Monitoring of Human Exposure to Radiation With the Binucleated Lymphocyte Micronucleus Assay

HE JI-LIANG, JIN HAI-YAN, JIN LI-FEN, AND GAO SHENG-YONG

School of Public Health, Zhejiang University (Hu Bin Campus), Hangzhou 310006, Zhejiang, China

The micronuclei (MN) of peripheral blood lymphocytes from radiation-exposed people were monitored with the binucleated lymphocyte micronucleus assay (CBMN). MN rates in people with radiation-disease, radiation exposed and a control group were 12.57‰, 4.20‰ and 3.26‰, respectively. The MN rate of patients with radiation-disease was significantly higher than those of other groups ($P<0.01$). The difference between the radiation-exposed group and control group was not significant ($P>0.05$). Meanwhile, chromosome aberrations (CA) of 3 groups were determined. The results were similar to those seen while the MN assay. CA rates were 2.06%, 0.93% and 0.69%, respectively. CA rate of the radiation-disease group was significantly higher than that of other groups ($P<0.05$, $P<0.01$). The difference between the radiation-exposed group and the control group was not significant ($P>0.05$). The study indicates that the CBMN assay is a rapid, sensitive and accurate method which can be used to monitor a large population exposed to radiation.

INTRODUCTION

Radiation is extensively applied in the medical field and other research areas. Humans are often exposed to environmental ionizing-radiation at different levels. It is important to consider the genetic effects caused by low levels of radiation in addition to the acute effects (Cai, 1995). Conventional biological indicators of genetic damage caused by radiation are chromosome aberrations (CA), sister chromatid exchanges (SCEs) and micronuclei (MN). Recently, the binucleated lymphocyte micronuclei assay (cytokinesis-blocking method, CBMN) has been used to study the genetic damage caused by radiation, especially *in vitro* tests. It was showed that the CBMN assay is more sensitive and convient than the conventional MN assay (Kormas and Koteles, 1988; Balasen and Ali, 1991; Muller *et al*., 1996). In order to detect the genetic damage in a population exposed to radiation with this sensitive test, in this experiment we tried using CBMN to monitor humans exposed to radiation and patients with radiation-disease, respectively. By observing MN rates in binucleated lymphocytes, we shall able to monitor a population exposed to environmental radiation with CBMN in the future. Meanwhile, the chromosome aberrations were also monitored in the groups and the results were compared with CBMN.

MATERIALS AND METHODS

Subjects: Patient group: 21 people, who were occupationally exposed to X radiation (average year exposure-dose: 1.89 ± 0.03 mSv), and were clinically diagnosed as patients

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with radiation-disease with an average age of 55 years. Radiation exposed group: 15 members of the medical staff, who were occupationally exposed to X-ray (average year exposure-dose: 0.68 ± 0.09 mSv), and were not clinically diagnosed as patients with radiation-disease, with an average age of 45 years; Control group: 15 people, average age of 40 years.

Method: Heparinized whole-blood (0.4 ml) was added to 4.6 ml of RPMI 1640 (Sigma) containing 20% fetal calf serum, with 0.2 mg/ml phytohemagglutinin (PHA) and antibiotics (100 IU penicillin and 0.1 mg/ml streptomycin). Two independent cultures were set up for CA and MN scoring. For CA analysis and MN analysis, the cultures were incubated at 37℃ for 48 h and 72 h, respectively. Colchicine was added to cultures (0.01 mg/ml) 2 h before harvesting for CA. Metaphase spreads were prepared according to conventional methods, and slides were stained with giemsa stain. MN were analyzed in cells in which cytokinesis had been blocked by Cytochalasin B (Cyto-B, 4.5 μg/ml, Sigma) 28 h before harvesting (Fenech *et al.*, 1985). Cells were then processed according to the method described and modified to enable the use of whole-blood cultures (Barale *et al.*, 1993). 100 metaphase spreads and 1000 binucleated (BN) lymphocytes were scored for the presence of CA and MN respectively. MN≥8‰ of cells served as an abnormal MN rate (Gao, Xu and Hao, 1995, Fenech and Morley 1985).

Cyto-B was used in CBMN assay so that the binucleated cells were formed after cell division. The binucleated cells were analyzed for microclei in order to increase the sensitivity and accurancy of micronucleus assay. It was relatively simple to observe micronuclei due to the enlarged cellsize because of the hypotonic solution (Shen and Xue 1995). When compared with the MN assay, the CA test requires slide preparations with good quality and skillful scorers to observe the end-points. Because of the simplicity of the technique and rapid assessment of the binucleated cells, we suggest the use of the CBMN test as an alternative procedure in a large scale study of a population exposed to radiation pollution.

Table 1 shows the results of binucleated lymphocyte MN assay. The mean micronuclei rate (12.57‰) in the patient group was 4 times greater than that (3.26‰) of the control group ($P<0.01$), and significantly higher than that (4.2‰) in the medical staff group ($P<0.01$). The mean MN rate in the medical staff group was slightly higher than that of the controls, but not significantly ($P>0.05$).

TABLE 1

The Micronucleated Cell Rate (MNC Rate) of Human Lymphocytes

Group	Number of Subjects	Number of Cells Scored	Number of Subjects With MN Rate ≥8	MNC Rate ($\bar{x} \pm s$)‰
Patients[a]	21	21 000	19	13.23 ± 4.22
Medical staffs[b]	15	15 000	2	4.20 ± 3.45
Controls[c]	15	15 000	0	3.20 ± 1.44

[a-c] $P<0.01$; [a-b] $P<0.01$; [b-c] $P>0.05$.

The number of individuals with an MN rate ≥8‰ in the patient group was 19 (90%); in the medical staff group there were 2 (13%); and there were none in the control group.

Table 2 shows the number of binucleated cells with microclei in patient group. It was found that the total number of BN cells with micronuclei was 278; but the total micronuclei number was 348. The number of BN cells with one micronucleus was 235; and the number of BN cells with 2-10 micronuclei was 43 (15.5%). Two BN cells had 10 micronuclei.

TABLE 2

Micronuclei Number of BN Cells in Patients Group

No.	Number of Micronucleated Cells	Number of Cells Carrying 1-10 Micronuclei										Total Number of MN
		1	2	3	4	5	6	7	8	9	10	
1	18	12	5								1	32
2	12	8	4									16
3	9	8	1									10
4	11	10			1							14
5	14	11	2		1							19
6	14	13			1							17
7	17	15	2									19
8	11	9	1								1	21
9	13	11	2									15
10	13	11	1	1								16
11	20	16	4									24
12	13	13										13
13	14	11	3									17
14	15	12	2	1								21
15	14	13	1									15
16	20	16	3	1								25
17	14	14										14
18	17	13	4									21
19	5	5										5
20	3	3										3
21	11	11										11
Total	278	235	35	2	3	1					2	348

Note. Means in the same column not sharing a common superscript are significantly different at $P<0.05$.

The chromosome aberrations (CA) detected in the peripheral lymphocytes for the three groups are shown in Table 3. The chromosome structure aberrations observed were mainly chromatid breakage, chromosome breakage and fragments. The mean CA rate (2.06%) in the patient group was significantly higher than that (0.69%) of the controls ($P<0.01$) and that (0.93%) of the medical staff group ($P<0.05$). The difference between the control and medical staff groups was not significant ($P>0.05$).

TABLE 3

The Frequencies of Chromosome Aberrations of Human Lymphocytes (%)

Group	Number of Subjects	Number of Cells Scored	Type of CA			CA Rates ($\bar{x}\pm s$) %
			b	B	F	
Patients[a]	18	1800	9	23	5	2.06 ± 1.80
Medical staff[b]	15	1500	7	5	2	0.93 ± 0.70
Controls[c]	13	1300	8	1	0	0.69 ± 0.48

[a-c] $P<0.01$; [a-b] $P<0.05$; [c-d] $P>0.05$.

Note. b: chromatid breakage; B: chromosome breakage; F: fragment

It is well known that ionizing radiation can induce diverse types of chromosome damage, depending on exposure dose, duration and individual sensitivity. It is important to select a suitable assay for assessing the genetic effects of radiation. Specially, a

rapid, simple and sensitive assay should be used for human monitoring. However, the chromosome aberration test (CA) and micronucleus test (MN) are the most conventional assays used at present. In this study, the cytokinesis-blocked micronucleus assay (CBMN) was used to detect MN in human binucleated lymphocytes. The results showed that the mean MN rate (13.23‰) in the patient group was significantly higher than that (3.26‰) of the control and (4.20‰) in the medical staff groups ($P<0.01$). Subjects with an MN rate >8‰ in the patient, medical staff and control groups were 19 (90%), 2 (13%) and 0, respectively. Micronuclei are formed from the condensation of lagging acentric chromosome, or chromatid fragments, or entire chromosomes, so these results indicate that the patients had chromosome damage because of exposure to large doses of radiation. Although the MN rate in the medical staff group was slightly higher than that of the controls, the difference between the two groups was not significant ($P>0.05$). This may be due to exposure to low-dose radiation, damage rapair and radio-adaptive response (Venkat *et al*., 1996).

Recently, more micronucleus tests aimed to detect the genetic effects of radiation were performed with CBMN method *in vitro*. The results showed that there was a strong positive correlation between the incidence of chromosome aberration and presence of micronuclei (Vijavaxmi, Deahl and Meltz, 1995). In this study, both MN and CA were detected in all three groups, and the results indicate that the CA rate in the patient group was significantly higher than that of the control group ($P<0.01$) and the medical staff group ($P<0.05$) group; however, the difference between the later two groups was not significant ($P>0.05$). The CA rate corresponded to the results of MN.

In the patient group, the total number of micronucleated cells was 278, and the total number of micronuclei was 348. The number of binucleated cell with 2-10 micronuclei was 43 (15.5%); of these two binucleated cells had 10 micronuclei. It micronuclei was 43 (15.5%), among them, two binucleated cells had 10 micronuclei. It was difficult to detect binucleated lymphocytes with more micronuclei using the conventional MN assay.

CONCLUSION

The binucleated lymphocyte micronucleus assay is a rapid, sensitive and accurate method especially useful in monitoring large sample population exposed to radiation.

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