BIODIVERSITAS
Volume 19, Number 5, September 2018
Pages: 1676-1682

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190512

Association between *XRCC1* exon 10 (*Arg399Gln*) gene polymorphism and micronucleus as a predictor of DNA damage among radiation workers

HARRY NUGROHO EKO SURNIYANTORO♥, YANTI LUSIYANTI, TUR RAHARDJO, SITI NURHAYATI, DEVITA TETRIANA

Center for Technology of Safety and Radiation Metrology, National Nuclear Energy Agency of Indonesia. Jl. Lebak Bulus Raya, Pasar Jum'at Jakarta Selatan 12070, Jakarta, Indonesia. Tel.: +62-21-7513906, Fax.: +62-21-7657950, ♥email: harry_nes@batan.go.id

Manuscript received: 1 July 2018. Revision accepted:12 July 2018.

Abstract. *Surniyantoro HNE, Lusiyanti Y, Rahardjo T, Nurhayati S, Tetriana D. 2018. Association between* XRCC1 *exon 10 (*Arg399Gln*) gene polymorphism and micronucleus as a predictor of DNA damage among radiation workers. Biodiversitas 19: 1676-1682.* This study was aimed to examine the association between *XRCC1* exon 10 gene polymorphism and micronucleus frequencies in radiation workers and their relation to the confounding factors. This study involved 37 radiation workers and 37 controls from several hospitals in Indonesia. Genotyping of X-ray cross-complementing group 1 (*XRCC1*) exon 10 gene polymorphism and micronucleus assay were performed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and Cytokinesis-Block Micronucleus assay (CBMN assay), respectively. The results indicated that MN frequencies were not significantly higher in the exposed workers than in controls (20.46±6.42 versus 16.89 ±9.72; P=0.07). The micronucleus frequencies of radiation workers with mutant genotype showed not significantly higher than controls in the same genotypes (22±6.64 versus 11.75 ± 8.13; P=0.11). The confounding factors, like age, years of employment and equivalent doses were significantly associated with micronucleus frequencies (P<0.05). The equivalent dose has a significantly positive correlation with micronucleus frequencies among radiation workers, increasing the MN frequencies by 16.3 per 1 mSv of equivalent dose (P=0.001). The genetic polymorphism of *XRCC1* gene exon 10 demonstrated no association with the extent of DNA damage in the hospital radiation workers. The MN frequencies were strongly associated with age, equivalent dose and years of employment.

Keywords: Gene polymorphism, *XRCC1* exon 10, micronucleus, ionizing radiation, radiation workers

Abbreviations: BER: Base Excision Repair; CBMN: cytokinesis-block micronucleus; DNA: deoxyribonucleic acid; MMR: Mismatch Repair; MN: micronucleus; NER: Nucleotide Excision Repair; PARP: poly-ADP-ribose polymerase; SNP: Single nucleotide polymorphisms; *XRCC1*: X-ray cross-complementing group 1

INTRODUCTION

Experts who work in radiobiology have studied the health risks of radiation workers exposed to low-dose ionizing radiation. The risk is not only in the form of the disease, but also the inherited mutations that indirectly increase the risk of a disease in offspring. Factors affecting health risks from low-dose radiation exposure include interactions between radiation with mutagen and carcinogens, varying repair mechanisms, cell sensitivity to radiation exposure and adaptive response variations that depend on antioxidants and radiation doses (Prasad et al. 2004). Individual survival requires genetic stability. Genetics stability maintaining requires an accurate mechanism of DNA replication and mechanisms to correct errors that occur continuously during the replication process. Less than one per thousand basic changes produce a permanent mutation, while the others will be fixed by DNA repair (Alberts et al. 2002).

Systems available in the human body, such as Mismatch Repair (MR), Base Excision Repair (BER), and Nucleotide Excision Repair (NER) can prevent the DNA damage. It can be repaired by enzymes through the BER pathway. X-ray cross-complementing group 1 (*XRCC1*) is one of the Poly (ADP-ribose) polymerase (PARP) family proteins that play a role in BER pathway by binding a single-strand of DNA and recruiting DNA repair protein (Wood et al. 2001). The *XRCC1* gene is located on chromosome 19q13.2-13.3, 33 kb in length, consisting of 17 exons, encoding a 2.2 kb transcript and 633 amino acids. Some studies (Norjmaa et al. 2016)) have shown that the *XRCC1* gene is related to single-strand breaks and base excision repair pathways. The *XRCC1* gene also plays an important role in DNA damage repair caused by ionizing radiation, X-rays, gamma rays, oxygen and alkylation agents. The *XRCC1* gene as the coder of *XRCC1* protein will produce three important functional enzymes, namely poly-ADP-ribose polymerase (PARP), DNA ligase III, and DNA polymerase β (Audebert et al. 2004).

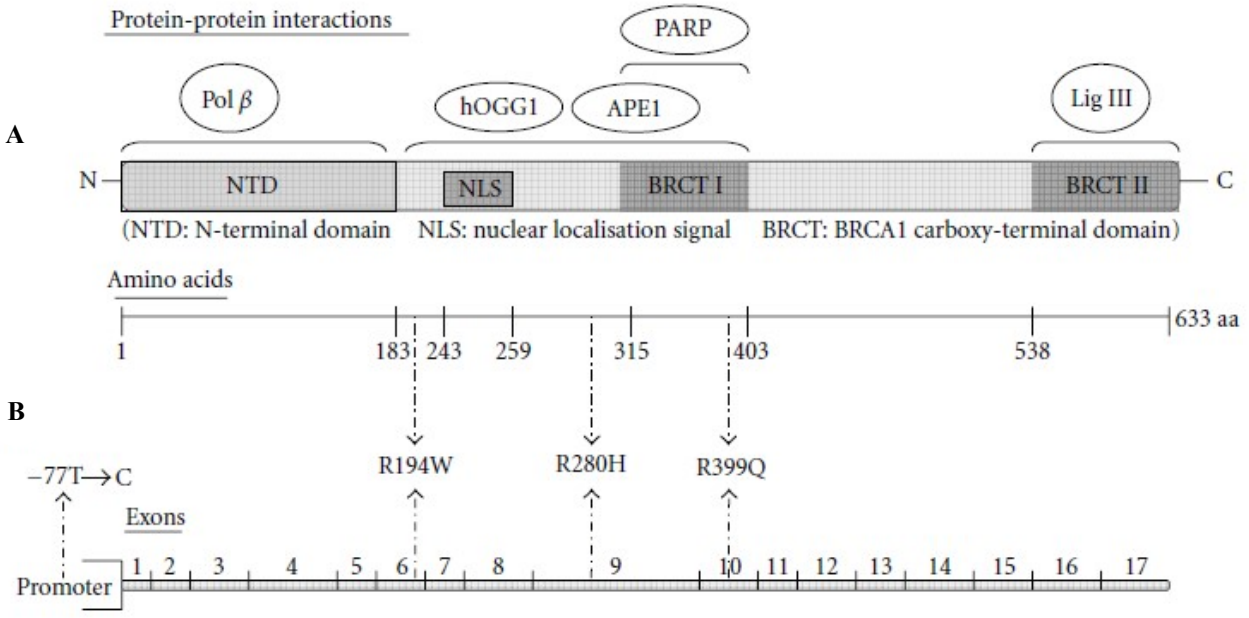

Figure 1.A. The domains of *XRCC1* gene exon 10 and it's interaction with other components of BER. B. The position of Single nucleotide polymorphisms of *XRCC1* gene (-77T>C, Arg194Trp, Arg280His and *Arg399Gln*) (Sterpone and Cozzi 2010)

Single nucleotide polymorphisms (SNPs) are the most common polymorphisms in humans with a frequency ≥ 1% in the population. SNPs that occur in DNA repair genes resulting in decreased DNA repair ability, increased mutation rate and cancer risk (Ochiai 2015). SNPs that occur in the *XRCC1* exon 10 gene change a nitrogen base G to A at codon 399 resulted in changes of amino acid arginine to glutamine (Norjmaa 2016). Some studies have shown an association between *XRCC1* gene polymorphism and DNA damage (Jiang et al. 2006; Zhao et al. 2006; Zhang et al. 2012; Saad-Hussein et al. 2017). One of DNA damage predictors is micronucleus, which is a biomarker of chromosomes damage or loss (Fenech 2007). Micronucleus is formed from fragments of chromosomes left in anaphase during the process of cell division. The cytokinesis-block micronucleus (CBMN), cytome assay method, is used to measure the frequency of micronucleus because it is more specific, accurate and the most widely used for measuring DNA damage in human populations (Heddle 1973; Fenech 2000).

Micronucleus occurrence is an important biomarker as a result of a response to the environment including diet and ionizing radiation exposure. Micronucleus measurements in radiation-working populations or people living in areas with high natural radiation exposure can be used to determine the impact of exposure to the DNA damage (Chang et al. 1997). This study was aimed to examine the correlation between *XRCC1* exon 10 gene polymorphism and micronucleus frequencies in radiation workers and their relation to gender, age, smoking status, years of employment, and an equivalent dose of ionizing radiation.

MATERIALS AND METHODS

Procedures

Study population

The study included 37 medical workers occupationally exposed to low doses of ionizing radiation in the Radiology and Radiotherapy Department at several hospitals in Indonesia and 37 controls who had never been occupationally exposed to ionizing radiation. Questionnaires were given to the subjects to find out complete information about gender, age, smoking status, years of employment, equivalent dose and history of disease ever suffered. Exclusion criteria included diagnostic X-rays, and radiotherapy underwent six months before sampling, which could have affected to the equivalent dose and/or DNA damage. The characteristics of the subjects are shown in Table 1.

There are five different working division among radiation workers: doctor, radiologists, radiotherapists, radiographers, and nurses. Each participant was briefed about the study protocol, with specific written information about the cytogenetic test, the aims of the study and signed informed consent. Blood samples (5 mL) was obtained from each subject (radiation workers and controls) and taken to the laboratory of Molecular Biology for further analysis.

Micronuclei assay

The CBMN-assay was performed as described by Fenech (2007) with some modifications. Lymphocyte cultures were incubated for 48 hours at 37°C, cytochalasin-

B (Sigma-Aldrich, St. Louis, MO) at a final concentration of 3 g/mL was added to cultures to block cytokinesis after 44 hours of incubation. The cultures were stopped at 72 hours, treated with a hypotonic solution for 4 minutes and fixed with two changes of methanol: acetic acid (3: 1, v/v). The fixed cells were spread onto clean glass slides and stained for 10 minutes with a 4% Giemsa solution. Microscopic analysis was performed under a light microscope with a 40 × 10 magnification and CBMN-assay parameters of micronucleus, verified under 1000× magnification. A score was obtained for slides from each subject. The frequency of binucleated cells containing one or more micronuclei was scored in 1000 cells per subject, to determine cytotoxicity following published CBMN-Cyt scoring-criteria refers to IAEA manual (Fenech 2007).

DNA isolation

DNA was isolated from lymphocytes extracted from whole blood using the QIAamp DNA Kit (Qiagen) according to the manufacturer's instructions. The obtained DNA was stored at -20°C.

Genotyping of XRCC1 exon 10

Genotyping of *XRCC1* exon 10 gene polymorphism was performed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), as described previously by Andreassi et al. (2009) with the forward primer was 5'- AGTAGTCTGCTGGCTCTGG-3' and the reverse primer 5'-TCTCCCTTGGTCTCCAACCT-3'. The PCR reactions were carried out with a denaturation of 95°C for 3 minutes, followed by 35 cycles of 15 seconds at 95°C (denaturation), 15 seconds at 60°C (annealing) and 15 seconds at 72°C (extension) and finally 1 minute at 72°C (final extension). Following amplification, PCR products were digested using 10 U of restriction enzyme *Msp*I (BioLabs, Inc.) for 16 hours at 37 °C, and electrophoresed on a 3% agarose gel. The wild-type GG genotype for codon 399 was determined by the presence of two bands at 269 and 133 bp, the mutant heterozygous GA genotype was determined by the presence of three bands at 402, 269 and 133 bp, while the mutant homozygous AA genotype was determined by the presence of the uncut 402 bp band (indicative of the absence of the *Msp*I cutting site).

Data analysis

The data analysis was conducted with SPSS version 16.0 for Windows. All of the data were expressed as mean±standard deviation. Independent sample T-test was used to test micronucleus frequencies difference between exposed workers and controls and to test a significant relationship between micronucleus and various genotypes. Linear regression analysis was performed to assess the relationship between years of employment, equivalent dose, and micronucleus frequencies on exposed workers. Poisson regression analysis was applied to evaluate the influence of gender, age, smoking status, years of employment, and the equivalent dose of ionizing radiation at micronucleus frequencies in the whole population and both groups separately. The genotype and allele frequencies were showed on frequencies distribution table and were checked for consistency with Hardy-Weinberg equilibrium and compared between the radiation workers and controls group by $\chi 2$ tests. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

In this study, single nucleotide polymorphisms (SNPs) of *XRCC1* gene *Arg399Gln* was investigated. The genotype analysis of this SNPs of *XRCC1* gene, for medical radiation workers from several hospitals in Indonesia, was performed using PCR-RFLP method. The characteristic of subjects according to age and gender data was displayed at Table 1, and the genotyping results are shown in Figure 2. Based on Figure 2, GG genotype (wild-type) was showed with 269+133 bp fragment length, GA genotype with 402+269+133 bp, and AA genotype (mutant homozygous) with 402 bp.

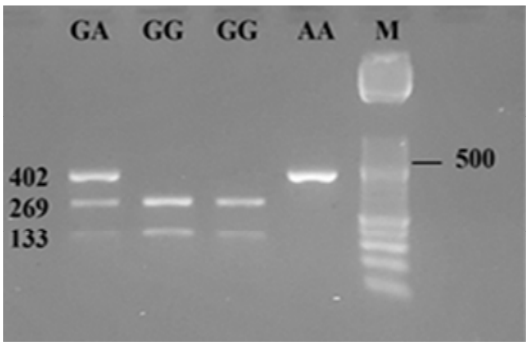

Figure 2. Results of genotyping for *Arg399Gln* polymorphism of *XRCC1* gene on 3% electrophorese gel. GG (wild-type), GA (mutant heterozygous), AA (mutant homozygous), M (DNA ladder 100 bp)

Table 1. Demographic characteristics of the study population

Parameters	Radiation workers	Controls	Total
Sample size (N)	37	37	74
Gender			
Females (%)	19 (51.35%)	18 (48.65%)	37 (50%)
Males (%)	18 (48.65%)	19 (51.35%)	37 (50%)
Age (years)			
Mean±SD	45±8.01	43.08±11.47	44.04±9.94
Range	29-59	20-58	20-59
Smoking status			
Never (%)	31 (83.78%)	30 (81.08%)	61 (82.43%)
Current (%)	6 (16.22%)	7 (18.92%)	13 (17.57%)
Years of employment (years)			
Mean±SD	20.78±7.47	-	-
Range	7-35		
Equivalent Dose (mSv)			
Mean±SD	0.21±0.2	-	-
Range	0.022-0.731		

This study was used totally 74 subjects which consisted of 37 radiation workers and 37 controls. Statistically, the characteristics of subjects, including gender, age, smoking status, years of employment and equivalent dose between radiation workers and controls did not show any differences among the subjects. The range of age was 29-59 years, the range of exposure duration (years of employment) to ionizing radiation for radiation workers was 7-35 years and the range of equivalent dose was 0.022-0.731 mSv (Table 1).

Micronucleus analysis

Micronucleus test results were reported as a total number of micronucleus per 1000 binucleated (BN) cells (Table 2). MN frequencies were not significantly higher in the hospital radiation workers compared to the controls (20.46 ± 6.42 versus 16.89 ± 9.72, P = 0.07). The frequency of MN in the never-smokers group was higher than in the smokers group, both in radiation workers (20.58 ± 6.42 versus 19.83 ± 6.57) and controls (17.77 ± 9.72 versus 13.14 ± 9.85), although the difference was not significant (P=0.19). The present study showed that the MN frequencies being higher in the AA genotypes group compared to the homozygous GG (wild-type) group (22±6.64 versus 21.39±6.42) in radiation workers. On the contrary, a decrease in MN frequency was observed in controls with AA genotype as compared to homozygous GG (wild-type) group (11.75 ± 8.13 versus 16.77 ± 9.84).

The years of employment for radiation workers were 7-35 years with an average working period was 20.78 ± 7.47 and the equivalent dose for radiation workers were 0.022-0.731 mSv with an average dose was 0.21 ± 0.2 (Table 1). Linear regression analysis was used to examine the effect of years of employment and equivalent dose to MN frequencies. There are a significant relationship between equivalent dose and MN frequency (β = 0.508, P = 0.001; Figure 3). On the contrary, no significant relationship between years of employment and MN frequency (β = 0.064, p = 0.706; Figure 4).

Poisson regression analysis results on gender, age, and smoking status are shown in Table 3. Gender has a significant effect on MN frequencies in control (P <0.0001), Age has a significant effect on MN frequencies in radiation workers (P=0.02) and controls (P <0.0001), smoking status has no significant impact on MN frequencies. Both of years of employment and equivalent doses have a significant effect on MN frequencies with P-value 0.044 and 0.001, respectively. Poisson regression analysis in the present study showed that the effect of gender on MN frequencies was higher in males than in females.

Correlation between equivalent dose and years of employment to MN frequencies

Linear regression analysis was used to examine the correlation between equivalent dose and years of exposure to MN frequencies. The result showed positive correlation between equivalent dose and MN frequencies among radiation workers. It means that MN frequency tended to rise with equivalent dose. This correlation have a statistically significant relationship (β = 0.508, P= 0.001) as shown in Figure 3. Correlation between years of employment and MN frequencies have no statistically significant (β = 0.064, P= 0.706) as shown in Figure 4.

Poisson regression analysis was also applied to evaluate the influence of age, gender, smoking status and dose equivalent of ionizing radiation on MN frequencies in the overall population and in both groups separately. The results showed that significant effects are indicated by age (P<0.0001), years of employment (P0.044) and equivalent dose (P=0.001) in the overall population, whereas in the controls group, the confounding factors influenced significantly to the MN frequencies are gender (P<0.0001) and age (P<0.0001), and in the radiation workers group is age (P=0.02), as shown in Table 3.

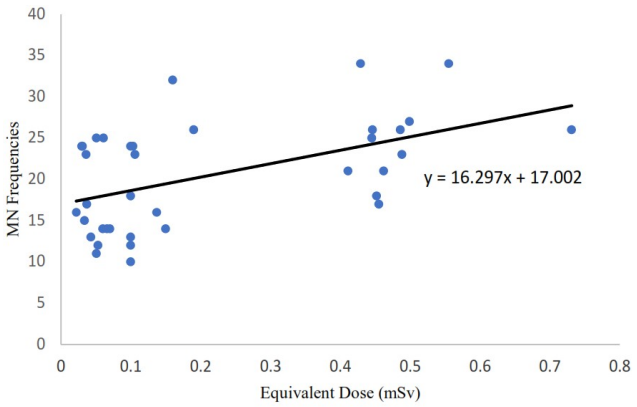

Figure 3. The relationship between DNA damage, assessed as MN frequencies in peripheral lymphocytes, and equivalent dose (mSv). The thick line is the result of linear regression analysis of the data. β = 0.508 , P = 0.001

Figure 4. The relationship between DNA damage, assessed as MN frequency in peripheral lymphocytes, and years of employment to ionizing radiation. The thick line is the result of linear regression analysis of the data. β = 0.064 , P = 0.706

Table 2. Micronucleus frequencies in the study population

	Radiation workers			Controls			P
	Subjects	**MN±SD**	**95% CI**	**Subjects**	**MN±SD**	**95 % CI**	
`All	37	20.46±6.42	18.39-22.53	37	16.89 ±9.72	13.76-20.02	0.07
Never smokers	31	20.58±6.42	18.32-22.84	30	17.77 ± 9.72	14.29-21.25	0.19
Current smokers	6	19.83±6.57	14.57-25.09	7	13.14 ± 9.85	5.84-20.44	0.19
XRCC1 exon 10							
GG	18	21.39±6.42	18.42-24.36	13	16.77 ± 9.84	11.42-22.12	0.14
GA	15	18.93±6.32	15.73-22.13	20	18 ± 9.85	13.68-22.32	0.73
AA	4	22±6.64	15.49-28.51	4	11.75 ± 8.13	3.78-19.72	0.11

Table 3. Poisson regression analysis of confounding factors on MN frequencies in peripheral lymphocytes of the study groups.

Confounding factors	**IRR**	***P***	**95% CI**
All			
Gender (1,2)	1.104	0.09	0.985-1.237
Age (years)	1.024	<0.0001	1.018-1.030
Smoking status (1,2)	1.087	0.296	0.929-1.273
Years of employment (years)	0.981	0.044	0.963-0.999
Equivalent dose (mSv)	1.978	0.001	1.345-2.910
Controls			
Gender (1,2)	1.455	<0.0001	1.213-1.745
Age (years)	1.034	<0.0001	1.026-1.043
Smoking status (1,2)	0.898	0.41	0.695-1.160
Radiation workers			
Gender (1,2)	1.050	0.56	0.891-1.237
Age (years)	1.021	0.02	1.003-1.039
Smoking status (1,2)	0.974	0.81	0.786-1.207

Note: IRR: Incidence Rate Ratio; [a]Gender: 1. Females; 2. Males; Smoking status: 1. Never; 2. Current

Table 4. Genotype and allele frequencies of *Arg399Gln* in the study population

	N (74)	**%**	**Radiation workers (n=37) (%)**	**Controls (n=37) (%)**	***P value***
Genotype codominant					
GG	31	41.89	18 (48.65)	13 (35.13)	
GA	35	47.29	15 (40.54)	20 (54.05)	
AA	8	10.82	4 (10.81)	4 (10.82)	0.39
Allele					
pG			0.69	0.62	
qA			0.31	0.38	

Genotype analysis

The genotype distribution in this study was consistent with the Hardy-Weinberg equilibrium for all the SNPs studied, both in radiation workers and controls, as shown in Table 4. The frequencies of genotypes for radiation workers were GG (48.65%), GA (40.54%) and AA (10.81%) with frequencies of G allele were (0.69) and A (0.31). The frequencies of genotypes for controls were GG (35.13%), GA (54.05%) and AA (10.82%), with frequencies of alleles, were G (0.62) and A (0.38). The results of the χ2-test showed no significant difference in the same genotype between radiation workers and controls (P=0.39).

Discussion

The strength and novelty of the present study is the investigation of biological markers of effect and susceptibility on the same population exposed to the chronic low level of ionizing radiation. To our best knowledge, this research has not been done in Indonesia. The present population study is the first in vivo study that combines genotype analysis in DNA repair genes with both exposure and micronucleus frequency in somatic cells of hospital workers, occupationally exposed to low levels of ionizing radiation, and controls. The use of micronuclei as one of the cytogenetic damage biomarkers has been done in previous research and proven to be a reliable biomarker of molecular DNA damage in the population exposed to ionizing radiation (Sakly et al. 2012). This approach offers the opportunity to complete cancer prevention programs for health surveillance of radiological workers, still based mainly on physical dosimetry.

The results of our study showed that MN frequencies were higher in the radiation workers compared to controls, although the difference was not significant (20.46 versus 16.89, P=0.07). The MN frequencies in current smokers-radiation workers were also higher than current smoker-controls (20.58 versus 17.77, P=0.19). The MN frequencies based on the division of the genotype group also showed the same results. The MN frequencies were higher in the radiation workers compared to controls, although not statistically significant. In the present study, MN frequencies were lower in the current-smoker subjects compared to never-smokers, both in the radiation workers and controls. This fact was in accordance with the previous study which states that nicotine can protect against reactive carcinogens contained in tobacco smoke (Nersesyan et al. 2011).

The subjects used in this study never received exposure exceeded the permitted dose limit, recommended by the

Nuclear Energy Regulatory Agency of Indonesia for radiation workers (20 mSv per year). The results of this study indicate a positive correlation between years of employment and an increase in MN frequency of 0.055 per 1 year, but it is not significant (P=0.706). On the other hand, the radiation workers group will be significantly increased in MN frequencies of 16.3 per 1 mSv of equivalent dose (P=0.001). This is similar to the study by Sakly et. al. (2012) which states an increase of MN frequency in hospital radiation workers in Tunisia, and the previous study which indicates any direct relation between MN frequencies in lymphocytes and radiation dose which can be used for purposes of biological dosimetry (Ozdal et al. 2016).

MN frequencies can indicate the confounding factors such as gender, age, and lifestyle inducing the chromosomal damage. Bonassi et al. (2011) stated that female subjects will experience higher MN frequencies than in male lymphocyte cells, but not significantly different in exfoliated buccal cells. In the other hand, Ferraz et al. (2016) stated that the gender factor did not affect the frequencies of MN, except for age, which is the most influential factor among all of the confounding factors. In our study, gender is significantly influenced only in the control group (P<0.0001), while in the radiation workers did not affect substantially (P=0.56), as well as in the whole population (P=0.09). The previous studies have shown that individuals at age range from 23-50 years will have an increase in MN frequencies, followed by a decline in age above 50 years (Orta & Gunebakan, 2012). The occurrence of decreased MN frequencies in individuals over 50 years is due to decrease cell proliferation ability with increasing age (Milosevic-Djordjevic et al. 2002). In our study, age is significantly influenced by the MN frequencies both in controls (P<0.0001), radiation workers (0.02) and the whole group (P<0.0001). The smoking habit also as a factor that affects the MN frequencies. Bonassi et al. (2003) stated that in most reports, the results are unexpectedly negative, and in many cases, smokers had lower MN frequencies compared to non-smokers subjects. This fact only occurs in smokers with less than 30 cigarettes per day, whereas for smokers with greater than 30 cigarettes or more per day will experience a significant increase in MN frequencies. The current-smokers subjects in this study spent cigarettes less than 16 cigarettes per day, so their MN frequencies were lower than never-smokers subjects. The smoking habit in our study is not significantly influenced to MN frequencies in all of the groups. The influence of these three factors showed the different results in many studies, so it is necessary to do a more in-depth study of these confounding factors.

Radiation workers with 7-35 years of employment and equivalent doses between 0.022-0.731 mSv were also studied in this study using Poisson regression analysis. The results show that years of employment and equivalent doses have a significant influence on MN frequencies with P value 0.044 and 0.001, respectively. This is due to the longer working period caused the increase in equivalent doses accumulation which received by radiation workers. A previous study by Qian et al. (2015) stated that radiation workers with >20 years of exposure time had higher MN frequencies compared to radiation workers with <20 years of exposure time. Syaifudin et al. (2017) also stated that the MN frequencies after irradiation of lymphocytes increased with the increased radiation dose, mainly for higher doses (>2 Gy). This is in accordance with the previous study which showed that the DNA damage is significantly affected by equivalent doses of ionizing radiation exposure and the length of the work period. Higher equivalent doses produced higher MN frequencies (Tucker, 2008).

Previous studies suggested that DNA repair (*XRCC1* and XRCC3) and folate-metabolism genes (MTHFR) also influence MN formation (Iarmarcovai et al., 2008). The genetic polymorphisms of DNA repair genes play an important role in the sensitivity of the individual genomes exposed to ionizing radiation (Damiola et al. 2014). The genotype analysis in this study showed no association between *XRCC1* gene polymorphism in all of genotypes groups and MN frequencies (Table 2). This result may be due to insufficient sample size thereby decreasing the strength of the research statistics. There are some limitations to this study. First, this retrospective study is characterized by some disadvantages including insufficient sample size, unavailable data, no randomization and limited accuracy of medical records. Second, the average of equivalent doses in this study was collected from the health records of workers exposed to ionizing radiation, and these data may be unstable since the data may be influenced by factors such as the population background. Finally, a population with 7-35 years of employment was selected, which may affect the precise measurements of the equivalent dose, consequently creating variances of the MN frequencies.

In conclusions, our study reported that radiation workers had higher MN frequencies compared than controls. The genetic polymorphism of *XRCC1* gene exon 10 demonstrated no association with the extent of DNA damage in the hospital radiation workers. The equivalent dose has a significantly positive correlation with micronucleus frequencies among radiation workers. Furthermore, it was found that the MN frequencies were strongly associated with age, equivalent dose and years of employment. In the subsequent studies, it is necessary to examine the DNA repair genes polymorphism in populations with controlled non-genetic factors, such as lifestyles, environments, and exercises that affect the MN frequency as a biomarker of DNA damage.

ACKNOWLEDGEMENTS

We would like to thank our co-workers in Radiobiology Laboratory, Nuclear Medicine Technique and Radiation Biology Division, Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency of Indonesia (BATAN), Jakarta for technical support. This study was fully supported by the Center for Technology of Safety and Radiation Metrology, National Nuclear Energy Agency of Indonesia.

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