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Short communication

Cytogenetic analysis of peripheral blood lymphocytes, many years after exposure of workers to low-dose ionizing radiation

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ABSTRACT

The present study aims to measure chromosomal aberrations and micronuclei in peripheral blood lymphocytes from 25 subjects exposed to 0.10–0.33 Gy external or internal irradiation 32–41 years ago using conventional cytogenetic analysis methods. The frequencies of total chromosome-type aberrations and micronucleus significantly increased in the exposed group compared with that in age-matched control group ($p < 0.001$); chromatid-type aberrations showed no difference between the two groups ($p > 0.05$). When exposed subjects were divided into two groups based on exposure dose, higher levels of dicentric plus translocation frequencies were observed in the ≥0.15 Gy dose group compared with those in the <0.15 Gy dose group, though the difference was not significant. Borderline association between exposure dose and dicentric frequency was detected in the exposed group ($r = 0.358$; $p = 0.079$). These results suggest that the genotoxic effects of ionizing radiation remain in subjects exposed to low-dose radiation even decades after exposure.

1. Introduction

Determination of chromosomal aberrations (CAs), especially dicentrics, induced by ionizing radiation in human peripheral blood lymphocytes has been considered to be the most sensitive and reliable biodosimetry method [1]. Scoring of micronucleus (MN) formation is another cytogenetic assay commonly used in biodosimetry. Many studies have investigated the effect of low-dose radiation on occupationally exposed hospital staff, residents of high-background radiation areas, Chernobyl cleanup workers, and retired workers of nuclear facilities using conventional cytogenetic analysis [2–9]. Higher frequencies of CAs and MN have been observed in all individuals exposed to ionizing radiation in the aforementioned studies compared with those of the corresponding control groups. These frequencies in human peripheral blood lymphocytes have been used as the specific biological parameters to evaluate environmental radiation and long-term health effects of ionizing radiation [10,11].

The present study aimed to measure the chromosomal aberration and MN frequencies in lymphocytes of 25 male subjects who had been exposed to 0.10–0.33 Gy internal or external irradiation 32–41 years ago. Results were compared with age-matched control individuals using conventional cytogenetic analysis. The effects of smoking status and exposure dose level on chromosomal aberration and MN frequencies were also investigated.

2. Materials and methods

2.1. Subjects

Twenty-five male subjects, who had been exposed to low-dose radiation 32–41 years ago, were enrolled in the present study. Five cases were uniformly exposed to single whole-body external radiation (dose range, 0.10–0.24 Gy) in their workplace between 1966 and 1975 [12]. The other 20 cases engaged in digging after underground nuclear testing in 1971 and received internal exposure to fission products of ^{137}Cs (dose range, 0.10–0.33 Gy) [12]. Personal exposure doses in externally and internally exposed subjects were calculated based on film or glass dosimeters and whole-body counting, respectively [12]. A total of 25 age-, gender-, and health status-matched subjects were registered in the experiment as a control group. All subjects were questioned in detail to determine whether they were systemically healthy. The questionnaire also included information about smoking habits, medical history, drug intake, and diagnostic medical irradiation. The subjects exhibited no current infections and did not receive medical irradiation within the last 6 months prior to sampling. The study was conducted at Henan Institute of Occupational Medicine (HIOM). The scope of this study was explained to each subject, and the Ethics Committee of HIOM approved all of the experiments. After written

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informed consent was obtained, approximately 3 mL of peripheral blood samples were collected from each subject using heparinized vacutainer tubes.

2.2. Lymphocyte culture

Whole blood lymphocyte cultures were set up according to the standard method [1] with minor modifications. Three separate culture vials were set up for each individual: two for CAs and one for MN. Whole blood (0.3 mL) from each subject was added to 5 mL of culture medium consisting of RPMI medium 1640 (Gibco, Grand Island, NY, USA) supplemented with 20% fetal calf serum (SunBao Biotech. Co., Ltd., Shanghai, China), 100 IU/mL penicillin, 100 μg/mL streptomycin, 200 mM L-glutamine (Sigma–Aldrich, Santa Clara, USA), and 200 μg/mL phytohemagglutinin (PHA; Mbchem Technol. Group Co., Ltd., Guangzhou, China) for mitogenic stimulation. Cultures were incubated at 37 °C in an incubator for 48 h for chromosomal aberration analysis and 72 h for MN assay.

2.3. Chromosome aberration analysis

Colchicine (Sigma–Aldrich, Santa Clara, USA) was added at a final concentration of 0.05 μg/mL 24 h before the end of incubation. After 48 h of incubation, cultures were harvested by centrifugation, suspended in hypotonic solution of 0.075 M KCl, incubated for 30 min at 37 °C, and fixed in three exchanges with a fresh mixture of methanol/acetic acid (3:1). Cell suspensions were dropped on wet, hot slides (37 °C) and dried at room temperature. Two slides were prepared for each culture and stained with 10% Giemsa (Sigma–Aldrich, Santa Clara, USA), and each slide was coded before being scored. 200 complete metaphases, i.e., those with 46 or more pieces, were recorded for each subject. Chromosomal aberrations, including acentric fragment, dicentrics, translocation, chromatid break, and chromatid exchange, were scored and verified by two experienced analyzers. The results were expressed as the number of chromosomal aberrations per 100 cells.

2.4. MN assay

Cultures were incubated for 72 h, and cells were collected by centrifugation and treated with hypotonic solution containing 0.075 M KCl for 1 min. Cells were fixed in a fresh solution of methanol/acetic acid (3:1) for 20 min after centrifugation and removal of the supernatant. The cells were centrifuged, resuspended, dropped on wet, cold slides (4 °C), and dried at room temperature. Two slides were prepared for each culture and stained with 10% Giemsa (Sigma–Aldrich, Santa Clara, USA), and each slide was coded before being scored. A total of 2000 lymphocytes transformed with PHA stimulation per subject were analyzed for the presence of MN, and the results were expressed as the number of MN per 1000 cells. MN scoring was limited to transformed lymphocytes with preserved cytoplasm according to the criteria proposed by Wang et al. [13].

2.5. Data analysis

Statistical analysis was performed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). The Mann–Whitney *U* test was used to compare the frequencies of CAs and MN between exposed subjects and controls. Spearman correlation coefficients were calculated to evaluate the effects of exposure dose and smoking status on the different cytogenetic assays. All reported *p* values were two-sided, and a significance level of 0.05 was used to determine significance.

3. Results

3.1. Cytogenetic analysis in exposed and control groups

The ages, exposure doses, and chromosomal aberration and MN results of exposed and control groups are presented in Table 1. Exposed and control subjects did not significantly differ in age ($p > 0.05$). The frequencies of total chromosome-type aberrations, including acentric fragment, dicentrics, and translocation, significantly increased in the exposed group ($p < 0.001$) compared with those of the control group. The differences in chromatid-type aberration frequencies per 100 cells were not significant between the two groups ($p > 0.05$). Moreover, chromatid exchange was only observed in the exposed group. MN frequency increased in the transformed lymphocytes of the exposed group compared with that in the control group ($p < 0.001$). Fig. 1 shows the different types of chromosomal damage observed in the exposed and control subjects.

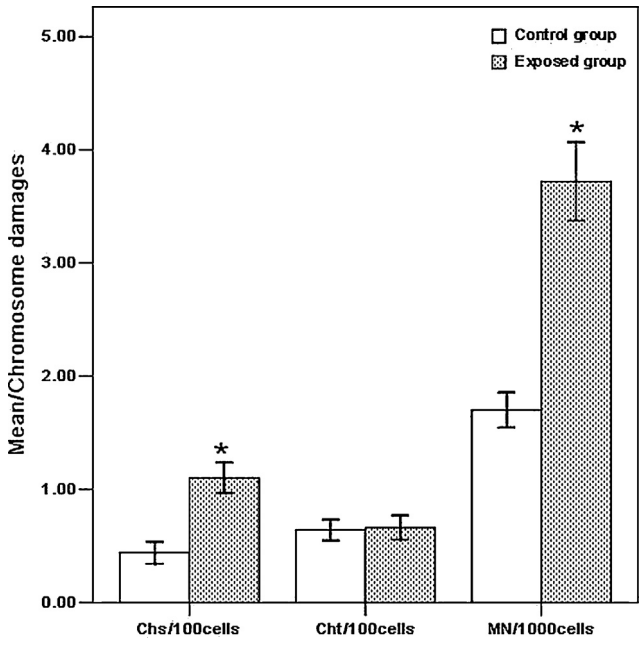

Fig. 1. Frequencies of chromosome-type aberration (Chs), chromatid-type aberration (Cht), and micronucleus (MN) in exposed and control groups (error bars indicate the standard error). $^{*}p < 0.001$.

3.2. Effects of exposure dose and smoking habits on chromosomal damage

Table 2 shows the effects of exposure dose and smoking habits on chromosomal aberration and MN frequencies. The 25 subjects were divided according to exposure dose into <0.15 Gy and ≥0.15 Gy dose groups. Higher chromosome-type aberration and MN frequencies were observed in the ≥0.15 Gy dose group compared with those in the <0.15 Gy dose group (Fig. 2), but differences observed were not significant ($p > 0.05$). Increases in total chromosome-type aberration rates were attributed to elevated dicentric and translocation frequencies in the ≥0.15 Gy dose group (Table 2; 0.71 versus 0.27 dic plus *t*/100 cells; $Z = 2.083$, $p = 0.110$).

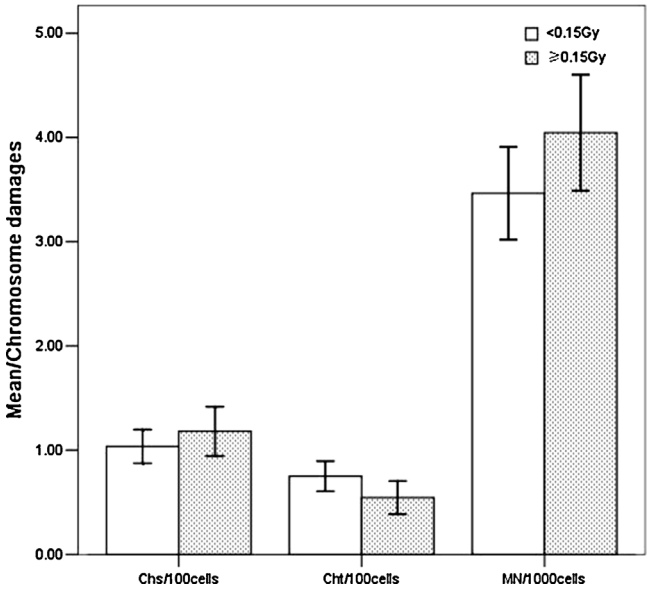

Fig. 2. Effects of exposed doses on chromosome-type aberration (Chs), chromatid-type aberration (Cht), and micronucleus (MN) frequencies in lymphocytes of the exposed groups (error bars indicate the standard error).