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PROCEEDINGS

**2nd International Conference on the Sources, Effects and
Risks of Ionizing Radiation (SERIR 2016)**

in conjunction with

**14th Biennial Conference of the South Pacific Environmental
Radioactivity Association (SPERA 2016)**

**Sanur Paradise Plaza Hotel
Bali, 5-9 September 2016**

Organized and hosted by



National Nuclear Energy Agency (BATAN)

in cooperation with



South Pacific Environmental Radioactivity Association

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National Nuclear Energy Agency (BATAN)**

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Australian Radiation Protection and Nuclear Safety Agency



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Royal Australian Chemical Intitute

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PREFACE

For the second time the Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency of Indonesia (BATAN) was held the 2nd International Conference on the Sources, Effects and Risks of Ionizing Radiation (SERIR2) in Sanur Paradise Plaza Hotel, Sanur, Bali, Indonesia, which was the continued event that already held in last 2013. Similar as previously, Conference dealt with the efforts to enhance data collection and disseminate scientific findings related to the issues of sources, effects and risks of the ionizing radiation, as well as to seek the way of communication among stakeholders (scientific communities, regulatory authorities, and general public) on those issues. This conference was in conjunction with the 14th biennial conference of the South Pacific Environmental Radioactivity Association (SPERA2016) that provides a platform for discussion among scientists on the occurrence, behaviour, impact and measurement of radioactive species present in the environment through natural processes, or resulting from human activities. This international conference also facilitated knowledge sharing on environmental radioactivity and related topics of local and global significance.

In the SERIR2 there were three keynote speakers presented their own expertise : Dr. Stephen Solomon (Principal Scientific Adviser to the CEO, Australian Radiation Protection and Nuclear Safety Agency (ARPANSA), Prof. Yoshiyuki Suzuki (Department of Radiation Oncology, Fukushima Medical University), and Dr. Ferhat Aziz (National Nuclear Energy Agency of Indonesia (BATAN)).

In this conference there was a press conference that was attended by local and national journalists. This event was handled by Bureau of Legal, Public Relation and Cooperation (BHKK), BATAN. The speakers were : Dr. Andreas Bollhöfer (President of SPERA), Dr. Justin Lee (Deputy Head of Mission, Department of Foreign Affairs and Trade of Australian Embassy for Indonesia), Dr. Gillian Hirth (ARPANSA), Prof. Dr. Djarot Sulistio Wisnubroto (Chairman of BATAN), and Prof. Dr. Mohammad Nasir (Directorate General of Minister of Research, Technology and Higher Education (Menristekdikti).

In this conference, of 38 papers submitted by authors from three countries (Indonesia, India and Japan), 35 papers were presented as oral and poster presentation. For oral, there were 20 papers presented into two groups of paper (group A, Radiation Exposures and Instrumentation and group B, Occupational Exposures and Health Effects), and for poster there were 15 papers. Totally there were 35 papers that consists of 32 papers from BATAN, one paper from Pachhunga University College-India, one paper from University of Udayana, and one paper from Siloam Hospital.

We would like to thank all those who participated in the conference for the lively discussions as well as the director of the Center for Radiation Safety and Metrology, BATAN upon the opportunity to organize this event as well as the SPERA which was agree to conduct the events in the same venue. In addition, we are also grateful to all the authors for their valuable time and contributions to the conference. Last but not least, the conference would not have been possible without the great help of the staff of the Center and Australian Nuclear Science and Technology Organization (ANSTO), South Pacific Environmental Radioactivity Association (SPERA), Australian Radiation Protection and Nuclear Safety Agency (ARPANSA). We would like to thank all of them for their assistance.

WELCOME ADDRESS BY PRESIDENT OF THE CONFERENCE

His Excellency,

Dr. Muhammad Dimiyati, Director General Research and Development, Representing Minister Science, Technology and Higher Education, The Republic of Indonesia;

Prof. Dr. Djarot Sulistio Wisnubroto, Chairman of National Nuclear Energy Agency (BATAN);

Dr. Andreas Bollhöfer, President of South Pacific Environmental Radioactivity Association (SPERA);

Dr. Justin Lee, Deputy Head of Mission, Department of Foreign Affairs and Trade of Australian Mission for Indonesia; and

Dr. Hendig Winarno, Deputy Chairman of BATAN;

Distinguished keynote speakers,

Chairman of the organizing committee,

Participants, Ladies and Gentlemen,

Good Morning and Assalamu-Alaikum Wr.Wb.

On behalf of the National Nuclear Energy Agency (BATAN) of Indonesia, it is my great pleasure to welcome you to the “2nd International Conference on the Sources, Effects and Risks of Ionizing Radiation (SERIR) and 14th Biennial International Conference of SPERA”, jointly organized by South Pacific Environmental Radioactivity Association (SPERA) and National Nuclear Energy Agency (BATAN), particularly The Center for Radiation Safety Technology and Metrology. I wish to welcome you to be in a beautiful Bali Island here.

This second International Conference on the SERIR is a continued of the first scientific meeting that had been done here in the same place three years ago. As in the first SERIR, this Conference is held under an urgent need to give contribution to the works of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). The 2nd International Conference on the SERIR will be a 1-day conference (5 September). This conference is aimed to disseminate scientific findings related to the issues of sources, effects and risks of ionizing radiation, and to communicate with stakeholders (scientific communities, regulatory authorities and general public).

Ladies and Gentlemen,

Ionizing radiation is generated in a range of medical, commercial and industrial activities. The most familiar and the largest of these sources of exposure is medical X-rays. Natural radiation contributes about 88% of the annual dose to the population, and medical procedures contribute most of the remaining 12%. Natural and artificial radiation are not different in kind of effect. Ionizing radiation has always been present in the environment and in our bodies. However, we can and should minimise unnecessary exposure to significant levels of artificial radiation. Ionizing radiation is also very easily detected. There is a range of simple, sensitive instruments capable of detecting minute amounts of radiation from natural and anthropogenic sources.

The Organizing Committee has invited contributions, academic and practice-based paper on all aspects of the following two topics: Radiation Exposures and Instrumentation; and Occupational Exposures and Health Effects, induced by Medical Radiation uses and Environmental/Natural Radiation. Some of oral and poster presenters will deliver those topics in the afternoon.

This Conference has attracted more than 80 participants from 6 countries. About 39 scientific papers will be presented by their authors orally or as posters. This event will offer you plenty of opportunities for extensive discussions, making of new contacts and strengthening the existing relationships after the oral presentations, during the poster sessions, while visiting the exhibition by SPERA or at the other events.

For the SPERA 2016, the 14th Biennial Conference of the SPERA, to be held 6-9 September, will provide a platform for discussion and debate among scientists on the occurrence, behaviour, impact and measurement of radioactive species present in the environment through natural processes, or resulting from human activities.

The joint conference will include a one-day workshop on Trends in Environmental Sample Preparation on the 6th September, facilitated by The Radiochemistry Division of the Royal Australian Chemical Institute (RACI). The workshop will present an overview of the fundamentals, procedures, and applications of both historical and the most recently developed sample preparation techniques for the extraction, clean up, and concentration of radionuclides from environmental samples

Participants, Ladies and Gentlemen.

In this opportunity, I would like to thank to honorable three invited speakers who have been able to be here, Dr. Stephen Solomon, from Australian Radiation Protection and Nuclear Safety Agency (ARPANSA)-Australia, Prof. Dr. Yoshiyuki Suzuki, MD, from Fukushima University-Japan; and Dr. Ferhat Azis, from BATAN-Indonesia. All of them are the prominent scientists in their own field and will provide a comprehensive overview of the current status of the global sources, effects and risks of ionizing radiation.

I look very much forward to this Conference and hope there will be warm discussion, because this Conference is open for everybody to give a view, and certainly we will do our best to make sure that the floor is really life. So, please be prepared to give comments and questions for the topics to be delivered by the speakers and presenters.

In this occasion, I would also like to thank the organizer and resource persons who have made this event possible, and who I am sure will be working tirelessly to ensure the success of the conference and workshop over the next few days.

Finally, I wish all of you will enjoy being in Bali, which is one of 16 Most Beautiful Islands in the World. Bali is a feast for the senses. Bali's spirit will wash over you like a warm, tropical wave.

Thank you and Wassalamu-Alaikum WrWb.

President of the Conference
Susetyo Trijoko, M.App.Sc.

OPENING REMARKS
MINISTER OF RESEARCH, TECHNOLOGY AND HIGHER EDUCATION
(Represent by Director General Research and Development)

Honorable;

1. Dr. Andreas Bollhofer (President of SPERA)
2. Deputy Head of Mission, Department of Foreign Affairs and Trade of Australian Embassy for Indonesia
3. Prof. Dr. Djarot Sulistio Wisnubroto, Chairman of Batan
4. All Experts, Participants
5. Distinguish Guest, Ladies and Gentlements

First of all, let us thanks Allah SWT for His blessings; we can be here to attend this International Conference. On behalf of Ministry of Research, Technology and Higher Education, I would like to express my gratitude to all of you, for participating 2th International Conference on the Sources, Effects and Risk of Ionizing Radiation (SERIR) and 14th Biennial International Conference on SPERA in the beautiful island of Indonesia...called BALI.

Delighted Ladies and Gentlemen,

The development of science and technology in the field of health, food, and energy is very progressive. Many researchers doing very sportive competitions to express their knowledge to support the human being. In the other hand, there are many obstacles should be break it out by the researchers to reach the research goals. This forum can be use as an arena to prove that we are capable of doing it. But we need to keep our awareness that whatever the level of research we present now; we should not merely stop at research paper or conceptual design. We must continue and create research outputs that are ready to be commercialized and giving positive impact to the people. Therefore, the benefit of research can be optimized for the good and prosperity of Indonesian people and the world. And with this spirit, the Ministry of Research, Technology and Higher Education support the mutually-benefit linkage between researchers and industries, in order to minimize their mismatch.

To move further, the Ministry of Research, Technology and Higher Education has had and will continue to push and facilitate research outputs that are ready to be used by the people, to be synergized with other research outputs, to give greater benefits and multiplier effects to the community. For example, Indonesian Institute of Science (LIPI) has invented fertilizer that can make paddy stands out of many pests, while Bogor Agricultural Institute has invented new paddy variety that can yield more than 10 ton per hectare. Research and Development of Ministry of Agriculture had invented paddy field management with Jarwo-system that can improve paddy field productivity. Each of the inventions is directly benefiting the user, but synergizing them through government support, will create much greater benefits, and direct impact for the people, mainly local people.

Delighted Ladies and Gentlemen,

Through this conference, hopefully the discussion will lead toward acceleration of people prosperity. We should not put too much effort on just debates that only satisfying researchers themselves. We have to do more than that. Scientific debates outputs that have been perfectly completed can be posted in the international journals, so they could be used to push forward the acceleration of science development in the world.

Once again, I hope that the conference output will provide positive impact through science and technology development, that is benefiting the community.

To all overseas participants, I welcome you in Bali, a beautiful and peaceful island. Enjoy your stay and hope that the serenity of the island inspires you to create changes for a better future.

Finally, by saying BISMILLAH.... I open 2nd International Conference on the Sources, Effects and Risk of Ionizing Radiation (SERIR2) and 14th Biennial International Conference on SPERA2016.

May Allah SWT, the God Almighty give us His Blessing.
Wabilahi Taufiq Walhidayah, Wassalamualaikum Wr. Wb.

Bali, 5 September 2016
Minister of Research, Technology and Higher Education
Mohamad Nasir

Press Conference (organized by BHHK)




	<p>Dr. Andreas Bollhöfer (President of SPERA)</p>
	<p>Dr. Justin Lee (Deputy Head of Mission, Department of Foreign Affairs and Trade of Australian Mission for Indonesia)</p>
	<p>Dr. Gillian Hirts (ARPANSA)</p>
	<p>Prof. Dr. Djarot Sulistio Wisnubroto (Chairman of BATAN)</p>
	<p>Dr. Muhammad Dimyati (Director General Research and Development, Ministry of Research, Technology and Higher Education, Kemenristekdikti)</p>

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Dr. Stephen Solomon (*Principal Scientific Adviser to CEO ARPANSA*)
“An ARPANSA Perspective on Radiation Protection of The Environment”

Keynote Speaker II



Prof. Dr. Yoshiyuki Suzuki, MD. (*Fukushima Medical University, Japan*)
“Cutting Edge Radiotherapy” Including Combination Therapy
with Immunotherapy)

Keynote Speaker III



Dr. Ferhat Aziz (*National Nuclear Energy Agency of Indonesia*)
“Environment Radioactivity Monitoring Activities in Indonesia and
Its Public Concerning”

Evaluation of Mitotic and Nuclear Division Indexes in Peripheral Blood Lymphocytes of Botteng Village, Mamuju Inhabitants

Masnelli Lubis, Sri Sardini and Dwi Ramadhani

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Abstract. *The biological effects of chronic natural low level ionizing radiation exposure on human population have been a thrust area of study in radiation biology. Botteng village in Mamuju a western coastal city in West Sulawesi, Indonesia, has areas with some of the highest levels of natural radiation measured to date compared to other areas throughout this country. Botteng inhabitants exposed to low radiation dose on their daily basis. Radiation exposure can causes a delay in the cell cycle, thus it will decrease the cell proliferation rate. Aim of this study was to evaluate the mitotic and nuclear division indexes in peripheral blood lymphocytes among people living in Botteng, an area with high natural background radiation in Indonesia. Blood samples were collected from thirty six healthy adult subjects in Botteng and thirteen healthy adult subjects in normal background radiation area. Mitotic index was calculated as the proportion of metaphase for 500 cells for each sample. By cytokinesis block micronucleus assay the assessment in proportion of mononucleated, binucleated, trinucleated and tetranucleated cells/500 cells were conducted to calculate nuclear division index. Study results showed that in this study the MI (8.98 ± 2.50 vs 9.09 ± 2.84) and NDI (1.35 ± 0.15 vs 1.40 ± 0.15) in Botteng was lower compared to control group. Statistical analysis revealed that the different was insignificant ($P > 0.005$). It is possible that peripheral blood lymphocytes in Botteng inhabitants were under the toxic effect of mutagenic agent that was probably radon exposure in this study suffer DNA damages and cannot survive the division cellular cycle that made cells enter in a process of necrosis or apoptosis before the finish of the first division. This study also showed that there was insignificant difference of MI and NDI in respect to age for all samples. Based on the results in can be concluded that chronic low radiation dose exposure in Botteng has no significant effect on lymphocytes proliferation. Further study using larger sample number should be conduct in further study to ensure the effect of chronic low radiation dose exposure on lymphocytes proliferation.*

Keywords: *Lymphocytes, Mitotic Index, Nuclear Division Index, Botteng, High background radiation.*

Introduction

Mitotic index (MI) as the proportion of metaphases among harvested lymphocytes is a cytogenetic test that used both *in vivo* and *in vitro*. This assay requires the addition of colchicine or colcemid to arrest the progression of cells from metaphase to anaphase ensuring sufficient number of metaphases for cytogenetic analysis. The MI was used to characterize proliferating cells and identify compounds that inhibit or induce mitotic progression. There are two factors that can influence the MI. First is the proportion of the cell population that participates in the whole cycle of interphase leading to division, and second the relative lengths of interphase and recognizable mitotic stages (Alakoç & Eroğlu 2011). Depression of the mitotic index is usually a consequence of a reduced rate of cell proliferation (mitotic delay).

Radiation exposure can stops the mitosis mechanism at various phases and causes cell death. Radiation can causes a delay in the cell cycle at G1, S and G2 phases of the interphase (Akbas et al. 2003; Zhao et al. 2001). The delay of G2 can induce by the decrease in the expression of the gene that codes the protein cyclin B1 and p34cd2, which arranges the transition from G2 to mitosis (Akbas et al. 2003). In this study we predicted that the mitotic of Botteng inhabitants should be lower compared to control samples, since people living in Botteng exposed to low

radiation dose on daily basis that can made the mitotic mechanism slower.

Another cell proliferation marker in blood cultures is the nuclear division index (NDI) which is considered a measure of general cytotoxicity, the relative frequencies of the cells may be used to define cell cycles progression of the lymphocyte after mitogenic stimulation and how this has been affected by the exposure. The hypothesis behind it, is that cells with greater chromosomal damage will either die before cell division or may be less likely to enter this phase. The lowest NDI value is 1.0, which occurs if all of the viable cells have failed to divide during the cytokinesis-block period and so, all will be mononucleotide. If all viable cells complete one division there will be all binucleated, the NDI value is 2.0. An NDI value can be greater than 2.0 if certain viable cells have completed more than one nuclear division during the cytokinesis-block phase and therefore contain more than two nuclei (Al Amili et al. 2013; Ionescu et al. 2011).

Aim of this study was to evaluate the MI and NDI in peripheral blood lymphocytes of Botteng village, Mamuju inhabitants living in West Sulawesi Province. People living in Botteng village, Mamuju received radiation exposure higher than other areas in Indonesia. This because Botteng village in Mamuju area has been reported for the high radiation dose rate due to the

Naturally Occurring Radioactive Material (NORM). Several areas in Mamuju have a radiation dose rate more than 5 mSv y^{-1} (Syaeful et al. 2014). An annual effective dose (H_E) in Botteng village, Mamuju was reach $6,15 \pm 0,81 \text{ mSv y}^{-1}$ (Alatas et al. 2012). Here the evaluation of MI and NDI of inhabitants compared with people live in normal background radiation area was conducted to investigate the effect of low radiation dose on the cell proliferation in circulating lymphocytes. Circulating lymphocytes are commonly used surrogate cells to measure the cellular function or events of other healthy somatic cells.

Material and Methods

Dosimetry

The measurement of gamma ray exposure level in Botteng was carried out using portable gamma spectrometer Exploranium GR-135 Plus. Outdoor radiation levels was measured using a portable plastic scintillimeter (PM1405, Polimaster) measuring micro-Sievert per hour ($\mu\text{Sv h}^{-1}$), with an accuracy of ± 10 , calibrated using different radiation sources (from Environmental Survey Laboratory, Radioecologic Division, Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency of Indonesia) (Figure 1).

Blood Sampling

Thirty six healthy adult subjects from Botteng and thirteen healthy adult subjects from normal background radiation area were included in this study. Peripheral blood samples were collected by venipuncture using heparinized vacutainer tubes (BD Vacutainer systems). The study was approved by Ethics Committee of the National Institute of Health Research and Development, Indonesian Ministry of Health, number LB.02.01/5.2.KE.051/2015 date of January 29, 2015. Informed consent was obtained from all donors. A detailed questionnaire was used to obtain information on age and occupation.



Figure 1. An environmental radiation monitoring instrument showing a dose rate of $0.72 \mu\text{Sv h}^{-1}$ in area with high natural radiation of Botteng.

Blood Culture and Mitotic Index Evaluation

Blood cultures were set up according to the IAEA standard procedures with minor modifications. One ml of whole blood samples were cultured for 48 hours in the incubator at 37°C containing 5% CO_2 . The culture medium consisted of 7.5 ml of Rosewell Park Memorial Institute (RPMI) 1640 supplemented with 20% heat inactivated fetal bovine serum (FBS), 1% streptomycin/penicillin, 2.5% ml of phytohemagglutinin (PHA). Colchicine was added to the culture for the last 3 hours of culture at a final concentration of $0.05 \mu\text{g/ml}$. The cells then were centrifuged for 10 minutes at 1500 rpm and re-suspended in 10 ml of 0.075 M KCl (pre-warmed to 37°C) for 25 minutes. The cells then were centrifuged again for 10 minutes at 1500 rpm and re-suspended in 2 ml of fresh fixative that was prepared from methanol and acetic acid (3:1). The fixation step was repeated four times until white sediment was obtained. The cell suspension then was stored in -20°C at least for one night until the slide preparation was conducted. Fixed cells were dropped onto clean, wet slides, dried and stained with 4% Giemsa solution (pH 6.8) for 10 minutes. MI then was calculated based on protocol in IAEA publication in 500 cells for each sample (Figure 2).

Micronucleus Assay and Nuclear Division Index Evaluation

Micronucleus assay was conducted based on protocol in IAEA publication (Anonymous 2011). Whole blood samples were cultured for 72 hours in the incubator at 37°C containing 5% CO_2 . The culture medium consisted of 4.5 mL of Rosewell Park Memorial Institute (RPMI) 1640 supplemented with 20% heat inactivated fetal bovine serum (FBS), 1% streptomycin/penicillin, 2.5% ml of phytohemagglutinin (PHA). Cytochalasin-B was added to the culture at 44 hours at a final concentration of $6 \mu\text{g/ml}$. The cells then were centrifuged for 10 minutes at 1000

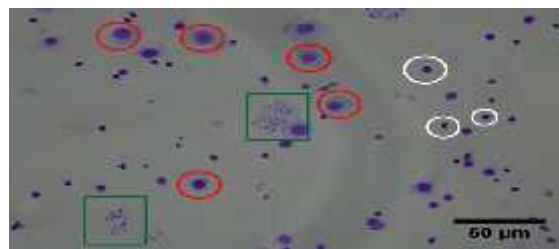


Figure 2. A microscopic view of Giemsa stained slide that also containing metaphase chromosome spread (boxes), blast cells (red circles) and nuclei which are not counted (white circles) as base for the evaluation of mitotic index of a respondent living in HBRA (magnification of 400X).

rpm and re-suspended in freshly made fixative consisting of methanol: acetic acid (10:1) diluted 1:1 with Ringer's solution. The cells then were washed with two to three further changes of freshly prepared fixative consisting of methanol:acetic acid (10:1) without Ringer's solution, until the cell suspension is clear. The cell suspension then was stored in -20°C at least for one night until the slide preparation was conducted. Fixed cells were dropped onto clean, wet slides, dried and stained with 4% Giemsa solution (pH 6.8) for 12 minutes. For each slide in each sample, the proportion of mononucleated, binucleated, trinucleated and tetranucleated cells per 500 cells scored was assessed. Nuclear Division Index (NDI) was calculated based on follows formula where M1, M2, M3 and M4 indicate the number of cells with one, two, three and four nuclei and N is the total number of cells analyzed. N = 500.

$$NDI = \frac{M1 + 2M2 + 3M3 + 4M4}{N}$$

Statistical Analysis

Unpaired *t*-test was used to compare the MI and NDI between Botteng and control samples using SPSS 22.0 statistical software, if the data have a normal distribution. The Kolmogorov-Smirnov test was applied to know the distribution of data.

Results and Discussion

The mean MI and NDI values in Botteng inhabitants (8.98±2.50; 1.35±0.15) was lower compared to mean MI and NDI values in control group (9.09±2.84; 1.40±0.15) (Tabel 1 and 2). An example of slide containing metaphase and blast cells for MI is presented in Figure 2. Statistical analysis revealed that the different was not significant ($p>0.05$). Table 3 shows the mean MI and NDI values with respect to smoking habit and age in Botteng and control groups. There was insignificant difference between smokers and nonsmokers in all samples. Our study revealed that Botteng inhabitants exhibited a lower number of mono- and binucleated cells and an increased number of polynucleated cells. This finding was in contrast with other study (Sinitsky & Druzhinin 2014). Sinitsky & Druzhinin (2014) found that the proliferation index, which characterizes the rate of cell division, was higher in people living in high radon concentrations area compared to control samples. They proposed that a compensatory mechanism to promote a more rapid renewal of the proliferative pool under the genotoxic

effects of radon might cause the higher rate of cell division in people living in high radon concentrations area.

It is well known that more than 50% of the average annual background radiation dose is due to radon (Druzhinin et al. 2015). Alpha particles represent the predominant form of radiation emitted as a result of the decay of radon. Ionizing radiation in the form of alpha particles can cause DNA damage and generate reactive oxygen species (ROS) resulting in cell cycle shortening, apoptosis and an increased potential for carcinogenesis. A link between radon and lung carcinogenesis has already been established and radon is thought to be the second leading cause of lung cancer in the UK after smoking, with evidence of a synergistic effect between radon and tobacco smoke (Robertson et al. 2013). A study conducted by Abo-Elmagd et al. in 2008 also found that the mitotic activity of bone marrow cells in Swiss albino mice decrease with increase radon dose.

MI and NDI are marker for estimates the general toxicity. The proportion of metaphase cells and binucleated cells may be used as a biomarker of the lymphocytes mitogen response, immune functions and cytostatic effects of various studied agents included the low radiation dose exposure. There are several hypotheses that can explain why in this study the mean MI and NDI values in Botteng inhabitants were lower compared to control group. First, the circulating lymphocytes under the toxic effect of mutagenic agent (which was probably radon exposure in this study), suffer DNA damages, cannot survive the division cellular cycle and enter in a process of necrosis or apoptosis before the finish of the first division.

Study in Sweden that used a comet assay to assess the primary DNA damage in single cells analysed the blood samples from 125 people living in 45 houses with different indoor air radon concentrations (35-1025 Bq/m³), as well as with different concentrations of radon in the drinking water (10–2410 Bq/l). They found that increased radon concentration in the air (>200 Bq/m³) was associated with increased DNA damage in lymphocytes ($P<0.05$) (Hellman et al. 1999). Second hypothesis is there may be an induction of mitotic delay, which, by not allowing the repair of genotoxic lesions, will modify the number of the cells entering mitosis and modify the proportion of mono-/bi-/tri- and tetranucleated cells. Thus, a lower NDI as fewer cells divide. Last, there is the hypothesis of a clastogenic effect of mutagens with an aneugenic action, inducing some degree of blockade of the cell cycle. Therefore, more cells will not divide and NDI will again be low (Ionescu et al. 2011).

In conclusion chronic low radiation dose exposure in Botteng has no significant effect on lymphocytes proliferation. Further study using larger sample number should be conduct to ensure the effect of chronic low radiation dose exposure on lymphocytes proliferation. Other cell proliferation marker should be use in further study like replication index (RI). Replication index commonly calculate in 500 cells per culture according to the following formula. Where M1, M2, and M3 represent for the number of cells in the first metaphase, second metaphase and third or more metaphases, respectively (Eroglu 2011).

$$RI = \frac{(1 \times M1 + 2 \times M2 + 3 \times M3)}{500}$$

Conclusion

It can be concluded that based on this study the mean of MI and NDI Botteng inhabitants was lower compared to control group even the different was not significant. It was possible that the circulating lymphocytes in Botteng inhabitants were under the toxic effect of mutagenic agent and suffer the DNA damages. Thus, it cannot survive the division cellular cycle and enter in a process of necrosis or apoptosis before the finish of the first division. Further study using larger sample number should be conduct to ensure the effect of chronic low radiation dose exposure on lymphocytes proliferation. Other cell proliferation marker should be use in further study.

Table 1. The mean MI value in Botteng and control groups.

Group	Number of sample	Total counted cells (metaphase + blast)	Total number metaphase cells	Mean MI ± SD (%)
Botteng	36	18,000	1618	8.98 ± 2.50*
Control	13	6,500	591	9.09 ± 2.84
All samples	49	24,500	2209	

* Students t-test: P > 0.05 (not different from control group)

Table 2. The mean NDI value in Botteng and control groups.

Group	n*	Total M1	Total M2	Total M3	Total M4	Total N	Mean NDI ± SD
Botteng	13	4526	1533	135	195	6500	1.35 ± 0.15*
Control	13	4467	1526	194	250	6500	1.40 ± 0.15
All samples	36	8993	3059	329	445	13000	

* Students t-test: P > 0.05 (not different from control group)

Table 3. The mean MI and NDI values with respect to age in Botteng and control groups

Parameter	n*	Mean MI ± SD (%)	n*	Mean NDI ± SD
Age (Botteng)				
0 – 30	10	9.48 ± 2.60	3	1.34 ± 0.21
31 – 50	17	9.62 ± 2.29	8	1.38 ± 0.13
>51	9	7.24 ± 2.14	4	1.33 ± 0.18
Total	36		15	
Age (Control)				
0 – 30	1	10.8	3	1.42 ± 0.17
31 – 50	4	8.60 ± 2.65	4	1.39 ± 0.13
>51	8	9.12 ± 3.20	6	1.39 ± 0.17
Total	13		13	

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Discussion

Q : Teja Kisnanto

Which one the most sensitive measure the genotoxic effect in lymphocytes of Mamuju Inhabitants?

A : Dwi Ramadhani

Both of Mitotic Index and Nuclear Division Index have the same sensitivity in assess the genotoxic effect caused by radon exposure in Mamuju.