PP-25

Construction of dose response curves for cytogenetic biodosimetry in the low dose range based on five persons

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ABSTRACT

In terms of biological dosimetry at the time of radiation exposure, the dicentric chromosome (Dic) assay (DCA) is the gold standard for assessing for the acute phase and chromosome translocation (Tr) analysis is the gold standard for assessing the chronic phase. It is desirable to have individual dose–response curves (DRCs) for each laboratory because the analysis criteria differ between laboratories. We constructed the DRCs for radiation dose estimation (with three methods) using peripheral blood (PB) samples from five healthy individuals. Aliquots were irradiated with one of eight gamma-ray doses (0, 10, 20, 50, 100, 200, 500 or 1000 mGy), then cultured for 48 h. The number of chromosome aberrations (CAs) was analyzed by DCA, using Giemsa staining and centromere-fluorescence in situ hybridization (centromere-FISH) and by chromosome painting (chromosome pairs 1, 2 and 4) for Tr analysis. In DCA, there was large variation between individuals in the frequency of Dics formed, and the slopes of the DRCs were different. In Tr analysis, although variation was observed in the frequency of Tr, the slopes of the DRCs were similar after adjusting the background for age. Good correlation between the irradiation dose and the frequency of CAs formed was observed with these three DRCs. However, performing three different biological dosimetry assays simultaneously on PB from five donors nonetheless results in variation in the frequency of CAs formed, especially at doses of 50 mGy or less, highlighting the difficulty of biological dosimetry using these methods. We conclude that it might be difficult to construct universal DRCs.

BACKGROUND

Several methods have been reported for rapid biological dosimetry immediately following exposure to low and high doses of radiation, of which the most reliable for international standardized biological dosimetry is the chromosome aberration (such as dicentric chromosome (Dic) and translocation (Tr)) assay. These assay is typically used following acute radiation exposure of between 100 mGy and 5 Gy, although recent studies report that chromosomal abnormalities such as Dics can be detected following chronic or low-dose radiation exposure. However, the accuracy of estimation methods using the dose-response curves (DRCs) following exposure to the low doses remains unclear.

We therefore irradiated samples from five healthy individuals with eight gamma-ray irradiations doses from 0 to 1000 mSv. Here we present the three types of standard DRCs compatible with three methods. The first is a classical method for DCA, Giemsa staining. The second is the centromere-fluorescence in situ hybridization (centromere-FISH) method, which likely provides higher accuracy than Giemsa staining. The third is a painting method for chromosome translocation (Tr) analysis using three probes (one each for chromosome pairs 1, 2 and 4).

CONCLUSIONS

- ✓ We constructed the dose-response curves that both Dic and Tr analysis following gamma-ray irradiation focusing on the low-dose range, especially of 100 mGy or less.
- ✓ The Tr frequency showed variations in the intercepts considered to be the effects of aging. However, the slopes of DRCs of the five individuals showed no difference due to age or gender.
- ✓ The DRCs that we constructed has poor dose responsiveness especially of 50 mGy or less. Therefore, we consider that 5000 or more cells analysis is necessary to increase accuracy.

RESULTS

Construction of dose–response curves for DCA (Giemsa staining and centromere-FISH)

Table 1. Average of dicentric chromosome results from five donors (Giemsa staining)

Dose (mGy)	Number of cells analyzed	Number of Dics	Frequency of observed Dics[#1]	95%CI[#2] (Dic-frequency)
0	2023.4	2.8	0.001	-0.0005-0.003
10	2026.4	4.2	0.002	0.0005-0.004
20	2020.8	2.6	0.001	0.0002-0.002
50	2051.8	4	0.002	0.0007-0.003
100	2020.4	4	0.002	0.0006-0.003
200	2026	7.2	0.004	0.001-0.006
500	2010.2	27.6	0.013	0.008-0.019
1000	2066.8	78	0.038	0.029-0.045

#1 Dicentric chromosome per cell analyzed.
#2 Confidence interval.

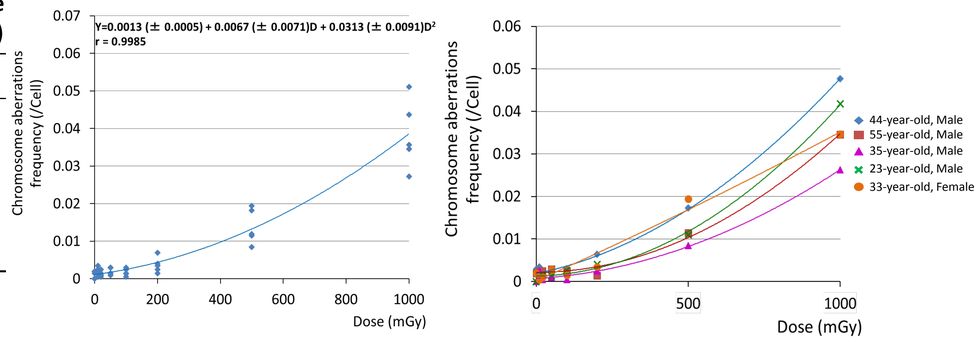


Figure 1. Dose–response curve for DCA analyzed by Giemsa staining. The frequencies of chromosome aberrations per 2000 cells in PB from five individuals induced by gamma ray irradiation were plotted. Regression analysis using DoseEstimate ver. 4.1 software was calculated from the average value of the five samples.. [Y = 0.0013 (±0.0005)+ 0.0067 (±0.0071)D + 0.0313 (±0.0091)D², r =0.9985] (Y: yield of chromosome aberrations, D: dose (Gy), r = correlation coefficient.) (Left) Dose response curves plotted from the analysis results of 5 individuals.(Right)

Table 2. Average of dicentric chromosome results from five donors (Centromere-FISH)

Dose (mGy)	Number of cells analyzed	Number of Dics	Frequency of observed Dics[#1]	95%CI[#2] (Dic-frequency)
0	2011.2	1.6	0.001	-0.0005-0.002
10	2015.2	2.2	0.001	0.0005-0.002
20	2018.8	3.2	0.002	0.0004-0.003
50	2026.2	4.8	0.002	0.001-0.003
100	2021	5.6	0.003	0.001-0.004
200	2026.6	12	0.006	0.004-0.008
500	2023	37.6	0.019	0.013-0.024
1000	2057	108	0.053	0.048-0.057

#1 Dicentric chromosome per cell analyzed.
#2 Confidence interval.

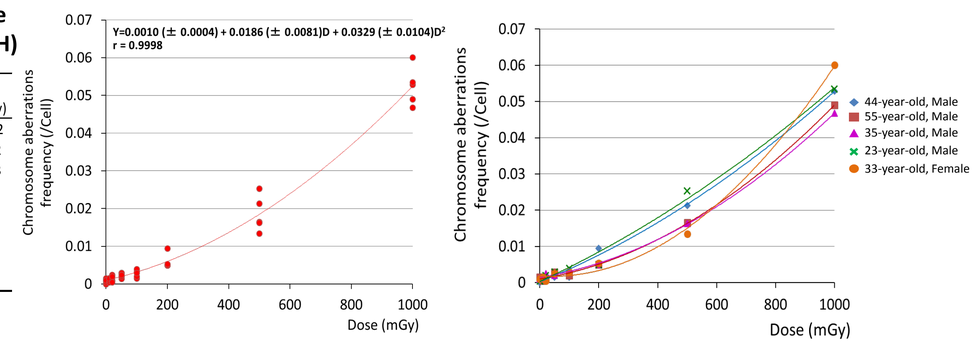


Figure 2. Dose–response curve for DCA analyzed by Giemsa staining. The frequencies of chromosome aberrations per 2000 cells in PB from five individuals induced by gamma ray irradiation were plotted. Regression analysis using DoseEstimate ver. 4.1 software was calculated from the average value of the five doners.. [Y = 0.0010 (±0.0004)+ 0.0186 (±0.0081)D + 0.0329 (±0.0104)D², r =0.9998] (Y: yield of chromosome aberrations, D: dose (Gy), r = correlation coefficient.) (Left). Dose response curves plotted from the analysis results of 5 individuals.(Right)

Construction of a dose–response curve for chromosome translocation analysis -comparison of effect of age-

Table 3. Average of chromosome translocation analysis of five donors

Dose (mGy)	Number of cells scored: Cell count of analysis	Number of cells scored: Cell equivalent[#1]	Number of translocations	Frequency of observed translocation[#2]	95%CI[#2] (Tr-frequency)
0	5551.6	2176.2	9.6	0.004	-0.00005-0.002
10	5652.6	2215.8	13.4	0.006	0.0005-0.002
20	5564.4	2181.2	13	0.006	0.0004-0.003
50	5436.4	2131.1	14.6	0.007	0.001-0.003
100	5424	2126.2	19.2	0.009	0.001-0.004
200	6058.4	2374.9	34.2	0.0014	0.004-0.008
500	5701.6	2235	81.4	0.036	0.013-0.024
1000	5197.4	2037.4	235.8	0.116	0.048-0.057

#1 equal to full genome cell count (The formula is provided in Material and M
#2 per 100 cells equivalents
#3 Confidence interval

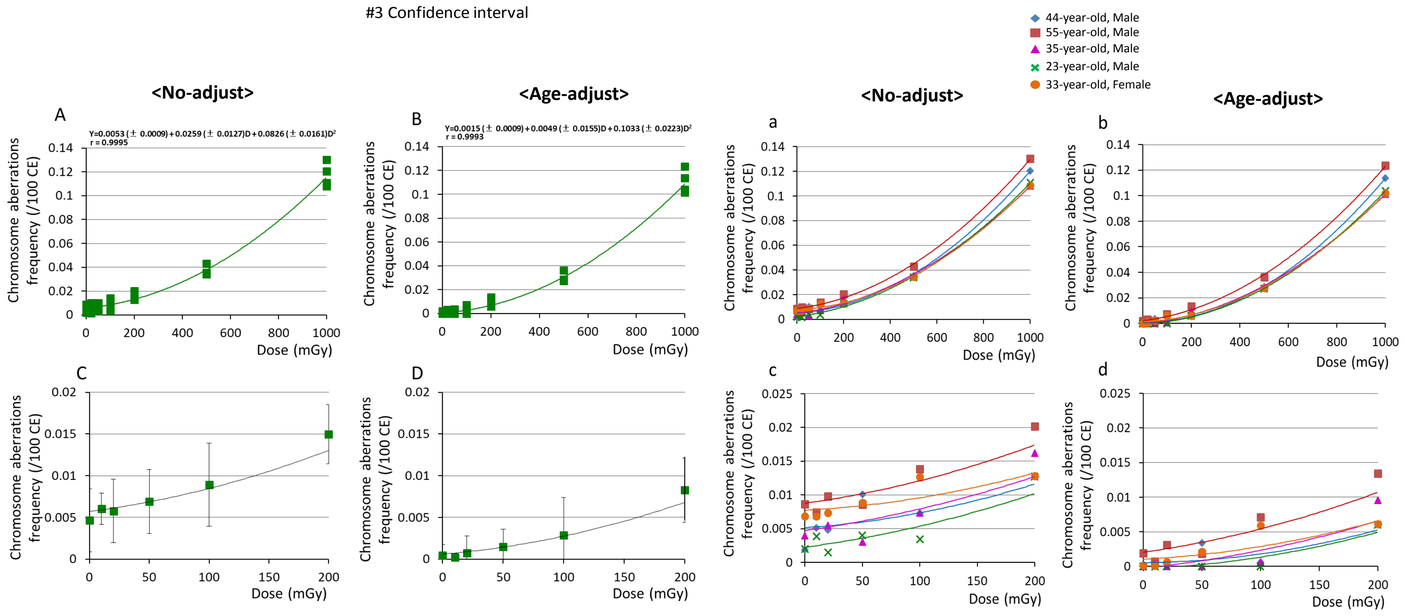


Figure 3. Dose–response curves for chromosome translocation analysis. The frequencies of chromosome aberrations per 2000 cells equivalents (Ces) in PB from five individuals induced by gamma-ray irradiations were plotted. (A) The dose–response curves before age-adjustment. Regression analysis using DoseEstimate ver. 4.1 software was calculated from the average value of the five samples. [Y = 0.0053 (±0.0009) + 0.0259 (±0.0127) × D + 0.0826 (±0.0161) × D2, r = 0.9995] (Y: yield of chromosome aberrations, D: dose (Gy), r = correlation coefficient). (B) The dose–response curves following age-adjustment. The regression analysis was [Y = 0.0015 (±0.0009) + 0.0049 (±0.0155) × D + 0.1033 (±0.0223) × D2, r = 0.9993]. (C) The dose–response curve before age-adjustment focusing on the low-dose range. (D) The dose–response curve following age adjustment focusing on the low-dose range.

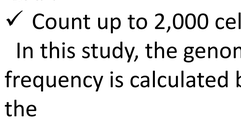
Figure 4. Dose–response curves for chromosome translocation analysis from five individuals. The dose–response curves in Figure 3 were plotted for individuals. (a) The dose–response curves before. (b) The dose–response curves following age-adjustment.]. (c) The dose–response curve before age-adjustment focusing on the low-dose range. (d) The dose–response curve following age adjustment focusing on the low-dose range.

MATERIAL AND METHODS

The samples and the medical records used in our study have been approved by the Ethics Committee of the Fukushima Medical University School of Medicine (approval number 1577). Written informed consent was obtained from all participants for analysis of PB samples.
Hiroshima University ran from blood collection to fixation.
Fukushima Medical University School of Medicine went from chromosome preparation to analysis.

Hiroshima Univ. operated

Gamma-ray irradiation and lymphocyte culture



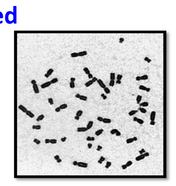
[0, 10, 20, 50, 100, 200, 500, 1000 mGy]

- ✓ ^{60}Co radiations source (1.11 TBq).
- ✓ Dose rate of 26.26t + 6.42 mGy (t: time(min)).
- ✓ 5 donors × 8 doses, total 40 samples.

Isolated lymphocytes were used, culture, harvest, and chromosome preparation were made according to a standard cytogenetic procedure.

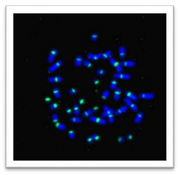
Fukushima Medical Univ. operated

Giemsa staining



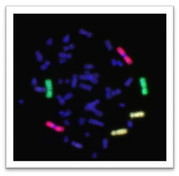
- ✓ Use 5% Giemsa (Merck Millipore, Darmstadt, Germany) solution.
- ✓ Count up to 2,000 metaphases.

Centromere-fluorescence in situ hybridization (Centromere-FISH)



- ✓ Use Poseidon probe (KRATECH, Amsterdam, The Netherlands)
- ✓ Nuclei were counterstained with Vectashield Mounting Medium with DAPI (Vector, Burlingame, USA)
- ✓ Count up to 2,000 metaphases.

Chromosome painting



- ✓ Use Customized XCP-Mix probe (Mix-#1R-#2G-#4RG; MetaSystems, Altlussheim, Germany)
- ✓ Nuclei were counterstained with Vectashield Mounting Medium with DAPI
- ✓ Count up to 2,000 cell equivalent.

In this study, the genomic translocation frequency is calculated by using the formula for the painted fractions of the genome as follows:

$$F_G = F_{P(1+2+4)} / 2.05\ [f_1(1-f_1)+f_2(1-f_2)+f_4(1-f_4) - (f_1f_2+f_1f_4+f_2f_4)]$$
$$= F_P \times 2.567 \text{ (Female)}$$
$$= F_P \times 2.533 \text{ (Male)}$$

(Lucas et al, Cytogenet Cell Genet. 1993)
(IAEA manual 2011, Cytogenetic Dosimetry)
where:
F_G is the full genome aberration frequency.
F_p is the translocation frequency detected by FISH.
$f_{1,2,4}$ is the fraction of genome hybridized, taking into account the gender of the subjects.
In order to unify the analysis cell numbers by each analysis method, we determined the frequency of abnormal whole genome using a 2,000 cell equivalent (obtained by dividing the observed number of cells in this coefficient respectively.

Chromosome analysis was performed by three trained.

ACKNOWLEDGMENTS

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