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Micronuclei and Chromosomal Aberrations as Biomarkers: A Study in an Arsenic Exposed Population in West Bengal, India

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In the past few years the incidence of arsenic (As) contamination in ground water and the consequent suffering of people from chronic arsenic toxicity resulting from drinking the contaminated water have increased to a great extent. There are many clinical manifestations but the most commonly observed signs and symptoms that identify people suffering from chronic arsenic poisoning are arsenical skin lesions (Chakraborti et al, 2002). Major arsenic incidences have occurred in Taiwan, Antofagasta in Chile, Mexico and Argentina. Smaller population groups have been reported in Poland, Minnesota, USA, Ontario, Canada, Nova Scotia, Hungary, Nokajo, Japan, Millard County, Utah, Lane County, Oregon, Lassen County, California and Fairbanks, Alaska (Das et al 1995). Ground water arsenic contamination in West Bengal (WB, India) was first reported in December 1983, when health officials identified 63 people from three villages of two districts as suffering from As toxicity. As of October 2001, 2700 villages in a total of nine districts in WB are identified as As-affected. More than 6 million people are drinking water containing $\geq$50 mg/l arsenic and >300000 people are suffering from arsenical skin lesions. Overall, 42.7 million people live in nine arsenic affected districts (Chakraborti et al, 2002).

Arsenic is a potent carcinogen, but the exact mechanism of arsenic-induced carcinogenicity still remain unclear; however short term assays indicate that As does not induce point mutations but rather it acts as a clastogen, that causes breaks in the chromosomes (Biswas et al 1999). Majority of the exposed population who had an exposure history for a relatively longer period showed higher incidences of micronuclei formation (Dulout et al 1996) and chromosomal aberrations (Ostrosky-Wegman et al 1991). A micronucleus (MN) is a small extranucleus separated from the main nucleus, generated during cellular division by a whole lagging chromosome or by an acentric chromosome fragments. The micronucleus test is used as an indicator of genotoxic exposition, since it is associated with chromosome aberrations (Roberts 1997). The study carried out by Ramirez and Saldanha (2002) showed an increase in oral mucosal cell MN frequency in person suffering from cancer in compare to the controls and concluded that MN are a product of early events in human carcinogenic processes, especially in oral regions. Smith et al (1993) came to the conclusion that the exfoliated oral cells MN frequencies serve as an appropriate index to

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monitor the genotoxicity induced by arsenic because these cells are in direct contact with the carcinogen. Chromosomal aberrations (CA) in peripheral blood lymphocytes have long been used as a biomarker of early effects of genotoxic carcinogens (Hagmar et al 2004). The study carried out by Hagmar et al (2004) showed that high levels of CA at test were clearly associated with increased total cancer incidence in the Nordic cohorts and increased total cancer mortality in the Italian cohort. The reports on cytogenetic survey of arsenic exposed population in West Bengal are very limited. Considering the widespread effects of arsenic induced toxicity in human beings, in the present study we selected both CA and MN as biomarker to assess the effect of arsenic in our study population.

MATERIALS AND METHODS

The arsenic exposed populations (n=45, 33 females and 12 males) were selected from the residents of Baduria block in Atghara in the district North 24Parganas. This district is well documented as one of the most arsenic affected district in West Bengal (Chakraborti et al 2002). A person using their present drinking water source at least for the past 10 years was an inclusion criterion for the exposed population as well as control population. Age and sex matched control populations (n=25, 19 females and 7 males) were selected from the arsenic unaffected area of West Bengal (Howrah, Kolkata). The socio-economic condition of both control and exposed population were same and the age range for both groups was 14-67 years. Cohorts of 70 persons (25 control individuals and 45 exposed individuals) were subjects of the present study.

Each subject was first undergone a standardized questionnaire interview which reveals the information on demographics, life-style factors, occupation, diet, source of daily water intake, medical and residential histories assessment of exposure and level of exposure. Physicians examined the study participants. Water and other bio samples were collected same day from the subjects and code numbers were given. The selected subjects provided informed consent to participate and they fulfill the inclusion criteria. It was found from the interview that arsenic in drinking water was the principal source of exposure in this region. The samples that were collected for arsenic estimation include drinking water (~100ml), nails (~250-500mg) and hair (~300-500mg). The samples were analyzed at the School of Environmental Studies, Jadavpur University, Calcutta. Drinking water was collected in acid-washed [nitric acid: water (1:1)] plastic bottles into which nitric acid (1.0ml/l) was added later on as a preservative .For collection of hair and nail samples ceramic blade cutters were used. Hair samples that were collected are of similar size and were taken from more or less similar region of head (behind the ear close to the scalp with a diameter of about 1cm; Agahian et al 1990).

For micronuclei (MN) study oral mucosal cell was collected from each subject. The subjects were asked to rinse their mouths with water and a premoistened wooden spatula was used to collect cells from the oral mucosa. The spatula was applied to a pre cleaned microscope slide. Smears were air dried and fixed in 80%

methanol. All slides were coded. Slides were stained by the Giemsa solution and the MN frequency was scored using the criteria described by Tolbert et al (1992). Thousand cells were scored blindly in each case to determine the MN percentage.

5ml of peripheral blood samples were taken from each subject in heparinised vials by venipuncture. The blood samples were coded, kept at 4°C in a cooling device, and brought to the laboratory within 2h of collection to study the complete haemogram like Hb, MCV, MCH, MCHC etc. and to carry out lymphocyte culture. Lymphocyte culture was carried out for CA analysis by the method of Sharma and Talukder (1974). For each subjects duplicate cultures were maintained. Leucocyte rich plasma (0.5ml) was added to 5 ml culture media supplemented with 20% fetal bovine serum and phytohaemagglutinin M (0.04ml/ml of culture media). The cultures were incubated at 37° C. The harvesting was done at 72 hrs after initiation of the culture. At 70 h of culture colchicine was added. Two hours later cells were centrifuged at 1000rpm for 10 min, treated with pre-warmed KCl (0.075M) for 15 min, centrifuged at 1000rpm for 10 min and fixed in methanol:acetic acid (3:1).Fixatives were removed by centrifugation and two more changes of fixative were performed. Fixed cell suspension was laid on clean grease-free glass slide and air-dried. The preparation was stained with aqueous Giemsa. All slides were coded and 100 metaphase plates were scored randomly for chromosomal aberrations (chromatid and chromosome types) per individual.

Before estimation, the nail and hair samples were digested with 5ml of concentrated nitric acid and 3ml of concentrated sulfuric acid following the method of Agahian et al 1990. Water was not subjected to any pre-treatment before analysis. Flow injection-hybride generation-atomic absorption spectrometry (FI-HG-AAS) was used for estimation of arsenic in the collected bio-samples. A Perkin-Elmer Model 3100 AAS equipped with a Hewlett-Packard Vectra 386/25N computer with GEM software, Perkin-Elmer EDL System-2, arsenic lamp (lamp current 400mA) was used for this purpose.

The significance of the differences between arsenic contents obtained in the control and exposed groups was calculated by Fisher's *t*-test. Different hematological parameters, CA and MN assays were also analyzed using Fisher's *t*-test and the level of significance is presented in the respective tables (both sided p value).

RESULTS AND DISCUSSION

A comparative data among the control and exposed population in terms of the arsenic contents (mean±SE) in water, hair and nail samples of the individuals were given in Table 1. The first group i.e. the control group contains 25 persons (19 females and 6 males) who were drinking water which contains arsenic within the permissible limit as given by WHO guidelines i.e. 10 μg/l (WHO 1996) and the second group i.e. the exposed group contains 45 persons (33 females and 12 males) with arsenic level in their drinking water above the permissible limit. The

persons belonging to both the control and exposed groups were mainly daily wage earners, students, house-wives, farmers by profession. No statistically significant difference was found between the mean (±SE) age of the control group (33.8±2.97 years) and the exposed group (33.93±2.11 years). The mean (±SE) arsenic content

Table 1. A short history and comparison of the mean values of arsenic content in water, hairs and nails of the control and exposed group.

Type	No.of individuals	Sex		Age (yrs) (Mean± SE)	Arsenic level in drinking water (µg/l) (Mean ± SE)	Arsenic level in hairs (µg /kg) (Mean± SE)	Arsenic level in nails (µg /kg) (Mean± SE)
		Female	Male				
Control	25	19	6	33.8± 2.97	6.44± 0.21	337.7± 19.99	378.13± 32.45
Exposed	45	33	12	33.9± 2.11	66.75± 2.50*	1451.46± 123.21*	2149.35± 168.12*

* $p \leq 0.01$ (Fisher's *t*-test)

in water of the exposed group was 66.75± 2.50 µg/l, whereas in control group it was found to be 6.44±0.21µg/l. The exposed group contained 1451.46±123.21 µg/kg arsenic (mean±SE) in hairs and 2149.35±168.12 µg/kg arsenic (mean±SE) in nails, whereas in control group the arsenic (mean±SE) content in hairs and nails were 337.7±19.99µg/kg and 378.13±32.45µg/kg respectively. The mean (±SE) arsenic contents of water, hair and nail samples were significantly high ($p \leq 0.01$) in the exposed population. Arsenic levels in nails and hairs have been noted by various researchers as reliable biological indicators of exposure to this element. The high concentration of arsenic in nails and hair is attributed to the high content of keratin in these tissues (Agahian et al 1990).

Table 2. Mean values (mean±SE) of the different blood parameter in control and exposed group.

Type	TC ($X10^3$/ µl)	Hb (g/dl)	RBC ($X10^6$/ µl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	7.8± 0.40	11.5± 0.37	3.78± 0.10	32.16± 0.94	85.24± 1.52	30.54± 0.70	35.74± 0.31
Exposed	8.65± 0.35	11.3± 0.25	3.77± 0.09	32.62± 0.66	87.0± 0.84	30.19± 0.34	34.70± 0.19

Table 3. Comparison of the mean values of micronuclei frequencies and chromosomal aberrations in control and exposed group.

Type	Mitotic Index MI(%) (Mean±SE)	Chromosomal Aberrations CA(%)# (Mean±SE)	Micronuclei frequencies MN(%) (Mean±SE)
Control	3.78±0.13	1.28±0.19	0.29±0.01
Exposed	2.4±0.09*	4.93±0.26*	0.98±0.07*

* $p \leq 0.01$ (Fisher's *t*-test); # excluding gaps

A detailed picture of different blood parameters like Hb, TC, RBC, MCV, MCH and MCHC were given in Table 2. There were no significant changes found in blood picture among the control and exposed population.

In our present study we found that there was a significant change in chromosomal aberrations (CA) and micronuclei (MN) frequencies of exposed individuals when compared to that of the control group as indicated in Table 3. There was a 3.34 fold increase in micronuclei, 3.85 fold increase in CA in exposed population in compared to that of the controls. These results clearly showed that due to exposure of arsenic in drinking water there was a significant increase in cytogenetic damage in lymphocytes as well as in oral mucosal cells. Among all type of stable CA found, chromatid breaks and gaps were the main CA observed and unstable CA was reflected as MN. Chromosome type aberrations such as chromosomal breaks, dicentrics were also observed. Beside this spindle disturbances like polyploidy were observed in few cases. Here we only present the average results and gaps were not included while calculating CA. For calculation of CA, chromatid breaks were taken as one change and chromosome breaks, dicentrics as two changes. In the present study, a decrease in the mean mitotic index value (MI) of the exposed population was noted which suggest a slower progression of lymphocytes from S to M phase of the cell cycle (Gonsebatt et al 1994) as every known clastogen disturbs the orderly progression of cells towards division. Arsenic is a known clastogen (Biswas et al 2001) and as all the cells are harvested at similar time period so this effect may be due to chronic exposure to arsenic. The gradient of MN frequencies in the oral mucosal cells of exposed population was confirmed in comparison with the controls, revealing a 3.34 fold increase in MN frequency in the exposed individuals.

The data of our study was in agreement with the earlier study carried out by Mahata et al (2003) in West Bengal who found a statistically significant increase in CA frequency in arsenic symptomatic individual in compare to the control group. However, the study carried out by Maki-Paakkanen et al (1998) found no statistically significant increase in CA, both including and excluding gaps in exposed group in compare to that of the control group. Ostrosky-Wegman et al (1991) in a pilot study in Mexico found that, 9 women and 2 men chronically highly exposed to well water As (390 μg As/l) showed a non-significantly higher

CA frequency in comparison to the respective control (11 women and 2 men, As in well water 19–60 μg As/l). This result was dissimilar to our findings.

In our study we found a 3.34 fold increase in MN in oral mucosal cells in our exposed group in compare to control group and this elevated level of MN in the exposed group was found to be statistically significant ($p \leq 0.01$). This result tallies with the study done by Tian et al (2001) who found a 3.4 fold increase in MN in the exposed populations who were drinking arsenic contaminated water with mean arsenic concentration of 527.5 ± 24μg/l. However, the study carried out by Martinez et al, 2005 in northern Chile found elevated but no statistically significant increase in MN frequency in exposed population in compare to the control individuals. This result was not in agreement with our study.

The present study can be concluded with our findings that MN estimation in buccal mucosa provides an easy, non-invasive technique of genotoxic damage. Environmental exposure to arsenic causes genotoxic effects that can be easily assessed by MN assay. The incidences of oral cancer in India are high and its relation to arsenic intake can be assessed by this method. In our study we found a significant increase in oral MN frequency in compare to matched controls. The CA of blood provides further support to this study. The prevalence of significantly high frequencies of MN and CA in our arsenic exposed study population is an alarming one and calls for immediate remedial and preventive measures against arsenic induced carcinogenicity.

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REFERENCES

Agahian B, Lee JS, Nelson JH, Johns RE (1990) Arsenic levels in fingernails as a biological indicator of exposure to arsenic. American Ind Hyg Assoc J 51:646-651

Biswas S, Talukder G, Sharma A (1999) Protection against cytotoxic effects of arsenic by dietary supplementation with crude extract of *Emblica officinalis* fruit. Phytother Res 13: 513-516

Chakraborti D, Rahman MM, Paul K, Chowdhury UK, Sengupta MK, Lodh D, Chanda CR, Saha KC, Mukherjee SC (2002) Arsenic calamity in the Indian subcontinent What lessons have been learned? Talanta 58:3-22

Das D, Chatterjee A, Mandal BK, Samanta G, Chakraborti D (1995) Arsenic in ground water in six districts of West Bengal, India: the biggest arsenic calamity in the world. II. Arsenic concentration in drinking water, hair, nails, urine, skin-scale and liver tissue (biopsy) of the affected people. Analyst 120:

917-924
Dulout FN, Grillo CA, Seoane AI, Maderna CR, Nilsson R, Vahter M, Darroudi F, Natarajan AT (1996) Chromosomal aberrations in peripheral blood lymphocytes from native Andean women and children from Northwestern Argentina exposed to arsenic in drinking water. Mut Res 370:151-158
Gonsebatt ME, Vega L, Montero R, Guzman P, Garcia-Vargas G, Del Razo LM, Albrores A, Cebrian ME, Ostrosky-Wegman P (1994) Lymphocyte replicating ability in individuals exposed to arsenic via drinking water. Mut Res 313: 293-299
Hagmar L, Stromberg U, Bonassi S, Hansteen IL, Knudsen LE, Lindholm C, Norppa H (2004) Impact of types of lymphocyte chromosomal aberrations on human cancer risk Results from Nordic and Italian cohorts. Cancer Res 64:2258-2263
Mahata J, Basu A, Ghosal S, Sarkar JN, Roy AK, Poddar G, Nandy AK, Banerjee A, Ray K, Natarajan AT, Nilsson R, Giri AK (2003) Chromosomal aberrations and sister chromatid exchanges in individuals exposed to arsenic through drinking water in West Bengal, India. Mut Res 534:133-143
Maki-Paakkanen J, Kurttio P, Paldy A, Pekkanen J (1998) Association between the clastogenic effect in peripheral lymphocytes and human exposure to arsenic through drinking water. Environ Mol Mutagen 32:301-313.
Mandal BK, RoyChowdhury T, Samanta G, Basu GK, Chowdhury PP, Chanda CR, Lodh D, Karan NK, Dhar RK, Tamili DK, Das D, Saha KC, Chakraborti D(1996)Arsenic in ground water in seven districts of West Bengal, India-the biggest arsenic calamity in the world. Curr Sci 70:976-986
Martinez V, Creus A, Venegas W, Arroyo A, Beck JP, Gebel TW, Surralles J, Marcos R (2005) Micronuclei assessment in buccal cells of people environmentally exposed to arsenic in northern Chile. Toxicol Lett 155: 319-327
Ostrosky-Wegman P, Gonsebatt ME, Montero R, Vega L, Barba H, Espinosa J (1991) Lymphocyte proliferation kinetics and genotoxic findings in pilot study on individuals chronically exposed to arsenic in Mexico. Mut Res 250: 477-482
Ramirez A, Saldanha PH (2002) Micronucleus investigations of alcoholic patients with oral carcinomas. Genet Mol Res 1: 246-260
Roberts DM (1997) Comparative cytology of the oral cavities of snuff users. Acta Cytol 41: 1008-1014
Sharma A, Talukder G (1974) Laboratory procedures in human genetics, Chromosome Methodology, vol 1. The Nucleus, Calcutta.
Smith AH, Hopenhayn-Rich C, Warner M, Biggs ML, Moore L, Smith MT (1993) Rationale for selecting exfoliated bladder cell micronuclei as potential biomarkers for arsenic genotoxicity. J Toxicol Environ Health 40:223-234
Tolbert PE, Shy CM, Allen JW (1992) Micronuclei and other nuclear anomalies in buccal smears: methods development. Mut Res 271: 69 – 77
Tian D, Ma H, Feng Z, Xia Y, Le XC, Ni Z, Allen J, Collins B, Schreinemachers D, Mumford JL (2001) Analyses of micronuclei in exfoliated epithelial cells from individuals chronically exposed to arsenic via drinking water in inner Mongolia, China. J Toxicol Environ Health A 64: 473-484