เส้นกราฟการตอบสนองต่อปริมาณรังสีสำหรับความผิดปกติของโครโมโซมแบบไดเซ็นทริกที่เกิดจากรังสีแกมมา เพื่อการเตรียมพร้อมรับมือเหตุฉุกเฉินทางรังสีในประเทศไทย

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บทคัดย่อ

การประเมินปริมาณการได้รับรังสีก่อไอออนอย่างแม่นยำมีความสำคัญอย่างยิ่งในการใช้มาตรวัดรังสีทางชีวภาพ โดยเฉพาะในพื้นที่ที่ปัจจัยทางสิ่งแวดล้อมและประชากรศาสตร์เฉพาะถิ่นอาจมีผลต่อการตอบสนองต่อรังสี การศึกษานี้นำเสนอการพัฒนาเส้นกราฟการตอบสนองต่อปริมาณรังสีสำหรับความผิดปกติของโครโมโซมแบบไดเซ็นทริกที่เกิดจากรังสีแกมมาในลิมโฟไซต์ของมนุษย์ ซึ่งมีเป้าหมายเพื่อเสริมสร้างความพร้อมในการรับมือเหตุฉุกเฉินทางรังสีในประเทศไทย โดยใช้ตัวอย่างเลือดจากอาสาสมัครเพศชายอายุ 39 ปีและเพศหญิงอายุ 32 ปี มาฉายรังสีแกมมาจากต้นกำเนิดโคบอลต์-60 อัตรารังสี 0.574 เกรย์ต่อนาที ในช่วงปริมาณรังสี 0-5 เกรย์ จากนั้นเพาะเลี้ยงลิมโฟไซต์และวิเคราะห์โครโมโซมแบบไดเซ็นทริกตามวิธีมาตรฐานที่แนะนำโดยทบวงการพลังงานปรมาณูระหว่างประเทศ (IAEA) สร้างเส้นกราฟการตอบสนองต่อปริมาณรังสีโดยใช้แบบจำลองเชิงเส้น-กำลังสองด้วยซอฟต์แวร์ Biodose Tools เวอร์ชัน 3.6.1 การประเมินความถูกต้องของเส้นกราฟที่สร้างขึ้นทั้งแบบกราฟแยกเพศและกราฟรวม พบว่า ค่าปริมาณรังสีที่ประเมินได้จากกราฟทั้งสองมีความใกล้เคียงกับปริมาณรังสีที่ได้รับจริง การวิเคราะห์ทางสถิติด้วย paired *t*-test และ ANOVA พบว่า ไม่มีความแตกต่างอย่างมีนัยสำคัญระหว่างค่าปริมาณรังสีที่ประเมินจากกราฟแยกเพศและกราฟรวม ผลการศึกษานี้ช่วยเสริมสร้างความสามารถของประเทศไทยในการประเมินการได้รับรังสีในสถานการณ์ฉุกเฉิน และยังเป็นการสนับสนุนความพยายามระดับโลกในการพัฒนามาตรวัดรังสีทางชีวภาพ โดยการจัดทำกราฟอ้างอิงที่เชื่อถือได้ขึ้นภายในประเทศ

คำสำคัญ: การเตรียมพร้อมรับมือเหตุฉุกเฉินทางรังสี มาตรวัดรังสีทางชีวภาพ ความผิดปกติของโครโมโซมแบบไดเซ็นทริก เส้นกราฟการตอบสนองต่อปริมาณรังสี เหตุการณ์ทางรังสี

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Dose-Response Curves for Gamma-Ray-Induced Dicentric Chromosomal Aberrations for Radiological Emergency Preparedness in Thailand

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Abstract

Accurate assessment of ionizing radiation exposure is essential in the realm of biological dosimetry, particularly in regions where unique environmental and demographic factors may influence radiation responses. This study presents the development of dose-response curves for gamma-ray-induced dicentric chromosomal aberrations in human lymphocytes, intending to enhance radiological emergency preparedness in Thailand. Blood samples from a 39-year-old male and a 32-year-old female were irradiated with Co-60 gamma rays at a dose rate of 0.574 Gy/min across a dose range of 0-5 Gy. The lymphocytes were cultured and analyzed for dicentric chromosomal aberrations following standard procedures recommended by the International Atomic Energy Agency (IAEA). The dose-response curves were generated using a linear-quadratic model with Biodose Tools v3.6.1 software. Accuracy assessment of the generated curves, for both individual and pooled datasets, showed that the estimated radiation doses closely aligned with the actual delivered doses. Statistical analysis using paired *t*-test and ANOVA revealed no significant difference between the estimated doses from the individual and pooled curves. These findings strengthen Thailand's capability to assess radiation exposure in emergency situations and support global efforts in biological dosimetry by establishing a reliable, locally generated reference curve.

Keywords: Radiological emergency preparedness, Radiation biodosimetry, Dicentric chromosomal aberration, Dose-response curves, Radiological incidents

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Introduction

Radiological emergency preparedness (REP) encompasses proactive measures aimed at mitigating the impact of radiological incidents or emergencies, which may result from nuclear accidents, radiological terrorism, or industrial mishaps. These events pose major threats to public health, the environment, and societal well-being, necessitating a comprehensive approach to planning, organizing, and implementing measures to address them effectively.

Central to REP is radiation biodosimetry-the assessment of radiation exposure in individuals through the analysis of biological samples such as blood or urine. This process is crucial for several reasons. First, it enables the accurate estimation of absorbed radiation doses in affected individuals, facilitating the evaluation of potential health risks and the implementation of appropriate medical management strategies. Second, biodosimetry aids in identifying individuals with high radiation doses, allowing emergency responders and healthcare professionals to prioritize medical treatment for those at increased risk of radiation-related health effects.[1] Third, radiation biodosimetry contributes to the broader public health response by providing data on radiation exposure and assisting public health authorities in assessing the overall impact of radiological incidents on affected populations. This information informs decisions regarding resource allocation, medical team deployment, and the implementation of protective measures, such as evacuation or sheltering, to reduce the risk of exposure.[2]

The evolution of radiation biodosimetry capabilities and infrastructure in Thailand has been influenced by past radiological incidents, such as the Co-60 radiation accident in Samut Prakan province in 2000[3] and the recent Cs-137 incident in Prachinburi province in 2023.[4] These incidents underscore the importance of preparedness efforts and highlight the need for accurate dose estimation tools, such as dose-response curves, to assess radiation exposure accurately. In addition to these well-documented events, Thailand faces potential risks from radiation-related incidents in medical and industrial contexts. While specific reports or publications on such events in Thailand are limited, similar incidents reported in other countries provide insight into potential risks. For instance, accidental overexposure in hospitals caused by equipment malfunctions or operator errors during radiotherapy procedures has been documented in several countries.[2, 3] Likewise, improper handling of radioactive materials in industrial applications, such as non-destructive testing or equipment calibration, poses a significant risk of radiation exposure if not managed appropriately.[2, 3]

Although these incidents are often less severe than large-scale radiological emergencies, they underscore the ongoing risks associated with mismanagement of radiation

sources. Addressing these risks requires sustained efforts to enhance radiological emergency preparedness in Thailand, including the development of reliable biological dosimetry tools such as dose-response curves. These tools are essential for accurately assessing potential radiation exposure and guiding effective emergency responses.

To address these challenges, Thailand established the Thai Biodosimetry Network in 2015, with the Office of Atoms for Peace (OAP) playing a pivotal role as the main agency and coordinator. The Network's activities focus on action planning, public knowledge dissemination, and expertise advancement in biodosimetry through research. Central to these efforts is the construction of dose-response curves, essential tools for accurately estimating absorbed radiation doses and assessing associated health risks during radiological incidents or accidents.

Numerous techniques exist for radiobiological evaluation[5], with the dicentric chromosome assay (DCA) being widely recognized as the gold standard due to its high accuracy.[6] DCA entails identifying abnormalities in the chromosome structure, particularly dicentric chromosomes within lymphocytes, and quantifying structural abnormalities to construct dose-response curves. Standard dose-response curves from Co-60 radiation sources using the DCA technique have been established in various countries, including Malaysia,[7] Spain,[8] Germany,[9] Vietnam,[10] Brazil,[11] India,[12] and China.[13] In Thailand, there is a notable scarcity of standardized radiation dose-response curves. While some standard curves exist for gamma rays from Cs-137 sources using the DCA and premature chromosome condensation (PCC) techniques,[14] these do not encompass the diversity of radiation sources used in various fields. Consequently, there is a pressing need to enhance personnel capabilities and maintain preparedness. Previous studies have suggested that biological factors, such as gender, may influence the frequency of chromosomal aberrations after radiation exposure, which could lead to variations in dose-response relationships.[15-17] This study aimed to address this need by detailing the development of standardized Co-60 dose-response curves using the DCA technique, with a specific focus on gender comparisons to account for potential variations in radiation responses. Additionally, the paper rigorously assessed the accuracy of these curves by evaluating their performance in estimating unknown radiation doses.

Materials and methods

1. Blood collection

Approval for the research was obtained from the Ethical Clearance Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (Ref: COA. MURA2022/489), ensuring compliance with ethical guidelines. All blood donors provided informed consent prior to participation.

To minimize confounding factors in the development of dose-response curves, we carefully selected two healthy individuals-a 39-year-old male and a 32-year-old female-with normal complete blood count results and no recent history of radiation exposure.[6,18] Additionally, we accounted for key variables that could influence chromosomal radiosensitivity, including the donors' occupational, lifestyle, environmental, and medical history. Both donors were non-smokers, had no history of chronic medical conditions, were not taking any medication that could affect radiation response, and had no recent exposure to diagnostic or therapeutic ionizing radiation. The donors were not engaged in occupations involving radiation or chemical exposure. Venous blood was collected using a needle into vacuum tubes containing lithium heparin as an anticoagulant, ensuring the integrity of samples for analysis.

2. Irradiation

Whole blood samples were collected in lithium heparin tubes and placed in a water-filled phantom to maintain a temperature of 37˚C.[6] Irradiation was conducted at the Bureau of Radiation and Medical Devices, Department of Medical Sciences, Ministry of Public Health, using a Co-60 radiation source at a dose rate of 0.574 Gy/min. Doses ranging from 0 to 5 Gy were administered. The irradiation doses were verified using a calibrated ionization chamber dosimeter positioned at the same location as the samples during irradiation. The dosimeter was calibrated according to the standards set by the IAEA to ensure the accuracy and consistency of the delivered doses. Following irradiation, the blood samples were incubated at 37˚C for 2 hours to facilitate DNA repair before cell culturing.[14]

3. Lymphocyte culturing and slide preparation

Short-term lymphocyte cultures were established using whole blood and aseptic techniques were applied throughout the process to maintain sample integrity. The culture medium consisted of RPMI1640 supplemented with 20% fetal bovine serum, 1% Penstrep, and phytohemagglutinin, along with Colcemid at final concentrations of 0.06 mg/mL and 0.05 μg/mL, respectively.[14] The cultures were placed in a CO_2 incubator at 37˚C for 48 hours to initiate cell division.[6]

Following incubation, the cells were harvested using a hypotonic solution (0.075M KCl), which helps to swell the lymphocytes and facilitate chromosome spreading. The harvested cells were then fixed with Carnoy's solution, spread onto slides, and stained with a 5% Giemsa solution in Wise buffer for analysis.[14]

4. Chromosome aberration scoring

An automated metaphase finding system, the Carl Zeiss Axio Imager and Metafer (MetaSystems; Germany), was utilized to locate and save cell images for

analysis. Chromosomes from metaphase cells were selected for analysis if they exhibited 45-46 centromeres, which corresponds to the expected number of centromeres in a normal diploid human cell. A typical healthy human cell contains 46 chromosomes, each with one centromere, but radiation exposure can induce the formation of dicentric chromosomes-chromosomes with two centromeres-leading to cells with 45 centromeres being included in the analysis.[6] At least 100 dicentrics were examined from metaphase cells, or a minimum of 1,000 cells were analyzed per analysis, following standard procedures for biological dosimetry.[6]

5. Statistical Analysis

Linear-quadratic dose-response curves were generated using the Biodose Tools program (version 3.6.1), an open-source software developed under the umbrella of the Running the European Network of Biological and Retrospective Physical Dosimetry (RENEB).[19] This software facilitated the analysis and interpretation of data obtained from the experiments, considering the linear-quadratic relationship between the number of chromosomal abnormalities and radiation dose.

The dispersion index (σ^2/y) and its normalized unit (u) for each dose were calculated to assess whether the dicentric frequency conformed to the anticipated Poisson distribution following gamma irradiation. The calculation utilized Equation (1) provided in the International Atomic Energy Agency (IAEA) manual,[6] where N represents the number of analyzed cells and X denotes the number of detected dicentrics.

$$u = \left(\frac{\sigma^2}{y} - 1\right)\sqrt{\frac{N-1}{2\left(1-\frac{1}{X}\right)}} \qquad (1)$$

Dispersion index values approaching 1 and u values within the range ±1.96 suggest compliance with the Poisson distribution, whereas u values exceeding 1.96 indicate data overdispersion, and u values below -1.96 signify underdispersion.[6]

The goodness-of-fit test for homogeneity was conducted using the Biodose Tools software. The Pearson's correlation statistic was used to establish the correlation between the delivered dose and dicentric frequency, with significance assessed at the 5% level ($p \leq 0.05$).

6. Dose estimation in blind samples

To validate the accuracy of the generated dose-response curves, peripheral blood samples were collected from six volunteers (three males aged 30, 39, and 40 years, and three females aged 22, 22, and 32 years), with no history of occupational radiation exposure. Careful consideration was given to potential confounding factors related to the donors and blood sampling. All donors were non-smokers, free from chronic medical conditions, and not taking any medications that could influence radiation response.

The blood samples were exposed to blind doses of gamma radiation from a Co-60 source, with three doses equally distributed within the 0-5 Gy range for both sexes to facilitate comparative analysis. After a 48-hour incubation period, slides were meticulously prepared, and dicentric scoring was performed blindly to minimize bias, ensuring that scorers were unaware of the dose information. The radiation doses were then estimated using the established calibration curve equations. This comprehensive methodology was designed to rigorously evaluate the reliability and precision of the dose-response curves in accurately estimating radiation doses from the blind samples.

Results

1. Generation of gamma radiation dose-response curves

Blood samples from a male volunteer aged 39 years and a female volunteer aged 32 years were exposed to gamma radiation from a Co-60 radiation source at a rate of 0.574 Gy/min. Analysis revealed that at a radiation dose of 0 Gy (non-irradiated samples), no cytogenetic abnormalities were detected. However, as the radiation dose increased, a higher number of cytogenetic abnormalities were observed (Fig. 1), showing a clear dose-response relationship (Fig. 2).

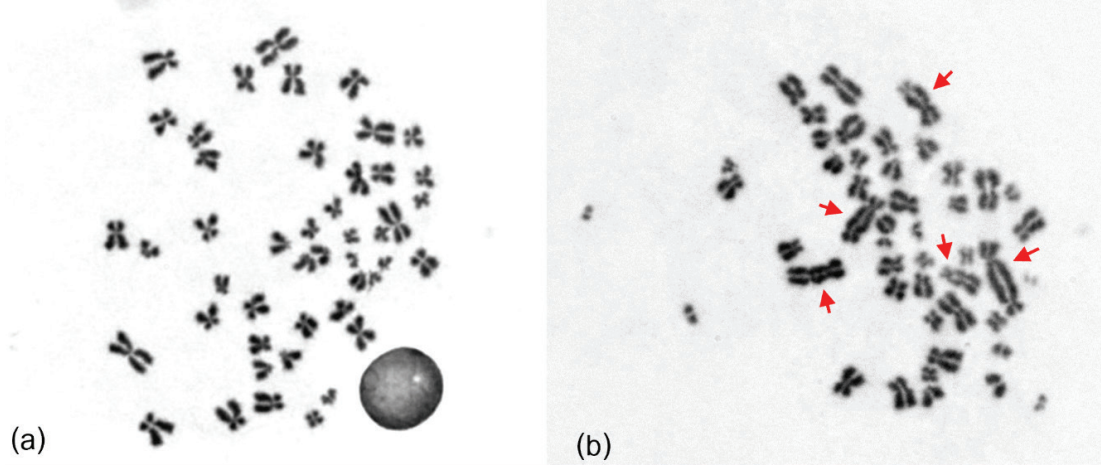

Fig. 1 Metaphase cells from (a) control group (0 Gy) and (b) those irradiated with 5 Gy showing dicentric chromosome abnormalities (indicated by pointed arrows).

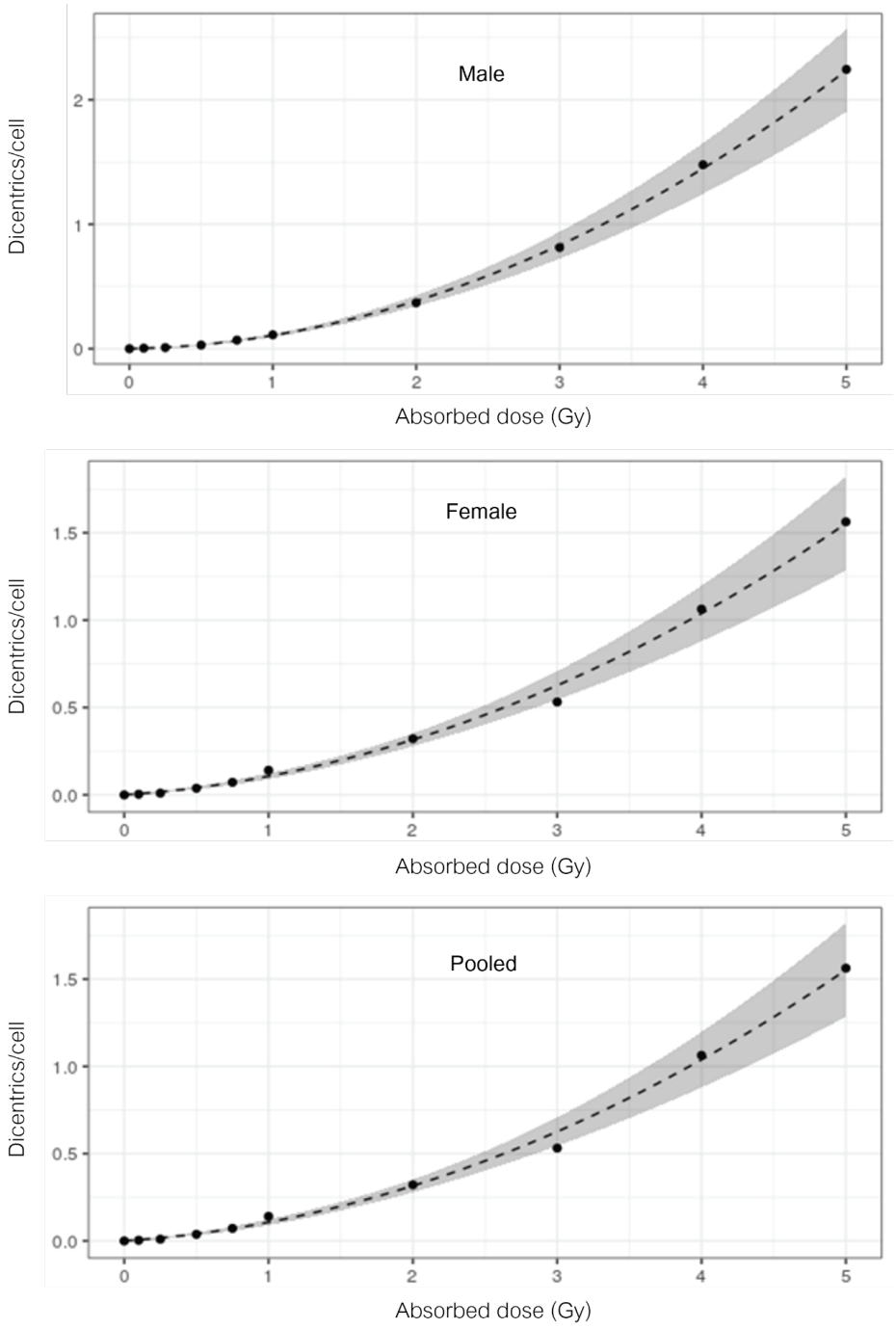

Fig. 2 Dose-response curves generated using Biodose Tools program for male, female, and combined male and female datasets.

The analysis of dicentric distribution in the blood samples from the male and female volunteers (Tables 1 and 2) demonstrated an association between dicentric frequency (Y) and an elevated radiation dose (D). The dicentric distribution was consistent with a Poisson distribution, with dispersion index (σ^2/Y) values mostly close to 1.

The population mean (*u* value) fell within the range ± 1.96, with values ranging from -1.55 to 1.58 for males and from -1.82 to 0.99 for females, indicating an appropriately distributed dataset. Upon combining data from Tables 1 and 2, it was evident that the results were consistently aligned in the same direction. Specifically, the majority of dispersion index values were close to 1, with the population average remaining within the range ± 1.96. An exception occurred at a radiation dose of 0.75 Gy, where the *u* value equaled -2.22, since a *u* value less than -1.96 implies an underdistribution (Table 3).

Table 1 Frequencies and distributions of dicentrics in male lymphocytes following 0-5 Gy ^{60}Co gamma irradiation.

Dose	Cells scored	Dicentrics	Distribution of dicentrics								Dicentrics/ cell (Y)	Dispersion index (σ^2/Y)	*u* value
			0	1	2	3	4	5	6	7			
0.0	1,000	0	1,000	0							0.000	-	-
0.10	1,000	4	996	4							0.004	1.00	-0.08
0.25	1,000	9	991	9							0.009	0.99	-0.19
0.50	1,000	29	972	27	1						0.029	1.04	0.93
0.75	1,000	69	931	69							0.069	0.93	-1.53
1.0	896	100	799	94	3						0.112	0.95	-1.08
2.0	271	100	193	59	16	3					0.369	1.13	1.58
3.0	123	100	59	35	22	7					0.813	1.06	0.44
4.0	69	102	13	22	25	7	1	1			1.478	0.75	-1.48
5.0	45	101	1	14	13	9	6	2			2.244	0.67	-1.55

Table 2 Frequencies and distributions of dicentrics in female lymphocytes following 0–5 Gy ^{60}Co gamma irradiation.

Dose	Cells scored	Dicentrics	Distribution of dicentrics								Dicentrics/ cell (Y)	Dispersion index (σ^2/Y)	*u* value
			0	1	2	3	4	5	6	7			
0.0	1,000	0	1,000								0.000	-	-
0.10	1,000	4	996	4							0.004	1.00	-0.08
0.25	1,000	10	990	10							0.010	0.99	-0.21
0.50	1,000	38	962	38							0.038	0.96	-0.84
0.75	1,000	72	928	72							0.072	0.93	-1.60
1.0	710	100	618	84	8						0.141	1.02	0.39
2.0	311	100	226	70	15						0.322	0.98	-0.23
3.0	188	100	104	69	14	1					0.532	0.81	-1.82
4.0	95	101	32	34	20	9					1.063	0.88	-0.85
5.0	64	100	14	21	16	9	2	1		1	1.562	1.18	0.99

Table 3 Frequencies and distributions of dicentrics in human lymphocytes following 0–5 Gy ^{60}Co gamma irradiation (pooled data from two donors).

Dose	Cells scored	Dicentrics	Distribution of dicentrics								Dicentrics/ cell (Y)	Dispersion index (σ^2/Y)	*u* value
			0	1	2	3	4	5	6	7			
0.0	2,000	0	2,000								0.000	-	-
0.10	2,000	8	1,992	8							0.004	1.00	-0.12
0.25	2,000	19	1,981	19							0.009	0.99	-0.29
0.50	2,000	67	1,934	65	1						0.033	1.00	-0.10
0.75	2,000	141	1,859	141							0.070	0.93	-2.22
1.0	1,606	200	1,417	178	11						0.124	0.99	-0.39
2.0	582	200	419	129	31	3					0.344	1.06	0.99
3.0	311	200	163	104	36	8					0.643	0.96	-0.50
4.0	164	203	45	56	45	16	1	1			1.238	0.84	-1.44
5.0	109	201	15	35	29	18	8	3		1	1.844	0.97	-0.18

Dose-response curves were generated using the Biodose Tools program for the analysis of data from Tables 1, 2, and 3 for radiation dose and dicentric frequency. The resulting curves conformed to a linear quadratic equation, $Y = C + \alpha D + \beta D^2$ where Y represents dicentric frequency and D represents the radiation dose (Fig. 2). The coefficients α and β, calculated from male, female, and combined data (Table 4), were validated for significance using the *t*-test. High *t*-values with low *p*-value (< 0.001) indicate that the coefficient is statistically significant. The *t*-value for both linear (α) and quadratic (β) terms are relatively high, meaning the model's relationship with D is well-supported. The intercept (C), with its low *t*-value and high *p*-value, suggests it can be considered negligible or statistically insignificant.

Comparison of the dose-response curves between male and female groups using ANOVA, resulting in a very low *F*-statistic (6.46×10^{-6}) and a very high *p*-value (0.998), indicates that there is no statistically significant difference between the linear (α) and quadratic (β) coefficients of the male and female groups.

Table 4 Fitted linear (α) and quadratic (β) yield coefficients, goodness-of-fit parameters, and ANOVA comparison for male and female dose-response curves.

Donor	Coefficient	Estimate	Standard error	*t*-statistic	*p*-value
Male	C	0.00000	0.00000	0.000	1.000
	α	0.02225	0.00722	3.080	<0.001
	β	0.08499	0.00567	14.98	<0.001
Female	C	0.00000	0.00356	0.000	1.000
	α	0.05623	0.01212	4.638	<0.001
	β	0.05085	0.00547	9.299	<0.001
Pooled	C	0.00000	0.00202	0.000	1.000
	α	0.03964	0.00818	4.846	<0.001
	β	0.06621	0.00422	15.68	<0.001
ANOVA	**Male and female comparison**			$F = 6.46 \times 10^{-6}$	$p = 0.998$

2. Testing accuracy of generated dose-response curves

The accuracy of the generated dose-response curves was assessed separately for the male, female, and combined datasets (Table 5). The analysis shows that the estimated radiation doses from individual and pooled curves are relatively close, with variations from the actual dose delivered ranging between slight underestimations and overestimations. The largest negative variation is observed in Test 1 (M) for the pooled curve (-27.76%), while the individual curve shows a smaller deviation (-18.90%). In Test 2 (M) and Test 3 (M), pooled estimates tend to have a slightly higher positive deviation (e.g., 12.91% for Test 2) compared to individual estimates (e.g., 7.55% for Test 2). For the female group, the variations for both individual and pooled curves show smaller differences compared to the male group, indicating more consistent dose estimation. Notably, Test 6 (F) shows minimal variation in the individual curve (-0.13%) but a larger negative deviation in the pooled curve (-7.06%).

These deviations from the delivered doses indicate that while pooled data can provide a general estimation, individual fitted curves potentially yield more tailored results. However, the statistical analysis of these estimates using a paired *t*-test show a large *p*-value (0.8955), and a *t*-value much smaller than the critical value. This finding is reinforced by the ANOVA test, where the *p*-value is also very high (0.9899), and the *F*-value is much smaller than the critical value. Both the paired *t*-test and ANOVA results indicate that there is no significant difference between the individual and pooled estimated doses.

Table 5 Estimation of absorbed dose in blind samples.

Test no.	Sex	Dose delivered (Gy)	Cells scored	Dicentrics	Dicentrics/ cell	Estimated dose (Gy)			
						Individual fitted curve (95%CI)	Variation from dose delivered	Pooled fitted curve (95%CI)	Variation from dose delivered
1	M	0.5	1,000	23	0.023	0.41 (0.30-0.52)	-18.90%	0.36 (0.26-0.48)	-27.76%
2	M	1.50	389	100	0.257	1.61 (1.44-1.79)	7.55%	1.69 (1.50-1.89)	12.91%
3	M	3.0	127	100	0.787	2.92 (2.62-3.23)	-2.81%	3.16 (2.83-3.52)	5.44%
4	F	0.5	1,000	42	0.042	0.51 (0.40-0.64)	2.20%	0.55 (0.44-0.67)	10.20%
5	F	1.50	482	100	0.207	1.54 (1.35-1.74)	2.75%	1.49 (1.32-1.68)	-0.27%
6	F	3.0	160	100	0.625	2.99 (2.66-3.35)	-0.13%	2.79 (2.49-3.10)	-7.06%
Paired *t*-test: *t*-value = -0.1381, *p*-value (two-tail) = 0.8955, *t* crit (two-tail) = 2.5706									
ANOVA: *F*-value = 0.0002, *p*-value = 0.9899, *F* crit = 4.9646									

95% CI refers to the range of estimated radiation doses in Gy at the 95% confidence level.

Discussion

The findings of this study provide valuable insights into the generation and accuracy testing of gamma radiation dose-response curves, highlighting the dose-dependent nature of cytogenetic abnormalities. The observed increase in dicentric chromosome frequency with escalating radiation dose aligns with prior research, confirming a predictable biological response to gamma radiation exposure.[20-21] This trend supports the robustness of dicentric chromosome analysis as an effective biomarker for assessing radiation exposure. The analysis demonstrated a clear association between dicentric frequency and increasing radiation dose, underscoring the stochastic nature of radiation-induced DNA damage. The consistency of dicentric distribution with a Poisson distribution, characterized by dispersion index values close to 1, aligns with the inherent randomness of chromosomal aberrations induced by ionizing radiation.[22] This stochastic characteristic was further supported by population mean (u) values remaining mostly within the range of ± 1.96, validating the distribution's appropriateness.

The generation of dose-response curves using the linear quadratic equation ($Y = C + \alpha D + \beta D^2$) demonstrated the model's suitability for characterizing the relationship between radiation dose and dicentric frequency. The significance of the linear (α) and quadratic (β) coefficients, as confirmed by high *t*-values and low *p*-values (< 0.001), reinforced the reliability of the model. The *F*-test results further validated the use of this equation, confirming the appropriateness of linear-quadratic curves for describing biological responses to radiation exposure.[6,23] These findings were consistent with earlier studies using gamma rays from cobalt-60 at a similar dose rate within the 0-5 Gy range,[11,24-25] supporting the broader applicability of the study's methodology.

An interesting observation in this study was a *u* value of -2.22 at a radiation dose of 0.75 Gy, observed in pooled data, indicating underdistribution in the dicentric chromosome frequency. Underdistribution may arise due to several factors, including biological variability, experimental conditions, or methodological limitations. Biological factors, such as variations in cellular radiosensitivity or DNA repair mechanisms, may have contributed to this finding.[17] Experimental conditions, including inconsistencies in cell handling or scoring, could also introduce variability. Additionally, methodological constraints, such as the number of scored cells at this dose level, might have amplified variability.[6] Future studies should consider increasing the number of scored cells at intermediate doses and incorporating a larger pool of donors to better understand and address such anomalies.

The current study adhered to standard biodosimetry procedures, analyzing at least 100 dicentrics or a minimum of 1,000 cells per sample, consistent with established guidelines. However, at lower radiation doses, where dicentric frequencies are low, scoring a larger number of cells becomes essential to reduce variability and improve the reliability of dose estimations.[6] Increasing the number of scored cells at lower doses to enhance the precision and robustness of dose-response curves should be considered in further research.

Another limitation of this study is the small number of donors, which may impact the statistical power and generalizability of the findings. The current study included only two donors (a 39-year-old male and a 32-year-old female), selected to limit variability introduced by age-related factors. While this approach was suitable for a preliminary study, a larger sample size encompassing a broader range of donor ages and biological characteristics is needed for future research. Including at least three donors, as recommended by biodosimetry guidelines, would enhance the robustness of the dose-response curves. Expanding the demographic diversity of donors could also provide deeper insights into factors such as age- or gender-specific variations in radiation response, improving the applicability of the results.[15]

The accuracy testing of the generated dose-response curves revealed that estimated radiation doses from both individual and pooled data showed close alignment with actual delivered doses. While deviations were observed (e.g., -27.76% in pooled male estimates and -18.90% in individual male estimates), they remained within acceptable ranges for biological dose estimation. Notably, female-specific data exhibited smaller variations, suggesting more consistent dose estimations. These findings partially align with prior research, such as Narendran *et al.*,[15] which identified potential gender-specific differences in radiation-induced chromosomal damage. They suggested that females exhibit lower frequencies of chromosomal aberrations, potentially due to biological factors such as hormonal influences or DNA repair mechanisms. The smaller percentage errors observed in female-specific estimations in this study corroborate these findings, although the limited sample size precludes definitive conclusions.

This study extends the work of Rungsimaphorn *et al.*,[14] who previously established dose-response curves for Cs-137 gamma rays in Thailand using the dicentric chromosome assay. By focusing on Co-60 gamma radiation, this study broadens the scope of standardized dose-response curves available for use in Thailand, addressing different radiation sources relevant to various fields. Furthermore, this study introduces gender-specific analyses and validates the generated curves using blind samples, enhancing their practical applicability in real-world radiological emergencies. These contributions not only fill critical gaps in Thailand's radiation biodosimetry capabilities but also provide a foundation for further research.

While the current findings contribute to understanding the generation and application of gamma radiation dose-response curves, further research is needed to enhance generalizability. Increasing the sample size in future studies would provide a more robust dataset to better explore potential gender-specific responses. Additionally, expanding the range of radiation doses studied could improve the understanding of dose-response relationships across different exposure scenarios, providing greater flexibility in practical applications of biodosimetry. These steps would significantly strengthen the reliability of dose-response curves and their application in diverse radiological contexts, ultimately enhancing radiological emergency preparedness in Thailand.

Conclusion

The successful development of standardized Co-60 dose-response curves using the dicentric chromosome assay marks a significant advancement in radiological emergency preparedness in Thailand. By providing a robust tool for accurately estimating radiation exposure, this work has strengthened the country's ability to respond effectively

to radiological incidents. The validated dose-response curves offer a reliable method for radiation dose estimation, ensuring precise assessments for individuals in the event of a radiological emergency. This advancement in radiation biodosimetry capabilities is crucial for prompt and effective response efforts and safeguarding public health and safety. In summary, the development of standardized Co-60 dose-response curves based on the dicentric chromosome assay represents an important contribution to enhancing radiological emergency preparedness in Thailand and ensuring the country's readiness to mitigate the impact of radiological incidents on its population.

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