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EFFECTS OF IONISING RADIATION ON MICRONUCLEUS FORMATION AND CHROMOSOMAL ABERRATIONS IN CHINESE RADIATION WORKERS

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This study is aimed to investigate the effects of ionising radiation (IR) on micronuclei (MN) formation and chromosome aberrations (CAs) in Chinese radiation workers. The study was conducted using peripheral blood lymphocytes from 1392 radiation workers from Public Hospitals of the city of Tangshan (the exposed group), and 143 healthy individuals as the control group. Fluorescence *in situ* hybridisation (FISH) was used to detect the unstable and stable nuclear CAs on metaphase. The MN assay was performed using the cytochalasin B method for cytokinesis-block. The MN and CA frequencies were significantly higher in the exposed group than in healthy controls (both $p < 0.001$). Examination of the incidence rates of MN and CA showed an increasing trend among workers in some occupations compared with the others (all $p < 0.05$). There were also significant differences in MN and CA rates among workers with different exposure times (all $p < 0.05$). Stable CA rates demonstrated an increased trend among workers with different exposure times (all $p < 0.05$), while no significance of unstable CA rates was found among workers with different exposure times (all $p < 0.05$). Importantly, the frequencies of CA and MN increased among different cumulative radiation dose groups (all $p < 0.05$). Correlation analysis showed that the frequencies of MN and CA were positively associated with the cumulative radiation dose. Long-term exposure to IR may have harmful effects on the health of radiation workers. The data obtained here show an increased risk of genetic instability that correlated with occupation, exposure time and equivalent dose among Chinese radiation workers.

INTRODUCTION

Ionising radiation (IR) is absorbed by living cells and disrupts normal cell function by inducing chemical and biological changes in cells[1–3]. In addition, IR has 'bystander effects' induced by signals from irradiated cells, which decreases clonogenic survival, induces genetic instability by elevated sister chromatid exchanges and promotes apoptosis as well as significant alterations in gene expression[4]. Exposure to IR leads to cell death or apoptosis through DNA damage by inducing DNA single-strand breaks (SSBs) and double-strand breaks (DSBs)[4–6]. Diagnostic and therapeutic applications, such as X rays and other medical devices, are an important source of IR and pose a significant risk of occupation-related exposure to IR. This poses a huge problem with regard to health of the exposed occupational group and for hospitals in managing occupational health risks[2, 6, 7]. Considering the serious radiological effects of IR on human health, the health risk to Chinese workers in different occupations, particularly in a healthcare setting, is important to understand[2]. Chromosome aberrations (CAs) are part of the broad spectrum of DNA mutations generated during DSB repair and are visible within few cell division cycles[8]. Micronuclei (MN) are fragments or whole centric chromosomes or chromatids resulting from non-repaired or mis-repaired DSBs in anaphase, and serve as an important index for measuring damage caused by IR[9]. Previous studies have shown that CA and MN frequency, detected in peripheral blood lymphocytes, is directly linked to damage caused by IR. CA and MN are both crucial predictors of the degree of radiation damage[6, 10]. However, an overall estimation of IR risk is complicated in an occupational setting and depends on the category and dose of radiation, irradiation conditions, body sensitivity and different exposure dose, all of which hugely impact the degree of damage[11]. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) concluded that, after an acute dose of IR, the risk rate of death from all tumours was up by 4.3–7.2 % (up to 1 Sv)[12, 13]. Previous studies have also shown that radiation workers exposed to low doses of IR (ranging from 20 to 40 mSv with a upper bound of 200 mSv) exhibited MN and CA, while high-dose IR led to gross interferences in cell and tissue functions[14–16]. Moreover, evidence suggests that 80 % of radiation workers exposed to IR for 10 y or more had elevated MN and CA[17]. Current mean occupational radiation doses are 14-fold less than those recorded during 1989–92, suggesting that policies and practices to ensure adequate radiation protection have helped in reducing the radiation exposure among workers[18]. This study examined the health records of subjects exposed to IR, with an aim to understand IR-induced rates of MN and CA among radiation

workers. The study results provide a scientific basis to discuss the effects of IR in the context of the current policies and practices in place, and to provide an effective strategy for protecting radiation workers from occupation-related/occupational IR exposure.

METHODS AND MATERIALS

Ethics statement

The study was performed and approved by the Institutional Review Board of Public Hospitals of the Tangshan City. The informed written consent form was obtained from each eligible patient and all procedures were conducted according to the Declaration of Helsinki.

Subjects and study design

A retrospective analysis accessed information on 1392 radiation workers in a healthcare setting in the city of Tangshan in 2010 as the exposed group. A total of 143 healthy subjects, matched for sex ratio and mean age, were enrolled as the control group. Medical histories of subjects in the control group were extensively reviewed to exclude individuals with significant diagnostic and therapeutic radiation exposures. All participants completed a questionnaire that included information such as name, sex, age, occupation, history of disease, history of exposure to toxic and harmful substances, and smoking history. Ray exposure types, cumulative exposure time and cumulative radiation dose were collected from health records of radiation workers. Specifically, the cumulative radiation dose in the health records before and after 1987 was estimated by Principles of Estimate on Personal Dose from External Exposure in Radiation Accident[18] and monitored by a thermoluminescence dosemeter MODEL 469 applied with LiF (Mg, Cu, P) powder according to The Specifications of Individual Monitoring for Occupational External Exposure, respectively[19].

Cell culture

Venous blood (0.3 ml) samples were obtained from each subject, and inoculated into chromosomal medium (Tianjing Rui Ai Jin Company) under sterile conditions and cultured for 40–44 h at 37°C. Colchicine (0.03 μg/ml) was added into the medium and cell culture was continued for an additional 4–6 h. After centrifugation (8 min, 2000 rpm) to collect the lymphocytes, the supernatant was discarded and the harvested cells were suspended in a hypotonic 0.075 mol l^{-1} KCL solution and fixed with methanol/glacial acetic acid (3:2). Subsequently, the cells were dropped on clean slides and air-dried. The slides were observed under a microscope, to verify if well-spread metaphases (containing full chromosome number) with chromosomes in good morphology were obtained, and subsequently, the slides were stored at −20°C until further use.

Chromosomal specimen preparation

Fluorescence *in situ* hybridisation (FISH) on metaphases was performed using a pancentromeric DNA probe and biotinylated DNA probes specific for chromosome 1 (Cambia). Briefly, the slides were placed in the oven with a constant temperature of 50°C for 30 min. The chromosome specimens with probe solution (10 μl) were covered with glass coverslips, sealed with rubber cement and denatured (73°C, 3 min). The slide was hybridised in a humidified chamber at 37°C for 5–12 h and the coverslips were removed, washed in a buffer containing 0.4 × SSC at 72°C for 5 min and again immersed in 2 × SSC buffer (0.05 % Tween-20) at 37°C for 30 s with air-drying. The slides were then treated with a mixture (80 μl) of fluoresceinated avidin and biotinylated goat anti-avidin, sealed under a coverslip for 20 min each at 42°C in a humidified chamber. 4′,6-diamidino-2-phenylindole (10 μl) was added and covered with glass coverslips and placed to hybridise in darkness for 10 min. Well-dispersed metaphase spreads were observed under a fluorescence microscope. Stable and unstable aberrations only involving chromosome 1 were scored.

Standard of judgement of CAs

Aberration loss was adjusted by using the standard 3-y disappearance half-time for unstable aberrations, and performing multiple testing by means of the Bonferroni procedure. The cell numbers of unstable CAs [dicentric (dic), centric ring (r) and acentric fragment (ace)] and stable CAs [translocation (t), inversion (inv), insertion (ins) and deletion (del)] were recorded. A total of 100 lymphocytes were counted in each case, and the results were confirmed by two observers. Results were converted to full genome equivalence based on the formula: $Fp = 2.05fp(1 - fp)FG$[20], where Fp is the CA rate detected by FISH (cell number of aberrations/100), fp is the target chromosome/full genome and FG is the CA rate of a full genome. The results were expressed as percentage. Based on the standard curve of a normal population, the normal range of the CA rate was set as 0–2 %. The predictive values of particular CA rates were calculated using data from Morton[21].

MN specimen preparation and analysis

The MN assay was performed using the cytochalasin B method[22]. Lymphocytes were cultured as described above. Cytochalasin B (6 μg ml^{-1}) was added at 44 h of culture. After a total of 72 h at 37°C, the cells were harvested as described above. Slides were prepared according to standard cytogenetic

procedures and stained with 4 % Giemsa (Sigma Chemical Co., USA). For each case, 1000 binucleated lymphocytes with well-preserved cytoplasm were scored. MN were identified according to the following criteria: MN was in cytoplasm with a diameter $<1/3$ of the whole nucleus; the shape of MN was circle or oval and the MN staining and refractivity were in accordance with that of the whole nucleus; and structures were similar to the whole nucleus with completed separation and no other fragments and impurities in the vicinity. Any difficulty in identification of MN was discussed and resolved by the two observers. MN rate (‰) = (MN cell numbers/cell number observed) × 1000 ‰. Based on the standard curve of a normal population, the normal range of MN cell rate and MN rate was between 0 and 6 ‰.

Statistical analysis

All statistical analyses were carried out using SPSS 16.0 software. Data are presented as means ± SD (continuous variables), or as frequencies and percentages (categorical variables). A Chi-square test and *t* test were applied for the comparison of categorical variables and continuous variables, respectively. Correlation analysis was performed with the Spearman analysis. The analysis results with $p < 0.05$ were considered statistically significant.

RESULTS

Baseline information

Among the 1392 workers exposed to IR, there were 859 males and 533 females with an average age of 40 ± 12 (range, 18– 70 y), including 621 radiation workers from the healthcare industry (379 medical personnel in the radiation diagnosis group and 242 in the radioimmunoassay radiotherapy group), and 771 radiation workers from industrial firms (568 in the industrial flaw detection group and 203 in the industrial radiation source group). The 143 healthy subjects included 96 males and 47 females with an average age of 43 ± 9 (range, 23– 70). There was no significant difference in the sex ratio and mean age between the exposed group and the control group (all $p > 0.05$). The average exposure time of the exposed group was 15.6 ± 7.1 y (range, 2 months to 47 y), including 723 workers with exposure time <10 y, 532 workers between 10 and 20 y, and 137 >20 y. The mean cumulative radiation dose was 33.759 ± 23.97 mSv; and the cumulative radiation doses were as follows : <10 mSv group ($n = 528$), 10– 19 mSv group ($n = 432$), 20– 50 mSv group ($n = 315$) and >50 mSv group ($n = 117$).

Comparison of CA rate and MN rate between groups

As presented in Table 1, the CA number in the exposed group is 947, including 543 t (57.33 %), followed by dic and ace, and r and ins are in much lower proportion. The control group showed 33 CA in cells, consisting of 18 t (54.54 %), and the frequencies of CA rate in the exposed group were significantly higher than the control group (0.68 vs. 0.22 %, $p < 0.05$). No statistical significance was found between observed CA values and the predictive value on r, ace, dic, t and ins (all $p > 0.05$). A higher MN rate was also observed in the exposed group in comparison with the control group (2.44 vs. 1.72 ‰, $p < 0.05$) (Table 2).

Table 1. Comparison of CAs between the exposed group and the control group.

Group	Cases (*N*)	Cells analysed (*N*)	CA types (observed value/predictive value)					CA number (observed/predicted)	Total CA rate (%)
			r	ace	dic	t	ins		
Exposed group	1392	139 200	14/16	92/104	293/331	543/613	5/6	947/1069	0.68[a]/0.77
Control group	143	143 00	0/0	3/3	11/12	18/20	1/1	33/37	0.22/0.26

CA, chromosome aberration; r, centric ring; ace, acentric fragment; dic, dicentric; t, translocation; ins, insertion.
[a]Compared with the control group, $p < 0.05$.

Table 2. Comparison of MN between the exposed group and the control group.

Groups	Cases (*N*)	MN		
		Numbers (*N*)	MN numbers (*N*)	MN rate (‰)
Exposed group	1392	1 392 000	3395	2.44[a]
Control group	143	143 000	246	1.72

MN, micronuclei.
[a]Compared with the control group, $p < 0.05$.

Comparison of CA and MN among radiation workers from different occupations

The results of MN and CA analysis in radiation workers from different occupations are presented in Table 3. The incidence rates of cells exhibiting MN and CA showed an increasing tendency between the various occupational groups, with the medical personnel in the radiation diagnosis group showing the lowest, and trending upward in the industrial flaw detection group and the oil-well-logging group, with the medical personnel in the radioimmunoassay radiotherapy group showing the highest, with statistically significant differences in MN and CA between the groups (all $p < 0.05$).

Comparison of CA and MN among radiation workers with different exposure time

The results displayed in Table 4 of MN damage and CA, in radiation workers with different exposure time, showed significant differences in the incidence rate of MN and CA among the workers with exposure time <10 y, 10– 20 y and >20 y (all $p < 0.05$). Stable CA rates demonstrated an increased trend among the workers with exposure time <10 y, 10– 20 y and >20 y (all $p < 0.05$), which implied that stable CA was increased with different exposure times. No significance of unstable CA rate was found among workers with different exposure times (all $p < 0.05$), suggesting that unstable CAs disappeared with cumulative exposure times.

Relationship between cumulative radiation doses and abnormal rate of MN and chromosome

The relationship of CA rate and MN with cumulative radiation dose of radiation workers is summarised in Table 5. The frequencies of CA showed an increasing trend with increase in cumulative radiation doses as follows: <10 mSv group, 10– 20 mSv group, 20– 50 mSv group and >50 mSv group. Similar association was also observed between MN and cumulative radiation dose (all $p < 0.05$).

Correlation analysis on cell MN and CA and cumulated radiation doses

Results of the Spearman analysis showed that MN rate of the radiation workers had positive linear correlation with the cumulative radiation doses ($r = 0.76$, $p < 0.05$). Similarly, CA rate positively correlated with the cumulative radiation doses ($r = 0.64$, $p < 0.05$) (Table 6).

Table 3. Comparison of CAs and MN among workers from different occupation.

Group	Cases (*N*)	Chromosome			MN		
		Cell number (*N*)	CA number (*N*)	CA rate (%)	Cell number (*N*)	MN number (*N*)	MN rate (‰)
Group A	379	37 900	139	0.37	379 000	634	1.67
Group B	568	56 800	401	0.71[a]	568 000	1151	2.03[a]
Group C	203	20 300	172	0.85[a,b]	203 000	629	3.10[a,b]
Group D	242	24 200	235	0.97[a,b,c]	242 000	981	4.05[a,b,c]

Group A, medic radiation diagnose group; Group B, industrial flaw detection group; Group C, oil-well-logging group; Group D, medic radioimmunoassay radiotherapy group; CA, chromosome aberration; MN, micronuclei.
[a]Compared with medical personnel in the radiation diagnosis group ($p < 0.05$).
[b]Compared with industrial flaw detection group ($p < 0.05$).
[c]Compared with oil-well-logging group ($p < 0.05$).

Table 4. Comparison of CA rate and MN rate among radiation workers with different exposure time.

Exposure time (y)	Cases (*N*)	Chromosome							MN		
		Cell number (*N*)	CA number (*N*)	Stable CA (*N*)	CA rate (%)	Unstable CA (*N*)	CA rate (%)	CA rate (%)	Cell number (*N*)	MN number (*N*)	MN rate (‰)
<10	723	72 300	324	134	0.18	190	0.26	0.45	723 000	1597	2.21
10–20	532	53 200	434	260	0.49[a]	174	0.33	0.82[a]	532 000	1316	2.47[a]
>20	137	13 700	189	154	1.12[a,b]	35	0.26	1.38[a,b]	137 000	482	3.52

CA, chromosome aberration; MN, micronuclei.
[a]Compared with exposure time <10 y, $p < 0.05$.
[b]Compared with exposure time of 10–20 y, $p < 0.05$.

Table 5. Relationship between cumulated radiation doses and MN and CA rate of the radiation workers.

Cumulative radiation dose (mSv)	Case (*N*)	Chromosome				MN	
		Cell number (*N*)	CA number (*N*)	CA rate (%)	Cell number (*N*)	MN number (*N*)	MN rate (‰)
<10	528	52 800	288	0.55	528 000	1030	1.95
10+	432	43 200	282	0.65[a]	432 000	1076	2.49[a]
20+	315	31 500	261	0.83[a,b]	315 000	901	2.86[a,b]
>50	117	11 700	116	0.99[a,b,c]	117 000	388	3.32[a,b,c]

CA, chromosome aberration; MN, micronuclei.
[a]Compared with <10 mSv group, $p < 0.05$.
[b]Compared with <20 mSv group, $p < 0.05$.
[c]Compared with <50 mSv group, $p < 0.05$.

Table 6. Correlation analysis of MN and CA with cumulated radiation doses.

Index	Cumulated radiation doses	MN	CA
Cumulated radiation doses	1	0.76[a]	0.64[a]
MN	0.76[a]	1	0.58[a]
CA	0.64[a]	0.58[a]	1.00

CA, chromosome aberration; MN, micronuclei.
[a]Compared with the control group, $p < 0.05$.

DISCUSSION

IR-based medical diagnoses and treatments are widely used in the medical profession and the risk of occupational exposure to radiation workers has concomitantly increased with more frequent and wider applications of IR-based medical procedures. The results obtained here indicate that the frequencies of MN and CA in workers exposed to IR are significantly higher compared with healthy controls, implying that IR exposure may cause a higher incidence of MN and CA in radiation workers. Owing to its universal applications in diagnostic and therapeutic methods, IR is a major contributor to man-made sources of radiation[23]. IR has negative effects on human health, especially in radiation workers, as reflected by the increase in a variety of peripheral haemograms, MN and CAs[17, 24, 25]. CAs and MN are reliable and useful biological markers for detecting and evaluating DNA damage induced by IR[13, 26]. DNA damage caused by IR may induce changes of peripheral haemograms, including the alterations of leucocyte numbers, the number of red blood cells and haemoglobin. The alterations of peripheral haemograms may result in severe occurrence of MN, and increase the incidence of CAs[27, 28]. Previous studies have shown that IR results in macromolecular ionisation and dissociation, and could directly or indirectly induce DNA damage, damage to leucocyte function and increase the aberration rate of leucomonocytes, leading to increased risk of MN and CAs[29–31]. Consistent with the results obtained here, Angelini *et al.* have demonstrated that IR may result in a higher frequency of MN and CAs, and lead to significantly higher DNA damage in radiation workers, compared with healthy controls[23]. The United Nations Scientific Committee on the Effects of Atomic Radiation estimated a global value of a mean annual effective dose of 0.5 mSv (UNSCEAR 2008 Report) for the optimisation of protection of occupationally exposed workers[32].

Importantly, this study revealed that radiotherapy physicians showed higher frequencies of MN and CAs compared with diagnostic radiologists, workers engaged in flaw detection and personnel in petroleum exploration. Hospitals workers are frequently exposed to IR from three linear accelerators used for radiotherapy, multi-slice CT scan and angiography[33]. In this regard, both patients and radiation workers may suffer from IR during diagnosis and treatment for various diseases. The radiotherapy physicians may be especially vulnerable to exposure to low daily doses of IR[34]. Previous studies showed that CAs in radiotherapy physicians and diagnostic radiologists are linked to ongoing DNA damage and repair, mainly caused by the IR exposure from diagnosis and treatment procedures[35–37]. Díaz-Valecillos *et al.* showed that radiation workers with 8 h of exposure time per week exhibited higher frequencies of CAs, but most radiation workers with CAs had longer exposure times, implying that chronic exposure to low-level IR may result in CAs, and CA largely depended on the exposure time[17].

Radiation workers with >20 y of exposure time were also studied and it was found that they exhibited higher frequencies of MN and CAs when compared with radiation workers with <20 y of exposure time, suggesting that the equivalent dose of radiation was associated with the frequencies of MN and CAs.

Consistent with this, other studies have also demonstrated that DNA damage is significantly correlated with the dose of IR exposure and the exposure time, and higher doses of IR result in a higher frequency of MN and CAs[13, 38]. Interestingly,it was also found that the stable CA rates increased with the cumulative exposure time, while no significance was detected in unstable CA rates and increased exposure time, which may be explained by the fact that stable CAs did not disappear with increase in exposure time, while unstable CAs disappeared with increased exposure time. IR generates ionising particles, which directly induce SSBs and DSBs or types of DNA damage, leading to accumulation of MN and CAs, mainly determined by the length of exposure and the equivalent dose of radiation[13, 39, 40]. It is known that the radiation dose equivalent in individuals is gradually accumulated and increases with prolonged exposure time, and inadequate repair of DNA damage increases the risk of MN and CAs[41].

There are some limitations in this study. First, this retrospective study is characterised by some disadvantages including unavailable data, no randomisation, limited accuracy of written records and restrictions due to institutional regulations. Secondly, the average effective doses in this study were collected from the health records of workers exposed to IR, and these data may be unstable since the data may be influenced by factors such as the population background. Finally, a population with a 20-y exposure time was selected, which may affect the precise measurements of the radiation dose, consequently creating variances of the frequencies of MN and CAs. Moreover, unstable CA had a 3-y disappearance half-time assumption, while in this text the exposure time for radiation workers was more than 10 y, and yet no adjustment was made with regard to the 3-y disappearance half-time of unstable CA.

In conclusion, this study strongly supports the view that IR is closely related to the observed frequencies of CAs and MN, and chronic exposure to low-level IR may have negative effects on radiation workers. Furthermore, it was found that the frequencies of MN and CAs were associated with radiation dose equivalent, occupational duties of workers and radiation exposure time.

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