The fluorescence plus Giemsa method was used for the scoring of sister chromatid exchanges, and the procedure of Perry and Wolff (3) was followed with slight modifications. All the slides were randomized with code numbers and were scanned by a single individual. The chromosomes were examined under a Nikon light microscope. The influence on the cell cycle proliferative index was evaluated by recording the percentage of cells in the first (M1), second (M2), and third (M3) cell divisions. A minimum of 100 cells were analysed from each culture. The cells in M2 phase were further examined and only the cells with well spread chromosomes were scored for sister chromatid exchanges (SCEs). A total of 25 cells from each culture were examined for SCE frequency. The Students 't' test was used for statistical analysis of SCE data.

Fluoride concentrations from the serum as well as from the water samples were determined with an Ion Selective Electrode Orion Model 701A.

Results

The frequencies of sister chromatid exchanges in fluoride affected individuals and in normal controls are shown in Table 1. The values are the average (mean) number of SCEs observed in minimum 25 M2 cells each with 46 chromosomes. Statistical analysis indicated a significant increase ($p<0.05$) in the SCE/plate in the study group when compared to the normal controls. The influence of fluoride toxicity on cell cycle proliferative index was also carried out. Cultures from both the fluoride affected individuals and normal controls showed presence of cells in 1st, 2nd and 3rd cell cycles indicating the existence of all three cell cycles in both the groups of cells. No significant variation was observed in the cell cycle proliferative index of fluoride affected individuals as compared to controls (Figure 1).

The serum fluoride level was significantly elevated ($p<0.001$) in persons residing in Gujarat as compared to those examined in Ahmedabad (Table 2).

Discussion

A number of investigators have utilized the SCE test to study the genotoxicity of fluoride (1,4-12). In the present study, human populations directly exposed to fluoride in drinking water in endemic regions of North Gujarat were investigated to evaluate the possible effect of fluoride on SCE. To the best of our knowledge this is the first report on genotoxic effects following long-term fluoride intake in an endemic area in India. In this study, the results from cultured human lymphocytes indicate a significant increase in the frequency of SCE in the study group compared to the normal control. *In vitro* studies have yielded inconsistent findings with regard to SCE in white blood cells. In human lymphocytes exposed to doses up to 4 mM (11) and 3 mM (12) of NaF, no significant variation in the frequency of SCE was reported. *In vivo* studies carried out in mice maintained for seven generations on a diet containing 1.2 mM NaF indicated no increase in SCE frequencies in bone marrow cells (8). On the other hand, an increase in the incidence of SCE was observed in cultured Red Muntjac cells and in cultured Syrian Hamster embryo cells at dosage levels of 0.5, 1.0, and 1.9 mM, respectively (9,10).

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TABLE 1. Frequencies of SCEs in control and study group

Sr.No.	Group	No. of cases investigated	No. of metaphases screened	No. of metaphases with 2nd cell cycle	SCE/ plate*
1	Control	21	2173	612	7.46 ± 0.52
2	Study	100	11005	3856	8.79 ± 0.26

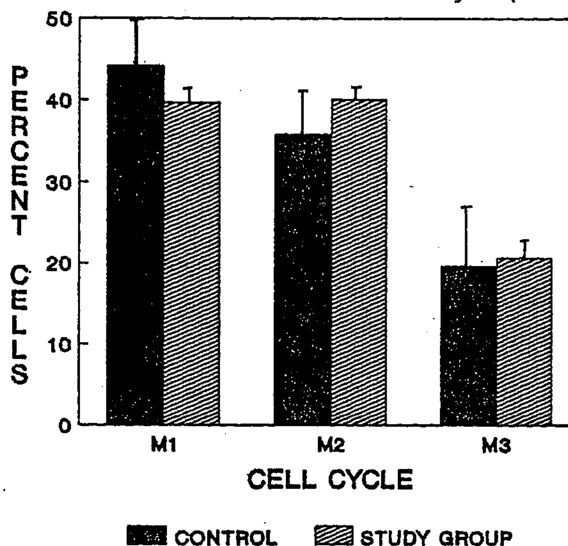
* = mean ± SE
The values are significantly different ($p < 0.05$).

TABLE 2. Fluoride concentration in drinking water samples and in serum of control and fluorotic cases screened for SCE

Area	Water Fluoride (ppm)	Serum Fluoride (ppm)
Control (Ahmedabad)	0.62 ± 0.02	0.04 ± 0.002
Range	0.60 - 1.04	0.03 - 0.05
n	24	25
Endemic Region (Mehsana District)	2.20 ± 0.05*	0.286 ± 0.02*
Range	1.949 - 2.211	0.177 - 0.539
n	36	24

Values are mean ± SE
n = number of samples
* = $p < 0.001$

FIGURE: Influence of fluoride on the cell cycle proliferation



Furthermore, no significant chromosomal damage was observed in lymphocytes of cattle with signs of chronic fluoride poisoning (13), in mouse oocytes at doses as high as 100 ppm, and ewe oocytes exposed to 10 ppm NaF (14). A significant increase in chromosome aberration was reported in human leukocytes (15), human fibroblasts (16,17), and in mice (18) maintained on fluoridated water.

It is, however, difficult to correlate the results of the present study with the earlier reports, since most of them have been carried out using either *in vitro* cultures or *in vivo* systems by treating the experimental animal with fluoridated compounds. Moreover, the doses of fluoride used for *in vitro* studies were much higher than those ever attained *in vivo* in body fluids of mammalian organisms (19), and the *in vivo* studies have been carried out at concentrations far in excess of those encountered in daily living (2). In the present study, the fluoride content in drinking water of the endemic villages (Table 2) was higher than the permissible level of 1 ppm, according to the WHO standards (20). Serum fluoride concentrations were also significantly higher in the fluorotic subjects of these areas, corroborating earlier data (21-23).

The results of the present investigation suggest that in fluoride-affected persons exposed to 1.95-2.2 ppm fluoride in drinking water chromosomal alterations as indicated by SCE frequency and chromosome aberrations were higher than in normal persons exposed to 0.6-1.0 ppm drinking water fluoride. However, analysis of samples from other endemic areas, and also use of a series of investigations including cytogenetic and mutagenic assays, are necessary to confirm the findings. Work is also needed on a large control group who are consuming 0 ppm or less than 0.2 ppm fluoride. However, the fluoride levels in drinking water in Ahmedabad and its vicinity range from 0.6 to 1.04 ppm (see Table 2).

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