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SISTER CHROMATID EXCHANGE FREQUENCY AND CHROMOSOME ABERRATIONS IN RESIDENTS OF FLUORIDE ENDEMIC REGIONS OF SOUTH GUJARAT

Sajayan Joseph, PK Gadhia[a]
Gujarat, India

SUMMARY: Peripheral blood lymphocytes of residents of three villages and one nearby township in South Gujarat with fluoride concentrations in the drinking water of 1.56 - 3.46 and 0.6 - 0.8 ppm, respectively, were examined for their frequency of sister chromatid exchanges (SCE) and chromosome aberrations. The rates of SCEs and chromosome aberrations in persons living in one of the endemic villages were significantly higher than in the others, and their lymphocytes were more susceptible to the clastogen Mitomycin-C.

Keywords: Chromosome aberrations, Endemic fluoride area, Human lymphocytes, Sister chromatid exchanges, South Gujarat.

INTRODUCTION

Although explored for some time, *in vitro* studies on genotoxic effects of fluoride in human cells remain inconclusive. In combination with certain alkylating agents, fluoride suppresses chromosome aberrations.[1] In cultured human lymphocytes, sodium fluoride (NaF) is reported to induce chromosome aberrations.[2] In contrast, no increase in chromosome aberrations was found in human diploid fibroblast.[3] Recently, Gadhia and Joseph[4] observed an increase in chromosome aberrations but not in sister chromatid exchanges (SCE) in cultured human lymphocytes exposed to 30 ppm of NaF. Few *in vivo* studies have been carried out in endemic fluoride areas, and those on SCE frequency in human lymphocytes yielded contradictory results. Thus, an increase in SCE has been reported in fluorosis patients[5,6] but a reduction in residents of endemic fluoride areas.[7]

As far as we are aware, no study has been reported on chromosome aberrations in residents of an endemic fluoride area. We therefore thought it worthwhile to analyze chromosome aberrations and SCE frequencies in a population exposed to higher concentration of fluoride (1.56 - 3.46 ppm) than the current permissible 1 ppm level. The study also included clastogenic testing with Mytomycin-C (MMC) to assess the sensitivity of the residents living in an endemic fluoride area to chromosome aberrations.

MATERIALS AND METHODS

A total of 42 blood samples from 7 adult males and 7 adult females (ages ranging between 28 and 35 years) were collected from each of the three villages of Gamtalav, Amalsadi, and Khedpur. For comparison with a lower fluoride village, 14 blood samples were collected from Mandvi township, which is 2 to 3 km away from these three endemic fluoride villages. Metaphase chromosome preparations from blood samples of fluoride-exposed and control indi-

[a]For Correspondence: Department of Biosciences, South Gujarat University Surat 395 007, Gujarat, India. E-mail: pankaj_gadhia@hotmail.com

viduals residing in Mandvi township were carried out by routine phytohemaagglutinin (PHA) stimulated cultures as described elsewhere.[4] The fluorescence plus Giemsa (FPG) technique was used for SCE.[8] Separate cultures were set up from each sample for chromosome aberrations, SCE and MMC. Bromodeoxyuridine (BrdU) (10 µg/mL) and MMC (30 ng/mL) were added to two sets of cultures 24 hr after initiation of culture. All slides were coded and scored by a single individual. A total of 100 second metaphases for SCEs and 100 metaphases for chromosome aberrations were scored from each culture. The cell replicative index (RI) was also calculated by recording the percentage of cells in first (M1), second (M2) and third (M3) cell division using the formula RI = (1 x % of M1 + 2 x % of M2 + 3 x % of M3)/100. The results were analyzed by two-tailed Student's 't' test using SSPS/PC+ statistical package.

RESULTS

The frequencies of sister chromatid exchanges (SCE), cell proliferative index, and chromosome aberrations of residents living in the endemic fluoride area are shown in Tables 1 and 2. Table 3 shows MMC-induced chromosome aberrations in residents to determine whether or not they are more sensitive to a clastogenic agent than healthy normal individuals.

A significant increase ($P < 0.01$) in frequencies of SCE was observed in one of the three villages. However, no significant variation was observed in the cell cycle proliferative index of fluoride-affected individuals as compared to the lower fluoride control (Table 1).

Table 2 shows that the frequency of chromosome aberrations was significantly higher ($P < 0.001$) in the study group than in the control and also higher in one of the endemic villages. MMC-induced chromosome aberrations showed a significant increase ($P < 0.05$) in this village compared to the control and the other villages (Table 3).

Table 1. Sister chromatid exchange (SCE) frequencies and cell replicative index in lymphocytes of residents of endemic fluoride area

Village	Fluoride in water mg/L	No. of samples	Mean age	No. cells scored	% of cells in M1	M2	M3	Replicative index	SCE/Cell
Control (Mandvi)	0.6 - 0.8	14	28	1400	37	37	30	2.01	7.50 ± 0.30
Gamtalav	2.36 - 3.06	14	29	1400	37	39	24	1.87	7.56 ± 0.19
Amalsadi	2.64 - 3.46	14	29	1400	41	35	26	1.89	7.51 ± 0.24
Khedpur	1.56 - 2.90	14	35	1400	36	49	15	1.79	9.35 ± 0.85*
Range of 3 endemic villages	1.56 - 3.46	42	31	4200	38	38	22	1.80	8.14 ± 0.42

*Significantly different ($P < 0.01$) from control and the other two endemic villages.

Table 2. Chromosome aberrations in blood of residents of an endemic fluoride area

Village	Fluoride in water (mg/L)	No. of samples	No. of meta-phases scored	Chromosome type aberrations/100 cells			Chromatid type aberrations/100 cells)			Total aberrations minus gap
				D	R	F	G	B	I	
Control (Mandvi)	0. 6 - 0. 8	14	1400	0.0	0.0	3.0	32.0	0.0	0.0	3.0
Gamtalav	2.36 - 3.06	14	1400	4.8	1.5	2.0	36.0	1.4	0.7	10.4†
Amalsadi	2.64 - 3.46	14	1400	3.3	0.7	2.0	33.0	1.2	1.9	9.1*
Khedpur	1.56 - 2.90	14	1400	5.3	1.9	4.0	48.0	0.6	2.7	14.5†
Range of three endemic villages	1.56 - 3.46	14	4200	4.5	1.4	2.7	39.0	1.1	1.8	11.5†

Significantly different *($P < 0.05$) and †($P < 0.001$) from control. D - Dicentric; R - Ring; F - Fragment; G - Gap; B - Break; I - Interchange.

Table 3. Chromosome aberrations in Mitomycin-C treated lymphocytes of residents of endemic fluoride area

Village	Fluoride in water (mg/L)	No. of sam-ple/metaphase scored	MMC dose ng/mL	Chromosome type (aberrations/100 cells)			Chromatid type (aberrations/100 cells)			Total aberrations minus gaps
				D	R	F	G	B	I	
Control (Mandvi)	0. 6 - 0. 8	14/1400	30	3.5	3.0	2.7	57.0	3.5	2.5	15.2
Gamtalav	2.36 - 3.06	14/1400	30	7.0	2.5	1.9	51.0	1.0	1.7	14.1
Amalsadi	2.64 - 3.46	14/1400	30	5.5	2.1	3.9	55.0	1.0	0.9	13.4
Khedpur	1.56 - 2.90	14/1400	30	11.0	5.3	7.4	59.0	4.1	1.9	29.7†
Range of three endemic villages	1.56 - 3.46	14/4200	30	7.8	3.3	4.4	55.0	2.0	1.5	19.0*

Significantly different *($P < 0.01$), †($P < 0.001$) from control. D - Dicentric; R - Ring; F - Fragment; G - Gap; B - Break; I - Interchange.

DISCUSSION

That fluoride causes genetic damage *in vitro* is generally acknowledged. Most of the *in vitro* studies carried out with sodium fluoride showed mainly chromatid gaps and breaks.[4,9,10] However, these indices are not considered reliable indicators of real damage to the genome.[11] Moreover, the fluoride concentrations used in the *in vitro* studies were many times higher than the level often present in drinking water.

On the other hand, *in vivo* studies have also yielded conflicting results. A significant increase in chromosomal aberrations in mice was found by Mohamad and Chandler.[12] However, Kram *et al*[13] reported no significant difference in SCE and chromosomal aberrations between mice raised on a high (50 ppm) and a low (1 ppm) fluoride diet. It is difficult to compare our findings with these studies, since the latter were conducted on experimental animals.

With regard to human lymphocytes, Sheth *et al*[5] reported a significantly elevated SCE rate in fluorotic individuals exposed to 1.95 - 2.2 ppm fluoride. Wu and Wu[6] also reported a significant increase in the SCE rate in fluorosis patients exposed to 4-15 ppm fluoride. On the other hand, Li *et al*[7] reported a reduction in SCE frequencies in persons exposed to fluoride concentration as high as 4 ppm.

The SCE results of the current study are in partial agreement with the first of the two foregoing reports.[5,6] As far as we are aware, no study on chromosome aberrations in human populations exposed to higher concentration of fluoride in drinking water has been reported. Our results indicate that there is a significant increase in the frequencies of chromosome aberrations and SCE in one of the village populations exposed to a fluoride concentration higher than the permissible limit. The lymphocytes of these residents were also more susceptible to a clastogen such as Mitomycin-C than the other populations and displayed a significant increase in chromosome aberrations.

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