Volume 21, No 3 | International Journal of Radiation Research, July 2023

Micronucleus assay in the blood of residents in Mamuju, West Sulawesi, Indonesia, a high background radiation area

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► ***Short report***

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Received: November 2022
Final revised: March 2023
Accepted: March 2023

Int. J. Radiat. Res., July 2023; 21(3): 601-604

DOI: 10.52547/ijrr.21.3.34

Keywords: *CBMN cyt assay, cytogenetic abnormalities, high background radiation area, Mamuju residents*

ABSTRACT

***Background*:** Mamuju is regarded as a high background radiation location (HBRA). The local population's cells may be harmed by the high ionizing radiation dosage in this region. Micronuclei (MN), nucleoplasmic bridges (NPB), nuclear buds (NBUD), and 8-shaped blood cells can be used to detect cytogenetic abnormalities. By comparing blood samples from HBRA to control site samples, this study focused on determining the impacts of high-risk levels of natural radiation in the cells. ***Material and Methods*:** The study employed a cytokinesis-block micronucleus cytome (CBMN Cyt) test. ***Results*:** In comparison to the control group, the study group had a higher nuclear division index (NDI) and a higher frequency of all abnormalities. The formation of MN and 8-shaped nuclei was significantly influenced by high natural radiation, whereas levels of NPB and NBUD were not significantly affected. Cytogenetic abnormalities were within normal limits and did not correlate with age or gender. ***Conclusion*:** Chronic high background radiation exposure impacted human lymphocyte cell growth and the levels of certain biomarkers.

INTRODUCTION

People are naturally exposed to radiation resulting from the decay of primordial radionuclides in the inner layer of the Earth, which varies according to the geographical conditions in each area [(1)]. Certain regions, such as Kerala (India), Yangjiang (China), Ramsar (Iran), Guarapari (Brazil), and Ramsar (India), have significant amounts of background radiation [(2,3)]. Mamuju is an HBRA in West Sulawesi, Indonesia with a Th-232 concentration of 3400 Bq/kg (the national average is 45 Bq/kg) and the highest K-40 of 1500 Bq/kg (the average is 142 Bq/kg) [(4)]. Mamuju has an average 699±65 nSv/h ambient dose rate; Ahu and Salletto villages within Mamuju have an average of gamma radiation dose of 4.3 mSv/year, while Topoyo village has 0.6 mSv/y [(5)]. Natural radiation can cause damage to cells and tissues which, if it occurs continuously, will lead to multi-organ failure and death. Several changes or types of damage that arise can be used to predict risk due to radiation, including damage to chromosomes, which has the potential to cause tumors or cancer [(6)].

Research on the biological effects of ionizing radiation can be carried out on cytogenetic biomarkers to gain an understanding of the mechanisms of damage occurrence. A commonly used technique to detect micronuclei (MN), nuclear buds (NBUD), nucleoplasmic bridges (NPB), and 8-shaped nuclei simultaneously is the cytokinesis-block micronuclei cytome (CBMN cyt) assay, since the findings may be acquired quickly [(7)]. Peripheral blood lymphocytes are the cell type most often used for the assessment of radiation effects. Blood lymphocytes have properties that are sensitive to radiation, so they are prone to DNA damage [(8)].

Several cytogenetic studies in Mamuju have been done previously by others [(9, 10)]. The aim of this study was to examine the cytogenetic alterations (MN, NBUD, NPB, and 8-shaped) that have occurred within the lymphocytes of the Mamuju local population as a result of natural radiation exposure. The micronucleus assay, which was used in this work to evaluate the DNA damage response and determine the degree of DNA damage in the Mamuju community blood samples, is the study's main strength and its innovative aspect. This study in Mamuju is the first of its kind to be carried out in Indonesia and addresses a crucial issue since radiation exposure-particularly ionizing radiation-can penetrate cells and result in

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[DOI: 10.61186/ijrr.21.3.601]

DNA damage.

MATERIALS AND METHODS

Subjects and ethical approval

The case group samples were obtained from 15 respondents from areas with high natural radiation (Salletto and Ahu villages) and the control samples were from 15 respondents from Topoyo village. This research was ethically approved by the Ethics Commission of Health Research, Health Research and Development Agency, Ministry of Health with certificate number LB.02-01/2/KE.063/2018 dated February 21, 2018.

Blood sampling and culture

Three mL of blood samples per respondent were taken intravenously using a sterile syringe and transferred to two vacutainer tubes containing heparin. For cultures, 4.5 mL of RPMI 1640 solution enriched with 0.1 mL of glutamine and 4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonic acid (HEPES) buffer (Gibco), 0.8 mL of fetal bovine serum, 0.1 mL of Penicillin-Streptomycin, 0.5 mL of blood samples and 0.1 mL of Phytohemagglutinin (Gibco) were put into the culture tube. The tube was then stored with the lid slightly loose in an incubator at 37°C supplied with 5% of CO_2 for 72 hours. At 44 hours, 15 µL of cytochalasin-B (Sigma) was added to the culture and incubated again. The blood cultures were harvested after 72 hours.

Lymphocyte cell harvest

Blood cultures were put into a 15 mL tube and spun at 800 rpm for 10 minutes. The supernatant was then discarded and 6 mL of hypotonic solution (0.56% (w/v) potassium chloride) was added. This solution was homogenized, and the centrifuge process was repeated at the same speed. After removing the supernatant, a fixative solution volume of 5 mL was added, and centrifugation was carried out for 8 minutes at 800 rpm. Following two more iterations of this phase, lymphocyte cells were kept overnight in a freezer.

Slide preparation, staining and observation

Lymphocyte cells were dropped onto the glass slide (a maximum of three drops). After drying, the slide was treated with 4% Giemsa dye for 10 minutes, then rinsed with distilled water and dried. The slide was covered with a glass cover using an entellant adhesive and observed under a Nikon Eclipse 100 light microscope. MN, NPB, NBUD, and 8-shaped nuclei were observed simultaneously and were identified according to the characteristics described by Fenech [7] and Kravtsov *et al.* [11]. The nuclear division index (NDI) was determined by counting mononucleate (M1), binucleate (M2), trinucleate (M3), and tetranucleate (M4) cells per 1000 cells and using the published standard formula [11,20]. The NDI is calculated as (M1+2M2+3M3+4M4)/N, where N is the total number of cells assessed. The numbers of MN, NPB, NBUD and 8-shaped nuclei observed per 1000 binucleated cells (BNC) were recorded.

Data analysis

NDI data and cytogenetic abnormalities were assessed using the independent sample T-test to calculate the mean values between the control and case groups. The impact of age and sex on cytogenetic abnormalities was examined using the one-way ANOVA. SPSS version 25 was applied to conduct all statistical tests with a 95% confidence level.

RESULTS

Fifteen residents from high background radiation locations (the cases group) and 15 residents from low background radiation areas (the controls group) participated in this study. Table 1 provides details on the average age, gender, and annual effective dose.

Table 1. Demographic information for the study population.

Parameter	Controls Group	Cases Group
Size of samples (n)	15	15
Age (years)		
Average ± SD	38.07 ± 13.87	43.47 ± 13.32
Range	15–58	23–65
Gender		
Male (%)	8 (53.33)	8 (53.33)
Female (%)	7 (46.67)	7 (46.67)
Annual effective dose (µSv/h)		
Mean ± SD	531.67 ± 70.41	3,956.6 ± 691.43
Range	339.89–639.48	2,803.2–5,045.76

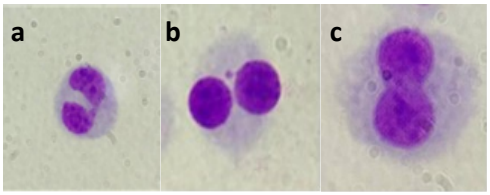


Figure 1. Binucleate lymphocyte cells with a microscopic view of **(a)** the nucleoplasmic bridge (NPB); **(b)** nuclear budding (NBUD), and **(c)** 8-shaped nuclei. Magnification is 1000 times.

Table 2. The average value of NDI of lymphocytes in the control and case groups of the Mamuju population.

Study Group	N	Total Number				n	NDI	Average of NDI±SD (%)	*p-value*
		M1	M2	M3	M4				
Control	15	7,277	6,686	508	549	15,020	24.34	1.62 ± 0.06	0.028
Case	15	6,354	6,524	592	631	14,101	25.12	1.67 ± 0.04	

Note: N: number of participants; M1: mononucleate; M2: binucleate; M3: trinucleate; M4: tetranucleate; NDI: nuclear division index; SD: standard deviation.

Table 3. The frequency (mean ± SD) of cytogenetic abnormalities in the control and case groups.

Group	MN	NPB	NBUD	8-Shaped
Control	4.33± 2.63	0.07±0.25	0.00±0.00	2.67±2.09
Case	10.93±4.81	0.13±0.35	0.13±0.35	7.53±2.13

Note: NBUD: nuclear bud; NPB: nucleoplasmic bridge; MN: micronucleus frequency.

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[DOI: 10.61186/ijrr.21.3.601]

DISCUSSION

The NDI in this investigation was determined to be within acceptable limits in both groups. There was a statistically significant difference ($p<0.05$) between the groups, and the NDI of the cases group was higher than that of the control groups (table 2). M1 cells, which indicate successful cell division, were less frequent in the case samples, leading to the prediction that the NDI of this group would be greater than the control samples. This finding is consistent with the research by Fenech [7]. The NDI is affected by changes in cell proliferation; more chromosomal damage will cause cells to either die before dividing or be less likely to undergo the cell growth phase. The number of cells participating in the interphase and the proportional length of the interphase during the mitotic stage are two variables that impact the NDI value [12]. According to research by Purnami *et al.* [(13], who investigated the effect of radiation exposure on cell proliferation, the NDI scores were higher in the samples from inhabitants of HBRA than those of the control group. Due to an adaptive response that causes cells to carry out proliferative renewals more rapidly under the genotoxic effects of natural radiation, an increase in NDI is predicted. This result is consistent with a study by Sinitsky and Druzhinin on NDI in the population of Kemerovo, an area of high radon concentration [14].

The frequencies of all cytogenetic abnormalities in the study group were higher than in the control. This frequency is reported as the number of cells containing abnormalities per total cells counted. However, as shown in table 3, statistical differences between the groups were only found in the levels of MN and 8-shaped nuclei ($p<0.05$). The MN frequency in both groups is still within the normal range, based on research by Fenech [7]. Our findings are also consistent with a report by Lindberg *et al.* [16], in which the MN frequency in normal lymphocyte samples was 2–36 cells per 1000 BNC. A lower number of MN in the blood of respondents from an HNRA was reported in previous research [15]. In the same study, the frequency of NBUD in the study and control groups were still within the normal threshold [15]. This threshold is in reference to the study by Lindberg *et al.* [16], who reported a mean NBUD frequency of about 19 cells per 1000 BNC.

In our study, the case group had a much higher frequency of 8-shaped nuclei than the control group (113 vs. 40). This extremely high number of 8-shaped nuclei should be assessed further to determine whether it is due to radiation or via another mechanism; for example, Kravtsov *et al.* [11] reported that this abnormality can occur in intensively dividing cells under nutrient deficiency. However, this type of nucleus can also be used as a marker of genome instability due to radiation and other genotoxic agents. There are limited scientific publications reporting the 8-shaped abnormality, and the expected frequency of such nuclei in normal cell populations is unknown. The 8-shaped formation is likely to be due to failure of lymphocyte cell nucleates to separate. Example microscope images of NPB, NBUD and 8-shaped cells at 1000 times magnification are shown in figure 1.

The statistical analysis showed that not all cytogenetic abnormalities were influenced by the characteristics of age and sex ($p>0.05$). This might be related to the radioadaptive response that occurs in residents living in areas of high natural radiation [17]. In accordance with the research of Ramadhani *et al.* [10], radiosensitivity is not related to age and sex in the resistant population. A previous study on inhabitants of Botteng village, Mamuju, also reported that age and sex had no significant correlation with the MN frequency [18]. In contrast to this finding, other studies reported that age influenced the MN. Karuppasamy *et al.* [19] revealed an increase in MN with increasing age due to an elevation in the levels of endogenous or clastogen-induced acentric fragments that cannot be repaired by cells. Salimi and Mozdarani stated that age and gender can be associated with genetic damage, which is influenced by unhealthy lifestyle activities such as smoking. The cumulative effect of DNA repair-related gene mutations, as well as chromosomal numerical and structural abnormalities, are variables that can raise the prevalence of MN [20].

CONCLUSION

According to the results, residents of Ahu and Salletto Villages, Mamuju, West Sulawesi, were exposed to high levels of natural radiation, which had an impact on the development of cytogenetic abnormalities (particularly MN and 8-shaped nuclei), as well as the NDI. Not all the cytogenetic abnormalities evaluated were impacted by age and sex variables.

ACKNOWLEDGEMENT

We are grateful to our colleagues working in the Molecular Radiobiology Laboratory, Research Center for Safety, Metrology and Nuclear Quality Technology, National Research and Innovation Agency (PRTKMMN, BRIN), Jakarta.

***Funding*:** The work is financially supported by the Research Center for Safety, Metrology and Nuclear Quality Technology, National Research and Innovation Agency (PRTKMMN, BRIN).

***Conflict of interests*:** There is no potential conflict of interest.

***Ethical consideration*:** The research was ethically approved by the Ethics Commission of Health Research, Health Research and Development Agency, Ministry of Health, certificate number LB.02-01/2/

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[DOI: 10.61186/ijrr.21.3.601]

KE.063/2018 dated February 21, 2018.

***Authors' contributions*:** N.K: performed the experiments. S.P: collected the samples, performed the experiments. D.T: performed the experiments. D: aided in correcting the manuscript. D.R: collected the samples, performed the experiments. H.N.E.S: drafted and revised the manuscript. M.S: collected the samples, performed the experiments, drafted and prepared the manuscript, processed and analyzed the data, designed the tables and figures, revised the manuscript.

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[DOI: 10.61186/ijrr.21.3.601]