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Rapid and High-Throughput Detection of Peripheral Blood Chromosome Aberrations in Radiation Workers

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Abstract

There is a pressing need to establish automated solutions for the rapid, high-throughput, and automatic detection of chromosome aberrations (CAs) in the occupational health surveillance of large-scale radiation workers. Here, we described and verified the accuracy of a new measurement system based on the automatic scanning and analysis of dicentric chromosomes (DICs). The effects of cell number on DIC detection by automatic scanning and analysis were studied, and the distribution of DIC values per cell was calculated. In total, 1088 cases were detected by automatic DIC scanning and analysis in 26 663 radiation workers, and 73 cases were further confirmed by a technician, including 5 cases in which radiation exposure lead to harmful medical consequences. Our approach reduces the workload by 96% and increases the speed of assessment approximately 7-fold. Overall, this study validates the utility of a novel rapid and high-throughput CA detection procedure as a means of occupational health surveillance of large-scale radiation workers.

Keywords

high-throughput, automatic dicentric chromosome analysis, radiation workers, occupational health surveillance

Introduction

The detection of chromosome aberration (CA) is not only used as a biomarker of radiation exposure but also in establishing the relationship between radiation exposure and cellular responses in vivo, in dose, and dose-rate responses, as well as potential health problems in humans.[1] The traditional measurement of CA requires hundreds of cells to be visually analyzed under the microscope for each tested sample, and each technician can only analyze 1 or 2 samples per workday. Automation offers an effective means to solve this problem. In the 1990s, with the development of the electron microscope and computer image processing technology, a CA automatic scanning analysis system was developed to automatically search for peripheral blood cells in metaphase and acquire high-resolution images, to identify cases of dicentric chromosomes (DICs). In 2009, automation DIC analysis has been achieved by Vaurijoux and his colleagues through establishing the dose–effect curve for automatic DIC analysis, and the analysis time was greatly reduced.[2] At present, according to the literature, automation DIC analysis is usually for estimation of the biological dose, but its application in occupational health examination of the large-scale radiation workers is rarely reported. The main purpose of the present study was to design and achieve a rapid, accurate, and high-throughput CA detection method for the occupational health surveillance of radiation workers using an analytical approach. We present a detection procedure, which is considerably less time-consuming than previous methodologies.

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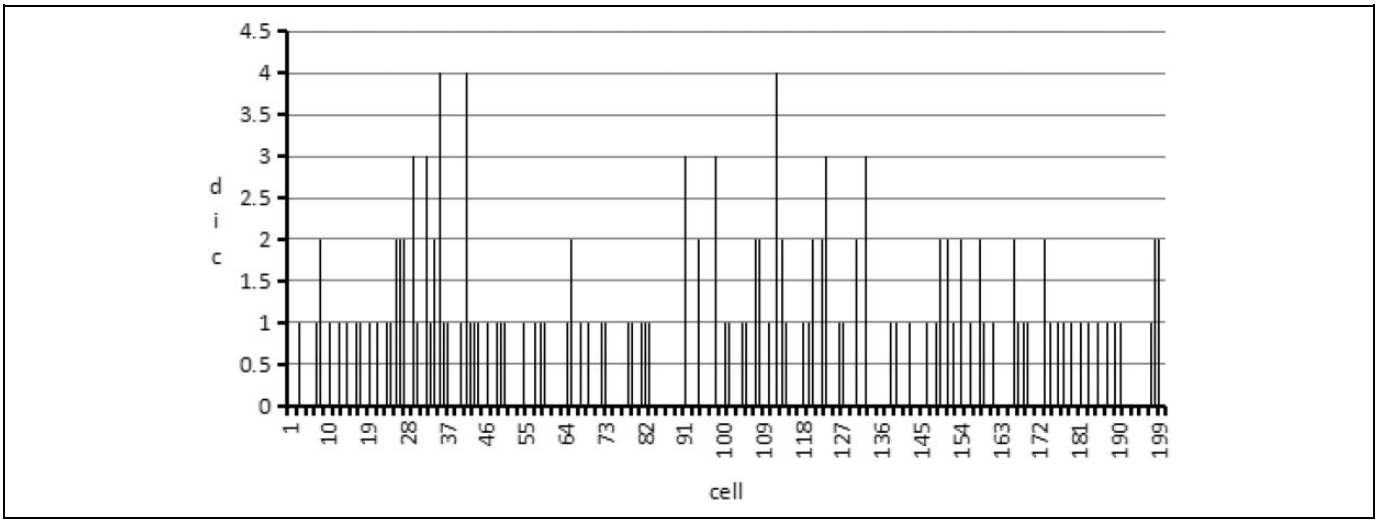

Figure 1. Distribution of DIC values on 0th to 199th cells. DIC indicates dicentric chromosomes.

Materials and Methods

Blood Sample Collection

In 2012, peripheral blood samples were collected from 20 healthy volunteers for analysis of the automatic detection of DIC rate and establishment of a high-throughput detection method. From 2013 to 2017, peripheral blood samples were collected from 26 663 radiation workers in batches for analysis by the high-throughput detection method. The 26 663 radiation workers consisted of 718 radiation workers from industrial testing and medical institutions whose average annual effective dose was 0.097 ± 0.020 mSv, 25 945 workers from nuclear power plants whose average annual effective dose was 0.243 ± 0.100 mSv. In the 25 945 workers who were from nuclear power plants, there were 10 664 from operation department, 6343 from maintenance department, 3198 from equipment management department, 1546 from radiation protection department, 1607 from production planning department, 1884 from technical support department, and 703 from security department.

Irradiation Conditions

The blood samples which from healthy volunteers were irradiated in the IAEA/WHO Network of Secondary Standard Dosimetry Laboratories Shanghai, China. Three dose points (0.5, 2, 4 Gy) were set, and the absorbed dose rate was 0.39 Gy/min.

Cell Culture and Chromosome Specimen Preparation

The blood samples of healthy volunteers were placed in a water bath of $37°C \pm 0.5°C$ for 2 hours after irradiation, while those of radiation workers were not irradiated and were cultured for less than 48 hours. Next, lymphocytes were cultured in Roswell Park Memorial Institute (RPMI) 1640 culture medium containing fetal bovine serum, phytoagglutinin (PHA), 1% penicillin and 100 μg/mL streptomycin, and 0.04 g/mL colchicine at 37°C in 5% CO_2 in a humidified incubator (Thermo Scientific, Scotts Valley, California) for 50 hours.[3] The whole blood culture method was used, and the proportion of blood to culture medium was 1:10. Heparin lithium (0.5 mL) was added to 5 mL lymphocyte culture medium as an anticoagulant. Cell suspensions were prepared using a CP-II-64 automatic cell harvester (Lechen Biotechnology, Shanghai, China). Cells were subjected to hypotonic treatment by 5 mL KCl solution twice for 30 min/time, then fixed 4 times with Carnoy solution for 5 minutes each. Slides were produced using a CP-AS-40 automatic slide-making machine (Lechen Biotechnology) and subjected to Giemsa staining using a CP-G-24 automatic dyeing machine (Lechen Biotechnology). The parameters of the instrument were set according to the results of preliminary experiments.

Effective Cell Rate Analysis

The effective cell rate was calculated according to the following formula:

$$\text{Effective cell rate } (\%) = (\text{Artificial cell number}/\text{Photographed cell number}) \times 100.$$

Automatic Detection of DIC Rate Analysis

The Metafer 4 (V.3.11.6) chromosome scanning and analyzing system (MetaSystems, Altlussheim, Germany) was used to search for cells in metaphase and acquire high-resolution images. The sensitivity parameter for automatic metaphase cell searching was set to 6, and 3 regions were set for the search window, with the 15% area proximal to tab, 35% area of the central slide, and 50% area distal to tab (Figure 1). The collected high-resolution images were subjected to DIC analysis using DCScore software (MetaSystems). The detected DIC was further confirmed by a laboratory technician. The numbers of

Table 1. Automatic Detection of DIC Rates Analyzed at Each Dose Point.

Absorbed Dose (Gy)	Sample	High-Resolution Images	Automatic DIC Number	Artificial DIC Number	DIC Automatic Rate (%)
0.5	1	1243	11	16	69
0.5	2	697	5	9	55
0.5	3	509	3	6	50
0.5	4	1420	11	16	63
0.5	5	1285	13	18	72
2	6	1032	61	81	75
2	7	1111	36	65	55
2	8	862	54	94	57
2	9	594	40	61	66
2	10	1262	77	124	62
4	11	200	62	112	55
4	12	300	92	133	69
4	13	300	64	121	53
4	14	300	65	115	57
4	15	420	105	146	72

Abbreviation: DIC, dicentric chromosomes.

DIC and marked cells were recorded after the elimination of false positives. Dicentric chromosome was confirmed using experience and/or Ikaros software (MetaSystems) based on the following principles: (1) no count for those not suggested by the software, (2) not considering whether the 46 chromosomes were complete in metaphase cells, (3) only the DICs in the main cell were counted when more than one cell was present in a high-resolution image, and (4) scattered DICs released from the broken cells were not counted. The numbers of DICs artificially confirmed and the numbers of marked cells by software were recorded. Then, the high-resolution images were artificially analyzed the same, and the artificial analysis of the DIC number was recorded. The DIC automatic rate was calculated according to the following formula:

$$\text{Automatic detection of DIC rate } (\%) = (\text{Automatic DIC number}/\text{Artificial DIC number}) \times 100.$$

Dicentric Chromosome Distribution Interval Analysis

We analyzed 142 samples containing one automatic DIC per 200 cells, in our laboratory, and observed the distribution of DIC values on the 0th to 99th and 100th to 199th cells of each sample.

Results

Automatic Detection of DIC Rate

According to the DIC numbers acquired by the automatic scanning and analysis system, and confirmed by a technician, as well as the artificial DIC numbers from the same high-resolution images, the automatic detection of DIC rate was in the range of 50% to 75%. The results are shown in Table 1.

Table 2. Effective Cell Rate Analysis.

Samples	High-Resolution Images	Artificial Number	Mean Value	Effective Cell Rate (%)	Mean Value (%)
200	250	194-224	206	78-90	83

Effective Cell Rate

Some of the cell images acquired by the automatic scanning and analysis system were not suitable for analysis due to poor quality. We analyzed the cell morphology of 200 samples in our laboratory. In total, 250 high-resolution images of metaphase cells were collected in each sample, and some of them were artificially removed as they were deemed to be unsuitable for analysis. The number of metaphase cells was in the range of 194 to 224. The effective cell rate range was 78% to 90%. The results are shown in Table 2. In our laboratory, 250 high-resolution images collected met the requirement for analysis of 200 metaphase cells in each case. If the number of cell was less than 200, it was readded or slides were reproduced and rescanned. In practice, the lowest number of high-resolution images required for the automatic scanning and analysis system was decided by the individual laborator.

Dicentric Chromosome Distribution

We analyzed 142 samples containing one automatic DIC per 200 cells in our laboratory and marked the locations of DIC on the 0th to 99th and 100th to 199th cells in each sample. There were 72 cases in which DIC appeared on the 0th to 99th cell, and 70 cases in which DIC appeared on the 100th to 199th cell (Figure 1). Therefore, some DICs were missed when only 100 cells were analyzed by the automatic scanning and analysis system. We therefore suggest that at least 200 cells should be analyzed in each case, and 250 or more high-resolution images should be acquired.

Aberration Rate Distribution

We examined 26 663 peripheral blood lymphocyte chromosome samples from radiation workers between 2013 and 2017, in our laboratory using the novel method outlined above. In total, 1088 cases were detected, and 73 cases were further confirmed by technicians, ultimately including 5 cases in which 4 cases undergone radiotherapy and an interventional radiologist with an individual cumulative dose of 3.6 mSv for 3 years. In these cases, at least 3 DIC were found per 200 cells (Figure 2).

Efficiency Comparison Between the New and the Traditional Method

The number of samples containing DIC ≥ 1 accounted for about 4% of the total cases, in our laboratory, as determined by the automatic scanning and analysis system. Three in 80 cases

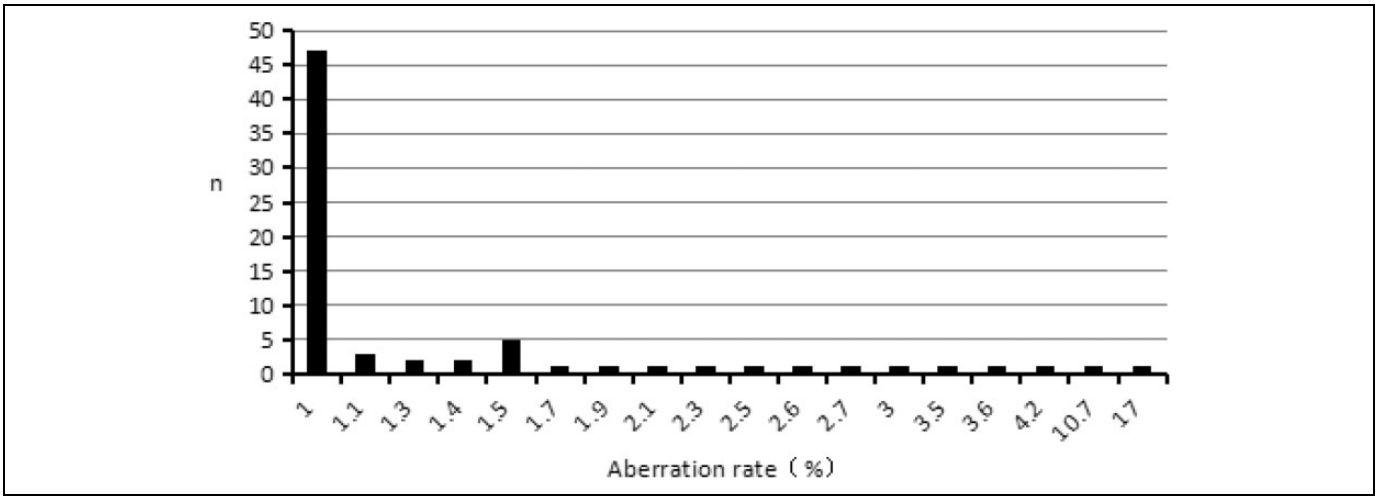

Figure 2. Aberration rate distribution in abnormal cases.

Table 3. Efficiency Comparison Between the New and the Traditional Method.

Method	Sample	Culture Time (d)	Slide Time (d)	Image Time (d)	Automatic DICs (d)	Artificial DICs (d)	Report (d)
New	80	2.000	1.000	1.670	0.069	1.500	6.239
Traditional	80	2.000	1.000	-	-	-	43[a]

Abbreviation: DIC, dicentric chromosomes.
[a]It takes 1 day to finish the report of 2 cases by the traditional method.

required confirmation by a technician, and the process took about 1.5 days. It only took 6.23 days in total for a technician to finish the report of the 80 cases, while the traditional method requires 43 days. The workload has therefore been reduced by 96%, and the speed has been increased about 7-fold. A comparison of the time required for the new and traditional methods is shown in Table 3.

Discussion

The traditional measurement of CA requires hundreds of cells to be visually analyzed under the microscope for each tested sample, and each technician can only analyze 1 or 2 samples per workday. Automation offers an effective means to solve this problem. The commercialized DIC automatic analysis software (DCScore software, MetaSystems, Altlussheim, Germany) has been on the market for 20 years since 1998. It can only analyze one of the DIC distortion automatically, and false positive or false negative might be present, so it was considered valueless. Automatic biological dose estimation has been realized by Vaurijoux and his colleagues through establishing the dose–effect curve by the software, and the analysis time was greatly reduced.[2] At present, according to the literature, automation DIC analysis is usually for estimation of the biological dose, but its application in occupational health examination of the large-scale radiation workers is rarely reported.

In this study, DIC is used as a marker to screen out those who have DIC in their blood samples by the software, then confirmed by artificially analysis, the results showed that the workload was reduced by 96%, and the speed has been increased about 7-fold.The detection accuracy of DIC by a technician will also decrease if the technician is having fatigue, especially when the number of samples is very large; however, the method outlined herein obviates this difficulty, it is less likely than traditional analysis to miss detection.

The DIC analysis technology is the method which DIC is recognized by the computer image analysis technology basing on the morphology of DIC from the collected chromosome digital images, and which has been used to evaluate radiation damage for more than 40 years. It is well known that the number of DIC can be changed by different dose, but there is no research to claim that the morphology of DIC can be affected by the dose. In theory, no matter how the dose, the morphology of the DIC must be same as long as the DIC appear. In fact, it is possible to choose any dose for irradiation, as long as it can produce sufficient DICs for analysis. In our study, we think that the morphology of the DIC is same as that high dose, although the average annual effective dose of radiation workers is very low. In order to analyze automatic detection rate of DIC of the software, we selected 0.5, 2, and 4 Gy for irradiation in vitro to obtain sufficient DICs. It is different from fitting dose–response curve with more than 10 irradiation dose points. In fact, it is feasible to choose low dose (<0.1 Gy). But the number of DIC produced under low dose will be very few compared with high dose in the same time. If we want to obtain enough number of DICs to analyze automatic detection rate of DIC of

the software quickly, it would be time-consuming to acquire a large number of metaphase digital images of chromosomes for detaching DIC.

Radiation-induced chromosomal structural aberrations include acentric fragments, double minutes, dicentric fragments, acentric fragments, dicentric and polycentric chromosomes, inversions, and reciprocal translocations. Analyses of these 7 kinds of CA are required for the long-term personal monitoring of radiation workers. However, currently, software can only analyze DIC events automatically and cannot detect the other forms of aberrations. Dicentric chromosome and acentric fragments account for a much larger portion of the radiation-induced CA, while the others smaller. It plays an important role in the diagnosis of chronic radiation injury, while centric rings and reciprocal translocations together account for 1% or more.[4] If the exposure dose exceeds a certain threshold level, DICs are expected to appear. As long as DIC exists, it can be detected by automated software. Therefore, it is feasible to use DIC as the primary marker for whether chronic or nonchronic radiation injury has occurred. The data for an abnormal case report are ultimately acquired by a chromosome scanning and analysis system and confirmed by a technician, so it coincides with the standard of the GBZ/T248-2014.[3]

Although the chromosome auto-scanning analysis system for DIC displays a high degree of reproducibility, and it is a powerful tool for dose-estimation as reported by Wang et al,[5,6] the data of the automatic DIC cannot be directly used in the assessment of CA for the occupational health surveillance of radiation workers. However, a previous report has shown that there is a good correlation between the dose–response curve established by automatic analysis and the artificial dose–response curve, and the automatic detection of DIC rate is about 50% to 70%.[7] Results from our study indicated that the automatic detection of DIC rate was similar to previous reports and was about 50% to 75%. In theory, if the automatic detection of DIC rate is 50%, one DIC may be missed by the new method. According to the standard of GBZ/T248-2014, one DIC is typically found in 200 analyzed cells.[4] Therefore, when DIC cannot be found in 200 analyzed cells, the test and assessment of CA for radiation workers is considered normal.

When only acentric fragments but no DIC occur, as in some cases of chronic radiation injury, the injury may not be correctly diagnosed. However, according to GBZ/T248-2014, CA is not a specific index of external chronic radiation disease, and the diagnosis must be based on clinical symptoms; for example, using the white blood cell count.[8] Therefore, the new method may miss some cases of CA but will not affect the diagnosis. In this study, 5 cases exposed to medical radiation and being suffered harm were successfully diagnosed among the 26 663 radiation workers. However, not all 26 663 cases were analyzed individually by technicians, so further research is needed in the future.

Rapid and high-throughput analysis of CA is probably an ideal screening tool for peripheral blood CAs in radiation workers. In this study, the results showed a higher sensitivity of diagnosis of external chronic radiation disease with our approach. Furthermore, the workload was reduced by 96%, and the speed has been increased about 7-fold. This technical scheme can meet the requirements of fast and accurate package solution with practical value for the rapid and high-throughput detection of CA for the occupational health surveillance of radiation workers, especially when the number of sample is very large.

Authors' Note

J.B. and H.D. contributed equally to this work.

Declaration of Conflicting Interests

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