MICRONUCLEUS AND SISTER CHROMATID EXCHANGE FREQUENCY IN ENDEMIC FLUOROSIS

D Q Wu and Y Wu
Hohhot, Inner Mongolia, China

SUMMARY: Inhabitants of the Hohhot Region in Inner Mongolia who drink high-fluoride (4-15 mg/L) water were compared for their micronucleus (MN) rate and sister chromatid exchange (SCE) frequency in their peripheral blood lymphocytes. In persons with fluorosis as well as those considered "healthy", the MN rate and SCE frequency were significantly higher (t test) than in a neighbouring control group drinking low-fluoride water.

Key words: Endemic fluorosis; Inner Mongolia, Hohhot region; Micronucleus (MN) rate; Sister chromatid exchange (SCE) frequency.

Introduction

Although widespread in occurrence, fluorine (as fluoride ion, F^-) does not have any known physiological requirement. It is generally accepted, however, that long term overintake of fluoride may cause skeletal as well as dental fluorosis. Many studies on other toxic effects of fluoride have been made, including whether it alters human genetic material and ultimately leads to more serious harm.[1] At present, various test systems and methods have been developed to detect fluoride toxicity, but with mixed results. Here we use the technique of micronucleus (MN) and sister chromatid exchange (SCE) to detect mutagenicity and potential carcinogenicity from fluoride in fluorosis patients who drink elevated concentrations of fluoride in water.

Materials and Methods

Subjects investigated: A total of 53 fluorosis patients, aged 16-59, were selected from the inhabitants of villages and towns in the Hohhot District where the water they drank contained fluoride in the range of 4-15 mg/L. From the same regions, 20 healthy persons were also chosen for study by gross clinical and X-ray examination. A formal control group consisted of 30 healthy persons of similar age, drinking low-fluoride water (< 1 mg/L), who lived near the endemic regions.

Lymphocyte micronucleus test: A 0.4-mL sample of blood was examined with an oil-immersion microscope, and the number of lymphocytes and micronuclei counted.

Lymphocyte sister chromatid exchange: A similar 0.4-mL sample of blood was incubated for 24 hr at 37°C. 5-Bromodeoxyuridine was added, and the culture was allowed to stand for an additional 72 hr. Colchicine (final concentration, 0.08 μg/mL) was added 4 hr before harvesting the chromosomes. Sister chromatid differentiation on staining was performed by the FPG technique.[2] Counting was done in oil immersion.

Results

SCE frequency: A total of 1590 cells of the patients with fluorosis were examined and sister chromosome exchange (SCE) was observed 12729 times. The mean value was 8.01 ± 1.78 per cell, and the range was between 6 and 19. The mean value of SCE in the healthy people was 4.82 ± 0.98 per cell, and the range

Inner Mongolia Sanitary and Anti-epidemic Station, Hohhot 010020, China.
Presented to the XXth Conference of the International Society for Fluoride Research, Beijing, China, September 5-9, 1994.

was between 3 and 11. The mean SCE value of the normal control group was 4.28 ± 0.67 per cell, and the range was between 2 and 5. There was a significant difference ($p < 0.001$) observed by t test. This showed that the DNA of the patients with fluorosis was seriously damaged, and that the DNA of the healthy people in the endemic regions was also damaged in varying degrees.

TABLE 1. Comparison of SCE frequencies between endemic fluorosis patients healthy volunteers from high fluorine regions and normal control groups

Groups	Fluorine content of drinking water (mg/L)	No. of cases	No. of cells observed	No. of SCE	SCE rate/cell mean ± SD
Fluorosis patients	4 - 15	53	1590	12729	8.01 ± 1.78
Healthy volunteers of high F regions	6.2	20	600	2893	4.82 ± 0.98
Normal control	0.6	30	1170	5005	4.28 ± 0.67

Micronucleus rates: The mean value of the MN was 1.94 ± 0.86% in the patients with fluorosis and the range value was 1-15%, which was much higher than the mean value of 0.57 ± 0.44% in the controls and the mean value of 1.05 ± 0.68% in the healthy exposed people. The results showed that there was a significant difference ($p < 0.005$). Thus chromosome breakage caused by fluorosis might lead to chromosome mutation and aberration.

TABLE 2. Comparison of MN between the endemic fluorosis patients healthy volunteers of high fluorine regions and normal control group

Groups	No, of cases	No of cells observed	MN observed	MN% Mean ± SD
Fluorosis patients	34	3400	66	1.94 ± 0.86
Healthy volunteers	20	2000	21	1.05 ± 0.68
Normal control	30	3000	17	0.57 ± 0.44

Discussion

In recent years, SCE analysis has been considered to be a sensitive method for detecting DNA damage. There is a clear relationship between a substance's ability to induce DNA damage, mutate chromosomes, and cause cancers. The SCE frequency in the human body in peripheral blood lymphocytes is very steady, and does not vary with age or sex.[3] Any increase of the SCE frequency is primarily due to chromosome damage. Thus using a method to detect SCE for exploring the toxicity and harm caused by fluoride is of great importance.

The results in this paper showed an obvious increase in the SCE frequency of the patients with fluorosis, indicating that fluorine had some mutagenic effects, and could give rise to DNA damage. The fact that the SCE frequency of the healthy people in the endemic regions was also higher than that of the controls in the non-endemic regions suggests that early harm by fluorine can be cytogenetically detected in the sub-clinical patients with fluorosis who could not be given an early diagnosis clinically.

Under normal circumstances, the incidence rate of micronucleus is very low, usually 0-2%.[4] The normal value checked in this paper is 0-2%, which agrees with that reported in the literature.[5] The results show that the mean value of the micronucleus rate of the fluorine-toxic patients was $1.94 \pm 0.86\%$ (range 1-15%) which is 2-3 times more than that of $0.57 \pm 0.44\%$ in the controls. The appearance of the micronucleus in peripheral blood indicates that fluorine may be a mutagenic agent. Hence we conclude that the micronucleus experiment can be used as a testing procedure to detect fluorosis, with fluoride being one of the dangerous factors inducing mutagenesis. After human peripheral blood lymphocytes were cultured by use of various concentrations of NaF, Lu Wenqing[6] found that the increased micronucleus rate may cause a higher chromosome malformation frequency. The main types of chromosome malformation were breaks and crevices. These findings support the results obtained in this paper.

To sum up, the rise of SCE and MN in the peripheral blood lymphocytes of the fluorine-intoxicated patients indicates that fluorine is a mutagenic agent which can cause DNA and chromosomal damage.

References

1 Tao Maoxuan, Gao Wenhuahua, Zao Zunliang. A cytogenetics study on farmers who drink contaminated well water. *Environment and Health 9* (1) 24-25 1992.

2 Li Hongyu, Zu Junzhen. Sister chromatid methodology. *Medicine in Hebei 8* (1) 35 1986.

3 Tsutsui T, Susuki N, Ohmori M. Sodium fluoride-induced morphological and neoplastic transformation, chromosome aberrations, sister chromatid exchanges, and unscheduled DNA synthesis in cultured Syrian hamster embryo cells. *Cancer Research 44* (3) 938-941 1984.

4 Liu Weijun. Improvement of micronucleus test method in peripheral blood lymphocytes and preliminary observations in clinical use. *Chinese Journal of Medical Testing 15* (4) 233-234 1992.

5 Tang Deping, Fong Lunda, Yin Baoli. Improvement in testing human lymphocyte micronucleus. *Industrial Hygiene and Occupational Disease 9* 107-108 1983.

6 Lu Wenqing, Zao Maiyin, Yan Hun, Shi Lei. Genetic toxicity of fluoride and antagonistic effect on fluoride toxicity. *Public Hygiene in China 10* (3) 157-159 1991.

Published by the International Society for Fluoride Research
Editorial Office: 81A Landscape Road, Mount Eden, Auckland 4, New Zealand