Micronucleus Frequencies and DNA Repair Gene *XRCC3* Polymorphism in Radiation Workers of Center for Multi Purpose Reactor (PRSG), BATAN

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ABSTRACT

The carcinogenic effects of low radiation dose have not been fully understood until now. Studies on individuals that occupationally exposed to low radiation dose can help to address this question. Aim of this study was to assess the micronucleus frequencies as indicator of DNA damage in radiation workers that are occupationally exposed to low radiation dose. The influence of single nucleotide polymorphism in *XRCC3* gene on the frequency of micronuclei was also evaluated in this study. The confounding factors effect like gender, age and smoking status in micronucleus frequencies was assess in all samples. A total 60 subjects consisted of 30 radiation workers from Center of Multi Purpose Reactor, National Nuclear Energy Agency of Indonesia and 30 control samples were enrolled in this study. Results showed that the different of MN frequencies in radiation workers compared to control samples were not statistically significant [0.019 *vs* 0.021; p = 0.549]. Age and smoking status did not affect micronucleus frequencies in all samples (p = 0.723 and 0.828). Micronucleus frequencies in females were higher compared to males, even the different was not significant (p = 0.3). Radiation workers with variant alleles for *XRCC3* polymorphism was not showed a higher MN frequencies compared than controls with the same genotypes. The small numbers of samples that have *XRCC3* variant alleles found in this study may contribute that there was no significant different of MN frequencies between wildtype allele (*Thr/Thr*) and mutant allele (*Thr/Met* or *Met/Met*). Further study using a larger samples and MN assay in combination with human pan-centromeric probe should be conduct to ensure this study results. Other SNP in *XRCC3* gene also should be examining in further study to know whether those SNP can contribute to the increase of MN frequencies.

INTRODUCTION*

Radiation workers received occupational exposure to ionizing radiation in their works. Irradiation exposure of this group is mainly external, and come from γ-radiation, with a negligible exposure to β-radiation and neutrons. The occupational exposure is a prolonged exposure to low doses and low dose rates. It is now known that the possible adverse health effects of such radiation exposure are stochastic effect like a malignant diseases [1]. Until now several studies already conducted to investigate the effect of occupational exposure in hospital staff, people living in high background radiation areas (HBRA), Chernobyl cleanup workers, and radiation workers of nuclear facilities using conventional cytogenetic analysis [2].

Among variety of methods exist for cytogenetic analysis, a micronucleus (MN) assay in peripheral lymphocytes allows a much faster detection of chromosomal damages [3]. Micronucleus assay has many advantages compared to other cytogenetic assays since it is rapid and requires less specialized expertise, and can be applied to monitor a big population. Micronucleus has been used in many biomonitoring studies and considered as a valuable biomarker of occupational exposure to

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ionizing radiation in human population [4].

The occurrence of MN is also an important biomarker for DNA repair capacity. It is well known that ionizing radiation can induce double strand breaks (DSBs) in DNA and most of DSBs were repair rapidly and accurately [5]. Genetic variation in DNA repair genes may influence individual DNA repair capacity and risk of developing cancer. Polymorphisms in genes that participate in different DNA repair pathways have been identified and they are believed to be related to cancer susceptibility and carcinogenesis [6]. The association of single nucleotide polymorphism (SNPs) in relevant genes with MN frequencies in peripheral blood lymphocytes (PBLs) represents a valuable tool for a better understand the role of SNPs in the pathogenesis of diseases, because the latter is one of the best-validated DNA damage biomarker that is sensitive to a wide range of endogenous, environmental and lifestyle factors that can harm the genome [7].

Among many genes involved in DNA repair pathways *XRCC3* was selected to analyze in the presents study. The *XRCC3* gene is located on the long arm of chromosome 14q32.33. *XRCC3* (x-ray repair cross complementation group 3) encodes a member of the RecA/Rad51-related protein family that participates in DSBs repaired via the homologous recombination (HR) repair pathway. A transition of cytosine (C) to thymine (T) (C → T) in exon 7 of the *XRCC3* gene, have been shown to be associated with malignancies. A polymorphism of *XRCC3* gene can affect the enzyme's function and its interaction with other proteins that involved in DNA damage repair [8,9].

Aim of this study was to evaluate the micronucleus frequencies in lymphocytes of 30 radiation workers from Center for Multi Purpose Reactor (PRSG), National Nuclear Energy Agency of Indonesia (BATAN) and 30 controls samples. This study also examines the influence of genetic polymorphisms T241M *XRCC3* on the MN frequencies in radiation workers and controls. As we concern this is the first study in Indonesia that examines the influence of genetic polymorphisms T241M *XRCC3* on the MN frequencies in radiation workers.

EXPERIMENTAL METHOD

Blood sampling and Culture

Blood samples from 30 healthy adult subjects from Reactor Operational Division of Center for Multi Purpose Reactor (PRSG), National Nuclear Energy Agency of Indonesia (BATAN) and control samples were collected by venipuncture using heparinized and EDTA vacutainer tubes (BD Vacutainer systems). The controls samples were those who had never been occupationally exposed to ionizing radiation. Reactor Operational Division was choose because based on the report in 2011, it was known that this division have the highest mean of effective dose for whole body exposure in 2007 until 2011. Informed consent was obtained from all donors. A detailed questionnaire was used to obtain information on age smoking habits. The ethical study was obtained from Komisi Etik Penelitian Kesehatan, Fakultas Kedokteran Universitas Diponegoro dan RSUP Kariadi Semarang number 357/EC/FK-RSDK/2016 date April 1, 2016. Peripheral blood lymphocytes then were cultured according to the micronucleus assay protocol in IAEA publication [10]. The scoring criteria were based on Fenech publication in 2007 [11]. One thousand binucleated lymphocytes (BNC) were scored at the magnification of 400× for each sample.

Extraction of Genomic DNA

Genomic DNA was purified from whole blood using the QIAamp DNA Blood Midi Kit (Qiagen) according to the manufacturer's instructions. All DNA preparations then stored at 4°C until use.

Genotype analysis by PCR-RFLP

Isolated DNA was amplified with a PCR mixture consisting of PCR Master Mix 10 µL, Forward Primer (0.5 µM) 5 µL, Reverse Primer (0.5 µM) 5 µL and DNA (50 ng) 5 µL. The primer pairs used were F5'-GGTCGAGTGACAGTCCAAAC-3' and R5'-CTACCCGCAGGAGCCGGAGG-3'. The PCR conditions were as follows: a 10 min denaturation step at 95°C followed by 30 cycles of 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, respectively, and a final extension step at 72°C for 10 min. Polymorphisms of *XRCC3* (Thr241Met) (rs861539) was determined by using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. The PCR amplicons were digested at 37°C for 3 h with discriminating restriction enzyme that recognized and cut either the wild-type or variant sequence site. The restriction enzyme used was *NlaIII*. The digested PCR products were analyzed on 3%

agarose gels and stained with ethidium bromide. Detail of PCR product and restriction fragment sizes are outlined in Table 1.

Table 1. Details of PCR and RFLP fragment sizes of *XRCC3* T241M

PCR	Restriction digestion		
Product Size (bp)	Enzyme	Genotype	Fragment sizes (bp)
415	*NlaIII*	Thr/Thr	274, 141
		Thr/Met	274, 170, 141, 104
		Met/Met	170, 141, 104

Statistical Analysis

The Kolmogorov-Smirnov test was applied to know the distribution of data. Since the distribution of MN frequencies was not normal then a Mann-Whitney non parametric test was used as a statistical test using SPSS 22.0 statistical software. Hardy-Weinberg equilibrium was tested on genotypic data using Michael H Court's online calculator for determine whether the observed genotype frequencies are consistent with Hardy-Weinberg equilibrium [12].

RESULTS AND DISCUSSION

MN frequencies in radiation workers and controls

The mean MN frequencies in radiation workers and control groups were presented at Table 2. Statistical analysis revealed that the different of MN frequencies was not significant (p = 0.549). This result was in agreement with another study that found the insignificant different in MN frequencies between radiation workers and control samples [3]. In contrast a study in Tunisian revealed that there was a significant increase of the MN frequencies in the lymphocytes of the hospital radiation workers compared to the control group [13]. The different in MN assay technique used in this study compared to study in Tunisian may affected the different result of MN frequencies. Study in Tunisian used a MN centromere assay that combines MN assay with pan-centromeric probe to detect MN derived from acentric chromosome fragments. It is well known that most of the radiation induced MN originates primarily from acentric fragments while spontaneous MN contains especially whole chromosomes. There is now some evidence that centromere identification in MN can increases the sensitivity of the CBMN assay in the low dose range [10,13].

Another factor that may contributed the insignificant different of MN frequencies between radiation workers and control samples was the accumulated dose of radiation. Based on report in 2011 the mean of effective dose for whole body exposure or *Hp(10)* from 2007 until 2011 in Reactor Operational Division workers of Center for Multi Purpose Reactor was 3.27 mSv [14]. This value was much lower compared to other study that found a significant different in dicentric, ring and fragment chromosomes when the accumulated doses value was more than 300 mSv [1]. Another study that evaluated the radiation workers in Hospital also showed a similar result with this study. Ropolo et al. did not found a significant different in MN frequencies between group that have an accumulated doses less than 2.5 mSv compared to those that have an accumulated doses between 2.5 mSv to 10 mSv and more than 10 mSv [3].

It also can be seen that the mean MN frequencies in radiation workers was lower compared to control groups. It is possible that the radioadaptive response phenomenon was developed in radiation workers. Radioadaptive response (RAR) defined as the biological response where an exposure of low dose radiation (priming dose) to organism can induces the protect mechanisms against the detrimental effects of a subsequent larger radiation exposure (challenging dose) [15,16]. Assessment of individual radiosensitivity in Reactor Operational Division workers of Center for Multi Purpose Reactor should be conduct in further research to prove the radioadaptive response phenomenon.

Table 2. The MN frequencies in radiation workers and control groups

Group	Mean MN Frequencies	p value
Radiation workers	0.019	0.549*
Control	0.021	

*>0.05

Gender, age and smoking habits effect in MN frequencies

Our study revealed there were insignificants different of MN frequencies in respect to gender, age and smoking status (Table 3). Interestingly MN frequencies in female were higher compared to males, even though the different was not significant. Researcher suspect

that the higher of MN frequencies in female correlated with greater tendency of the inactive X-chromosome to be lost as an MN relative to other chromosomes, and to the fact that females have two copies of the chromosome compared to only one in males [17,18]. A research conducted by Jones et al. showed that in 19.9% of the cells scored at least one gender chromatin positive MN was present [19]. Other research by Hando et al. found that X- chromosome present in 72.2% of the MN scored and a significant increase with age in the number of MN containing an X-chromosome [20].

Increase of MN respect to age is due to a combination of several factors which were (i) the cumulative effect of acquired mutations in genes involved in DNA repair (ii) numerical and structural aberrations in chromosomes caused by exposure to endogenous genotoxins, inadequate nutrition, exposure to environmental or occupational genotoxins, as well as a wide range of unhealthy lifestyle factors [17]. Here in this study we did not found the increase of MN frequencies in respect to age. Smoking status in this study also did not affect the MN frequency in all samples. It is possible that using a larger sample number an age and smoking status effects on micronucleus frequencies can be found.

Table 3. The MN frequencies in respect to gender, age and smoking status in all samples

Variables	Mean MN Frequencies	*p* value
Gender		
Male	0.018	0.3*
Female	0.024	
Age		
>50	0.020	0.828*
<=50	0.020	
Smoking Status		
Yes	0.0205	0.753*
No	0.0204	

*>0.05

Distribution of T241M *XRCC3* polymorphism and its association with MN numbers

Distribution of *XRCC3* genotypes and variant allele frequencies in radiation workers and controls were presented in Table 4. There were no statistically significant differences in the genotype distributions between radiation workers and controls (p = 0.1607). The genotype distributions in all groups were in Hardy–Weinberg equilibrium. Allele frequencies were also in agreement with previous publication [6].

Table 4. Distribution of *XRCC3* genotypes in radiation workers and the controls

Gene	Genotype	Radiation Workers, n (%)	Controls, n (%)	Total, n (%)
XRCC3	*Thr/Thr*	28 (93.3)	29 (96.6)	57 (95)
	Thr/Met	2 (6.6)	1 (3.3)	3 (5)
	Met/Met	0	0	0
Allele frequency	*Thr*	0.50	0.48	0.98
	Met	0.009	0.011	0.02

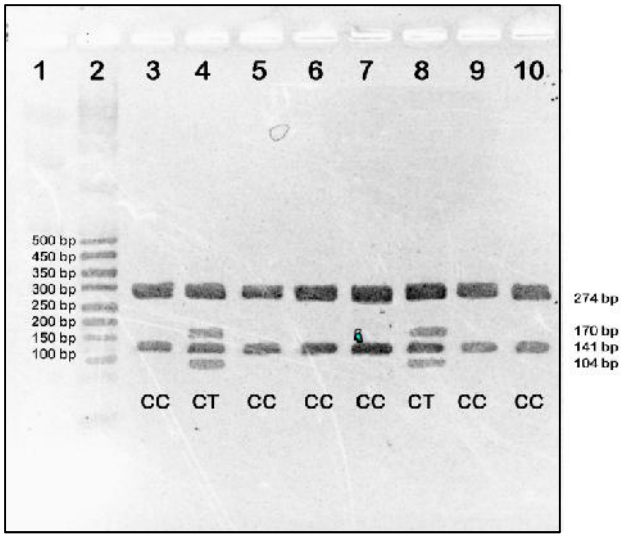

Fig. 1. PCR-RFLP analysis of *XRCC3* Thr241Met polymorphism. Lane 1: blank, lane 2 : molecular marker 50 bp DNA ladder, lanes 4 and 8 : heterozygous Thr/Met variant genotypes, lane 3,5,6,7,9,10: homozygous wild-type Thr/Thr genotype.

As can be seen in Table 4 and Figure 1 that we only found two samples in radiation workers and one in controls that have *Thr/Met* genotype. From all samples that have *Thr/Met* genotype, only one person in controls that have MN number more than the background frequency (0-40) which was reach 50 in 1000 BNC. Two others have a MN number below than 40 in 1000 BNC. Based on this finding it cannot be concluded whether T allele can increase the MN frequencies. In contrast with this study, Andreassi et al. found a correlation between T241M *XRCC3* and MN frequencies in interventional cardiologist [21]. Different in sample ethnic may be the reason why in this study the micronucleus and T241M *XRCC3* association was not found. Other factor that also contributed why in this study a correlation between T241M *XRCC3* and MN frequencies was not found is the samples used

were too homogeneous. Since most of the samples especially in radiation workers were a Javanese and in the same age ranges. It is also possible that the T241M in *XRCC3* was not a specific subtype of polymorphism that correlates with MN frequencies. Other polymorphism subtypes in *XRCC3* likes A4541G (rs1799794) and A17893G (rs1799796) maybe a more specific polymorphism correlate with MN frequencies induce by low radiation dose exposure. A recent meta-analysis study revealed that rs1799794 polymorphism may have a protective effect against late adverse effects of radiotherapy [22]. It is also possible that rs1799794 may have a protective effect against chronic low radiation dose exposure, since in our study the micronucleus frequencies in radiation workers was lower compared to controls. An examination on rs1799794 and rs1799796 polymorphism subtypes and it is correlation with MN frequencies should be conduct in further study.

CONCLUSION

In this study we did not found a significant different in micronucleus frequencies of radiation workers compared to control samples. A correlation between age, gender and smoking habit and MN frequencies also not existed in this study. An association between T241M *XRCC3* and MN frequencies also was not found in this study, since only a limited sample with *Thr/Met* or *Met/Met* genotype found. From three samples with *Thr/Met* genotype only one sample harbor MN numbers more than 40 and it was found in control group. Further study using MN assay in combination with pan-centromeric probe should be conduct to ensure this study results. Other SNP in *XRCC3* gene also should be examining in further study to know whether those SNP can contribute to the increase of MN frequencies.

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