ORIGINAL ARTICLE

Development of Dose-Response Calibration Curve for Dicentric Chromosome Induced by X-Rays

Yanti Lusiyanti, Mukh Syaifudin, Tuti Budiantari, Sofiati Purnami, Dwi Ramadhani

Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency of Indonesia, Jakarta, Indonesia

ABSTRACT

Chromosome aberration is a biomarker that has been used as a standard tool in biological dosimetry (biodosimetry) of individuals after exposure to ionizing radiation. It is based mainly on the induction of dicentric chromosomes – one of the radiation-induced biological effects, in order to correlate them with radiation dose. In this study, a dose calibration curve for X-rays was generated by using the dicentric assay and by fitting the data to both Chromosomal Aberration Calculation Software and Dose Estimate programs to compare the output of each method. Peripheral blood samples from four nonsmoker healthy donors were irradiated with various doses ranging from 0 to 4 Gy with 250 kV or 122 keV X-rays at a dose rate of 0.17 Gy/min. The irradiated blood was cultured, harvested, and analyzed according to the standard procedure as described by the International Atomic Energy Agency with slight modifications. The dose-response calibration data for dicentrics were fitted with the linear-quadratic model ($Y_{dic} = 0.03987D^2 + 0.00651D$). The dose-response calibration curve obtained in this research was comparable to other estimations with similar radiation quality and dose rates. The results in this research convinced us in sustaining a biodosimetry using a dose-response calibration curve in our laboratory.

Key words: Biological dosimetry, dicentric, dose-response calibration curve, radiation, X-rays

Introduction

Biological dosimetry (biodosimetry) is a method to determine the absorbed dose in individuals with unplanned exposure to ionizing radiation when the physical dosimeter was unavailable.[1,2] Biodosimetry using chromosome aberration biomarker has been a valuable dose assessment method, especially when there are difficulties in interpreting the data given by physical dosimetry or in case of radiation overexposure. The dicentric chromosome assay (DCA) was the only available biodosimetry method for many years, either after accidental or occupational exposure.[3] It was used in various radiation accidents such as the Chernobyl disaster in 1986, Tokaimura nuclear accident in 1999 and recently in Fukushima accident in 2016.[3-5]

Dicentric chromosomes are categorized as unstable chromosome aberrations induced by ionizing radiation as a result of misrepair by nonhomologous end-joining whereby an exchange of material occurred on two damaged chromosomes. Previous studies have shown that there is a close correlation between the yield of radiation-induced dicentrics and the absorbed dose of ionizing radiation for either *in vivo* or *in vitro* exposures.[1,2] The number of cells containing unstable chromosome aberrations such as dicentrics will decline with time after exposure to radiation as a result of the selection process that occurs during cell proliferation. Therefore, analysis of the dicentric chromosome should be performed immediately after radiation exposure.[6] Biologically estimated dose using DCA is obtained by comparing the observed yield of dicentrics in peripheral blood lymphocytes of the irradiated person with a standard dose-response calibration curve constructed after *in vitro* irradiation.[3]

Dose interpretation using a calibration curve from different laboratories may introduce extra uncertainty. Even with the improvements in the laboratory techniques, differences in calibration curves between laboratories still exist. These can be due to diverse culture conditions or personal scoring experience. Laboratories that perform biological dose assessment using DCA are required to obtain their own dose-response calibration curve for different types and energies of radiation.[3] A number of reports illustrated that the yield of radiation-induced dicentrics is

Access this article online	
Quick Response Code:	Website: www.genome-integrity.org
	DOI: 10.4103/genint.genint_1_19

Address for correspondence:
Mrs. Yanti Lusiyanti,
Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency of Indonesia, Jalan Lebak Bulus Raya No. 49, Kotak Pos 7043 JKSKL, Jakarta 12070, Indonesia.
E-mail: k_lusiyanti@batan.go.id

How to cite this article: Lusiyanti Y, Syaifudin M, Budiantari T, Purnami S, Ramadhani D. Development of dose-response calibration curve for dicentric chromosome induced by X-rays. Genome Integr 2019;10:2.

dependent on a linear-quadratic model. For high-LET radiations, the shape of the dose-response is linear, with no quadratic term.[4,5] It was observed that the lower limit of dose detection by dicentrics for low-LET radiation is around 0.1–0.2 Gy in 500–1000 cells, whereas the background level of dicentrics for the general population is 0.5–1.0/1000 cells.[7,8]

Based on the ISO standards, DCA has become a routine component of many radiological protection programs,[9] and its methodological standard has been accepted internationally.[6] There is a need for a competent biodosimetry laboratory in Indonesia that is capable of performing cytogenetic analysis for dose estimation. In the previous study, our laboratory has constructed a standard dose-response calibration curve of dicentrics in gamma-radiation-induced human lymphocytes.[2] The curve for micronuclei induced by X-rays was also reconstructed as well.[10] In addition, a curve for gamma-ray exposure was obtained involving subjects with different ethnicity/population and limited radiation dose range.[11]

The aim of this study was to establish dose calibration curve for X-rays using the dicentric assay and to fit the data to both the Chromosomal Aberration Calculation Software (CABAS) and Dose Estimate programs to compare the output of each method. This curve will be used for predicting radiation absorbed dose received by individual who gets an overexposure of ionizing radiation in Indonesia.

Materials and Methods

Blood sampling and irradiation process

Peripheral blood samples were collected in heparinized vacutainers from four nonsmoking healthy donors aged 25–48 years with no previous exposure to ionizing radiation for at least in the past 6 months. Informed consent was obtained from all these donors as approved by the Institutional Ethics Committee. The blood samples were irradiated *in vitro* to X-rays (YXLON MG 325) at 250 kV using filters of 1.66 mm Cu and 1 mm Al with HVL of 2.52 mm Cu and having the dose rate of 0.17 Gy/h at the Secondary Standard Dosimetry Laboratory of the Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency. The radiation doses ranging between 0 and 4.0 Gy were used in the experiments. After irradiation, the blood samples were maintained at 37°C for 1 h to enable repair of chromosomal damages. Measurement of air kinetic energy released in material (air KERMA) for 250 kV X-ray was carried out using the Farmer type electrometer of NE 2570 connected to IC type of NE 2571. Air-KERMA was measured at the source to detector distance of 100 cm and radiation field diameter of 10 cm. The air-KERMA was calculated by following the International Atomic Energy Agency (IAEA) Technical Reports Series No 277 protocol.[12]

Cell culture set up

Cell culture was done according to previous reports.[2,6,11] From each sample, two blood cultures were setup. Two milliliters of the whole blood samples (irradiated and control) were cultured for 48 h in an incubator at 37°C with a humid atmosphere of 5% CO_2. The culture medium consisted of 8.0 mL of RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum and 1% streptomycin/penicillin antibiotics (Gibco). For stimulating cell division, 3.0% mL of phytohemagglutinin (Gibco BRL, Grand Island, NY, USA) was added. Colcemid (Gibco BRL) was added for the past 4 h of culture at a final concentration of 0.1 mg/mL to block the mitotic process of the cells at the first metaphase stage. After the culture was completed, the content of each tube was then transferred into 15 mL centrifuge tubes. The tubes were then centrifuged for 10 min at 1500 rpm. The precipitate was resuspend in 8 mL of 0.075 M KCl (pre-warmed at 37°C) for 20 min, which was followed by the addition of 2 mL of cold fresh Carnoy's fixative solution (3:1 methanol: glacial acetic acid mixture). This fixation step was repeated 2–3 times until white sediment was obtained. The fixed cells were stored in the freezer for at least one night until the preparation of the slide was made.

Scoring chromosomal aberrations

Frequencies of unstable chromosome aberrations (dicentrics, ring, and acentric fragments) were scored in metaphase cells that contained complete 46 chromosomes. At least 1000 metaphase cells for each donor, and each dose point were analyzed. Metaphases were analyzed using four independent scorers using the light microscope, and all chromosomal aberrations scored were taken into account.

Data analysis

Dose-response calibration curves were constructed using the CABAS version 2 (developed at the Swietokrzyska Academy, Kielce, Poland)[13] and Dose Estimate software then was used to conduct the goodness-of-fit and Chi-squared test for homogeneity.[8,14] A Poisson distribution, the dispersion index (σ^2/y) and the normalized unit of this index (u) were obtained for each dose using an equation described in the IAEA manual[6] where N indicates the number of cells analyzed and x is the number of dicentrics detected. Dispersion index values closed to 1 and u values between ± 1.96 indicated conformity with the Poisson distribution. Overdispersion of data was showed by the u values higher than 1.96. In contrast, u values lower than −1.96 indicated an underdispersion.[6]

Results

Frequency and distribution of dicentrics in blood samples obtained following exposure of eight different radiation doses of X-rays are shown in Table 1. In total, 2061 dicentrics, 135

Table 1: Frequency and distribution of dicentrics in blood samples exposed *in vitro* to X-rays

Dose (Gy)	Metaphase scored	Total of dicentrics	Total of rings	Total of acentric
0	3250	0	0	0
0.25	2000	9	0	20
0.5	2000	22	2	40
0.75	2000	55	3	115
1	1986	96	3	126
2	2000	336	29	499
3	1765	711	40	1046
4	1305	832	58	1052

ring and 2898 acentric chromosomes were found. Table 2 shows the frequency and distribution of dicentrics, the dispersion index (σ^2/y), and *u* index obtained in each different radiation dose point.

Dicentric chromosome formation was increased significantly after single X-ray exposure, and increased in this chromosome damage formation was found in all doses. A good correlation between the increment of dicentric formation determined by the analysis of 1305–3250 metaphases using Giemsa staining was found. Other chromosome-type or chromatid-type aberrations were also recorded, such as rings, acentrics (aces), breaks, and gaps.

The Chi-squared test for homogeneity results showed no inhomogeneity noticed between the donors at all doses as indicated by a value of $P > 0.05$. Since all the donors showed homogeneity, the results were pooled to construct the dose-response calibration curves for dicentrics.

Discussion

In the study reported here, there was a dose-dependent increase in the production of dicentrics following radiation exposure, as shown in Table 1, and other types of chromosomal abnormalities such as ring and acentric ring were also observed, but it had been included only to support the extent of the damage. In Figure 1, dicentric data were analyzed using the CABAS program taking into account the pooled data of the four donors and the resultant curve was fitted to $Y_{dic} = 0.03987D^2 + 0.00651D$, while for the curve generated using Dose Estimate, it was $Y_{dic} = 0.0391009D^2 + 0.0230046D - 0.00607194$. The present study demonstrates the goodness-of-fit test for the dicentric calibration curves. Therefore, the data were well represented by the linear-quadratic model ($\chi^2 = 6.78$; degrees of freedom = 5; $P = 0.99$); moreover, the correlation coefficients (r) were close to 1 indicating a very strong relationship between the fitted data points. As highlighted earlier, the yield of dicentrics for gamma and X-rays were sparsely distributed among cells following the Poisson distribution. Our data are consistent with a random distribution of cellular and molecular damage, where from Table 1, it can be seen that the dispersion index values were ~1 and *u* values were between ±1.96. It is shown that almost all data points followed a Poisson distribution. Interestingly, the dicentric distribution at 4 Gy was overdispersed as indicated by *u*. The overdispersion of data could be caused by nonhomogeneous irradiation of blood samples. Lemos-Pinto *et al*.[15] stated that the overdispersion can be caused by nonhomogeneous irradiation of blood samples, resulting in nonuniformity of radiation effects, and this phenomenon was uncommon for high doses but has been reported elsewhere.[1,16] In this study, the dose-response curve produced here showed a significance for the highest dose at 4 Gy. An overdispersion at the highest doses also was observed in other working groups.[16,17] Schröder and Heimers[17] stated that the application of 100 kV X-ray irradiation might be a possible reason for the observed overdispersion at the high dose. Since this study, we used 250 kV of X-ray, which could explain the overdispersion at 4 Gy founded supporting the phenomenon observed by Schröder and Heimers.[17] As dicentric production depends on the quality and energy of radiation source, it would be important to compare the curves obtained for different types of radiation.[3,16-19] Table 3 summarizes the results of the present

Table 2: Unstable chromosome aberrations in blood samples exposed *in vitro* to X-rays

Dose (Gy)	Metaphase scored	Total of dicentrics	Distribution of dicentrics					σ^2/y	*U*-test
			0	1	2	3	4		
0	3250	0	3250	0	0	0	0	1	0
0.25	2000	9	1991	9	0	0	0	0.996	−0.134
0.5	2000	22	1978	22	0	0	0	0.989	−0.340
0.75	2000	55	1947	51	2	0	0	1.050	1.460
1	1986	96	1895	86	5	0	0	1.060	1.780
2	2000	336	1699	268	31	2	0	1.050	1.670
3	1765	711	1191	453	106	14	1	1.030	0.920
4	1305	832	710	409	146	29	11	1.080	2.10

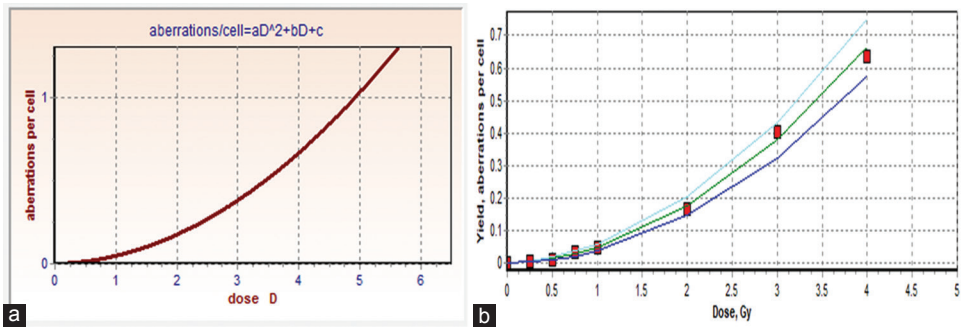

Figure 1: Linear-quadratic of dose-response curve for dicentric aberrations constructed using the dose-response calibration curves for dicentric yields induced by X-rays using Chromosomal Aberration Calculation Software (a) and dose estimate (b) software

study compared to the results obtained by other groups using different types of low-LET radiation, i.e., 100–250 kV X-rays. From Table 3, it could be seen that there was variability in the fitted coefficients, compared to these different dose-response calibration curves. It is possible to notice a certain degree of variability in the fitted coefficients (α and β) for the different radiations. The coefficients obtained in the present study agree well with other author's coefficients, especially result in data by Schröder and Heimers.[17]

Many factors were known to have an effect on resulting dose-response relationship curves, such as differences in the biological characteristics of donors lymphocytes and culture protocol slide preparation and scoring criteria. Therefore, each laboratory is required to generate its own calibration curve to achieve the accuracy of dose estimation. A dose-response calibration curve should be constructed by considering the basic physical parameters such as types of ionizing radiation – low- or high-LET and radiation dose rate.[15] Other factors that must be considered are dose detection limit and time interval.[20] The result may depend on the exposure conditions such as heterogeneous (partial-body) or homogeneous (whole-body) exposures, exposure time, and affected organs and organ systems.[21] As shown in Table 3, types of radiation, energy, and dose rate directly influence the values of α and β by considering the respective relative biological effectiveness of different energies in producing dicentric chromosomes.[13,22] It is clearly demonstrated that the linear coefficient is influenced by radiation quality where it is low at higher energies and higher at lower energies. In contrast, the coefficients obtained in this research are comparable to the values obtained by Schröder and Heimers.[17] According to Romm *et al.*[23] that the beta term is influenced by the applied dose rate, whereas the alpha term is linear energy transfer-dependent. Thus, the reason for this divergence in fitted coefficients probably lies in the dose rate used by investigators because lower dose rate changes the shape of calibration curves as the linear coefficient tends to dominate.[23] This might be due to the longer time required to irradiate the samples allowing time for DNA repair.[15] It may be important for a biodosimetry laboratory to generate several curves to cover all the types of radiations that are involved in accident scenarios. This curve may also be useful for *in vitro* dose reconstruction after accidental occupational exposures involving high energy X-rays, which is the basic recommendation in radiological emergency programs. In conclusion, the dose-response calibration curve is important for dicentric chromosome aberrations in human lymphocytes induced by X-rays. The generated dose-response calibration curves both by CABAS and dose estimated program show a good fit and give a good calibration curve [Table 4]. Overall, based on the results reported here, it gives us the confidence to apply the calibration curve for future biodosimetry requirements in case emergency preparedness in the radiological accident.

Table 3: Value of α and β coefficients of different energies and dose rates of X-ray

References	Source	Dose rate (Gy/min)	α	β
Lloyd *et al.*[16]	X-rays (250 kV)	1.0	0.036	0.067
Schröder and Heimers[17]	X-rays (100 kV)	0.4	0.035	0.070
Prasanna *et al.*[18]	X-rays (250 kV)	1.0	0.059	0.029
Beinke *et al.*[19]	X-rays (240 kV)	1.0	0.043	0.063
Pajic *et al.*[3]	X-rays (250 kV)	1.0	0.060	0.042
Present study (CABAS)	X-rays (250 kV)	0.17	0.039	0.0015
Present study (dose estimate)	X-rays (250 kV)	0.17	0.035	0.0018

CABAS: Chromosomal aberration calculation software

Table 4: Comparison of linear and quadratic yield coefficients calculated with chromosomal aberration calculation software and dose estimate

Program	C±SE	α±SE	β±SE	df	χ^2	*r*
CABAS	0	0.0085±0.0032	0.039±0.0015	5	6.78	-
Dose estimate	0	0.0085±0.0038	0.039±0.0018	5	6.78	0.99

SE: Standard error, CABAS: Chromosomal aberration calculation software

Acknowledgments

The authors gratefully acknowledge the National Nuclear Energy Agency of Indonesia (BATAN) for supporting this study through Center for Technology of Radiation Safety and Metrology (PTKMR), BATAN Annual Research Project. This research was also supported by the IAEA as an IAEA Contract Research Project (IAEA-CRP E3.50.08).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Martins V, Antunes AC, Monteiro Gil O. Implementation of a dose-response curve for γ-radiation in the Portuguese population by use of the chromosomal aberration assay. Mutat Res 2013;750:50-4.
2. Lusiyanti Y, Alatas Z, Lubis M, Suvifan VA, Ramadhani D, Purnami S. Dose-response curve of chromosome aberrations in human lymphocytes induced by gamma-rays. Atom Indon 2013;39:124-8.
3. Pajic J, Rakic B, Jovicic D, Milovanovic A. Construction of dose response calibration curves for dicentrics and micronuclei for X radiation in a Serbian population. Mutat Res Genet Toxicol Environ Mutagen 2014;773:23-8.
4. Hayata I, Kanda R, Minamihisamatsu M, Furukawa M, Sasaki MS. Cytogenetical dose estimation for 3 severely exposed patients in the JCO criticality accident in Tokai-Mura. J Radiat Res 2001;42 Suppl:S149-55.
5. Lee JK, Han EA, Lee SS, Ha WH, Barquinero JF, Lee HR, *et al.* Cytogenetic biodosimetry for Fukushima travelers after the nuclear power plant accident: No evidence of enhanced yield of dicentrics. J Radiat Res 2012;53:876-81.
6. International Atomic Energy Agency. Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies. Vienna Austria: International Atomic Energy Agency; 2011.
7. Barquinero JF, Barrios L, Caballín MR, Miró R, Ribas M, Subias A. Establishment and validation of a dose-effect curve for gamma-rays by cytogenetic analysis. Mutat Res 1995;326:65-9.

8. Ainsbury EA, Lloyd DC. Dose estimation software for radiation biodosimetry. Health Phys 2010;98:290-5.
9. Yoshida MA, Hayata I, Tateno H, Tanaka K, Sonta S, Kodama S, *et al*. The chromosome network for biodosimetry in Japan. Radiat Meas 2007;42:1125-7.
10. Lusiyanti Y, Alatas Z, Syaifudin M, Purnami S. Establishment of a dose-response curve for X-ray-induced micronuclei in human lymphocytes. Genome Integr 2016;7:7.
11. Syaifudin M, Lusiyanti Y, Purnami S, Lee YS, Kang CM. Assessment of ionizing radiation induced dicentric chromosome and micronuclei in human peripheral blood lymphocyte for preliminary reconstruction of cytogenetic biodosimetry. Atom Indon 2017;43:47-54.
12. International Atomic Energy Agency. Technical Reports Series No. 277, Absorbed Dose Determination in External Beam Radiotherapy. Vienna, Austria: International Atomic Energy Agency; 2000.
13. Deperas J, Szluinska M, Deperas-Kaminska M, Edwards A, Lloyd D, Lindholm C, *et al.* CABAS: A freely available PC program for fitting calibration curves in chromosome aberration dosimetry. Radiat Prot Dosimetry 2007;124:115-23.
14. Wang H, Liu Q, Wan D, Xiang J, Du L, Wang Y, *et al.* BioDoser: Improved dose-estimation software for biological radiation dosimetry. Comput Methods Programs Biomed 2012;108:402-6.
15. Lemos-Pinto MM, Cadena M, Santos N, Fernandes TS, Borges E, Amaral A. A dose-response curve for biodosimetry from a 6 MV electron linear accelerator. Braz J Med Biol Res 2015;48:908-14.
16. Lloyd D, Edwards AA, Prosser JS. Chromosome aberrations induced in human lymphocytes by *in vitro* acute X and gamma radiation. Radiat Prot Dosimetry 1986;15:83-8.
17. Schröder H, Heimers A. Chromosome aberrations induced in human lymphocytes by *in vitro* and *in vivo* X-rays. Mutat Res 2002;517:167-72.
18. Prasanna PG, Loats H, Gerstenberg HM, Torres BN, Shehata CW, Duffy KL. AFRRI's Gamma-ray, X-ray, and Fission-Neutron Calibration Curves for the Lymphocyte Dicentric Assay: Application of a Metaphase Finder System. Bethesda: Armed Forces Radiobiology Research Institute; 2002.
19. Beinke C, Braselmann H, Meineke V. Establishment of an x-ray standard calibration curve by conventional dicentric analysis as prerequisite for accurate radiation dose assessment. Health Phys 2010;98:261-8.
20. Sullivan JM, Prasanna PG, Grace MB, Wathen LK, Wallace RL, Koerner JF, *et al.* Assessment of biodosimetry methods for a mass-casualty radiological incident: Medical response and management considerations. Health Phys 2013;105:540-54.
21. Riecke A, Ruf CG, Meineke V. Assessment of radiation damage-the need for a multiparametric and integrative approach with the help of both clinical and biological dosimetry. Health Phys 2010;98:160-7.
22. Zhao L, Wu D, Mi D, Sun Y. Radiosensitivity and relative biological effectiveness based on a generalized target model. J Radiat Res 2017;58:8-16.
23. Romm H, Oestreicher U, Kulka U. Cytogenetic damage analysed by the dicentric assay. Ann Ist Super Sanita 2009;45:251-9.