Atom Indonesia Vol. 39 No. 3 (2013) 124 - 128

Dose-Response Curve of Chromosome Aberrations in Human Lymphocytes Induced by Gamma-Rays

Y. Lusiyanti*, Z. Alatas, M. Lubis, V.A. Suvifan, D. Ramadhani and S. Purnami
Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency
Jl. Lebak Bulus Raya No. 49, Jakarta 12440, Indonesia

ARTICLE INFO

Article history:
Received 8 November 2013
Received in revised form 27 December 2013
Accepted 31 December 2013

Keywords:
Chromosome aberrations
Dicentric assay
Standard calibration curve
Biodosimetry

ABSTRACT

Chromosome aberration is a biomarker to predict the level of cell damage caused by exposure to ionizing radiation on human body. Dicentric chromosome is a specific chromosome aberration caused by ionizing radiation and is used as a gold standard biodosimetry of individuals over exposed to ionizing radiation. In radiation accident the dicentric assays has been applied as biological dosimetry to estimate radiation absorbed dose and also to confirm the radiation dose received to radiation workers.The purpose of this study was to generate a dose response curve of chromosome aberration (dicentric) in human lymphocyte induced by gamma radiation. Peripheral blood samples from three non smoking healthy volunteers aged between 25-48 years old with informed consent were irradiated with dose between 0.1-4.0 Gy and a control using gamma teletherapy source. The culture procedure was conducted following the IAEA standard procedures with slight modifications. Analysis of dose-response curves used was LQ model $Y = a + \alpha D + \beta D^2$. The result showed that α and β values of the curve obtained were 0.018 ± 0.006 and 0.013 ± 0.002, respectively. Dose response calibration curve for dicentric chromosome aberrations in human lymphocytes induced by gamma-radiation fitted to linear quadratic *model.* In order to apply the dose response curve of chromosome aberration disentric for biodosimetry, this standar curve still need to be validated.

INTRODUCTION

Radiation exposure to the body may cause the interaction of radiation with biological materials where part of the cells will be damaged cytogenetically as the alterations of chromosome structure or aberrations in peripheral blood lymphocytes. Dicentric chromosome in human peripheral blood lymphocytes is the gold standard for radiation exposure and chromosome translocation is a cytogenetic biomarker for retrospective biodosimetry. Biodosimetry is a method to quantify an individual's absorbed dose in situations of occupational or accidental over-exposure to ionizing radiation when no physical dose-estimate is available and biological dosimetry is the only way to quantify the dose. In radiation protection, biodosimetry is an important and independent method that complements physical dosimetry, as well as a vital factor for diagnosis and for assessment of the prognosis of subjects who have been irradiated [1,2].

The dicentric assay technique has been shown as the most sensitive method for quantifying the radiation dose because of its ability to estimate the average whole-body dose. These aberrations can be an unstable form such as dicentric chromosomes and rings, and a stable form such as translocations. The biologically estimated dose is obtained by comparing the observed yield of unstable chromosomal aberrations (dicentrics and centric rings) in peripheral blood lymphocytes of the studied subjects, with a standard dose–response curve. The standar dose-response curve is obtained in *vitro* meaning that blood samples are irradiated in tubes [1,3,4]. When the chromosome aberration detection methods will be applied as radiation biodosimetry it is important to know that detection of chromosome aberration only performed on cells that had passed through the first division of cell cycle post-exposure. This is necessary in order to optimize the response of the quantity of the damage caused by radiation exposure [5,6].

* Corresponding author.
E-mail address: k_lusiyanti@batan.go.id

Dose–response curves have shapes and slopes that differ as a function of LET and relative biological effectiveness. For low- LET radiation (e.g. γ rays and X-rays), the dose–response curve for dicentrics fits better to a linear–quadratic model (LQ) $Y = a + \alpha D + \beta D^2$, where Y is the yield of dicentrics, a is the background frequency of dicentrics and α and β are the linear and dose squared coefficient [7-9].

According to the IAEA [8], each laboratory must have its own dose–response curve, since several factors can influence the dose–effect relationships such as culture conditions or sensitivity of cell and dicentric scoring efficiency [2,10,11]. In general the relationship has been shown to be linear for high-LET radiation and linear quadratic for low-LET radiation [12,13].

The purpose of this study was to generate a standard dose response curve of unstable chromosome aberration (dicentric) induced by radiation exposure to ^{60}Co for predicting radiation absorbed dose received by individual that over exposed to ionizing radiation.

EXPERIMENTAL METHODS

Blood sampling and irradiation process

Peripheral blood samples were collected in 4 ml heparinised vacutainers tube from three non smoking healthy volunteers aged between 25-48 years old. One of the aliquots was used as a control and the rest were exposed to ^{60}Co teletherapy machine at National Radiation Laboratory of Metrology PTKMR BATAN. The doses given were 0 (control), 0.1; 0.25; 0.5; 1.0; 2.0; and 4.0 Gy at a dose rate of 0.38 Gy/min. The irradiation procedure was proceedeed as described in IAEA TRS 277 [14] and performed twice. After irradiation, blood samples were kept at 37°C to allow for any chromosomal repair to take place.

Culture set up and fixation procedures

The culture procedures were conducted following the IAEA standard procedures [1,8] with slight modifications. All of the components used for culturing were obtained from Gibco. One milliliter of the whole blood samples were cultured for 48 hours in the incubator at 37°C containing 5% CO_2. The culture medium consisted of 7.5 mL of RPMI-1640 supplemented with 20% heat inactivated fetal calf serum and 1% streptomycin/penicillin, and 2.5% ml of phytohema-gglutinin was added to stimulate cell division. To block the mitotic process of the cells at the metaphase stage, colchicines was added for the last 3 hours of culture at a final concentration of 0.1 mg/mL. The cells were centrifuged for 10 minutes at 1500 rpm and resuspended in 10 ml of 0.075 M KCl (pre-warmed to 37°C) for 25 minutes. At this stage, 2 ml of fresh Carnoys fixative (methanol : acetic acid = 3 : 1) solution was added into the tube. This fixation step was repeated four times until white sediment was obtained. The cell suspention was stored in -20°C at least for one night until the slide preparation was conducted. Then the slides were stained with 5% giemsa solution (pH 6.8) for 4 minutes and observed using light microscope.

Scoring the metaphases cell of unstable chromosomal aberrations

The frequencies of unstable chromosome aberration (dicentric, ring and acentric fragments) were scored in complete metaphases with 46 centromers as described in the IAEA standard procedure. At least 500 first division metaphase cells were scored per irradiated samples and 500-1000 metaphase cells were analysed per control samples. The slides were also stained using Fluorescence Plus Giemsa (FPG) to analyze the different chromatid harlequin effect in non metaphase cells [1,8,15]. The number of aberrations and metaphase index were observed under a microscope connected to digital Camera System and Imaging (Fig. 1). Dose–response calibration curves were constructed by means of the Chromosome Aberration Dose Estimate software, v.5.1 [16]. The standar u-test described by Papworth and adopted by Savage was used to determine whether dicentrics followed a Poisson distribution probabilities [3,17].

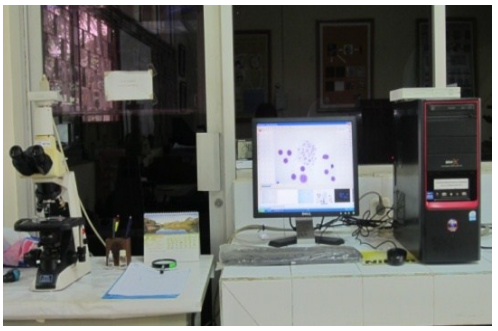
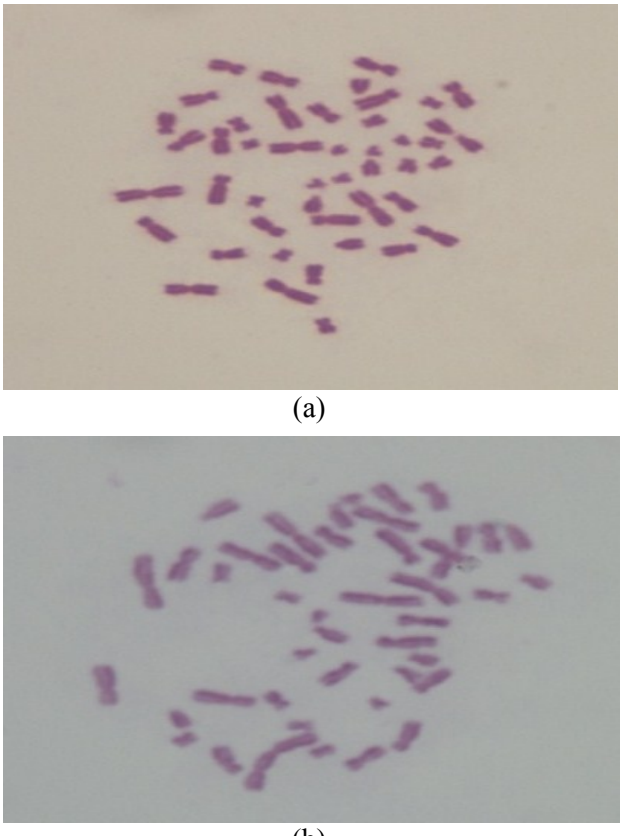


Fig. 1. Microscope connected to Digital Camera and Imaging System.

RESULTS AND DISCUSSION

Observation of chromosome aberration can be performed on blood lymphocytes cells that are most

sensitive cell to radiation. The frequency of dicentric chromosome as biomarker of chromosomal damage caused by exposure to radiation can be observed when the cells are at metaphase stage at the first cell division cycle. This is necessary in order to optimize the response of the quantity of the damage caused by radiation exposure. To implement the dicentric chromosome as biological dosimetry, it needs to make sure that the cell culture results obtained are mostly in metaphase at the first mitosis (M1) cells. From this research obtained that the percentage of M1 to M2 induced by ^{60}Co irradiation for incubation periods of 48 hours showed that frequency of M1 of M1 was above 50% compare to M2. Visualization of Metaphase in M1 and M2 presented in Fig. 2.



(a)

(b)

Fig. 2. Visualization of Metaphase spread in M1 (a) and M2 (b).

In this research the frequencies of unstable chromosome aberration (dicentric, ring and acentric fragments) were scored in complete metaphases with 46 centromers as described in the IAEA standard procedure [8]. Visualization of metaphase spread with dicentric chromosome is presented in Fig. 3.

The result indicated that the frequency of dicentric chromosome, which are specific indicators to ionizing radiation, increased with increasing dose. Due to its small number, ring chromosomes were not included in the analysis, therefore only the resulted data of dicentrics were fitted by LQ dose response curve model $Y = a + \alpha D + \beta D^2$ using Dose Estimate 5.1 Program [16]. The equation as the result of statistical calculations based on the data obtained is $Y = 0.0 + (0.018 \pm 0.006D) + (0.013 \pm 0.002D^2)$ with a correlation coefficient $r = 0.996$ (Fig. 4).

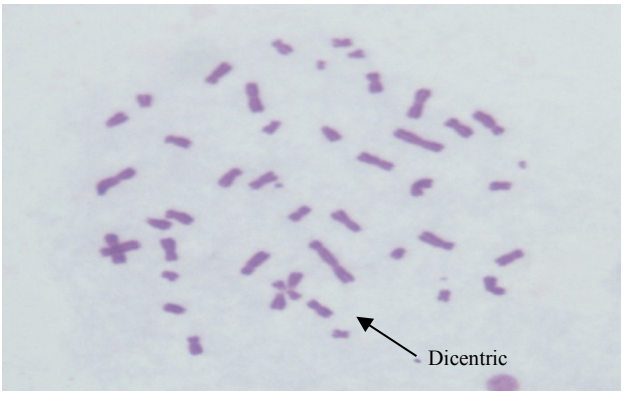


Fig 3. A metaphase spread with one dicentric.

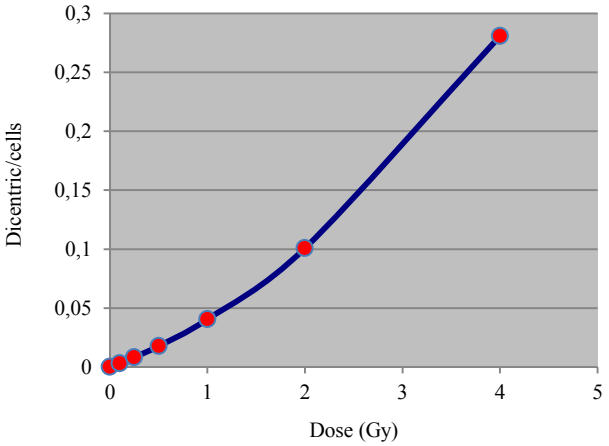


Fig. 4. Dose-response calibration curve of dicentric chromosomes as a function of dose of ^{60}Co.

In this research, the value of the coefficient α was 0.018 greater than β coefficient of 0.013 indicating that disentric formed by a single radiation tracks much higher than double track. Gamma-rays have a low LET means low ionization frequency for each unit distance or track. The probability of two ionizations by a single track that occurs in cells as a target will be low. At least two tracks of ionization needed to produce damage to the two chromosomes that would eventually merge to form a dicentric chromosome. The probability will be much higher when two damages caused by the ionization of the two traces obtained. Thus dicentric frequency caused by a single track will be equivalent to a linear function of the dose, while the result of two track have dicentric proportional to the square of the dose [1,8]. Comparation of the value of α and β coefficients of the LQ curve for induced dicentric

with several other published papers are presented in Table 1.

Table 1. Value of α and β coefficients and their standard errors (SE) for different types of gamma-rays ^{60}Co.

No	Reference	Dose Rate (Gy/min)	α ± SE	β ± SE
1.	Senthamizhchelvan et.al [13]	0.5	0.029 ± 0.008	0.05 ± 0.004
2.	Koksal et.al [12]	0.4	0.021± 0.005	0.07± 0.002
3.	Present paper	0.38	0.018 ± 0.006	0.013 ± 0.002

The result indicated that the the value of α and β coefficients showed a relatively similar coefficient value of α and β. According to IAEA manual, the dicentric induced by gamma rays produces a distribution damage which is very well represented using the Poisson distribution model u-test because curve fitting methods are based on Poisson statistics [7,8,17]. Data of dicentric frequencies for different doses and their distribution are presented in Table 2.

Table 2. Dicentric distribution obtain from blood samples irradiated with gamma-rays.

Dose (Gy)	Meta-phase scored	∑ dicen-tric	Cell distribution of dicentrics					u-Test	P*
			D0	D1	D2	D3	D5		
0	2000	0	2000	0	0	0	0	0	P
0.1	2000	2	1998	2	0	0	0	-0.01	P
0.25	2000	10	1990	10	0	0	0	-0.14	P
0.5	2000	18	1984	15	0	1	0	10.27	NP*
1	2000	58	1947	48	5	0	0	4.55	NP
2	2000	225	1798	183	15	4	0	4.04	NP
4	1853	506	1436	344	59	13	1	4.69	NP

P=Poisson NP=Non Poisson

The above calculation of u showed that the dicentric followed a Poisson distribution patterns ($u \geq \pm 1.96$) at the low dose whereas at the dose 0,5 – 4 Gy follow non Poisson distribution ($u \leq \pm 1.96$). The similar result also found in research conducted by Martin *et al*. whereas the dicentric distribution were consistent with Poisson at the lower doses but were over dispersed at the higher doses (1-3 Gy) [18]. This result is likely influenced by the sensitivity of each chromosome. The results showed that a number of specific chromosomes were more sensitive to radiation than other chromosomes resulting in more frequent exchange of fragments resulting chromosomal breakage. Distribution of chromosome fragments apparently is not random in the human genome [4,10,19]. Several factors that affecting the outcome of chromosome aberration induction, namely biological and physical factors under laboratory conditions. Physical factors that affect the formation of dicentric induction is LET, dose and dose rate, while biological factors such as the kinetics of lymphocytes, variety and sensitivity of cell culture media [12,20]. To apply dose response curves on future biological dosimetry management of radiological casualities the curves need to be validated.

CONCLUSION

Dose response calibration curve for dicentric chromosome aberrations in human lymphocytes induced by gamma-radiation fits to linear quadratic model. The calibration curves were used to estimation of radiation absorbed in situations of occupational or accidental over-exposure to ionizing radiation. In order to apply the disentric calibration curve as biodosimetry,our dose response curve needs further validation.

ACKNOWLEDGMENTS

The Authors gratefully acknowledge the BATAN for supporting this study via PTKMR-BATAN Annual Research Project.

REFERENCES

1. Anonymous, Technical Report Series, No. 405, IAEA, Vienna (2001).
2. V. Hadjidekova, R. Hristova, E.A. Ainsbury, *et al*., Health Phys. **98** (2010) 252.
3. A.S. Rodrigues, N.G. Oliveira, O. Monteiro Gil, *et al.*, Radiat. Prot. Dosim. **115** (2005) 1.
4. T. Iwasaki, Y. Takashima, T. Suzuki, *et al.*, Radiat. Res. **175** (2011) 208.
5. M.A.A. Bender, A.A. Awa, A.L. Brooks, *et al.*, Mutat. Res. **196** (1988) 103.
6. R.J. Purrott, N. Vulpis and D.C. Lyod, Experientia **37** (1981) 407.
7. E.J. Hall and A.J. Gioccia, Radiobiology for the Radiobiologist, 7th ed, J.B. Lippincott Company, Philadelphia (2012).
8. Anonymous, Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies, IAEA-EPR, Vienna (2011).
9. H. Romm, R.C. Wilkins, C.N. Coleman, *et al*, Radiat. Res. **175** (2011) 397.

10. D.C. Lloyd, R.J. Purrott, G.W. Dolphin, *et al.*, Int. J. Radiat. Biol. **1** (1975) 75.

11. J.F. Barquinero, L. Barrios, M.R. Caballin *et al.*, Mutat. Res. **326** (1995) 65.

12. G. Koksal, F.S. Pala and D.O. Dalci, Mutat. Res. **329** (1995) 47.

13. S. Senthamizhchelvan, G.S. Pant, G.K. Rath, *et. al.*, Radiat. Prot. Dos. **123** (2007) 241.

14. Anonymous, Technical Reports Series No. 277, IAEA, Vienna (2000).

15. P. Perry and S.Wolff, Nature **252** (1974) 739.

16. Ainsbury and D. Lloyd, Health Phys. **98** (2010) 276.

17. A.A. Edward, D.C. Llyod and R.J. Purrott, Rad. Env. Biophys. **16** (1979) 89.

18. V. Martins, A.C. Antunes and O. Monteiro Gil, Mutation Research **750** (2013) 50.

19. J.N. Lucas, F. Hill, C. Burk, *et al.*, Health Physics **68** (1995) 761.

20. M.S. Sasaki, Biological Aspects of Radiation Protection, T. Sugahara and O. Hug (Eds.), Springer, Berlin (1971).