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Biological Dosimetry Using Micronucleus Assay in Simulated Partial-Body Exposure to Ionizing Radiation

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ABSTRACT

In radiation accidents, it is common that only several parts of the body are exposed to radiation. As a consequence there is a mixture of exposed and unexposed lymphocytes in peripheral blood cells of the samples. This phenomenon will cause the dose value estimated using the exposed lymphocytes to be lower than the actual dose. In this study, an assessment of partial body exposures using micronucleus assay by estimating the partial body dose and fraction of irradiated blood was conducted. An optimal D_0 value also has been determined in this study to estimate the fraction of irradiated cells. Peripheral blood lymphocytes (PBLs) from three healthy donors were irradiated *in vitro* with 2 Gy of X-rays. Partial radiation exposure was simulated by mixing the irradiated and non-irradiated blood in different proportions. The proportions of mixtures of blood samples irradiated *in vitro* were 5, 10, 15, 20, and 30 %. Blood samples were then cultured and harvested based on micronuclei assay protocol. At least 2000 binucleated cells with well-preserved cytoplasm were scored for the MN frequency. Dose Estimate 5.1 software was used to calculate the dispersion index (σ^2/y) and normalized unit of this index (U) in each proportion of bloods. The fractions of irradiated cells were calculated with CABAS (Chromosomal Aberration Calculation Software) for several different D_0 values (2.7; 3.8; 5.4). The results showed that D_0 value at 5.4 gave the closest results to the actual proportion of irradiated bloods, while for the dose estimation the estimated doses value from all proportions in all donors were higher than the actual dose. The factor that may cause this phenomenon was that the dose response calibration curve used to predict the radiation dose was not constructed in the laboratory used. Overall it can be concluded that a biodosimetry using MN assay can be used to estimate the radiation dose in partial body exposure. In order to establish a biodosimetry using MN analysis the dose-response calibration curve MN analysis should be constructed first in the laboratory used.

INTRODUCTION

The radiation doses absorbed and distributed into the bodies of radiation accident casualties are important to guide immediate medical treatment, further health care, and prognosis of exposure casualties [1]. In radiation accidents, the exposure mostly occurs only to part of the body and as a result there is a mixture of exposed and unexposed lymphocytes in peripheral blood samples [2]. Since the exposed and unexposed lymphocytes are mixed, the dose received by the exposed lymphocytes and the dose delivered locally could be underestimated [3]. Discrimination between victims that are exposed for the whole body with those that are exposed partially is important as they will have different clinical outcomes, and it affects the medical care to treat them [4]. Until now, there are two mathematical models that have been developed to assess the dose according to the fraction of exposed lymphocytes, *i.e.*, Qdr and Dolphin's models. Both models have been validated *in vitro* by mixing irradiated and unirradiated blood in different

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proportions and have also been tested *in vivo* in accident situations, with promising results [3,5].

Several studies have been conducted to estimate the radiation dose that is received partially by radiotherapy patients. Silva-Barbosa *et al*., study showed that chromosomal aberrations and micronuclei (MN) quantification in lymphocytes from peripheral blood of cervical uterine cancer patients before and after receiving 0.08 Gy and 1.8 Gy was a potentially advantageous methodology of dose assessment especially in partial body exposure to ionizing radiation [6]. Another study, conducted by Senthamizhchelvan *et al*., revealed that biological dosimetry using micronuclei (MN) assays was applicable for dose estimation in therapeutic irradiation of cancer patients in acute high dose partial body irradiation [7].

Biological dosimetry (biodosimetry) is an investigation of biological effects (bioindicators) induced by radiation in order to correlate them with the radiation dose. Scoring of unstable chromosome aberrations (dicentrics, rings, and fragments) is considered as the most reliable method for evaluating individual exposure, and is specific to radiation exposure [8-10]. Another well known bioindicator of radiation damage is MN in peripheral blood lymphocytes [11]. Micronucleus are chromosome fragments that lag behind during anaphase and are not included in the main nucleus during telophase. They appear as small nuclei and can be identified during cell binucleation in the division of mitogen-activated human lymphocytes by blocking cytokinesis [5-7].

In this study, an assessment of partial-body exposures using MN assay was conducted, including the estimations of partial-body dose and fraction of irradiated blood. An optimal D_0 value has also been determined in this study to estimate the fraction of irradiated cells.

EXPERIMENTAL METHODS

Blood sampling, irradiation process and simulated partial-body irradiation

Since the focus of this study was to estimate the partial-body dose exposures using MN assay, we only used three samples that consisted of one male and two females without history of smoking habit and ionizing radiation exposure beyond routine diagnostic exposures. The reason that both male and female samples were used in this study was that it was decided that it was necessary to know whether the gender factor influenced the predicted dose value in partial-body exposure. Peripheral blood samples from all donors were collected in heparinized vacutainers. The characteristics of the donors are presented in Table 1. The blood samples were irradiated *in vitro* at the Secondary Standard Dosimetry Laboratory at Center for Radiation Safety Technology and Metrology, National Nuclear Energy Agency of Indonesia. Blood samples were exposed to X-ray (YXLON MG325) at 122 kV using additional filters of 1.66 mm Cu and 1 mm Al with an HVL of 2.52 mm Cu at 2 Gy with a dose rate 0.17 Gy/min. The radiation dose used in this study was 2 Gy because this is commonly used as a radiotherapy dose fractionation. After irradiation, the blood samples were maintained at 37 °C for one hour to enable repair of chromosomal damages. To simulate partial-body exposures the irradiated blood was mixed with non-irradiated blood to final proportions of 5, 10, 15, 20, and 30 %.

Table 1. Characteristics of the donor samples.

Gender	Age	Smoking Habit (yes; no)	X-ray medical diagnostics within the last year (yes; no)
Male	30	No	No
Female	43 & 52	Yes (0) No (2)	Yes (0) No (2)

Calibration of irradiation facilities

The measurement of air kinetic energy released in material (kerma) was done before the X-ray machines were applied for irradiating blood samples using an NE Technology type 2570 Farmer dosimeter and a type 2571 ionization chamber. Measurement of air kerma was done in the air at the source-to-sample-center distance of 100 cm, and the radiation field was 10 cm. The air kerma from X-ray track was calculated using the equation given in IAEA Technical Reports Series No. 277.

Blood culture

Blood cultures (0.5 mL) were set up in Roswell Park Memorial Institute (RPMI 1640) culture medium supplemented with HEPES and L-Glutamine, 15 % Fetal Bovine Serum (FBS), Penicillin-Streptomycin, and Phytohaemagglutinin (PHA). The cultures were maintained in a 5 % humidified CO_2 incubator at 37 °C for 72 h. After incubation for 44 h, 15 µl of cytochalasin B (Sigma-Aldrich) solution in DMSO (Sigma-Aldrich) was added to cultures, and cultivation was continued for another 24 h. The cultures were then treated with

cold hypotonic solution (0.075 M KCl) to lyse red blood. The fixative consisting of methanol:acetic acid (10:1) diluted with Ringer's solution (NaCl, KCl, $CaCl_2$) should be added to replace the hypotonic solution. Then, the supernatant was washed with fixative solution twice or three times until the cell suspension is clear. The cells were then resuspended gently and the suspension dropped onto clean glass slides and allowed to dry. The slides were then stained with 4 % Giemsa's solution in a potassium phosphate buffer (pH 7.3) and allowed to dry overnight. The slides were mounted with cover slip and allowed to dry completely before scoring. The slides were then analyzed, and identification of MN was conducted according to the scoring criteria in IAEA publication [5]. At least 2000 binucleated cells with well-preserved cytoplasm were scored for the MN frequency.

Statistical Analysis

The dispersion index (σ^2/y) and also the normalized unit of this index (U) were calculated for each proportion of blood using Dose Estimate 5.1 software. The fractions of irradiated cells were also calculated for the estimated dose (D) using several different D_0 value (2.7; 3.8; 5.4) using Chromosome Aberration Calculation Software (CABAS) version 2.0.

RESULTS AND DISCUSSION

The MN frequencies and distributions in all proportions of irradiated blood tested were presented at Table 2. It was clearly seen that in all proportion of irradiated bloods the u values were significant overdispersion (u > 1.96). Value of u varies from 7.93 to 26.2. In dicentric analysis it was well known that to determine the type of radiation exposure can be seen from u value evaluation. In case of whole body exposure the u value commonly follow Poisson distribution. A significant overdispersion is suggestive of partial irradiation for dicentric analysis and can be used to differentiate the homogeneously exposed samples from heterogeneously exposed samples [12]. In contrast for MNs assays the u values commonly overdispere, as it can be seen in table 2 even at control group (100 %) the u value still overdispere. The proportion of irradiated cells and estimated dose (D) with several different D_0 values (2.7; 3.8; 5.4) using CABAS 2.0 were presented at Table 3. It can be seen that D_0 value at 5.4 gave a results closest to the real proportion of irradiated bloods (Fig. 1).

Table 2. The MN frequencies and distributions in all proportions of irradiated blood.

Donor (Age, Sex)	% Irradiated Bloods	BNC	MN	Distribution of MN						$Y \pm SE$	$\sigma^2/y \pm SE$	U
				0	1	2	3	4	5			
1 (30, Male)	5 %	2160	35	2135	18	5	1	1	0	0.016±0.005	1.780±0.030	26.200
	10 %	2153	47	2114	33	4	2	0	0	0.022±0.004	1.400±0.030	13.400
	15 %	2157	82	2089	55	12	1	0	0	0.038±0.005	1.330±0.030	10.900
	20 %	2200	105	2118	64	14	3	1	0	0.048±0.008	1.510±0.030	16.800
	30 %	2204	171	2080	88	27	7	2	0	0.078±0.010	1.620±0.030	20.800
	100 %	2037	590	1599	313	102	19	4	0	0.290±0.016	1.330±0.031	10.600
2 (43, Female)	5 %	2209	60	2161	39	7	1	1	0	0.027±0.006	1.510±0.030	17.000
	10 %	2189	83	2122	56	8	1	2	0	0.038±0.006	1.520±0.030	17.200
	15 %	2171	100	2088	69	11	3	0	0	0.046±0.007	1.350±0.030	11.700
	20 %	2198	93	2123	61	11	2	1	0	0.042±0.006	1.450±0.030	15.100
	30 %	2298	152	2175	98	21	4	0	0	0.066±0.007	1.370±0.029	12.500
	100 %	2054	597	1612	317	101	19	4	1	0.291±0.016	1.350±0.031	11.300
3 (52, Female)	5 %	2157	76	2097	47	10	3	0	0	0.035±0.006	1.470±0.030	15.400
	10 %	2173	96	2099	58	11	4	1	0	0.044±0.008	1.560±0.030	18.600
	15 %	2140	97	2065	58	13	3	1	0	0.045±0.008	1.530±0.030	17.500
	20 %	2128	115	2029	84	14	1	0	0	0.054±0.006	1.240±0.031	7.930
	30 %	2227	162	2100	98	24	4	1	0	0.073±0.009	1.450±0.030	14.900
	100 %	2077	550	1664	304	86	18	5	0	0.265±0.015	1.350±0.031	11.400

Table 3. Predicted fraction of irradiated cells and estimated dose (D) with several different D_0 values (2.7; 3.8; 5.4).

Donor (Age, Sex)	% Irradiated Bloods	D_0 = 2.7		D_0 = 3.8		D_0 = 5.4	
		Estimated % Irradiated	Estimated Partial Dose (Gy)	Estimated % Irradiated	Estimated Partial Dose (Gy)	Estimated % Irradiated	Estimated Partial Dose (Gy)
1 (30, Male)	5 %	10.16	4.3237	6.66	4.3237	4.86	4.3237
	10 %	14.27	2.8037	10.99	2.8037	9.04	2.8037
	15 %	23.27	2.8037	18.36	2.8037	15.32	2.8037
	20 %	26.48	3.4517	19.96	3.4517	16.02	3.4517
	30 %	37.33	4.1589	27.66	4.1589	21.69	4.1589
2 (43, Female)	5 %	16.74	3.1624	12.55	3.1624	10.10	3.1624
	10 %	22.56	3.0624	17.36	3.0624	14.22	3.0624
	15 %	27.52	2.7504	22.07	2.7504	18.62	2.7504
	20 %	24.62	3.1127	18.98	3.1127	15.55	3.1127
	30 %	35.34	3.0624	28.28	3.0624	23.72	3.0624
3 (52, Female)	5 %	20.76	3.3090	15.55	3.3090	12.47	3.3090
	10 %	24.68	3.5909	18.27	3.5909	14.47	3.5909
	15 %	25.26	3.5449	18.81	3.5449	14.97	3.5449
	20 %	33.48	2.3592	28.13	2.3592	24.58	2.3592
	30 %	36.82	3.4045	28.85	3.4045	23.74	3.4045

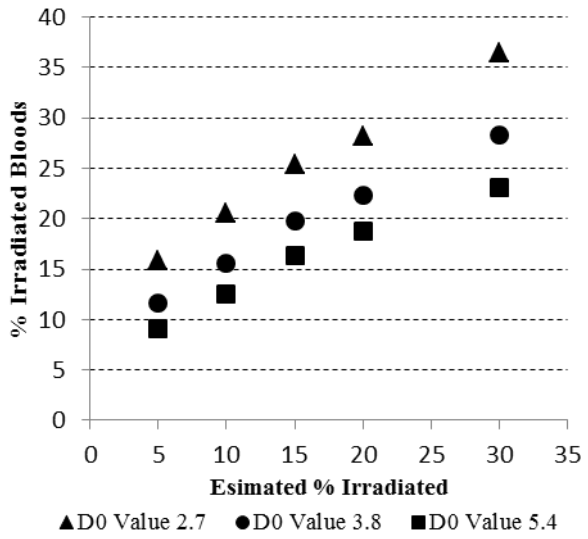

Fig. 1. Estimate of percentage (%) irradiated compared to actual values irradiated using three different D_0 values, namely 2.7 (triangles), 3.8 (circles) and 5.4 (squares).

Even though the D_0 value of 5.4 gave a better results compared to others D_0 values, the predicted proportions of irradiated cells are still inaccurate. The factor that may have caused this phenomenon was the dose-response calibration curve used to predict the fraction of irradiated cells. In this study the dose-response calibration curve was from Pajic *et al*., [13]. Since the laboratory where this study took place did not have its own dose-response calibration curve for MN, a calibration curve from another study is used here. Inaccuracy in fraction estimation was also reported using several D_0 value in the dicentric assay [14].

IAEA recommended that laboratories performing biological dose assessment using cytogenetic analysis should obtain their own dose-response calibration curve for different types and energies of radiation [5]. For that reason, and to obtain more accurate dose prediction (and prediction of proportion of irradiated cells in the case of partial body exposure) the laboratory used in this study now develops the dose-response calibration curve for micronuclei analysis. For dose estimation, the estimated doses value from all proportions in all donors was higher compared to the actual dose of 2 Gy (Fig. 2). A factor that may contribute to this result was the dose-response calibration curve used for dose estimation not being constructed in the laboratory. From the results of this study, it was clear that the radiation dose-response calibration curve for the MN analysis should be produced in the same laboratory. The laboratory previously successfully developed a dose-response calibration curve for dicentric analysis induced by gamma rays [15]. In order to establish a biodosimetry using MN analysis, the dose-response calibration curve for MN analysis must be constructed first.

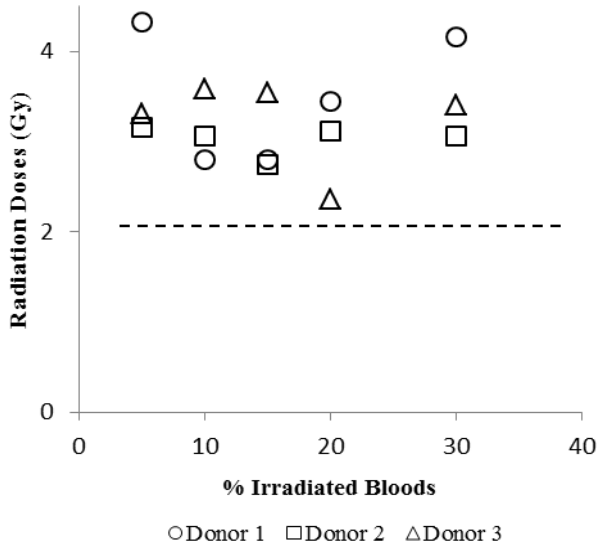

Fig. 2. Estimate of radiation dose values for all proportions of irradiated bloods from three different donors. The dashed line is the actual radiation dose of 2 Gy.

The results show that the induced MN freuencies are proportional to the percentage of irradiated lymphocytes. For example, the total number of MN for 30 % at donor 1 was 171 while for 100 % was 590. It can also be seen that most of binucleated cells contained a single MN. There are inter-individual differences among subjects that are influenced by different mutagen sensitivity and DNA repair capacity. It was known that MN frequency in females tends to be higher relative to males by a factor approximately 1.4. Researchers suspect that the higher MN frequency in females correlated with greater tendency of the inactive X-chromosome to be lost as an MN relative to other chromosomes, and to the fact that females have two copies of the chromosome compared to only one in males [16,17].

A study conducted by Jones *et al*. [18] showed that in 19.9 % of the cells scored at least one sex chromatin-positive MN was present. Another study by Hando *et al*. [19] found that X-chromosomes present in 72.2 % of the MN scored and that a significant increase occurred with age in the number of MNs containing an X-chromosome. The increase of MN with age is due to a combination of several factors, namely: (i) the cumulative effect of acquired mutations in genes involved in DNA repair, and: (ii) numerical and structural aberrations in chromosomes caused by exposure to endogenous genotoxins, inadequate nutrition, exposure to environmental or occupational genotoxins, and a wide range of unhealthy lifestyle factors [16].

Interestingly, from our results the total MN in the male was higher compared to average of total MN from the two females. Age also did not exhibit influence in our study as can be seen that the total MN in donor 3 was lower compared to other donors. Since in this study the number of sample donors only consisted of three people, it is possible that influence of sex and age to total MN cannot be seen from this study. Further studies should be conducted to verify the effect of sex and age factors to number of MN in human peripheral lymphocytes. Here an evaluation of micronuclei for estimating the dose of radiation to lymphocytes is described. Micronuclei are only expressed in cells that proceed to complete nuclear division. This assay has emerged as one of the preferred methods for assessing chromosomal damage because they enable both chromosome loss and chromosome breakage to be measured reliably.

Moreover, this technique is useful for determining irradiation dosage by examining a large number of binucleated cells faster and using simpler techniques than chromosome aberrations. The technique is very easy to use when a large number of cells must be examined for routine monitoring of workers exposed to radiation [20,21] as MN is a good tool for cancer risk prediction as well as for studying genotoxicity and cytotoxicity as well as studies on genomic damage by chemicals in general [22].

CONCLUSION

Overall, it can be concluded from this study that biodosimetry using MN assay can be used to estimate the radiation dose in partial body exposure. However, it was not possible to distinguish between partial or total body exposures using Poisson or overdispersion distribution as the basis when conducting biodosimetry using MN assay. An optimal D_0 value was also calculated in this study and it can be seen that from this study that a D_0 value of 5.4 gave more accurate results compared with 2.7 and 3.8. To obtain more accurate results the laboratory used should construct the dose-response calibration curve for MN analysis in further studies.

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