



Establishment of a Dose-response Curve for X-ray-Induced Micronuclei in Human Lymphocytes

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ABSTRACT

The cytokinesis-block micronucleus assay in peripheral blood lymphocytes is an established technique for biodosimetry. The aim of this project was to generate a X-ray induced micronuclei (MN) curve for peripheral blood lymphocytes taken from five healthy donors. The blood samples were irradiated with X-rays of 122 KeV at a dose rate of 0.652 Gy/min to doses of 0.5, 1, 2, 3, and 4 Gy. The blood samples were then cultured for 72 h at 37°C and processed following the International Atomic Energy Agency standard procedure with slight modifications. The result showed that the yields of MN frequencies were increased with the increase of radiation dose. Reconstruction of the relationship of MN with dose was fitted to a linear-quadratic model using Chromosome Aberration Calculation Software version 2.0. Due to their advantages, mainly, the dependence on radiation dose and dose rate, despite their limitation, these curves will be useful as alternative method for *in vitro* dose reconstruction and can support the preparedness for public or occupational radiation overexposure and protection. The results reported here also give us confidence to apply the obtained calibration curve of MN for future biological dosimetry requirements in Indonesia.

Key words: Biodosimetry, cytogenetic, micronuclei, radiological emergency, X-rays

Introduction

Establishing a laboratory, that is, competent enough to perform cytogenetic analysis for biodosimetry is very important in Indonesia, where large use of radiation sources for peaceful purposes is in place. Moreover, in Center for Technology of Radiation Safety and Metrology (PTKMR), National Nuclear Energy Agency of Indonesia (BATAN), radiation emergency preparedness has become a priority program to support the unfortunate event, if any, of mass radiation casualties. This is an attempt to have our own *in vitro* dose-response calibration curves for dose reconstruction to facilitate biological dosimetry.

Chromosomal aberration is widely known as cytogenetic indicators used in the field of radiobiology to evaluate the effects of exposure to ionizing radiation, especially dicentric chromosome analysis which has become the “gold standard” cytogenetic indicator specific to radiation.^[1] However, in

the recent past, additional assays have been worked out and validated including the now well-established cytokinesis-block micronucleus (CBMN) assay. Even though micronuclei (MN) can arise from exposure to a variety of clastogenic agents and are not necessarily radiation specific, ionizing radiation is considered as a clastogen and it is an efficient inducer of MN. The CBMN assay is established as a very reliable, thoroughly validated and is a standardized technique in the field of radiation biology to assess *in vivo* radiation exposure of occupational, medical, and accidentally exposed individuals and to determine individual *in vitro* radiosensitivity or cancer susceptibility.^[2-4] To improve our cytogenetic laboratory capability as the National Cytogenetic Biodosimetry Laboratory in Indonesia, we have established our own dose-response standard curves of unstable chromosome aberrations for gamma radiation^[5] and X-rays.^[6]

In vitro MN test with CBMN of human peripheral blood lymphocytes has been used extensively to study chromosomal damage induced by ionizing radiation or chemicals.^[7] Because radiation-induced MN showed a radiation dose and quality dependence, MN can be used as a biological dosimeter for radiation protection purposes^[8] and MN assay has been recommended by

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the International Atomic Energy Agency (IAEA).^[9] CBMN has been used by some researchers such as to conduct assessment of the radiation effects for workers in nuclear power plant or to ensure the radiation protection program running well.^[10,11]

This study aimed to generate dose-response curve for MN based on binucleated cells (BNCs) an additional method to support radiation emergency preparedness in Indonesia. The dose-estimation accuracy of the calibration curve was tested by performing an *in vitro* irradiation and blind scoring.

Materials and Methods

Individual background frequencies of micronuclei

A total of 30 blood samples were collected from nonsmoking and apparently healthy individuals to determine the frequency of MN in the background; the age range was between 25 and 55 years that consist of 15 males and 15 females. We divided the cohort into three groups with range of ages 23–35 years (Group A), 36–45 years (Group B), and 46–55 years (Group C). A questionnaire on medical history and letter of informed consent were given to all the donors. All data including type and number of medical exposures, such as X-ray exposure to the chest during a medical examination, were recorded. Blood samples were cultured according to the standard protocol based on the recommendation of the IAEA^[9] with slight modifications.

Research subjects and irradiation

Based on determination result individual background level of MN, a peripheral blood samples were collected in heparinized vacutainers from 5 nonsmoking healthy volunteers aged between 25 and 55 years old (mean 39.7 years) with informed consent from the volunteers and approval from the Institutional Ethics Committee and biographical data including history of illness and history of working with radiation. For *in vitro* irradiation, one of the aliquots was used as control and the rest were exposed to different doses (0.5–4 Gy) of X-rays (YXLON MG 325) at 240 kV voltage. The energy is 122 KeV with additional filters of 1.66 mm Cu, 1 mm Al. The irradiation was performed as per procedure described in IAEA TRS 405 after irradiation, blood samples were kept at 37°C to allow for any chromosomal repair to take place.

Lymphocyte culture and slide preparation

Blood culture and harvest procedures were conducted according to the instructions in the IAEA manual^[9] with minor modifications. Briefly, 0.5 ml of peripheral blood samples were cultured in 6 ml of RPMI 1640 medium enriched with L-glutamine and HEPES, 10%–15% fetal bovine serum, 2% penicillin-streptomycin, and stimulated with 2%–3% phytohemagglutinin in 37°C incubation for 72 h. At the 44 h of culture, 45 µl cytochalasin B (Cyt-B) (3 mg/ml) was added into the blood cultures. After incubation period, each blood sample was centrifuged at 800 rpm for 5 min and the upper layer (supernatant) was removed. About 6 ml of cold hypotonic solution (0.075 M KCl) was added, centrifuged at 800 rpm for 5 min. Then, the cells were fixed in methanol: Glacial acetic acid (10:1) diluted with ringers solution. After three times fixation, the cells were dropped on the glass

slide and allowed to dry. After staining with 4% Giemsa, cover glasses were applied onto the slides for scoring.

Micronuclei scoring

Scoring of MN was conducted using Nikon microscope with ×100 magnification. The MN were scored according to the criteria proposed by Fenech and Morley^[12] and IAEA.^[9] A minimum of 500–1000 BNCs were analyzed for MN detection. The dose-response calibration curve was obtained by iteratively reweighted least square regression analysis using Chromosome Aberration Calculation Software (CABAS) version 2 (Institute of Nuclear Chemistry and Technology, Warsaw, Poland).

Statistical analysis

A dose-effect calibration curve for MN induced by X-rays was constructed using CABAS version 2 software. MN frequencies of interindividual background were tested using MedCalc version 12.7.0.0 (MedCalc Software, Ostend, Belgium).

Results and Discussion

Biological dosimetry using biomarkers of chromosome damage such as MN is a valuable dose assessment method in cases of radiation overexposure with or without physical dosimetry data. To estimate dose by biodosimetry, any biological dosimetry service needs to have its own dose-response calibration curve.

Micronuclei background frequencies

From the preliminary study, we observed the optimal number of BNCs that were determined by testing several Cyt-B concentrations (3, 4.5, and 6 µg/mL) in three human lymphocyte cultures. The results showed that the highest percentage of BNC was found in 4.5 µg/mL Cyt-B with 1.7 - 2.5 fold increase in nuclear division index. Visualization of MN in BNCs found in healthy individuals was described in Figure 1. In this study, MN frequencies were within normal limit and ranged from 0.001 to 0.036 per cell (1–36/1000 BNCs), this finding is similar with the published papers by others.^[13] However, MN in each age group of 25–35 years, 36–45 years, and 46–58 years was relatively increased in accordance with increase in age, but no significant difference was found among groups as shown in Table 1 and Figure 2. This result is consistent with other studies that there is individual variation in MN and closely associated with age and gender.^[14] While the frequency and distribution of MN in BNCs based on gender group were shown in Table 2, we found that the mean MN frequencies for background level for male is 14.15 ± 0.005 MN/1000 binucleates and for female is 19.00 ± 0.008 MN/1000 binucleates as shown in Figure 3.

Table 1: Frequencies of micronuclei on peripheral lymphocytes observed based on three age of groups

Age group (years)	Total BNC	Distribution of MN				Total MN	MN/BNC
		0	1	2	3		
25-35	10,000	9882	116	2	-	120	0.012±0.004
36-45	10,000	9865	125	10	-	145	0.015±0.005
46-55	10,000	9827	155	15	3	194	0.019±0.007

BNC: Binucleated cell, MN: Micronuclei

In vitro dose-response relationship

MN scoring in peripheral lymphocytes has been suggested as an additional method for quantifying radiation-induced chromosome damage. This damage can be induced by radiation and observed in peripheral blood lymphocytes. In this research, MN were observed in BNCs in blood samples exposed to X-rays from 0.5 to 4 Gy. Visualization of MN in BNCs found in irradiated sample was described in Figure 3.

The frequencies and distribution of MN in BNCs are presented in Table 3. The spontaneous MN frequencies in lymphocytes of the nonirradiated control groups showed no significant difference among individuals. However, the baseline number of MN per cell in nonirradiated control group was 0.025 ± 0.02 . In all irradiated samples, we found significantly higher MN frequencies per 1000 BNCs than in control. The results indicate that MN was significantly more sensitive compared to the other cytogenetic damages over time that depends on various factors including the type of biomarkers and the severity of the outcome to the cells

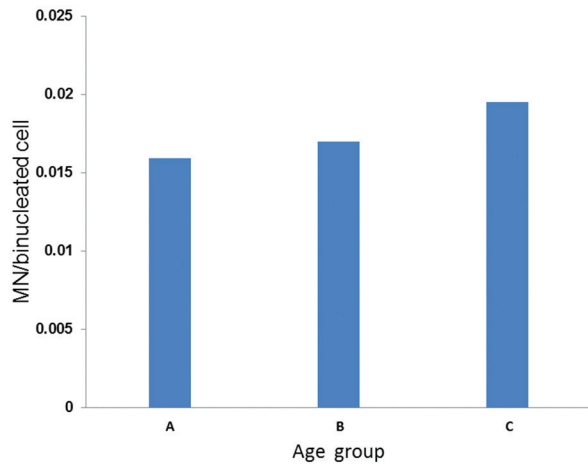


Figure 1: Frequencies of micronuclei based on three age groups of Group A (25–35 years), Group B (36–45 years), and Group C (46–55 years)

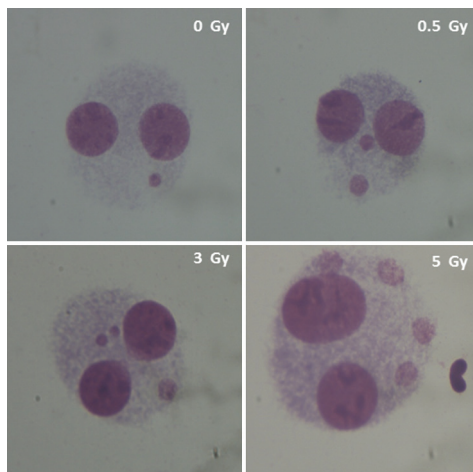


Figure 3: Micronuclei in binucleate cells in control (0 Gy), 0.5, 3 and 4 Gy of X-rays radiation dose-exposed blood samples

Table 2: Frequencies of micronuclei on peripheral lymphocytes that were observed based on gender in range 25-55 years

Gender	Total BNC	Distribution of MN				Total MN	MN/BNC
		0	1	2	3		
Female	15,000	9882	227	12	1	254	0.017±0.008
Male	15,000	9865	192	15	2	228	0.015±0.005

BNC: Binucleated cell, MN: Micronuclei

Table 3: Yield and distribution of micronuclei in binucleated cells after X-rays exposure

Dosis (Gy)	Number of BNC	Distribution MN							Number of MN
		0	1	2	3	4	5	6	
0	5000	4937	97	12	1	0	0	0	124
0,5	5097	4780	275	30	10	0	0	0	365
1	5162	4456	631	59	15	1	0	0	798
2	5041	3479	1150	319	53	13	1	0	2004
3	5785	3390	1472	760	133	23	7	0	3518
4	5005	2395	1545	780	304	101	34	0	4543

BNC: Binucleated cell, MN: Micronuclei

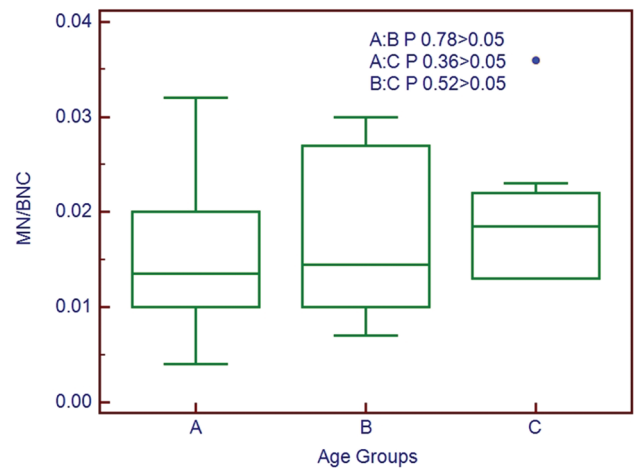


Figure 2: Statistical analysis of micronuclei frequencies based on the three age groups using MedCalc version 12.7.0.0

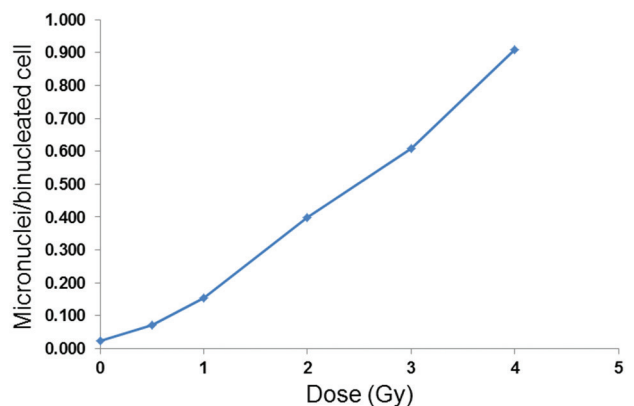


Figure 4: The dose-response curve for micronuclei induced for X-rays at 0.5–4 Gy. $Y = 0.022 + 0.112D + 0.028D^2$ ($r = 0.99$)

Table 4: Dose estimation for the three individuals for the validation of the dose-response curve

Sample	Real dose (Gy)	Curve yield	Estimated dose (Gy)	95% dose CI
A	1.5	CBMN	1.28	1.321-2.042
B	1.5	CBMN	1.40	1.007-1.878
C	1.5	CBMN	1.43	1.218-1.658

CI: Confidence interval, CBMN: Cytokinesis-block micronucleus

which can induce mitosis-linked cell death. There were more lymphocytes with multiple MN (up to 5) observed in samples which received higher doses up to 2 Gy.

The construction of dose-effect relationship of cells exposed to ionizing radiation was obtained by fitting the linear-quadratic model $Y = a + bD + cD^2$, where Y is the yield of MN/cell, a is the spontaneous of MN, b is the coefficient of the one-track component, c is the coefficient of the two-track component, and D is the dose in Gy. Using CABAS version 2 as shown in Figure 4, the result of the equation is $Y = 0.022 \pm 0.0069 + 0.112 \pm 0.019D + 0.028 \pm 0.006D^2$ ($r = 0.998$). The above equations showed that the value of α and β for MN are 0.112 and 0.028, respectively, and the background MN frequency was 0.022. There was a significant relationship between frequency of MN and radiation dose. To evaluate the accuracy of equation of dose-response curve, we performed an *in vitro* irradiation of peripheral blood samples from three donors simulating whole-body exposures of 1.5 Gy. Doses were estimated by referring MN frequency to the calibration curves after blind scoring in 500 CBMN on coded slides. Table 4 presents the values of irradiation and estimated doses with the corresponding 95%. The dose estimate was varied from 1.28 to 1.43 Gy. In this study, all samples are obtained only from adult individuals. A follow-up study which involves individuals from different age group is necessary.

In this study, we have used MN as biodosimeter. Nowadays, screening of MN in human lymphocytes was much developed aiming rapid imaging and automated counting for public health evaluation in case of human professional exposure or nuclear accident. The major limitations of the CBMN assay used in radiation biology are related to retrospective dosimetry and accidents involving partial body irradiation. The tendency to underestimate radiation doses in situations of delayed blood sampling is due to the fact that MN represents unstable chromosome aberrations which have limited life span *in vivo*, especially after high doses.^[15]

Conclusion

MN test using CBMN assay on lymphocytes is a relevant method to assess background level of MN frequency, both in terms of number as well as individual variation. The dose-response curves of MN for X-rays have been firstly established in our laboratory. Reconstruction of the relationship of these frequencies with

dose followed a linear-quadratic curve lines, and therefore, it is very important for dose estimation in radiation emergency and for evaluate or assess irradiation effect.

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Conflicts of interest

There are no conflicts of interest.

References

- Thierens H, Vral A, Barbé M, Meijaers M, Baeyens A, Ridder LD. Chromosomal radiosensitivity study of temporary nuclear workers and the support of the adaptive response induced by occupational exposure. *Int J Radiat Biol* 2002;78:1117-26.
- Thierens H, Vral A, de Ridder L, Touil N, Kirsch-Volders M, Lambert V, *et al.* Inter-laboratory comparison of cytogenetic endpoints for the biomonitoring of radiological workers. *Int J Radiat Biol* 1999;75:23-24.
- Sari-Minodier I, Orsière T, Auquier P, Martin F, Botta A. Cytogenetic monitoring by use of the micronucleus assay among hospital workers exposed to low doses of ionizing radiation. *Mutat Res* 2007;629:111-21.
- Fucic A, Brunborg G, Lasan R, Jezek D, Knudsen LE, Merlo DF. Genomic damage in children accidentally exposed to ionizing radiation: A review of the literature. *Mutat Res* 2008;658:111-23.
- Lusiyanti Y, Alatas Z, Purnami S, Suwifan VA, Ramadhani D, Lubis M. Dose-response curve of chromosome aberrations in human lymphocytes induced by gamma-rays. *Atom Indones J* 2013;39:101-56.
- Lusiyanti Y, Alatas Z, Purnami S, Ramadhani D. Dose-response curve of chromosome aberrations in human lymphocytes induced by x-rays. *J Math Fundam Sci* 2016. [In press].
- Mill AJ, Wells J, Hall SC, Butler A. Micronucleus induction in human lymphocytes: Comparative effects of X-rays, alpha particles, beta particles and neutrons and implications for biological dosimetry. *Radiat Res* 1996;145:575-85.
- Hall EJ, Giaccia AJ. *Radiobiology for the Radiobiologist*. 7th ed. Philadelphia: JB Lippincott Company; 2012.
- IAEA. *Cytogenetic dosimetry: Applications in preparedness for and response to radiation emergencies*. Vienna: IAEA-EPR; 2011.
- Little JB. What are the risks of low-level exposure to alpha radiation from radon? *Proc Natl Acad Sci U S A* 1997;94:5996-7.
- Thierens H, Vral A. The micronucleus assay in radiation accidents. *Ann Ist Super Sanita* 2009;45:260-4.
- Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. *Mutat Res* 1985;147:29-36.
- Bonassi S, Fenech M, Lando C, Lin YP, Ceppi M, Chang WP, *et al.* Human micronucleus project: International database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. *Environ Mol Mutagen* 2001;37:31-45.
- Fenech M. Cytokinesis-block micronucleus assay evolves into a "cytome" assay of chromosomal instability, mitotic dysfunction and cell death. *Mutat Res* 2006;600:58-66.
- Vral A, Fenech M, Thierens H. The micronucleus assay as a biological dosimeter of *in vivo* ionising radiation exposure. *Mutagenesis* 2011;26:11-7.