RESEARCH ARTICLE

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Preliminary Study of Spontaneous Micronuclei and Hematology Profile of Workers Exposed to Low-Dose Radiation

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Abstract

Objective: This study aims to evaluate the conditions suffered by radiation workers based on the concerned indication of complete blood tests and genotoxicity using genomic biomarkers of micronuclei. **Methods:** A comprehensive approach was taken in this study. A total of 5 ml of blood from every worker was taken for a genotoxic test using CBMN Assay and hemopoietic evaluation. The blood samples from 48 workers were divided into two subgroups, radiation workers and control group. Sub-group I comprised 24 samples that were used as radiation-exposed workers with an average age of 49.17± 8.37 years old and an average working experience of 17.66 ± 9.36 years, and sub-group II comprised 24 samples that were used as the controls with an average age of 39.13 ± 9.37 years old. **Results:** Our analysis revealed that the Count Blood Cells (CBCs) parameter of thrombocytes, MCV, and MCH obtained from the exposed subjects were slightly higher than those from the control subjects, but not significantly different ($p>0.05$). The spontaneous MNBNC from the exposed subjects was also slightly higher, but not significantly different. Importantly, we found a weak correlation between the individual annual dose received and work experience with both the hematology profile and micronuclei, suggesting potential long-term health implications for radiation workers. **Conclusion:** The data obtained here showed an increased risk of genetic instability correlated with occupation, exposure time, and dose received by radiation workers. This study's conclusions are significant, as they demonstrate that long-term exposure to ionizing radiation, even below the dose limits, was related to a significantly increased level of some blood biomarkers and genomic instability, highlighting the need for further research and potential changes in occupational health practices.

Keywords: CBMN assay- genomic instability- hematology profiles- micronuclei- radiation-exposed workers

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Introduction

Ionizing radiation (IR) is recognized as an occupational hazard in the workplace due to its potential for biological damage. Workplace exposure to ionizing radiation can result in both acute and chronic health effects, including genetic instability, potentially leading to various diseases. Assessing risks associated with occupational radiation exposure to radiation hazards is significant for worker health [1]. The sensitivity of cells to ionizing radiation is variable, with hematopoietic cells exhibiting the highest sensitivity [2]. The sensitivity of cells to radiation varies; the hematopoietic system is among the most radiosensitive due to the role of the functional cells in oxygen transport in the blood, which is crucial for immune defense against pathogens. Previous studies have demonstrated that radiation can induce cytological effects, lead to cell dysfunction, and alter hematological factors in blood cells. The quantity of blood cells in healthy individuals remains relatively stable, exhibiting variations in response to environmental and occupational hazards [3-5].

A hematology profile that assesses blood cell counts is a screening tool for a range of hematological and non-hematological conditions. CBC, despite its simplicity and low cost, is significant in the diagnostic and prognostic assessment during the latent period of certain diseases, particularly chronic conditions [6]. Micronuclei (MN) examination is another biomarker commonly utilized to evaluate damage induced by ionizing radiation (IR). The MN assay in PBL serves as an indirect assessment utilized as an alternative method to evaluate cytogenetic damage in groups exposed to ionizing radiation in occupational settings [7,8]. Multiple studies indicate a higher incidence of MN among hospital workers exposed to low levels of

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radiation in their occupations. The elevated frequency of MN signifies an increased risk of genetic damage and associated health concerns, a finding that warrants further investigation [9-13].

Hematological testing utilizing CBC parameters has indicated notable alterations in blood parameters, demonstrating significant differences between radiation workers and the control group [14, 15]. The assessment of cytogenetic studies utilizing the genomic biomarker MN, alongside the hematological profile derived from CBC parameters, serves as supplementary information that enhances physical dosimetry and facilitates a more comprehensive evaluation of radiation health effects. This research employs the genomic biomarker MN to assess the health conditions experienced by radiation workers, utilizing data from comprehensive blood tests and genotoxicity evaluations. This study aims to elucidate the effectiveness of CBC tests and genomic biomarkers in predicting radiation effects on the health of radiation workers, thereby improving their safety and well-being.

Materials and Methods

Ethical approval

The ethical approval certificate for this present study was issued by the Health Research Ethics Commission, Ministry of Health, Republic of Indonesia with certificate number LB.02.01/5.2.KE.079/2017.

Study population

A total of 48 workers were divided into two subgroups. Subgroup I comprised 24 direct contact radiation workers as the exposed subjects with an average age of 49.17 ± 8.37 years in the range of 33 - 66 years old. They worked as physicians, technicians, and nurses and got exposure doses in the range of 0.02-3.99 mSv with an average dose of 0.54 ± 0.82 mSv during the previous year. Sub-group II comprised 24 non-contact radiation workers as the unexposed subject and the control subject with an average age of 39.13 ± 9.37 years old in a range of 25-57 years old and an average of work experience of 17.66 ± 9.36 years in a range of 2-34 years. They worked as administrative workers and had no recorded over-exposure in their personnel documents. A blood sample was taken as much as 5 mL using a syringe and put into a tube containing anti-coagulant heparin from a BD vacutainer.

Hematological examination

Every blood sample in a vacutainer obtained from all subjects was mixed with EDTA anticoagulant and was examined using a hematology analyzer ABX Micros 60, with strict adherence to the Laboratory standards. Nine CBC parameters were considered and analyzed for all groups in this study such as hemoglobin (HB) in g/dL, white blood cells (WBC) in count $x10^3$ /mm^3, platelet count (PLT) in count $x10^3$ /mm^3, hematocrit (HCT) in %, red blood cells (RBC) in count $x10^3$/mm3, mean corpuscular hemoglobin (MCH) in pg, mean corpuscular hemoglobin concentration (MCHC) in g/dL, lymphocytes (LYM) in %, and neutrophils (NEUT) in %, ensuring the quality and reliability of the research.

Determination of CBMN assay

The Micronucleus (MN) assay utilized Cytochalasin B, which was purchased from Sigma Chemicals. Lymphocytes were cultured following the methodology outlined in previous research [16, 17], which has been a cornerstone of our scientific community. Cytochalasin B (3.5 μg/mL) was introduced after 44 hours of incubation. Subsequently, after 72 hours of incubation at 37°C, cells were harvested by centrifugation and removal of the supernatant. The cells were then fixed with a freshly prepared mixture of methanol/acetic acid (10:1) and Ringer's salt solution (1:1). This process of centrifugation and resuspension was repeated three times, and the cells were dropped onto clean microscope slides for the detection of micronuclei by conventional staining with 4 % Giemsa. The slides were coded and scored by light microscopy at 40 magnifications. For each sample, 2000 binucleated lymphocytes with well-preserved cytoplasm were scored [7].

Statistical analysis

All data of the hematology profiles and the frequency of micronuclei were carried out using the Medcalc version 12.7 software (Kolmogorov-Smirnov and Mann-Whitney test). The independent sample t-test ($p>0.05$) was considered to have a statistically significant difference. The relationship between dose, CBC characteristics, and cytogenetic data were performed by correlation of Spearman's.

Results

Baseline information

The total workers taken from the object of the study (n=48) were divided into two subgroups, which are workers who have direct contact with radiation exposure as the exposed subjects with the mean of age of 49.17± 8.37 years old and 24 no contact radiation workers as the unexposed subject or the control subject with the mean of age of 39.13± 9.37 years old. The mean work experience of the exposed subject was 17.66 ± 9.36 years old. In the exposed group, the cumulative effective dose ranged from 0.02-3.99 mSv to 0.54 ± 0.82 mSv, and the work experience ranged from 2- 34 years old was 17.66 ± 9.36 years old. This comprehensive study leaves no stone unturned, providing a detailed understanding of the impact of radiation exposure on workers.

Hematology

The result of this study showed that the mean value based on the selected complete blood count (CBC) parameter was calculated to examine low or high trends. Comparison of the mean value of CBC parameters with the normal range and comparisons based on the t-tests between the exposed subject and the unexposed subject workers are presented in Table 1. Results of this study showed that the mean values based on the selected CBC parameters of the mean values had a significant difference between the exposed subject and the unexposed subject. The most affected CBC parameter, which showed an increased trend and was relatively high, was lymphocyte

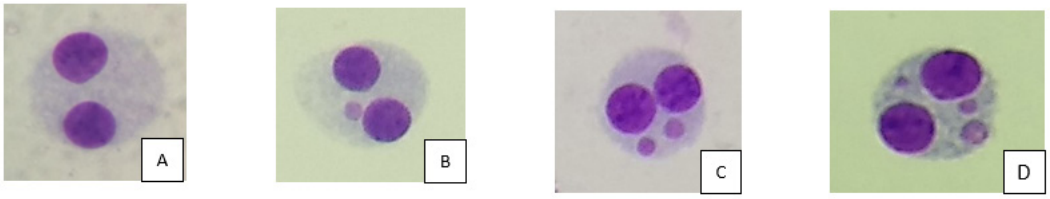

Figure 1. Binucleate Cells (A); Binucleate cells with 1 MN (B); Binucleate cells with 2 MN (C) Binucleate cells with 3 MN (D).

Table 1. Comparison of MN, Dose, and Work Experience between the Exposed and the Control

	Micronuclei/2000 BNC		Normal Range/1000BNC
Parameter	Exposed subject	Non exposed subject (control subject)	
Spontaneous MN			
Mean ± SD	19.0 ± 6.19	16.12 ± 5.02	20.5 ± 11.69
Range	6-33	7-24	1-35*
Dose (mSv/year)			
Mean ± SD	0.54 ± 0.8173	-	5
Range	0.025-3.99		
Work Experience (Years)			
Mean ± SD	17.75 ± 9.1		
Range	5-33	-	
<16 years	16.9 ± 3.56		
>16 years	20.76 ± 7.45		

Note: *, statistically significant with p value<0.05

count and was followed by platelet, MCH, and MCHC but did not have a significant difference (p>0.05). Meanwhile, the rest of the CBC parameters, including HB, leukocytes, and hematocrit, showed similar values between the exposed and the control subjects.

Micronuclei

The formation of micronuclei in binucleate cells (MNBNC) assay, reported as the total number of MN per 1000 BN cells, and the microscopic view of MNBNC were shown in Figure 1. Table 2 compares the mean value of spontaneous MNBNC in the exposed and the control subjects in binucleate cells. Spontaneous MNBNC frequencies relatively. From Table 2, It could be seen that the mean value of spontaneous MNBNC of the exposed subject (19.0 ± 6.19) was greater than that of the control subject (16.12± 5.02). Nevertheless, the IAEA Report, a crucial validation of our findings, confirms that the mean MN value for both values was relatively below the spontaneous MN limit value formed in healthy individuals. The work experience of workers with different exposure times showed significant differences in the incidence rate of MN among the workers with exposure time. <16 years and >16 years (p<0.05).

Relationship between Cumulative Radiation doses and Spontaneous MN

For the exposed group, the cumulative radiation dose ranged from 0.02-3.99 mSv with an average dose of 0.54 ± 0.82 mSv, and the age ranged from 5-34 years old with an average age of 17.66 ± 9.36 years old. The relationships between various blood parameters and micronuclei of radiation workers with their work experience and dose accumulation are presented in Table 3. The results, obtained

Table 2. Comparison of the Exposed and the Control Subjects in Term of Blood Parameters

Parameter	Expose Subject	Unexposed subject	Normal range
HB (g/dL)	14.95 ± 1.29	14.25 ± 1.57	11-16.5
WBC (10^3/mm^3)	7.45 ± 2.16	7.4 ± 1.59	4-10
Hematocrit (%)	44.23 ± 3.63	44.08 ± 5.77	35-50
Lymphocytes (%)	30.04 ± 7.11	27.23 ± 7.11	21.9-50.3
Platelets (10^3/mm^3)	299.54 ± 75.4	255 ± 71.2	150-390
MCV (mm^3)	84.05 ± 3.10	82.86 ± 7.06	80-97
MCH (pg)	28.38 ± 1.14	28.11 ± 2.79	26.5-35
MCHC (g/dL)	33.37 ± 2.05	31.79 ± 1.69	31.5-35
Erythrocytes (10^3/mm^3)	5.26 ± 0.39	5.29 ± 0.81	3.80-5.80

Table 3. Correlation of Different CBC Parameters and MN with Doses and Work Experience

		HB	WBC	Hematocrit	Lymphocytes	Platelet	MCV	MCH	MCHC	RBC	MN
Doses	Person correlation coefficient	0.04	-0.09	0.001	0.03	0.1	-0.19	-.0.07	0.1	0.1	0.45
	P-value	0.8	0.66	0.9	0.87	0.45	0.35	0.7	0.4	0.6	0.02*
Work experience	Person correlation coefficient	0.08	0.3	0.1	-0.03	0.08	0.06	0.05	0.2	0.07	0.02
	P-value	0.7	0.05	0.6	0.05	0.6	0.7	0.7	0.3	0.7	0.9

Note: *=statistically significant with p value<0.05

through a comprehensive and meticulous research process, showed that almost all CBC parameters showed a weak positive correlation except for WBC, MCV, and MCH, which showed a negative correlation to dose accumulation. The correlation between blood parameters and working experience has a significant positive correlation ($p<0.05$) except for lymphocytes, which had a negative correlation but were significantly different ($p<0.05$). Meanwhile, the MN rate of radiation workers had a positive correlation with the cumulative absorbed dose and a significant positive correlation ($r = 0.45$, $p<0.05$), as well as when it was associated with work experience that had a positive correlation ($r = 0.02$, $p>0.05$).

Discussion

Ionizing radiation influences human health by breaking chemical bonds of the molecules and causing DNA damage by producing free radicals; hence, proliferative cells can undergo apoptosis. The result of this study showed that from the exposed subjects is obtained out of nine Count Blood Cell (CBC) parameters were still in the range of normal range except out of four parameters such as Lymphocyte, platelet, MCV, and MCHC, had a number/trend to increase compared to those obtained from the control subject. Nevertheless, there is no significant difference among workers. The increase in the number of lymphocytes can be indicated as an indicator of people living with leukemia, while the decrease in the number of lymphocytes shows symptoms of anemia [18, 19]. In line with the result of this study, it was reported that the most affected CBC parameter that increased relatively high was lymphocyte count, followed by platelet and MHCH. Meanwhile, the rest of the CBC parameters, such as HB, leukocytes, hematocrit, and erythrocytes, showed similar values between the exposed and control subjects and did not have a significant difference [15].

In this research, we found that the platelet count in the exposed subject was significantly higher than that of the control subject. This result, in agreement with previous research, reported that higher platelet values were found in the exposed group than in the control group [18]. Platelet activity is associated with the initiation of coagulation cascades. When a blood vessel is damaged, the sub-endothelial surface becomes the primary target site of platelet action, where it is established hemostasis. Another researcher states that the increased level of platelet cells is associated with thrombocytopenia, and in addition, thrombocytopoiesis is one of the most radiosensitive hematopoietic cell lines in humans [20, 21]. However, these alterations depend on an effective radiation dose range and exposure time [22].

The MCV value is identified as a standard hematologic parameter since radiation-exposed workers also have evidence of abnormal deviation. Similarly, an abnormal MCV level can also be seen among direct and non-direct radiation-exposed workers. The MCV indicates microcytic (small average erythrocyte size), normocytic (standard average erythrocyte size), and macrocytic (sizeable average erythrocyte size. The results of some studies yielded an increased level of MCV value caused by chronic exposure [23, 24]. These findings have significant implications for the health and safety of radiation-exposed workers, highlighting the importance of regular health monitoring and safety measures.

The MCHC level commonly was due to the destruction or deformability of RBCs [25]. From the result of the exposed subject, the MHCH level tends to increase its value, although it is not significant compared to the result of the control subject value. As reported in other research, inconsistent results regarding the MCHC level decreased [11, 21]. The result significantly reduced the MCHC level obtained from the exposed subject compared to those from the control subject. Other research reported that the MCHC value from the exposed subject increased significantly compared to that of the control subjects [24]. Ionization radiations can damage stem cells of the hematopoietic system and alter the production of bone marrow-stormed elements, which are essential to maintaining this system [14, 26].

The principal target of ionizing radiation is the DNA molecule, and one of the biological effects of ionizing radiation is gene mutations, including micronucleus (MN) formation [27-29]. The cytokinesis-blocked MN assay, which measures the frequency of micronucleated binucleate cells (MNBNC), is a genomic biomarker for assessing cytogenetic damage and is valuable for monitoring genetic risk [30]. In this study, the occupational exposure of the test group resulted in a significant rise in spontaneous MNBNC frequency ($p<0.05$), which was relatively higher in the exposed subject compared to the control subject but was not significant. This result is consistent with other studies on occupational workers from China, Korea, and Romania, where a higher MN frequency has been reported after exposure to chronic low doses of ionizing radiation [10, 20, 31-33]. These findings highlight the potential health risks associated with chronic low doses of ionizing radiation.

Long-term exposure to low doses of ionizing radiation and exposure time have been reported to adversely affect

human cells, especially radiation workers [9]. Variation was observed in the MNBNC frequency between the exposed subjects concerning the length of service, which is considered a factor affecting cytogenetic endpoints in radiation professionals. The average MN frequencies about experience work/exposure time demonstrate that the work experience >16 years relatively has a higher MN frequency compared to that of <16 years of the exposed group but statistically no significant ($p>0.05$). Previous research reported that the exposure time >20 years was higher frequencies of MN than that <20 years [10]. An individual's health may be unaffected by a single exposure to low-dose radiation, but chronic exposure may result in significant health risks, underscoring the need for caution and further research [34]. The level of MN is also affected by sex, age, lifestyle, disease status of the donor, clastogenic agents, and aneugenic agents [35, 36].

The understanding of the influence of factors related was necessary to age, gender, lifestyle, and disease in MN rates [37]. While the correlations were positive, they were not statistically significant, indicating that the observed relationships could be due to chance rather than a true association. This underscores the need for further research to confirm these findings. The correlation for work experience based on the Sharman analysis showed that almost all CBC parameters had a positive correlation except WBC, MCV, and MCH, which had a negative correlation to dose accumulation. At the same time, the p-value was not significant. A positive weak correlation $r=0.02$ was shown but was insignificant (0.9). As for the frequency of MN with length of work is generally positively correlated ($r=0.4$) and significant $p<0.05$. Moreover, a consistent positive correlation exists between working experience and Hgb [38].

In this study, MN rates were positively but not significantly correlated with other reported studies. Based on the radiation dose that radiation workers received, the data showed that the frequencies of MN increased among the different cumulative radiation dose groups ($p<0.05$). However, the variation of dissimilarity found with other research might be due to the different absorbed radiation doses, workloads, and working hours per week. The study had two limitations. Firstly, the study population was relatively small, which could limit the generalizability of the findings. Secondly, radiation-absorbed dose data was not included in the study, which could affect the accuracy of the results. However, it's important to note that the absorbed dose of none of the radiation workers exceeded the dose limits provided by the International Commission on Radiological Protection (ICRP). Future research needs to assess the radiosensitivity level based on immunity and the individuals' oxidative stress level. Correlation between collective absorbed dose and changes in blood parameters is suggested for future studies. This study and other studies show that long-term exposure to low radiation may change blood parameters.

In conclusion, long-term exposure to low radiation levels may change blood parameters. Occupational exposure to low doses of ionizing radiation to the workers caused genetic instability independently, affecting age, gender, and bi-nucleate cells. A hematological profile based on the CBC test can be used to follow up on the overall health status of radiation workers. A weak correlation was found between individuals' annual doses and their work experience with hematological profiles and micronuclei. Therefore, regular and periodic complete blood count tests and radiation workers' absorbed dose monitoring are recommended, providing a sense of reassurance and security. We suggest that more studies are required on the effects of low doses of ionizing radiations on the blood parameters, with more excellent samples for both females and males, as well as immune status.

Author Contribution Statement

Yanti Lusiyanti: Conceptualization, methodology, investigation, and writing the original draft; Devita Tetriana: Conceptualization, methodology, and reviewing; Viria Agesti Suvifan: Investigation; Teja Kisnanto: Methodology and investigation; Darlina Yusuf: Methodology and investigation; Caecilia Tuti Budiantari: Investigation and reviewing; Harry Nugroho Eko Surniyantoro: English editing, reviewing and final revision; Iin Kurnia Hasan Basri: Methodology and reviewing.

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Ethical Declaration

The ethical approval certificate for this present study was issued by the Health Research Ethics Commission, Ministry of Health, Republic of Indonesia with certificate number LB.02.01/5.2.KE.079/2017.

Conflict of Interest

All of authors state that this study was conducted without any financial relationship construed as a potential conflict of interest.

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