ORIGINAL ARTICLE

Enhancing Cytogenetic Biological Dosimetry Capabilities of the Philippines for Nuclear Incident Preparedness

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ABSTRACT

The utility of a biological dosimeter based on the analysis of dicentrics is invaluable in the event of a radiological emergency wherein the estimated absorbed dose of an exposed individual is crucial in the proper medical management of patients. The technique is also used for routine monitoring of occupationally exposed workers to determine radiation exposure. An *in vitro* irradiation study of human peripheral blood lymphocytes was conducted to establish a dose-response curve for radiation-induced dicentric aberrations. Blood samples were collected from volunteer donors and together with optically stimulated luminescence (OSL) dosimeters and were irradiated at 0, 0.1, 0.25, 0.5, 0.75, 1, 2, 4, and 6 Gy using a cobalt-60 radiotherapy unit. Blood samples were cultured for 48 h, and the metaphase chromosomes were prepared following the procedure of the International Atomic Energy Agency's Emergency Preparedness and Response – Biodosimetry 2011 manual. At least 100 metaphases were scored for dicentric aberrations at each dose point. The data were analyzed using R language program. The results indicated that the distribution of dicentric cells followed a Poisson distribution and the dose-response curve was established using the estimated model, $Y_{dic} = 0.0003\ (\pm 0.0003) + 0.0336\ (\pm 0.0115) \times D + 0.0236\ (\pm 0.0054) \times D^2$. In this study, the reliability of the dose-response curve in estimating the absorbed dose was also validated for 2 and 4 Gy using OSL dosimeters. The data were fitted into the constructed curve. The result of the validation study showed that the obtained estimate for the absorbed exposure doses was close to the true exposure doses.

Key words: Absorbed dose, biodosimetry, dicentric chromosomes, dose response, linear quadratic

Introduction

The Philippine government has a national preparedness plan whose objective is to establish an organized response capability for timely and coordinated action in the event of an emergency. Nuclear incident is one of the four major categories of emergencies included in the plan. Realizing the critical role that the cytogenetics group will play during radiation emergency, the Philippine Nuclear Research Institute conducted a study to establish our own dose-response calibration curve for dicentrics assay.

Biodosimetry is an essential tool for providing timely assessments of radiation exposure, particularly when physical dosimetry is unavailable or unreliable.[1] Maznyk *et al.*[2] argued that for mass-casualty events involving public exposure to ionizing radiation, it is paramount to rapidly provide this dose information for medical management of casualties. For proper dose estimation, each biodosimetry laboratory should establish its own dose-response curves to prevent any erroneous calculation.

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Materials and Methods

Subject recruitment

Eight healthy persons, three males and five females, aged 21–55 years old were recruited as blood donors. They were required to sign an informed consent form after being explained of the study. All donors were requested to accomplish a questionnaire to assess their general physical condition, lifestyle, previous X-ray examinations, diets, use of medications, etc.

Blood collection and irradiation

Ten milliliters of blood was drawn from each donor and 1 ml each was transferred to nine sterile glass vials. Two sets of optically stimulated luminescence nanoDot dosimeters per dose were also placed in separate glass vials. The vial with the blood sample and

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the vial containing the dosimeters were placed side by side in an improvised container filled with water just enough to immerse the vials with the temperature maintained at 37°C ± 1. The blood samples and the dosimeters were irradiated using the cobalt-60 radiotherapy system of the Veterans Memorial Medical Center. The doses were 0.1, 0.25, 0.5, 0.75, 1, 2, 4, and 6 Gy at the dose rate of 91.98 cGy/min. Irradiation time was computed based on the dose rate of the source at the time of irradiation.

Blood culturing

About 0.5 ml of irradiated blood samples was then added to 5 ml PB-Max Karyotyping medium within 2 h from irradiation time, in duplicates. Samples were cultured in an incubator with 5% CO_2 for 48 h at 37°C.

Harvesting, slide spreading, and staining

Metaphase chromosomes were harvested, fixed, spread on slides, and stained as per guidelines of the International Atomic Energy Agency's (IAEA) Emergency Preparedness and Response: Biodosimetry 2011 manual.[1]

Dicentric scoring

The slides were first scanned at low magnification, and when metaphase spreads were found, the microscope was switched to higher magnification. The images were captured using Nikon Microscope Eclipse E200 with a Motic DS-Fi1 camera attachment. Images were saved on a flash drive for counting of chromosomes and dicentric scoring in the computer.

Validation study

To validate the accuracy of the established dose-response curve in estimating absorbed dose, blood samples from four donors, two males and two females, aged 23–52 years old were exposed to 2 Gy and 4 Gy. Processing, culturing, harvesting, slide preparation, and analysis were done following the procedure for the *in vitro* irradiation study of blood samples for the establishment of dose-response curve for dicentrics in the IAEA guidelines.[1]

Statistical analysis

The data were analyzed using R programming language, an open-source programming language designed by Ihaka and Gentleman,[3] and the R programming language by R Core Team.[4] The method used for estimating the coefficients is the iterative reweighted least squares, which is the method mentioned in the IAEA EPR 2011 manual[1] and stated that "the best fit value for each coefficient is achieved by assuming a Poisson distribution and maximizing the likelihood of the observations by the method of iteratively reweighted least squares."

Results and Discussion

Dose-response curve

The data obtained [Table 1] showed an increase in the frequency of dicentric chromosomes as the irradiation dose is increased. This observation is more pronounced at higher doses (1.0, 2.0, and 4.0 Gy). For 6.0 Gy, the number of dicentrics decreased. It was also observed that the number of metaphase cells decreases as the irradiation dose is increased. This observed decrease in metaphase cells at 6.0 Gy can be inferred from the study of Pujol *et al.*[5] who reported that most cells show difficulties in reaching mitosis at doses over 5 Gy and that this cannot be used to score dicentric chromosomes. It was also observed that the number of analyzable metaphase spreads decreased at the higher doses.

Statistical analysis showed that the cell distributions of dicentrics followed a Poisson distribution except for 0.1 Gy and 0.25 Gy, where both have not available (does not exist) U-test statistic. This is due to the dicentric count which is 1. As stated in IAEA EPR 2011 (equation 5, section 8.3),[1] the number of dicentrics detected should have at least 2 counts to compute the U-test statistic. However, at lower doses, it is difficult to achieve higher dicentric scores and instead several thousand cells per point should be scored.[1] Dicentric yield at 6 Gy showed overdispersed distribution considering that the estimated U-test statistic is more than 1.96. The dose-response curve [Figure 1] follows the linear-quadratic model. These results can be explained by the study of Vaurijoux *et al.*[6] which stated that low linear energy transfer radiation (X or γ rays) produces many tracks containing few primary events with a more randomized distribution of tracks and a more uniform distribution of damage between cells. The same authors also reported that the dose-effect relationship is linear in the low-dose range and becomes quadratic at high doses.

For Table 2, all estimates except the constant, C, are significant base on its *P* value. The Chi-square *P* value indicated that the fitted data points were not statistically different from the observed ones, confirming a good fit.

Table 1: Distributions of dicentrics

Dose (Gy)	Total cells (*n*)	Dicentrics (X)	Cell distributions of dicentrics							Dispersion index	U-test statistic
			0	1	2	3	4	5	6		
0	3076	1	3075	1	0	0	0	0	0	1.0000000	NA
0.1	332	2	330	2	0	0	0	0	0	0.9969789	−0.05496497
0.25	354	1	353	1	0	0	0	0	0	1.0000000	NA
0.5	157	8	149	8	0	0	0	0	0	0.9551282	−0.42365927
0.75	201	6	195	6	0	0	0	0	0	0.9750000	−0.27386128
1	300	16	284	16	0	0	0	0	0	0.9498328	−0.63351227
2	104	16	90	13	0	1	0	0	0	1.2330097	1.72699863
4	51	31	28	16	6	1	0	0	0	0.9922581	−0.03934955
6	26	24	12	10	1	1	1	1	0	1.8133333	2.93741482

NA: Not available

Table 2: Curve fitting: The followings are the summary from linear-quadratic model

C±SE	α (Gy^{-1})±SE	β (Gy^{-2})±SE	Chi-square *P* value	df
0.0003298±0.0003426	0.0336027±0.0110477	0.0236312±0.0053753	0.2400741	6
Z=0.963	*Z*=3.042	*Z*=4.396		
P=0.33568	*P*=0.00235	*P*=1.1e-05		

SE: Standard error

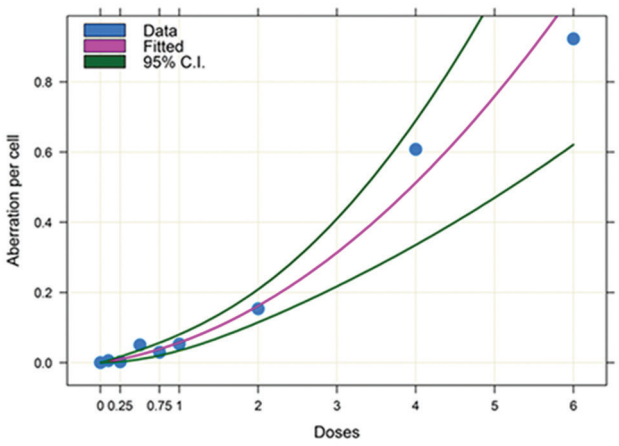

Figure 1: Dose-response curve: observed values dotted in blue and expected values in purple line; the 95% confidence interval in green line. The predicted values were extracted from the linear-quadratic model with complete coefficient

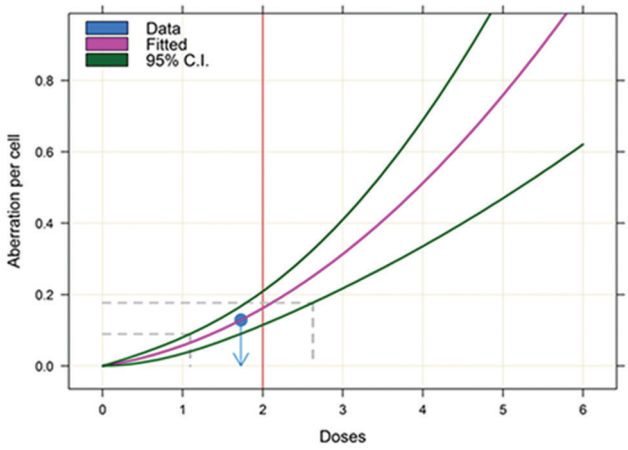

Figure 2: Interval estimates of dose for 2.0 Gy under 95% confidence interval with dose-response regression curve under 95% confidence interval

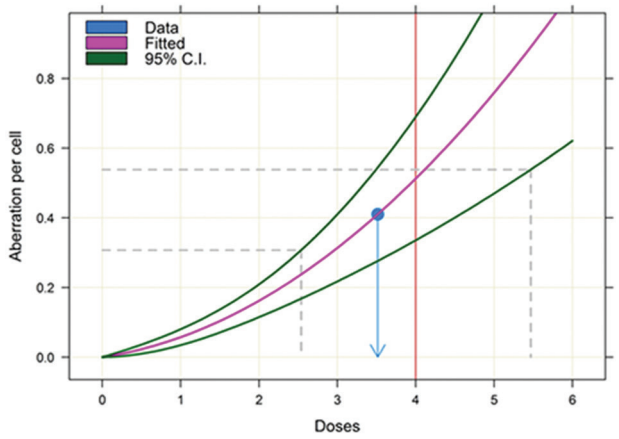

Figure 3: Interval estimate of dose for 4.0 Gy under 95% confidence interval with dose-response regression curve under 95% confidence interval

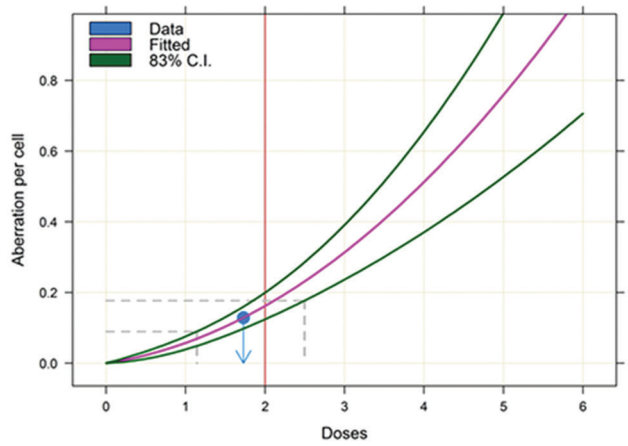

Figure 4: Dose interval estimates for 2.0 Gy under 95% confidence interval with dose-response regression curve under 83% confidence interval

The dose-response curve was established using the estimated linear-quadratic model, $Y = 0.0003298 + 0.0336027D + 0.0236312D^2$.

Validation of dose-response curve

Dose point estimate validation was performed using the estimated linear-quadratic model and is given by:

$$Y = 0.0003298 + 0.0336027D + 0.0236312D^2$$

The calibration is done by solving for D above, which is given by:

$$D = \frac{-\alpha + \sqrt{\alpha^2 + 4\beta(Y - C)}}{2\beta}$$

$$= \frac{-0.0336027 + \sqrt{0.0336027^2 + 4(0.0236312)(Y - 0.0003298)}}{2(0.0236312)}$$

Thus, for known dose 2 Gy [Figure 2], the estimate of the model for $X = 33$ (dicentrics) and $N = 256$ (cells), such that $Y = \frac{33}{256}$ is

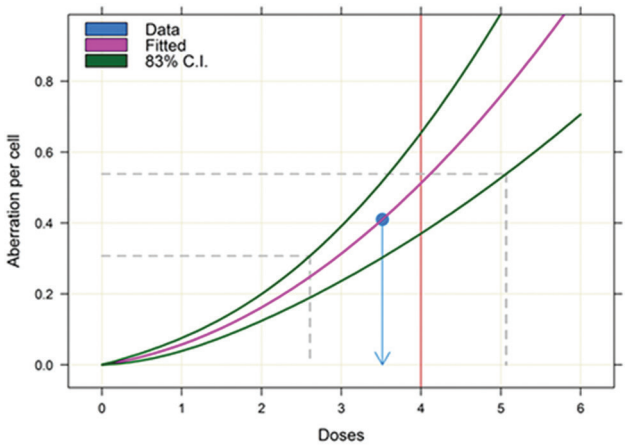

Figure 5: Dose interval estimates for 4.0 Gy under 95% confidence interval with dose-response regression curve under 83% confidence interval

Table 3: Distribution of dicentrics in blood samples exposed to 2.0 and 4.0 Gy

Dose (Gy)	Total cells (*n*)	Dicentrics (X)	Cell distribution of dicentrics						
			0	1	2	3	4	5	6
2.00	256	33	224	31	1	0	0	0	0
4.00	492	202	333	126	25	6	2	0	0
Total	1026								

Table 4: Preliminary statistics

Dose (Gy)	Total cells (*n*)	Dicentrics (X)	Index	Statistics
2.00	256	33	0.9353535	−0.7412793
4.00	492	202	1.1362949	2.1408340
Total	1026			

$$D = \frac{-0.0336027 + \sqrt{0.0336027^2 + 4(0.0236312)\left(\frac{33}{256} - 0.0003298\right)}}{2(0.0236312)}$$

$$= 1.727553\,\text{Gy}$$

For known dose 4 Gy [Figure 3], such that $Y = \frac{202}{492}$ is

$$D = \frac{-0.0336027 + \sqrt{0.0336027^2 + 4(0.0236312)\left(\frac{202}{492} - 0.0003298\right)}}{2(0.0236312)}$$

$$= 3.515786\,\text{Gy}$$

Dose interval estimate

The uncertainty can be calculated using the 95% confidence limit of the Poisson variable. The confidence limit is the gray dashed line intersecting the confidence interval of the dose-response curve. The value in the x-axis corresponds to the confidence limit [Figures 4 and 5]. Both 2.0 Gy and 4.0 Gy are inside the confidence limit, but 4.0 Gy has overdispersed distribution as indicated by its U-test statistics in Table 3. To account for this overdispersion, the confidence limit of 4 Gy was computed using some adjustments on its Y_1 and Yu. These Y_1 and Yu are the corresponding *y* values projected by the dashed lines onto the x-axis. The adjustment was done by multiplying a correction factor described in Equation 9 of EPR-Biodosimetry 2011.[1] To avoid overestimation of dose, 83% confidence limit of the regression curve was used instead of the 95% as suggested also in EPR-Biodosimetry 2011.[1] The statistical table from Crow and Gardner[7] was the reference used to obtain the Poisson 95% confidence limit of the dicentrics in 4 Gy of Table 4.

Conclusion

The established dose-response curve follows the linear-quadratic model. The goodness of fit test adjusted by the linear-quadratic mo (Chi-square $P = 0.24$ with df = 6) indicated that the model is appropriate and has passed the validity test. The uncertainty of the dose estimate, especially in cases of overdispersion in the dicentric distribution, can be corrected by adjusting the confidence interval from 95% to a lower confidence interval like 83% as in this case.

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Conflicts of interest

There are no conflicts of interest.

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