Research Article

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Expression of γ-H2AX Using Immunofluorescence Assay as an Adaptive Response of PBMC in Radiation Workers at Dharmais Cancer Hospital

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ABSTRACT

Background: Exposure ionizing of radiation in radiation workers has the potential to cause DNA damage in the form of double strand break as the beginning of genomic instability. DNA damage can be observed with γ-H2AX as the biomarker of DNA double strand breaks (DSBs). The formation of γ-H2AX in the nucleus can occur after radiation exposure of 1 mGy. This study aims to determine the radiation effects in radiation work environments as a study of adaptive responses of peripheral blood mononuclear cell (PBMCs) after radiation by observing γ-H2AX foci expression..

Methods: Blood samples from nine radiation workers and nine non-radiation workers were irradiated with doses 0 Gy, 1 Gy, 1.5 Gy, and 2 Gy. Detection of γ-H2AX foci was done by immunofluorescence assay. The mean of γ-H2AX foci was counted in 50 PBMCs per sample. The comparison mean of γ-H2AX foci was analyzed using t-independent test.

Result: Based on the result study, there were no significant differences in the number of γ-H2AX foci without treatment (p = 0.807). The results of study showed that the formation of 2-3 foci per cell after exposure of 2 Gy increases along with the increasing irradiation doses.

Conclusion: The mean of index of γ-H2AX foci in PBMCs within normal limits between non-radiation workers and radiation workers and level of risk DSBs damage is relatively similar after exposure at doses 1 Gy, 1.5 Gy, and 2 Gy.

INTRODUCTION

Biomarkers are tools to identify health risks from environmental influences such as radiation exposure, as an assessment of cancer risk and response to therapy. Therefore, it is necessary to evaluate the biomarkers occurring as a result of low dose ionizing radiation exposure and low dose rates including risk evaluation as a basic consideration for radiation protection standards (1,2). Ionizing radiation can cause damage to biological materials by ionizing or removing electrons to form positive ions and negative ions, and its power will decrease if the received energy becomes exhausted (3).

Ionizing radiation exposure to both low dose and high dose has the potential to cause deoxyribonucleic acid (DNA) damage in the form of double strand breaks (DSBs) or single strand break (SSB). The existence of lesions in DNA as the effect of ionizing radiation is the beginning of genomic instability that triggers cell death from radiation, cell transformation, and carcinogenesis (4,5). Basically DNA damage can be studied using various observational methods such as γ-H2AX foci, chromosome aberration, micronucleus, and others (6).

Observation of the γ-H2AX foci was first found in DNA that was damaged by the ionizing radiation exposure. γ-H2AX is the nucleosome of the histone that acts to regulate the response to DNA damage and continues

the activation of the repair signal. In this process, the occurrence of γ-H2AX phosphorylation around the DSB area formed the γ-H2AX foci. The formation of γ-H2AX in nucleus can occur after radiation exposure of 1 mGy and the number increases along with the increasing dose (7-9). The occurrence of phosphorylation of the H2A histone variant γ-H2AX in serine 139 may be mediated by different protein kinases, including ataxia-telangiectasia mutated (ATM) and phosphatidylinositol 3-kinase (PI3K). After DSBs repair, there will be γ-H2AX dephosporylation in serine 139 which can be done in the presence of wild type p53 and thus the foci γ-H2AX cannot be seen in nucleus (10,11).

This study aims to determine the radiation effects of radiation workers as an adaptive response study PBMCs (lymphocytes and monocytes) of radiation workers in Radiotherapy Installation Dharmais Cancer Hospital after radiation of 1 Gy, 1.5 Gy, and 2 Gy by observing the expression of foci γ-H2AX.

MATERIAL AND METHOD

Sample

Blood samples were obtained from nine radiation workers and nine non-radiation workers at the Dharmais Cancer Hospital, Jakarta. The Institutional Review Board at Medical Faculty of Indonesia had approved the study and all workers were provided informed consent before participating in this study (Number of Ethic Approval : 910/UN2.F1/ETIK/2017).

Place and Time of Research

Research activities were conducted in the cytogenetic and the molecular radiobiology laboratory, National Centre for Radiation Safety and Metrology Technology (PTKMR-BATAN), October-November 2017.

Irradiation of Blood Samples

For the treatment of irradiated samples prior to lymphocyte isolation, blood was irradiated with doses of 1 Gy, 1.5 Gy, and 2 Gy with gamma rays produced by Cobalt-60 at the Centre for Applications of Radiation Isotopes (PAIR- BATAN). The Cobalt-60 plane is set at a rate of 60 Gy/hour.

Detection of γ-H2AX Foci

Blood samples that were taken intravenously were isolated by standard procedures to obtain pure lymphocytes. The lymphocytes were dripped on the slide glass, fixed with 2% formaldehyde for 5 minutes, Triton-X 0.25% in PBS for 5 minutes, and with 1% BSA solution in PBS for 5 minutes. We then added the first antibody γ-H2AX in 2% BSA for 15 minutes and added first antibody (γ-H2AX) for 45 minutes on the moist chamber and put in the incubator on 37.50C. Next was washing with 1% BSA, 3 x 15 minutes, second antibody incubation for 30 minutes, PBS washing 3 x 15 minutes, drying wind and mounting. Detection of γ-H2AX foci was done with 100x magnification fluorescent microscope. For γ-H2AX calculation, we calculated the average number of foci or γ-H2AX foci group in 50 cells (6,12).

Data Analysis

Data analysis to compare the mean index of γ-H2AX foci was done by using SPSS ver 21. Hypothesis test with significance value is $p < 0,05$. Kolmogorov-Smirnov test was used to ensure data normality. Relationship between foci γ-H2Ax and dose variation was analysed using T-independent test.

RESULTS

Microscopic observations were performed to see the γ-H2AX foci expressing a bright green on PBMCs (Figure 1). The formation of foci means DNA DSB damage occurred in PBMCs.

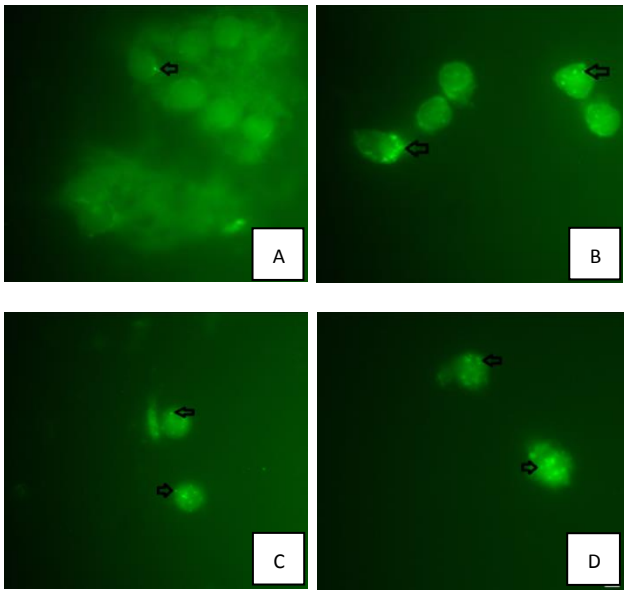

Figure 1. γ-H2AX foci PBMCs radiation workers with irradiated dose (A) 0 Gy or without treatment, (B) 1 Gy, (C) 1.5 Gy, and (D) 2 Gy (100x magnification).

Morphological changes due to irradiation do not provide a different morphological image of either radiation with a dose of 1 Gy, 1.5 Gy, or 2 Gy. A round, large, and slightly formed nucleus that formed a hollow on one side was seen in the PBMCs. Cells also appear to colonize and accumulate in some parts.

Table 1, generally explains the increase of foci γ-H2AX along with the increasing irradiated doses from 1 Gy to 2 Gy either in non-radiation workers or radiation workers. A low foci γ-H2AX indicates H2AX in PBMCs are within normal limits and minimal DSB DNA damage, and the mean of γ-H2AX foci indicates high DSBs due to ionizing radiation.

Table 1. Damage of DNA measured by histone γ-H2AX in PBMC radiation workers after irradiation 1-2 Gy using γ-rays

Subject	Tare Dose 1 Year (mSv)	0 Gy	1 Gy 60'	1.5 Gy 60'	2 Gy 60'
Radiation Workers					
A	0.26	0.26	2.90	1.21	3.28
B	0.00	0.14	1.08	1.68	1.72
C	0.22	0.42	1.24	2.20	1.58
D	0.18	0.16	1.98	1.76	1.96
E	0.17	0.42	2.00	1.74	3.06
F	0.06	0.72	1.78	2.26	2.52
G	0.15	0.54	1.06	2.02	2.00
H	0.17	0.42	2.76	2.94	3.40
Q	0.75	0.16			
Average	0.22	0.36	1.85	1.98	2.44
± SD	0.21	0.20	0.71	0.51	0.73
Non-radiation Workers					
I	0.27	0.22	1.02	1.62	1.26
J	0.00	0.26	1.22	1.40	1.70
K	0.30	0.40	2.24	1.54	3.34
L	0.00	0.28	1.46	2.64	1.82
M	0.11	0.48	2.72	2.24	2.94
N	0.22	0.42			
O	0.28	0.32	1.58	1.86	1.96
P	0.28	0.40	0.86	1.22	2.10
R	0.34	0.30	1.44	1.64	1.98
Average	0.20	0.34	1.57	1.77	2.14
± SD	0.13	0.09	0.62	0.47	0.68
p value		0.807	0.414	0.414	0.404

The result of statistical analysis in Fig. 2 shows no significant difference in mean number of γ-H2AX foci before radiation between non-radiation workers and radiation workers (p = 0.807). The difference in the distance of foci γ-H2AX in each sample occurs spontaneously or because of other factors affecting DSBs damage. At doses of 1 Gy to 2 Gy, there was no significant difference in the mean number of γ-H2AX foci at doses of 1 Gy to 2 Gy with p values of 0.414, 0.414, and 0.404, respectively. The difference in the distance of foci γ-H2AX in each sample occurs because of the differences in the biological response of each individual in receiving exposure to ionizing radiation resulting in DSBs damage.

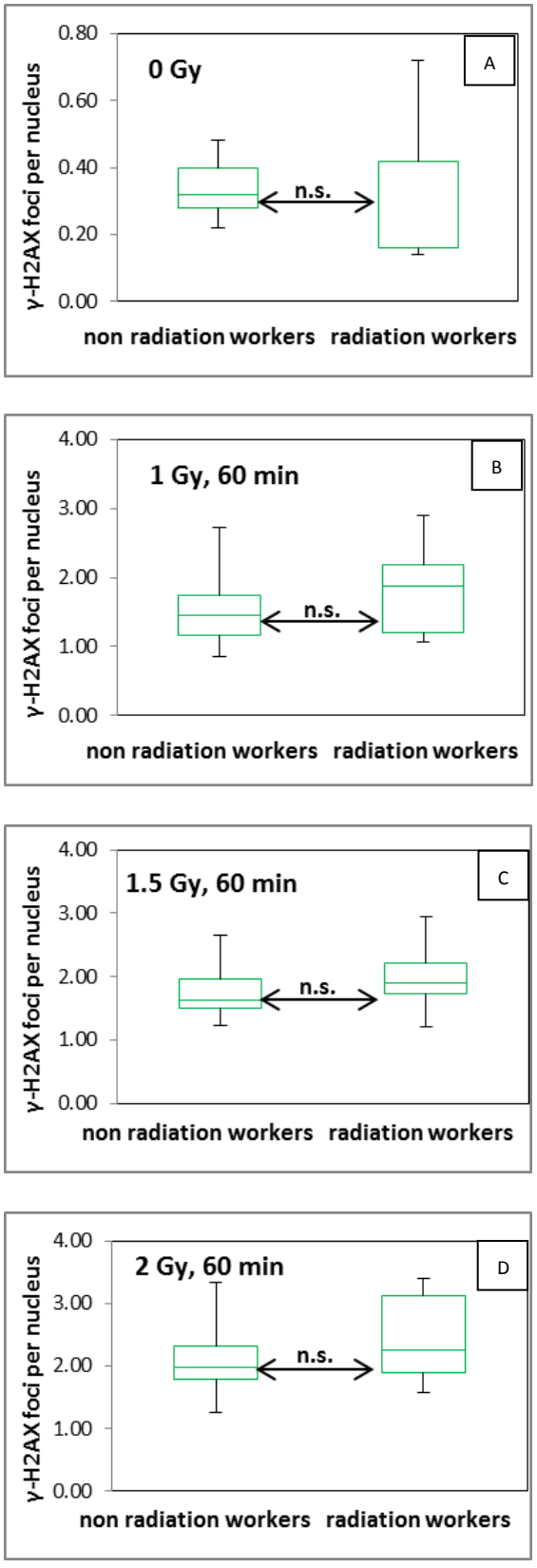

Figure 2. Comparison of foci γ-H2AX in radiation workers with doses (A) 0 Gy, (B) 1 Gy, (C) 1.5 Gy, and (D) 2 Gy.

DISCUSSION

In this study, PBMCs were isolated from non-radiation workers and radiation workers to be analyzed for DNA damage using γ-H2AX histones. Morphologically, the cells exhibit lymphocytes and γ-H2AX foci expression. There is no difference in morphological features of PBMCs. In each treatment the image of the nucleus is round, relatively large and slightly forming a hollow on one side. When viewed from the pattern of the distribution, foci γ-H2AX was seen to spread more evenly around the nucleus, this is because the ionizing radiation used is ray- γ which belongs to the low-LET category that can spread in all directions throughout the cell. Urushibara et al. (13) explains that any radiation beam at a particular wavelength gives rise to 1 lesion from 4 ionization and excitation on low-LET radiation and is associated with the formation of 1 DSB around the foci. It is known that H2AX histone may experience phosphorylation in non-lethal DSB regions. Foci formation can occur spontaneously in small amounts of metabolic processes. In the study of Mognato et al. (15), γ-H2AX foci was faster in DNA repair of about 80-90% for 24 hours induced by γ-rays.

Based on the increased dose, H2AX formed about 1-2 foci per nucleus after 1 Gy of radiation. While in the research conducted by Djuzenova et al. (6), normal people produce 2 foci per nucleus after given radiation 0.5 Gy. Other publications mentioned an increase in the number of foci 6 to 10 foci per cell after the irradiation of 1 Gy. The difference in the number of foci per cell is due to the number of cells observed, the time after radiation, the distribution of photon planes (X-rays or γ-rays), and the biological behavior of each individual (6).

The data showed that there was no significant difference between control and radiation workers at a dose of 0 Gy (Fig. 2). This can be seen from the p value of 0.807 which assumes that the lymphocyte cells between the control and the radiation workers are in normal condition. The mean γ-H2AX foci in normal individuals obtained per cell is 0.34 ± 0.09 with a range of 0.22 to 0.48. While in the research conducted by Fleckenstein et al. (16) shows the results of background value of about 0.07-0.08, which is 6 times lower than the value that has been presented.

Results of statistical analysis on all three doses were not significantly different. The data showed that there was no significant difference between non-radiation workers and radiation workers at doses of 1 Gy to 2 Gy (Fig. 2). This can be seen from the value of $p > 0.05$ which gives the assumption that between non-radiation workers and radiation workers have the same level of risk of DSBs damage after irradiation. In a study by Cholpon et al., a comparison of the number of foci between normal and abnormal cells was significantly different ($p < 0.05$) after 2 Gy exposure. This shows that in abnormal cells, when given 2 Gy irradiation treatment, it will show the expression of histon H2AX higher than normal cells (6).

CONCLUSION

Based on the results of this research, γ-H2AX foci in PBMCs is still within normal limits between controls and radiation workers.

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