arsenic exposure assessment

Study participants were provided with acid-washed [nitric acid–water (1:1)] plastic bottles for collection of drinking water (~100 ml) samples, into which nitric acid (1.0 ml/l) was added as preservative.[12] First morning voids (~100 ml) were collected in precoded polypropylene bottles for arsenic estimation. Water, urine, nail (~250–500 mg) and hair (~300–500 mg) samples were collected, and arsenic was estimated as described earlier.[17] Flow injection-hydride generation-atomic absorption spectrometry (FI-HG-AAS) was used for estimation of arsenic content in different biological samples (urine, nail and hair) and drinking water, as described previously.[12] Quality control was looked after meticulously, as elucidated previously.[17]

Alkaline comet assay

Alkaline comet assay was performed after the protocol modified from that of ITRC.[18] Briefly, glass microscope slides were coated with 1% normal melting-point agarose and dried. When the slides were fully dried, 20 μl of peripheral whole blood was mixed with 100 μl of 1.0% low-melting agarose and 80 μl of PBS (pH = 7.4), so that, the final concentration of low-melting agarose was 0.5%. Eighty microliters of this sample was spread on the precoated slide (per slide) at 37°C. The slides were placed briefly on ice to allow the agarose to coagulate. Then, 80 μl of 0.5% low-melting agarose was spread on the slides containing cells embedded in an agarose matrix. The slides were placed in ice-cold lysis buffer (2.5M NaCl, 100 mM EDTA, 10 mM Trizma base, 10% DMSO, 1% Triton-X; pH = 10) and cells were lysed overnight. After lysis of the cells, the slides were placed in horizontal electrophoresis tanks filled with electrophoresis buffer (300 mM NaOH/1 mM EDTA, pH ≥ 13.0) and DNA in the cells was allowed to unwind for 20 min. The electrophoresis was then started and the slides were electrophoresed at 25 V, 300 mA for 30 min. After electrophoresis, the slides were neutralized in neutralization solution (400 mM Trizma base, pH = 7.5; 3 times 5 min each) and dried on a slide warmer. The dried slides were stained with ethidium bromide (2 μg/ml) and scored under a fluorescence microscope using the Komet 5.5 (Kinetic Imaging Ltd, Liverpool) image-analysis system. Two slides were used per individual and a total of 100 readings were recorded (50 from each slide). Olive tail moment (OTM), percent of tail DNA and tail length were taken as the parameters for determining the extent of DNA damage.

CA assay

From each subject, 5–7 ml venous blood was drawn and lymphocyte culture was carried out following standard protocol as described earlier.[8] Whole blood (0.7 ml) was added to 7 ml of RPMI-1640 supplemented with L-glutamine, 15% FCS, penicillin (100 IU/ml), streptomycin (100 μg/ml) and 2% PHA-M form. Duplicate cultures were maintained for each sample. The cultures were incubated at 37°C and harvested at 72 hr. All the slides of CA assay were coded, and from each individual subject, depending on the availability of good scoring metaphase plates, 50–100 metaphases were randomly scored for CA.[8] Gaps were not included in the aberrations per cell. Results were expressed as CA per cell and also as percentage of aberrant cells.

Challenge assay

Challenge assay (assessment of DNA repair capacity) was performed using whole blood from study subjects (with hyperkeratosis and without arsenic-induced skin lesions), using comet assay. Whole blood samples from 24 subjects (12 with hyperkeratosis and 12 without skin lesion) were divided into 2 aliquots to assess baseline DNA damage, as also the DNA repair efficiency upon γ-irradiation. The 2 groups were well matched as far as age, sex and exposure to arsenic are concerned (data not shown). Baseline damage in blood samples was estimated by means of alkaline comet assay, as described above, from 1 aliquot. The second aliquot was subjected to DNA damage by irradiation of 100 cGy γ rays.[19] These aliquots were then incubated at 37°C for 1 hr to allow for repair of damaged DNA. Finally, efficiency of DNA repair system was assessed by measuring the extent of damaged DNA after repair period of 1 hr, by conventional alkaline comet assay.[16]

Statistical analyses

Mean, median, standard deviation, 2-tailed *p*-values, one-way ANOVA with Tukey–Kramer Multiple Comparisons Post-Test and Kruskal–Wallis Test with Dunn's Multiple Comparisons Post-Test were calculated by using GraphPad InStat Software (Graphpad Software, San Diego).

Results

Demographic characteristics of the study population are summarized in Table I, which shows that all the 3 study groups are well matched as far as age, sex and smoking status are concerned. However, both the exposed subgroups (with hyperkeratosis and without skin lesions) have significantly higher environmental arsenic exposure (arsenic content in drinking water) as well as urinary excretion of arsenic compared to the unexposed group. However, within the exposed population, neither subgroup differs significantly either in environmental arsenic exposure (arsenic content in drinking water) or in urinary arsenic excretion.

Table II shows the DNA damage status of the 3 study groups. Here also, both the exposed subgroups (with hyperkeratosis and without skin lesions) have significantly higher DNA damage, as can be seen from significantly higher values of each of the 3 parameters, that is, OTM, percent of tail DNA and tail length ($p < 0.001$ in each case) compared the unexposed group. However, within the exposed population, there was no significant difference in any of these 3 parameters, in between the 2 subgroups, that is, with and without skin lesion groups ($p > 0.05$).

Table III depicts the CA status of the 3 study groups. Here also, both the exposed subgroups (with hyperkeratosis and without skin lesions) have significantly higher incidence of CA (both CA/cell and percent of aberrant cells) [$p < 0.001$] than the unexposed group. In this case, however, within the exposed group, group of individuals exhibiting arsenic-induced precancerous hyperkeratosis have significantly higher incidence of CA (both CA/cell and percent of aberrant cells) [$p < 0.01$] than the group of individuals without skin lesion.

Efficiency of DNA repair system in each of the 2 exposed subgroups (with hyperkeratosis and without skin lesions) has been shown in Table IV. There is no significant difference in the basal DNA damage level, in terms of each of the 3 parameters used (OTM, percent of tail DNA and tail length) [$p = 0.58$, $p = 0.80$ and $p = 0.90$, respectively] in between the 2 subgroups. However, significant difference in the level of damaged DNA in each of the 3 parameters used (OTM, % of tail DNA and tail length) [$p < 0.001$ in each case] can be seen in between the 2 subgroups after the induction of damage and subsequent repair.

Discussion

Arsenic is recognized as a carcinogen, whose chronic ingestion leads to multitude of multisystemic cancerous developments. The

TABLE I – DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION EXPOSED TO ARSENIC THROUGH DRINKING WATER

Parameters	Unexposed ($N = 30$)	Exposed group without skin lesions ($N = 30$)	Exposed group with hyperkeratosis ($N = 30$)
Age in years (mean ± SD)	42.83 ± 12.99	37.57 ± 10.87	40.97 ± 11.69
Sex [N(%)]			
Male	13 (43.33)	12 (40)	15 (50)
Female	17 (56.67)	18 (60)	15 (50)
Occupation [N (%)]			
Males			
Cultivation	12 (92.31)	11 (91.67)	10 (66.67)
Business	0 (0)	0 (0)	2 (13.33)
Daily wage earners	0 (0)	0 (0)	1 (6.66)
Service	0 (0)	0 (0)	1 (6.66)
Student	0 (0)	1 (8.33)	0 (0)
Unemployed	1 (7.69)	0 (0)	1 (6.67)
Female			
Housewife	16 (94.12)	18 (100)	14 (93.33)
Cultivation	1 (5.88)	0 (0)	0 (0)
Service	0 (0)	0 (0)	1 (6.67)
Smoking Status [N (%)]			
Smoker	12 (40)	14 (46.67)	12 (40)
Nonsmoker	18 (60)	16 (53.33)	18 (60)
Arsenic content (mean ± SD)			
Drinking water (μg/l)*	4.88 ± 2.19	05.04 ± 193.24**	224.1 ± 232.80**
Urine (μg/l)*	12.69 ± 12.10	296.03 ± 322.09**	311.7 ± 448.78**

*$p < 0.001$ for Kruskal–Wallis Test.–**$p < 0.001$ when compared to unexposed group by Dunn's Multiple Comparisons Post-Test.

TABLE II – COMPARISON OF DNA DAMAGE STATUS IN THE THREE STUDY GROUPS

Parameters	Unexposed	Exposed without arsenic-induced skin lesion	Exposed with hyperkeratosis	p value for one way ANOVA
OTM (mean ± SD)	0.55 ± 0.83	2.76 ± 1.39*	2.51 ± 1.40*	<0.001
% of tail DNA (mean ± SD)	4.29 ± 1.49	14.05 ± 4.71*	13.40 ± 3.51*	<0.001
Tail length (mean ± SD)	2.20 ± 0.72	11.85 ± 5.51*	13.54 ± 4.38*	<0.001

One-way ANOVA with Tukey–Kramer Multiple Comparisons Post-Test.
*$p < 0.001$ when compared with unexposed group.

TABLE III – COMPARISON OF CHROMOSOMAL ABERRATION (CA) STATUS IN THE 3 STUDY GROUPS

Parameters	Unexposed	Exposed without arsenic-induced skin lesion	Exposed with hyperkeratosis	p value for Kruskal–Wallis test
CA/cell (mean ± SD)	0.018 ± 0.012	0.074 ± 0.020*	0.099 ± 0.021*,**	<0.001
% of aberrant cells (mean ± SD)	1.55 ± 0.850	6.96 ± 1.581*	9.35 ± 1.863*,**	<0.001

Kruskal–Wallis Test with Dunn's Multiple Comparisons Post-Test.
*p <0.001 when compared to unexposed group.–**$p < 0.01$ when compared to exposed group without arsenic-induced skin lesion.

most widely noted arsenic-induced precancerous skin lesion is hyperkeratosis, and, it has been reported previously, that skin cancers most often appear at the sites of existing hyperkeratosis.[8] Several plausible mechanisms for arsenic carcinogenicity have been suggested including CA, oxidative stress, altered growth factors, cell proliferation, altered DNA repair, and so forth.[20] However, little or no attempt has been made till date to elucidate the factors that might render certain persons susceptible to develop arsenic-induced premalignant skin lesions, while some others do not develop characteristic skin lesions even after consuming water containing comparable levels of arsenic, for a similar time period.

DNA damage has long been known to result in CA. The offending lesions most commonly comprise of changes to DNA sequence, including events effecting genomic regions such as deletion, duplication, amplification and translocation.[21] In fact, a 3-step model has been proposed for the induction of CA,[22] which involves

a. Persistent increase of single-stranded regions in the replicating DNA.
b. Creation of transient double strand breaks (DSBs) induced at sites opposite these regions by endogenous endonucleases.
c. Finally, the third step requires that improper repair of these DSBs occurs from either nonrepair or misrepair that then leads to the final CAs.[22]

Thus, it is evident that the third step is crucial in maintaining genomic stability. Even if any agent induces single strand or DSBs in the DNA, it might be taken care of by efficient DNA repair machinery, so that it cannot proceed to give rise to CAs. Any flaw in the repair system, thus, might lead to the generation of CAs even if the damage induced might be equal to the damage that can be easily corrected by an efficient DNA repair system. Again, literature shows that heightened CAs are the harbinger of carcinogenic outcomes.[23] Hence, faulty or suboptimal DNA repair systems might easily bring about precancerous lesions in individuals exposed to arsenic.

Studies reveal that much of the DNA damage brought about by arsenic is the consequence of generation of ROS during its cellular metabolism. Again, arsenic induces DNA adducts through calcium-mediated production of peroxynitrite, hypochlorous acid and hydroxyl radicals.[24] Studies also show that, a large portion of arsenite-induced DNA strand breaks come from excision of oxidative DNA adducts and DNA–protein crosslinks (DPCs).[25] Arsenic-induced DPC could hamper DNA replication, leaving

TABLE IV – COMPARISON OF DNA DAMAGE STATUS IN THE 2 STUDY GROUPS WITH AND WITHOUT γ-IRRADIATION CHALLENGE

Parameters hyperkeratosis	Basal DNA damage (without γ-irradiation)		DNA damage with γ-irradiation	
	Exposed without arsenic-induced skin lesion (n = 12)	Exposed with hyperkeratosis (n = 2)	Exposed without arsenic-induced skin lesion (n = 12)	Exposed with (n = 12)
OTM (mean ± SD)	2.30 ± 0.51	2.43 ± 0.69	3.66 ± 0.20	4.80 ± 0.34*
% of tail DNA (mean ± SD)	12.89 ± 1.76	12.75 ± 2.01	22.84 ± 1.95	31.02 ± 1.34*
Tail length (mean ± SD)	14.00 ± 1.55	14.07 ± 1.79	22.68 ± 2.01	35.67 ± 3.16*

Unpaired 2-tailed t test.
*$p < 0.001$ when compared with group of exposed individuals without arsenic-induced skin lesion.

unreplicated stretches that might result in chromosome- and chromatid-type aberrations.[26] All these damages induced by arsenic are corrected by excision repair system and involves both the nucelotide excision repair (NER) system as also the base excision repair (BER) system. Thus, if there is any alteration in the efficiency of the DNA repair system in any individual that results in suboptimal repair of damaged DNA, that individual will be unable to combat effectively the different types of DNA damages induced by arsenic. These damages will hence, continue to accumulate over a period of time, after which, the individual will exhibit symptoms of arsenic toxicity and possibly arsenic-specific premalignant skin lesions.

Previous studies, using comet assay, have shown that arsenic, as well as its metabolites, can induce DNA damage.[16,27] However, most of these studies were performed on cell lines or, were comparison between exposed and unexposed groups of individuals. None of these studies attempted to find out the difference between the 2 subgroups (with hyperkeratosis and without arsenic-induced skin lesion individuals) of the exposed population. We have selected lymphocytes for our study, as it has been shown previously that, arsenic induces considerable genetic damage in peripheral blood lymphocytes, by means of micronucleus assay and CA assay.

From our studies, it appears that both the exposed individuals (with hyperkeratosis and without skin lesions) have similar level of basal DNA damage caused by arsenic, directly or indirectly. However, the CA status is quite different in these 2 groups, with the group consisting of individuals with arsenic-induced premalignant hyperkeratosis having significantly higher CA in comparison to the group consisting of individuals without skin lesion. This observation is consistent with our previous findings.[14] This apparently anomalous observation can be easily explained, if we consider that this difference in CA status in the 2 groups reflects the relative efficiency of their DNA repair system. This leads to the hypothesis that the DNA repair mechanism in the former group might not be as effective as that of the latter group, and perhaps this difference in their DNA repair capacity is what makes those individuals susceptible to arsenic toxicity and subsequent carcinogenicity.

To verify this hypothesis, we performed the challenge assay. Challenge assay has long been used as a reliable technique to assess the efficacy of DNA repair systems in face of several types of challenges.[16,19] The dosage selected here (100 cGγ) is based on the previous studies.[19] In this particular study, our objective was to look at the comparative DNA repair efficiency of exposed individuals with arsenic-induced hyperkeratosis, and those without arsenic-induced skin lesions. Hence, we did not include unexposed controls in the particular experiment. There has been a previous study, which hypothesized that elevated levels of arsenic lead to depressed NER capacity.[16] In our study, we could see that the basal DNA damage level in these 2 groups were not significantly different. However, when they were challenged with γ-irradiation, DNA repair machinery of the individuals without arsenic-induced skin lesion could cope up with the genotoxic changes far more effectively than that of the individuals with arsenic-induced premalignant skin lesions. In the present experimental set up, we have allowed 1 hr for the treated cells to undergo repair process. It is well established that half-life of repair process in human lymphocytes as estimated from kinetic studies is ~5 min.[28] Therefore, a period of incubation of 1 hr is sufficient to detect the efficiency of DNA repair system without requirement of checking DNA damage and repair status at later time points. Our observation clearly points out that there is a discrepancy in the DNA repair capacities among the individuals of the 2 concerned groups (with premalignant hyperkeratosis and without arsenic-induced skin lesions). This difference in the DNA repair capacities might well be genetic in origin, as suggested by our previous work.[14,15]

To sum up, our work reveals that basal level of arsenic-induced damage is uniform in the exposed group, independent of the presence of arsenic-specific hyperkeratotic skin symptoms. However, the difference lies in the DNA repair capacities of the 2 groups, which is reflected in the increased incidence of CA in the group consisting of individuals with arsenic-induced hyperkeratosis. Thus, the present work addresses the problem of susceptibility to arsenic-induced carcinogenicity at the mechanistic level, which has seldom been done before, if ever, and projects differences in DNA repair capacity as a prime contender for arsenic carcinogenicity.

Acknowledgements

This study is supported by grants (CMM-0003) and research fellowship to Mr. Mayukh Banerjee from Council of Scientific and Industrial Research (CSIR), Govt. of India. Thanks are also due to Fogarty International Training Program in collaboration with University of California, Berkeley, for providing training to M.B.

References

1. U.S. EPA. EPA Drinking Water News. 2006 [accessed May 01, 2006]. Available at http://www.nesc.wvu.edu/ndwc/ndwc_news_EPA.htm/
2. WHO. WHO guidelines for drinking water quality. Health criteria and other supporting information, 2nd edn., vol. 2. Geneva: WHO, 1996. 940–9.
3. IARC. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans: some drinking water disinfectants and contaminants, including arsenic. IARC Sci Publ 2004;84:1–477.
4. Ahsan H, Chen Y, Wang Q, Slavkovich V, Graziano JH, Santella RM. DNA repair gene XPD and susceptibility to arsenic-induced hyperkearatosis. Toxicol Lett 2003;143:123–31.
5. Alain G, Tousignant J, Rozenfarb E. Chronic arsenic toxicity. Int J Dermatol 1993;32:899–901.
6. National Research Council (NRC). Arsenic in drinking water. Subcommittee on Arsenic in Drinking Water. Washington DC: National Academy Press, 1999.
7. Guha Mazumder DN, Haque R, Ghosh N, De BK, Santra A, Chakroborty D, Smith AH. Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal. India. Int J Epidemiol 1998; 27:871–7.
8. Ghosh P, Basu A, Mahata J, Basu S, Sengupta M, Das JK, Mukherjee A, Sarkar AK, Mondal L, Ray K, Giri AK. Cytogenetic damage and genetic variants in the individuals susceptible to arsenic-induced cancer through drinking water. Int J Cancer 2006;118:2470–8.
9. Liu SX, Athar M, Lippai I, Wladren C, Hei TK. Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity. Proc Natl Acad Sci USA 2001;98:1643–8.

10. Mouron SA, Grillo CA, Dulout FN, Golijow CD. Induction of DNA strand breaks. DNA-protein crosslinks and sister chromatid exchanges by arsenite in a human lung cell line. Toxicol Vitr 2006;20: 279–85.
11. Basu A, Som A, Ghoshal S, Mondal L, Chaubey RC, Bhilwade HN, Rahman MM, Giri AK. Assessment of DNA damage in peripheral blood lymphocytes of individuals susceptible to arsenic induced toxicity in West Bengal India. Toxicol Lett 2005;159:100–12.
12. Basu A, Ghosh P, Das JK, Banerjee A, Ray K, Giri AK. Micronuclei as biomarkers of carcinogen exposure in populations exposed to arsenic through drinking water in West Bengal, India: a comparative study in three cell types. Cancer Epidemiol Biomarkers Prev 2004; 13:820–7.
13. Mahata J, Basu A, Ghoshal S, Sarkar JN, Roy AK, Poddar G, Nandy AK, Banerjee A, Ray K, Natarajan AT, Nilsson R, Giri AK. Chromosomal aberrations and sister chromatid exchanges in individuals exposed to arsenic through drinking water in West Bengal, India. Mutat Res 2003;534:133–43.
14. Banerjee M, Sarkar J, Das JK, Mukherjee A, Sarkar AK, Mondal L, Giri AK. Polymorphism in the ERCC2 codon 751 is associated with arsenic-induced premalignant hyperkeratosis and significant chromosome aberrations. Carcinogenesis 2007;28:672–6.
15. De Chaudhuri S, Mahata J, Das JK, Mukherjee A, Ghosh P, Sau TJ, Mondal L, Basu S, Giri AK, Roychoudhury S. Association of specific p53 polymorphisms with keratosis in individuals exposed to arsenic through drinking water in West Bengal, India. Mutat Res 2006;601: 102–12.
16. Andrew AS, Karagas MR, Hamilton JW. Decreased DNA repair gene expression among individuals exposed to arsenic in United States drinking water. Int J Cancer 2003;104:263–8.
17. Ghosh P, Banerjee M, De Chaudhuri S, Chowdhury R, Das JK, Mukherjee A, Sarkar AK, Mondal L, Baidya K, Sau TJ, Banerjee A, Basu A, et al. Comparison of health effects between individuals with and without skin lesions in the population exposed to arsenic through drinking water in West Bengal, India. J Expo Sci Environ Epidemiol 2007;17:215–23.
18. ITRC. The SCGE/ Comet Assay Protocol. 2006 [accessed 13 April 2006]. Available at http://www.cometassayindia.org/Protocol%20for%20Comet% 20 Assay. PDF.
19. Au WW, Lane RG, Legator MS, Whorton EB, Wilkinson GS, Gabehart GJ. Biomarker monitoring of a population residing near uranium mining activities. Environ Health Perspect 1995;103:466–70.
20. Kitchin KT. Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol Appl Pharmacol 2001;172:249–61.
21. Ferguson DO, Alt FW. DNA double strand break repair and chromosomal translocation: lessons from animal models. Oncogene 2001;20:5572–9.
22. Wong RS, Dewey WC. Molecular mechanisms for the induction of chromosomal aberrations in CHO cells heated in S phase. Environ Mol Mutagen 1993;22:257–63.
23. Yunis JJ. The chromosomal basis of human neoplasia. Science 1983;221:227–36.
24. Wang TS, Hsu TY, Chung CH, Wang AS, Bau DT, Jan KY. Arsenite induces oxidative DNA adducts and DNA-protein cross-links in mammalian cells. Free Radic Biol Med 2001;31:321–30.
25. Tezuka M, Hanioka K, Yamanaka K, Okada S. Gene damage induced in human alveolar type II (L-132) cells by exposure to dimethylarsinic acid. Biochem Biophys Res Commun 1993;191:1178–81.
26. Vega L, Gonsebatt ME, Ostrosky-Wegman P. Aneugenic effect of sodium arsenite on human lymphocytes in vitro: an individual susceptibility effect detected. Mutat Res 1995;334:365–73.
27. Gomez SE, del Razo LM, Munoz Sanchez JL. Induction of DNA damage by free radicals generated either by organic or inorganic arsenic (AsIII. MMAIII, and. DMAIII) in cultures of B and T lymphocytes. Biol Trace Elem Res 2005;108:115–26.
28. Cebulska-Wasilewska A. Response to challenging dose of X-rays as a predictive assay for molecular epidemiology. Mutat Res 2003;544: 289–97.