Effect of Arsenic Contaminated Drinking Water on Human Chromosome: A Case Study

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Abstract Arsenic contamination of ground water has become a serious problem all over the world. Large number of people from Uttar Pradesh, Bihar and West Bengal of India are suffering due to consumption of arsenic contaminated drinking water. Study was carried out on 30 individuals residing in Ballia District, UP where the maximum concentration of arsenic was observed around 0.37 ppm in drinking water. Blood samples were collected from them to find out the problem related with arsenic. Cytogenetic study of the blood samples indicates that out of 30, two persons developed Klinefelter syndrome.

Keywords Arsenic · Water pollution · Blood · Chromosomal abnormalities

Introduction

Contamination of arsenic (As) in the ground water is one of the major threats to the human health due to highly toxic nature of arsenic(III). Arsenic is entering the ground water through natural weathering processes of the arsenic bearing rocks and minerals and by the discharge of effluents of various industries like petroleum refining, fertilizer, pesti-

cides, herbicides, glass and ceramics, wood preservatives, alloys, electronics, catalysts and feed additives/veterinary chemicals [1–3].

Ground water contamination with arsenic has been noticed in the West Bengal, Bihar, Uttar Pradesh, Jharkhand, Assam and Chattisgarh states of India. This problem is also prevalent in the countries like Bangladesh, Argentina, Canada, China, Mongolia, Taiwan and Saudi Arabia [4–8]. Arsenic exists in the aquatic environment in two forms viz- arsenite [As(III)] and arsenate [(As V)]. The former is more toxic than latter and by its methylated forms. The permissible limit of arsenic in the drinking water is 0.01 ppm [9]. Its presence in the ground water more than permissible limit causes various kinds of serious diseases viz- eczema of skin/mucous membranes, hyperkeratosis of palms and soles, warts, leukemia, acute renal failure, encephalopathy, sensory disorders, neuropathy and cancer, rhinopharyngitis, pulmonary insufficiency, interstitial fibrosis, melanosis, hypertension and peripheral vascular disease [1, 3, 8]. Thus arsenic acts as a silent killer. In the present paper effect of consumption of arsenic contaminated water has been studied on the human chromosomes.

Materials and Methods

Study Design and Group Selection

From the arsenic affected area of four villages (Baria, Maniar, Dubahar and Sikandarpur) of Ballia district, UP (India) 30 persons (13 male and 17 female) were identified who had symptoms of skin disease like hyperpigmentation and hypopigmentation (Table 1). They were screened for the study of chromosomal aberration. Detailed information was collected from them about their habits like smoking, tobacco

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Table 1 Effect of arsenic contaminated water on human chromosome in Ballia District, UP, India, during December, 2010

S. no.	Sampling site	Male/female	As (ppm)	Symptoms
1	Bairia	Female	0.018	LPSP
2	Bairia	Male	0.07	LPSP
3	Bairia	Female	0.08	LPSP
4	Bairia	Female	0.08	LPSP
5	Bairia	Female	0.09	LPSP
6	Bairia	Male	0.10	LPSP
7	Bairia	Male	0.19	MPSP
8	Bairia	Male	0.19	MPSP
9	Bairia	Female	0.21	MPSP
10	Maniar	Female	0.02	LPSP
11	Maniar	Female	0.02	LPSP
12	Maniar	Female	0.03	LPSP
13	Maniar	Male	0.04	LPSP
14	Maniar	Male	0.34	PSP
15	Maniar	Male	0.37	PSP
16	Dubhar	Male	0.04	LPSP
17	Dubhar	Male	0.04	LPSP
18	Dubhar	Female	0.04	LPSP
19	Dubhar	Female	0.05	LPSP
20	Dubhar	Female	0.05	LPSP
21	Dubhar	Female	0.05	LPSP
22	Dubhar	Male	0.06	LPSP
23	Dubhar	Male	0.06	LPSP
24	Dubhar	Male	0.08	LPSP
25	Sikandarpur	Male	0.01	LPSP
26	Sikandarpur	Female	0.01	LPSP
27	Sikandarpur	Female	0.02	LPSP
28	Sikandarpur	Female	0.03	LPSP
29	Sikandarpur	Female	0.04	LPSP
30	Sikandarpur	Female	0.07	LPSP

Name of the collected ground water samples serial number: 1. Baria (Jal Kal Bibhag) 2. Baria (Baria Gram Panchayat) 3. Baria (Sonbarasa) 4. Baria (Chikitalaya Colony) 5. Baria (Bus stand) 6. Baria (Baria Bazar) 7. Baria (Trilokinath colony) 8. Baria (Kunwar Toly colony) 9. Baria (Kamalesh Hotel) 10. Maniar (Primary Health centre) 11. Maniar (Hospital colony) 12. Maniar (Ramnath Hotel) 13. Maniar (Inter College) 14. Maniar (Bus Stand) 15. Maniar (Dainik Jagaran) 16. Dubhar (Vimalendu Mishra) 17. Dubhar (Thana) 18. Dubhar (Samudiak Health Centre) 19. Dubhar (Samudiak Health Centre Workers Colony) 20. Dubhar (Sanath Pandey ki chapra) 21. Dubhar (Bharsar gram-first) 22. Dubhar (Bharsar gram-Second) 23. Dubhar (Silki gram-First) 24. Dubhar (Silki gram- Second) 25. Ramgadh (Tiwari tola) 26. Ramgadh (Chaubey Chapra) 27. Sikandarpur Bazar 28. Sikandarpur (Gandhi Khadi Gramudogya) 29. Sikandarpur (Sohal) 30. Sikandarpur (College Road)

LPSP Less pronounced skin pigmentation, *MPSP* More pronounced skin pigmentation, *PSP* Pronounced skin pigmentation

chewing, chronic diseases and about their medical treatment. Blood sample were taken from non addicted people exposed to arsenic as well as from the healthy people.

Blood Culturing and Chromosomal Preparation

Two 15 ml autoclaved culture vials were taken in a laminar flow Model (DB-5511), Decibel, New Delhi and 0.5 ml Roswell Park Memorial Institute Medium (RPMI) (R6504) media was poured in each of them using sterile pipette tip. This media favours the growth of Human lymphoid cells. Subsequently 500 µl of fetal calf serum was then added in each of them as it contains growth factors and low antibodies. This was followed by the addition of 50 µl of phytohemagglutinin (PHA). PHA, a lectin is added to make lymphoid cells (T cells only) to divide a limited number of times before undergoing senescence by cross linking with cell surface receptors hence increasing cell signalling then 0.3 ml (8–9 drops) of freshly collected heparinised blood was added to them. Cultures were incubated in Biological Oxygen Demand (BOD) incubator with 5 % CO₂ concentration and at 37 °C temperature for 70 h in sterile conditions. About 7.5 µl of colchicine was added to both of the vials and they were again kept into the incubator. After 90 min of adding colchicine the cultures were transferred to glass centrifuge tubes. The tubes were then centrifuged at 1,300 Revolutions per minute (RPM) for 8 min at room temperature in a swinging basket centrifuge, Sigma model (1-15PK), made in Germany. Supernatant was discarded and it was tapped to break the pellet. Few drops of hypotonic maintained at 37 °C was added to the pellet and it was tapped to mix. It was repeated thrice to mix properly. Volume was made to 8 ml, and it was incubated at 37 °C for 15 min. Few drops of fixative was added and mixed by pipetting. It was then centrifuged at 1,300 rpm for 8 min at room temperature. Supernatant was discarded and pellet was mixed by tapping 2–3 times with 3 drops of fixative. The volume was maintained to 8 ml and tubes were incubated at room temperature for 5 min. The tubes were again centrifuged at 1,300 rpm for 8 min at room temperature. Supernatant was discarded carefully and the pellet was again resuspended in 8 ml of fixative for washing. Centrifugation was done at 1,300 rpm for 5 min at room temperature. About 6 ml of upper supernatant layer was discarded and 2 ml was retained. Few drops from the left out liquid were dropped over a clean slide from height and left for drying. The slides were then stained with Giemsa stain for 5 min and then washed in the distilled water in coupling jars. Cover slip was applied using dibutyl phthalate xylene (DPX) and left for drying. The slides were observed under the microscope with oil-emersion 100X objective [10].

G Banding of Chromosome

The slides made by air drop method were kept for 10 days for ageing [11]. After the aging they were kept in 30 mg/ml

trypsin solution made in 0.85 % NaCl solution of pH 7.4 for 45 s. Immediately they were washed in 1:1 solution of sodium hydrogen phosphate and potassium hydrogen phosphate solution. The slides were then washed 2–3 times in distilled water and transferred into Geimsa stain and kept for 5 min for staining. They were then washed in distilled water 2–3 times and dried and then cover slip was applied using DPX. After drying they were observed under the microscope with oil immersion 100X objective.

Karyotyping

Selected metaphase plates were karyotyped following the standard human chromosome nomenclature (ISCN 1995) from each individual [12].

Results and Discussion

The first visible symptoms caused by exposure to low arsenic concentrations in drinking water are abnormal black-brown skin pigmentation which is known as ‘melanoses’. However with severe exposure to arsenic there is hardening of palms and soles which leads to keratosis. If the exposure further continues skin depigmentation starts, resulting into white spots (leukomelanosis). The palms and soles advance thicken and painful cracks emerge. These symptoms are known as hyperkeratosis which may lead to skin cancer [13, 14].

In present study 20 people were suffering through keratosis. While nine people having pigmentation on limb, chest and tongue (Table 2). The distribution of the total arsenic concentration in drinking water is shown in the Table 1. Out of 30 blood samples studied from the arsenic affected area two samples have shown chromosomal abnormalities (Table 2). The normal chromosome complement in human is 46XX in female and 46XY in male. But during the present study two cases were observed with genetic anomalies. One had mosaic 47XXY while the other had 45XO chromosome complement. It was the case of highly arsenic exposed person. Skin lesions were more pronounced in the high arsenic exposed person than in the less exposed one. 47XXY is also known as Klinefelter syndrome [15]. This contention is also supported by the work of Ostrosky et al. [16] and Warner et al. [17] who reported CA due to chronic arsenic exposure. Dulout et al. [18] analyzed the structural aberrations (translocation) as well as the numerical aberrations (aneuploidy) due to consumption of arsenic contaminated water. The children exposed to high concentration of arsenic in Hungary have shown a significantly higher incidence of aberrant cells [19]. Positive correlation exists between CAs and arsenic concentration in their drinking water. Gebel (2001) opined that CA is a better indicator of arsenic genotoxicity [20].

Table 2 Dermatological lesions and chromosomal abnormalities

Disorder	No. of cases
Keratosis	
No	10
Yes	20
Location of keratosis	
No keratosis	10
Hands	6
Feet	5
Both hands and feet	9
Severity of keratosis	
No keratosis	10
Mild	12
Moderate	8
Pigmentation	
No	21
Yes	9
Location of pigmentation	
No pigmentation	21
Limbs and trunk	7
Limbs, trunk and tongue	2
Severity of pigmentation	
No pigmentation	21
Less	5
Medium	2
Pronounced	2
Chromosomal abnormalities	
No abnormalities	28
Klinefelter syndrome (KS)	2

Klinefelter syndrome (47, XXY) results from the meiotic nondisjunction of the X chromosome of the gametes from either of the parents. It is one of the most common chromosomal variations found in the infertile men. It occurs with a frequency of 1/500 male live births (Paulsen et al. [21]), accounting for 7–10 % of the men with non-obstructive azoospermia (Matsuda et al. [22]). Hamberton et al. [23] described the presence of an extra X chromosome in the male individual resulting in the sex chromosome make up being XXY. Karyotype of such individuals reveals 47, XXY chromosome complement. Mosaic form is caused by meiotic nondisjunction of the X chromosome after fertilization of the zygote. The associated phenotypes are sterility, gynecomastia, incomplete masculine body build, tallness and sometimes learning disability. They are characterized by small testis and azoospermia [24]. Among all the features of men with Klinefelter syndrome sterility is the most common [25]. Since arsenic is affecting the people at chromosomal level, therefore, there is an urgent need to carryout extensive and continuous research for its

remediation. This is only possible when common people, the doctors and the scientists come together to work on the same platform.

Conclusions

The present investigation has been carried out on the effect of consumption of arsenic contaminated water on the individuals in Ballia district of Uttar Pradesh. The arsenic distribution in the ground water is seen to vary from 0.01 to 0.37 ppm. Thirty individuals from the affected area were examined for the effect of arsenic and their blood samples were studied for any genetic aberration. The study indicate that individuals who consumed high arsenic contaminated water suffered keratosis, pigmentation and chromosomal abnormalities.

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References

- Hossain Masud SK, Anantharaman N. Studies on arsenic(III) biosorption using *Thiobacillus ferrooxidans*. Ind J Environ Prot. 2005;25:76–80.
- Kostal J, Yang R, Wu CH, Mulchandani A, Chen W. Enhanced arsenic accumulation in engineered bacterial cells expressing ArsR. Appl Environ Microbiol. 2004;70:4582–7.
- Singh AL, Sarma PN. Removal of arsenic(III) from waste water using *Lactobacillus acidophilus*. Bioremediation J. 2010;14:92–7.
- Rahman MM, Chowdhury UK, Mukherjee SC, Mandal BK, Paul K, Lodh D, et al. Chronic arsenic toxicity in Bangladesh and West Bengal India: a review commentary. J Toxicol Clin Toxicol. 2001;39:683–700.
- Tsang WP, Chu HM, How SW, Fong JM, Lin CS, Yeh S. Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. J Natl Cancer Inst. 1968;40:453–62.
- Rosas I, Belmont R, Ammeinta A, Baez A. Arsenic concentration in water soil forage and milk in Camara Languera, Mexico. Water air soil pollut. 1999;112:133–49.
- Grantham DA, Jones JF. Arsenic concentration in water of wells in Nova Scotia Canada. J Am Water Works Assoc. 1977;69:652–7.
- Katsoyiannis IA, Zouboulis AI. Application of biological processes for the removal of arsenic from groundwaters. Water Res. 2004;38:17–26.
- WHO. Guidelines for drinking water quality: Recommendations. Vol.1, 2nd ed. Geneva: World Health Org 1993.
- Washington IA. Collection, transport, and processing of blood cultures. Clin Lab Med. 1994;14:59–68.
- Summer AT, Evans HJ, Buckland RA. New technique for distinguishing between human chromosomes. Nature (London) New Biol. 1971;232:31–2.
- Mitelman F, editor. ISCN (1995): An international system for Human Cytogenetic Nomenclature. Basel: Karger; 1995.
- Chen CJ, Hsueh YM, Chiou HY, et al. Human carcinogenicity of inorganic arsenic. In: Abernathy CO, Calderon RL, Chappell WR, editors. Arsenic exposure and health effects. London: Chapman and Hall Ltd; 1997. p. 232–42.
- Abernathy CO, Liu YP, Longfellow D, Aposhian HV, Beck B, Fowler B, et al. Arsenic: health effects, mechanisms of actions, and research issues. Environ Health Perspect. 1999;107:593–7.
- Amory JK, Anawalt BD, Paulsen CA, Bremner WJ. Klinefelter's syndrome. Lancet. 2000;356:33–335.
- Ostrosky-Wegman P, Gonshebb ME, Montero R, Vega L, Barba H, Espinosa J. Lymphocyte proliferation kinetics and genotoxic findings in a pilot study on individuals chronically exposed to arsenic in Mexico. Mutat Res. 1991;250:477–82.
- Warner ML, Moore LE, Smith MT, Kalman DA, Fanning E, Smith AH. Increased micronuclei in exfoliated bladder cells of individuals who chronically ingest arsenic contaminated water in Nevada. Cancer Epidemiol Biomarkers Prev. 1994;3(7):583–90.
- Dulout FN, Grillo CA, Seoane AI, Maderna CR, Nilsson R, Vahter M, Darroudi F, Natarajan AT. Chromosomal aberrations in peripheral blood lymphocytes from native Andean women and children from north western Argentina exposed to arsenic in drinking water. Mutat Res. 1996;370:151–8.
- Paldy A, Farkas I, Markus V, Gundy S. Chromosomal aberration in children exposed to high concentration of arsenic in drinking water. Mutat Res. 1991;271:194–7.
- Gebel TW. Genotoxicity of arsenical compounds. Int J Hyg Environ Health. 2001;203:249–62.
- Paulsen CA, Plymate SR. Klinefelter's syndrome. In: King RA, Rotter JI, Motulsky AG, editors. The genetic basis of common diseases. New York: Oxford University Press; 1992. p. 876–94.
- Matsuda T, Horii Y, Yoshida O, et al. Unilateral obstruction of the vas deferens caused by childhood inguinal herniorrhaphy in male infertility patients. Fertil Steril. 1992;58:609–13.
- Hamberton JL, Canning N, Ray M, Smith S. A cytogenetic survey of 14,069 newborn infants. Incidence of chromosome abnormalities. Clin Genet. 1975;8:223–43.
- Griffin JE, Wilson JD. Disorders of the testes and the male reproductive tract. In: Wilson JD, Foster DW, editors. Williams Textbook of Endocrinology. 8th ed. Philadelphia: W.B. Saunders; 1992. p. 799–852.
- Rives N, Joly G, Machy A, Simeon N, Leclerc P, Mace B. Assessment of sex chromosome aneuploidy in sperm nuclei from 47, XY/47, XXY males: comparison with fertile and infertile males with normal karyotype. Mol Hum Rep. 2000;6(2):107–12.