PRINT: ISSN 0972-3757 ONLINE: 2456-6360
Int J Hum Genet, 13(1): 47-51 (2013)
DOI: 10.31901/24566330.2013/13.01.08

Study of the Effect of Tea in an Arsenic Exposed Population Using Micronuclei as a Biomarker

Tulika Chakraborty[1] and Madhusnata De[2]

[1]Department of Biophysics, Bose Institute, Centenary Building, P1/12 C.I.T. Scheme VII-M, Kolkata 700 054, West Bengal, India
[2]Department of Genetics, Ramakrishna Mission Seva Pratishthan,Vivekananda Institute of Medical Sciences, 99 Sarat Bose Road, Kolkata 700 026, West Bengal, India

KEYWORDS Toxic Metals. Micronuclei. Arsenic. Tea. Addiction

ABSTRACT Arsenic is a paradoxical human carcinogen. In West Bengal it affects around 42.7 million people. Arsenicosis is the effect of arsenic poisoning due to high level of arsenic in body, usually over a long period such as from 5 to 20 years. A total of 100 arsenic exposed individuals and 50 age and sex matched healthy controls were taken for this study. All the individuals have given information regarding their tea drinking habit and addiction history. Arsenic from water, hair and nail samples of all exposed group and control group individuals were studied. Oral mucosal cell was collected from each subject to study micronuclei (MN) from buccal smear. In exposed individuals the mean arsenic content in the drinking water was found to be 66.75µg/l which was above the WHO recommended level of 10µg/l. The mean arsenic content in hair and nails of the exposed individuals was found to be significantly higher (P≥0.01) than the control. In the present study we found that in the individuals belonging to exposure group had a significantly higher buccal smear MN percentage (P≥0.01) than the control group. It was also found from this study that the individuals who had tea drinking habit had a lower percentage of MN in compare to non-tea drinkers. The individuals who had addiction history, in case of both tea drinkers and non tea drinkers had a higher percentage of MN in compare to the non-addicted person. Therefore micronuclei can serve as an efficient biomarker of arsenic exposure and tea has got protective role against arsenic exposure.

INTRODUCTION

Arsenic is one of the most toxic metals derived from the natural environment. Arsenic was ranked first in a list of 20 hazardous substances by the Agency for Toxic Substances and Disease Registry and United States Environmental Protection Agency (Goering et al. 1999). Humans are exposed to arsenic (As) primarily from air, food and water. However, elevated inorganic arsenic in drinking water is the major cause of arsenic toxicity. The WHO recommends a limit of 0.01 mg/l of arsenic in drinking water. The two worst arsenic affected areas in the world are Bangladesh and West Bengal, India. 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. In October 2001, 2700 villages in a total of nine districts in WB are identified as arsenic-affected. More than 6 million people are drinking water containing ≥ 50 mg/l (Chakraborti et al. 2002). The problems of protection against exposure to arsenic through drinking water have assumed considerable importance, due to widespread effects of arsenic poisoning of large human populations numbering several millions in West Bengal and Bangladesh. The effects of arsenic induced toxicity in human beings can be assesed using a biomarker. Here for this study we selected micronuclei (MN) as a biomarker. A micronucleus (MN) is a small extra nuclear part 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). 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). Our earlier study in the arsenic affected area of West Bengal showed similar results (Chakraborty et al. 2006). 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

Address for correspondence:
Dr. Tulika Chakraborty,
Department of Biophysics,
Bose Institute,
Centenary Building,
P1/12 C.I.T. Scheme VII-M,
Kolkata 700 054,
West Bengal, India
Phone: 2355 9219/9416/9544, 2569-3334
Fax: 91-33-2350-6790
E-mail: tuli_8@rediffmail.com

controls and concluded that MN are a product of early events in human carcinogenic processes. MN study is a short non-invasive technique and can be repeated very easily.

Considering the widespread effect of arsenic toxicity there was always a search to provide protection against arsenic toxicity using a natural ingredient. Tea is a well-known beverage, brewed from leaf of *Camellia sinensis* and widely consumed throughout the world. Commercial tea is available in several forms, amongst which black tea is most prevalent in India and green tea in Japan and China. Consumption of tea has been associated with antimutagenic and possible anti-carcinogenic effects (Yang1997). In an earlier study by our lab co workers had shown that black tea when used along with freshly prepared solution of ferrous sulphate can reduce the cytotoxic effects of inorganic arsenic in mice (Poddar 2004).

In the present work we wanted to study the effect of tea in the arsenic exposed population using MN as a biomarker. The work is of special importance in view of the widespread exposure of human population of several districts of West Bengal, India and Bangladesh to arsenic through drinking water from deep tube wells.

METHODOLOGY

The arsenic exposed populations (n=100, 69 females and 31 males) were selected from the residents of Baduria block in Atghara, which is located in the North 24 Parganas district. 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 15 years was an inclusion criterion for the exposed population as well as control population. Age and sex matched control populations (n=50, 33 females and 17 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 15-39 years. Cohorts of 150 persons (50 control individuals and 100 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, source of daily water intake, diet, medical and residential histories assessment of exposure and level of exposure, addictions etc. 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, Kolkata. 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)]. Ethical committee of our Institute had approved the study.

Oral smears were obtained from the subjects as follows for micronuclei study:

The subjects were asked to rinse their mouths with water and a pre-moistened wooden spatula was used to sample cells from the oral mucosa. The spatula was applied to a pre-cleaned microscope slide. Smears were air dried and fixed in 80% methanol. Slides were stained by the Giemsa solution and the micronuclei frequency was scored using the criteria described by Sarto et al. (1990) and Tolbert et al. (1992). The same person scored 1000 cells blindly in each case to determine the micronuclei (MN) percentage.

RESULTS

A comparative data among the control and exposed population in terms of the arsenic contents (mean±SD) in water, hair and nail samples of the individuals were given in Table 1. The first group i.e. the control group contains 50 persons (33 females and 17 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 100 persons (69 females and 31 males) with arsenic level in their drinking water above the per-

Table 1: Detailed 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 group (in years)*			*Arsenic level in drinking water (mg/l)*	*Arsenic level in hair (mg/kg)*	*Arsenic level in nails (mg/kg)*
		Female	*Male*	*11-20*	*21-30*	*31-40*	*(Mean±SD)*	*(Mean±SD)*	*(Mean±SD)*
Control	50	33	17	24	15	11	6.44±0.23	361.23± 92.36	389.17±152.47
Exposed	100	69	31	42	33	25	66.75±2.53**	1398.74±519.53**	1989.43±734.75**

**Statistically significant at $P \geq 0.01$ (Fisher's *t* test)

missible limit. The persons belonging to both the control and exposed groups were mainly daily wage earners, students, housewives, farmers by profession. In the control group 24 persons belong to the age group of 11-20 years, 15 persons in the age group of 21-30 years and 11 persons in the age group of 31-40 years. In the exposed group the number of persons belonging to the age group of 11-20, 21-30 and 31-40 years were 42, 33 and 25 respectively. The mean (SD) arsenic content in water of the exposed group was 66.75±2.53 µg/l, whereas in control group it was found to be 6.44±0.23µg/l. The exposed group contained 1398.74±519.53 µg/kg arsenic (mean±SD) in hairs and 1989.43±734.75 µg/kg arsenic (mean±SD) in nails, whereas in control group the arsenic (meanSD) content in hairs and nails were 361.23±92.36 µg/kg and 389.17±152.47 µg/kg respectively. The mean (±SD) arsenic contents of water, hair and nail samples were significantly high (P≥0.01) in the exposed population.

Table 2 showed a detailed history of exposure, tea drinking habit and addiction history among the study participants. Of the 50 persons belonging to the control group, 46 persons drank water from tube well and 4 persons drank corporation water. Twenty five persons did not take tea, 15 persons took tea with milk and 10 of them took tea without milk. Five of them had chewed various tobacco preparations, 5 were smokers and one drank alcohol and no addiction was found in 43 subjects. All of the 100 persons residing in As affected areas drank water from tube well. Fifty-three of them did not take tea, 27 persons took tea with milk and 20 persons took tea without milk. Various forms of tobacco-chewing were found in 29 of them, 12 were smokers and one drank alcohol and no addiction was found in 60 subjects.

Table 3 showed a detailed MN frequency among the study individuals based on tea drinking habit. In the control group the average MN frequency was 0.32 whereas in the exposed group it was 0.73. In the control group, the individual who drink tea has a MN frequency of 0.31 whereas for non tea drinkers it was 0.33. In the exposed group, the individual who drink tea has a MN frequency of 0.71 whereas for non tea drinkers it was 0.74. These values are significantly higher than the control values (P≥0.01).

Table 3: Frequency of MN in the studied population based on tea drinking habit

Type	*Micronuclei requencies MN (%) (Mean ±SD)*		
	Average	*Drink tea*	*Do not drink tea*
Control	0.32 ± 0.032	0.31± 0.023	0.33 ± 0.025
Exposed	0.73 ± 0.033*	0.71± 0.039*	0.74 ± 0.041*

*Statistically significant at $P \geq 0.01$

Table 4 showed a detailed MN frequency among the study individuals based on tea drinking habit along with addictions. In the control group the average MN frequency of tea drinkers along with addiction was 0.35 whereas for the tea drinkers without addiction it was 0.29.

Table 2: Detailed history of exposure, tea drinking habit and addiction among the study participants.

Type	*Total no. of samples*	*Drinking water*		*Tea*			*Addiction*			
		Tube well	*Corp. water*	*No*	*With milk*	*Without milk*	*Tobacco chewing*	*Smoking*	*Alcohol*	*No addiction*
Control	50	46	4	25	15	10	5	5	1	43
Exposed	100	100	-	53	27	20	29	12	1	60

*Some persons had more than one kind of addiction.

Table 4: Frequency of MN in the studied population based on addictions

Type	*Micronuclei frequencies MN (%) (Mean±SD)*			
	Drink tea		*Do not drink tea*	
	Tea with addiction	*Tea without addiction*	*Tea with addiction*	*Tea without addiction*
Control	0.35 ± 0.027	0.29 ± 0.039	0.34 ± 0.035	0.32 ± 0.038
Exposed	0.78 ± 0.051*	0.70 ± 0.047*	0.73 ± 0.043*	0.69 ± 0.053*

*Statistically significant at $P \geq 0.01$

Again in the control group the average MN frequency of non tea drinkers along with addiction was 0.34 whereas for the non tea drinkers without addiction it was 0.32. In the exposed group the average MN frequency of tea drinkers along with addiction was 0.78 whereas for the tea drinkers without addiction it was 0.70. Again in the exposed group the average MN frequency of non tea drinkers along with addiction was 0.73 whereas for the non tea drinkers without addiction it was 0.69. These values are significantly higher than the control values (P≥0.01).

DISCUSSION

An environmental tragedy is developing in West Bengal, India where a large population is drinking arsenic-contaminated water, and an alarming number of toxicity cases associated with ingestion of arsenic-contaminated water have been reported (Guha Majumder et al. 1998).

Unstable chromosome aberrations can be studied in epithelial cells by the detection of MN and other nuclear aberrations in exfoliated interphase cells (Picker and Fox 1996). Various groups have found it that analysis of MN in buccal cells to be a sensitive method for monitoring genetic damage in human populations (Kayal et al. 1983; Foiles et al. 1989; Sarto et al. 1990). Micronuclei are suitable internal dosimeters for revealing tissue specific genotoxic damage in individuals exposed to carcinogenic mixtures (Stich et al. 1990).

In our present study we found that there was significant changes in MN frequencies in the exposed population in compare to control as indicated in Table 3 and 4. These results clearly showed that due to exposure of arsenic in drinking water there was a significant increase in cytogenetic damage in oral mucosal cells. The gradient of MN frequencies in the oral mucosal cells of exposed population was confirmed in comparison with the controls, revealing a 2.3 fold increase in MN frequency in the exposed individuals which was statistically significant at P≥0.01. In the tea drinkers group the average MN frequency was lower than the non tea drinkers group. It was also found that the persons, who had got any kind of addictions, had a higher percentage of MN frequency than the non addicted person both in tea drinkers and non-tea drinkers group as indicated in Table 4. This may be due to the fact that arsenic is a potent clastogen.

In our study we found a 2.3 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≥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 ± 24mg/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.

To the authors' best knowledge this was the first study of this kind to evaluate the effect of tea on a human population affected by arsenic.

CONCLUSION

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 and tea has got some protective effect against arsenic exposure. 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 signifi-

cant increase in oral MN frequency in arsenic exposed population in compare to matched controls. The prevalence of significantly high frequencies of MN in our arsenic exposed study population is an alarming one and calls for immediate remedial and preventive measures against arsenic induced carcinogenicity.

ACKNOWLEDGEMENT

The authors are grateful to Secretary, Ramakrishna Mission Seva Pratishthan and all the staff members of Vikas Kendra, Atghora. This research was partially funded by NTRF, Kolkata, India.

REFERENCES

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

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.

Chakraborty T, Das U, Podder S, Sengupta B, De M 2006. Micronuclei and Chromosomal aberrations as biomarkers, a study in an arsenic exposed population in West Bengal, India. *Bulletin of Environmental Contamination and Toxicology,* 76(6): 970-976.

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. *Mutat Res,* 370: 151-158.

Foiles PG, Miglietta LM, Quart AM, Quart E, Kabat GC, Hecht SS 1989. Evaluation of 32P- postlabeling analysis of DNA from exfoliated oral mucosa cells as a means of monitoring exposure of the oral cavity to genotoxic agents. *Carcinogenesis,* 10: 1429-1434.

Goering PL, Aposhian HV, Mass MJ, Cebrian M, Beck BD, Waalkes MP 1999. The enigma of arsenic carcinogenesis: role of metabolism. *Toxicol Sci*, 49: 5-14.

Guha Mazumder DN, Haque R, Ghosh N, De BK,Santra A, Chakraborti D and Smith AH 1998. Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal India. *Int J Epidemiol*, 27: 871-877.

Kayal JJ, Trivedi AH, Dave BJ, Nair J, Nair UJ, Bhide SV, Goswami UC, Adhvaryu SG 1993. Incidence of micronuclei in oral mucosa of users of tobacco products singly or various combinations. *Mutagenesis*, 5: 31-33.

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(2): 319-327.

Picker JD, Fox DP 1996. Do curried foods produce micronuclei in buccal epithelial cells. *Mutat. Res*, 171: 185-188.

Poddar S 2004. Dietary intervention with iron and black tea infusion in reducing cytotoxicity of arsenic. *Indian J Exp Biol*, 900-903.

Ramirez A, Saldanha PH 2002. Micronucleus investigations of alcoholic patients with oral carcinomas. *Genet Mol Res*, 1(3): 246-260.

Roberts DM 1997. Comparative cytology of the oral cavities of snuff users. *Acta Cytol*, 41: 1008-1014.

Sarto F, Tomanin R, Giacomelli L, Canova A, Raimondi F, Guiotto C, Fiorentino MV 1990. Evaluation of chromosomal aberrations in lymphocytes and micronuclei in lymphocytes, oral mucosa and hair root cells of patients. *Mutat Res*, 228: 157-169.

Stich HF, Acton AB, Palcic B 1990. Towards an automated micronucleus assay as an internal dosimeter for carcinogen exposed human population groups. *Cancer Res*, 120: 94-105.

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(6): 473-484.

Tolbert PE, Shy CM, Allen JW 1992. Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutat Res*, 271: 69-77.

WHO 1996. Guidelines for drinking water quality. In: *Health Criteria and Other Supporting Information*. 2nd Edition. Geneva: WHO, 2: 156-167.

Yang CS 1997. Inhibition of carcinogenesis by tea. *Nature*, 383: 134.