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Genetic polymorphism in DNA base excision repair gene XRCC1 among medical radiation workers

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ABSTRACT

X-rays repair cross-complementing group 1 (XRCC1) gene is one of the gene that plays an important role in base excision repair system (BER) and DNA repair both single and double strand breaks. Individuals with XRCC1 exon 10 (Arg399Gln) gene polymorphisms and carrying 399Gln allele variants (A allele) have a greater risk of DNA damage than their wildtype, 399Arg. The aim of this study was to examine the genotype frequencies of single nucleotide polymorphisms (SNPs) of XRCC1 exon 10 among medical radiation workers. This study involved 77 samples from several hospitals in Indonesia. Genotyping of XRCC1 exon 10 gene polymorphism was performed using PCR-RFLP. Individuals carrying A allele had lower frequency than that is carrying their wildtype of 399Arg (0.39 vs. 0.61). The results indicated that 39% of medical radiation workers had a risk of repair efficiency of DNA damage and might influence an individual's risk of cancer. Ionizing radiation induces many types of damage to DNA, requiring multiple repair pathways to restore genomics integrity. Other important genes/pathways, especially those for DNA double-strand break repair, might also play a role and should be further investigated. Furthermore, polymorphisms leading to inefficient DNA repair might also be associated with late reactions to radiotherapy.

ABSTRAK

Gen *X-rays repair cross-complementing group1* (XRCC1) adalah salah satu gen yang berperan penting dalam BER dan perbaikan DNA baik kerusakan untai tunggal maupun ganda. Individu dengan polimorfisme gen XRCC1 exon 10 (Arg399Gln) dan membawa varian alel 399Gln (alel A) memiliki risiko kerusakan DNA lebih besar daripada wildtype, 399Arg. Tujuan dari penelitian ini adalah untuk menguji frekuensi genotip polimorfisme nukleotida tunggal (SNPs) dari XRCC1 exon 10 pada petugas radiasi medis. Penelitian ini melibatkan 77 sampel dari beberapa rumah sakit di Indonesia. Uji genotip polimorfisme gen XRCC1 ekson 10 dilakukan dengan menggunakan teknik PCR-RFLP. Individu yang membawa alel A memiliki frekuensi lebih rendah daripada yang membawa wildtype 399Arg (0,39 vs 0,61). Hasil ini menunjukkan bahwa 39% pekerja radiasi medis memiliki risiko berkurangnya efisiensi perbaikan kerusakan DNA dan dapat mempengaruhi risiko kanker seseorang. Radiasi pengion menginduksi berbagai jenis kerusakan pada DNA dan membutuhkan beberapa jalur perbaikan untuk memulihkan integritas genomik. Gen/jalur penting lainnya, terutama untuk perbaikan untai ganda DNA, mungkin juga berperan dan perlu diteliti lebih lanjut. Selanjutnya, polimorfisme yang menyebabkan perbaikan DNA yang tidak efisien juga terkait dengan respon terhadap radioterapi.

Keywords:
DNA damage
XRCC1
genetic polymorphism
DNA repair
medical radiation workers

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INTRODUCTION

The role of ionizing radiation on the human body, especially against deoxyribonucleic acid (DNA) damage is very important to be investigated, because humans cannot escape from exposure to ionizing radiation in their daily activities. During this time, the use of ionizing radiation in diagnostic and treatment devices has been widely used. This contributes to the radiation dose in the population.[1] The most affected population group is radiation workers in hospitals that are consistently exposed to low doses of ionizing radiation.

Radiation exposure consists of two types, a high doses and low doses. High doses of radiation exposure are generally known to have an effect, including cancer induction. While exposure to low-dose radiation is still less clear.[2] Exposure to ionizing radiation to the human body causes many adverse effects, especially in cellular DNA. This DNA damage through various means and to overcome it requires the action of DNA repair enzymes to maintain the integrity of DNA. Thus, DNA repair enzymes play an important role in maintaining genomic integrity and various DNA functional damage.[3-5]

DNAdamage can be caused by endogenous and exogenous factors. The main endogenous factor is reactive oxygen species (ROS) whereas exogenous damage is caused by environmental factors such as ultraviolet rays, ionizing radiation and chemicals. DNA repair pathways consist of 3 types, namely direct reversal repair, single strand break repair and double strand break repair systems. Single strand break repair (SSBR) is the most important DNA repair system. The SSBR consists of 3 types, namely baseexcision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR). BER is a simple repair system that works in cells to repair single DNA damage caused by endogenous factors, NER is a complex repair system for repairing larger areas caused by exogenous factors, whereas the MMR plays a role for Fix base pair mismatch during the post-replication process.[6]

BER system, plays a very important role in the human body to maintain DNA integrity, prevent cancer and DNA damage. BER is involved in the repair of oxidized bases and single strand breaks DNA after exposure by ROS, including ionizing radiation. Both oxidized brass and Single strand break, formed by ROS, are a major cause of genetic stability and cellular viability. This will lead to faster mutation rates and increase chromosomal damage. ROS are produced from endogenous and exogenous sources, including ionizing radiation.[7,8]The BER pathway works by removing and replacing the damaged DNA base. This process begins with the release of DNA bases that are damaged specific glycosylase DNA enzymes, followed by sugarphosphate chain cutting, apurinic/apyrimidinic (AP) site cutting by endonuclease, DNA synthesis and ligation. This biochemical reaction involves the enzyme 8-oxoguanine DNA glycosylase 1 (*OGG1*), AP endonuclease 1 (*APE1* or *APEX1*), poly (ADPribose) polymerase-1 (*PARP*-1), polynucleotide kinase, DNA polymerase-β.[9]

X-rays repair cross-complementing group 1 (*XRCC1*) gene has a large role in DNA repair, which is as a scaffolding protein in BER, DNA repair of both SSBR and double-strand breaks, maintaining genomic stability in human cells.[10-12]XRCC1 protein deficiency may decrease the ability of DNA repair and lead to increased hypersensitivity to agents that damage DNA and ionizing radiations.[13] *XCRR1* gene is located on chromosome 19q13.2 and consists of 17 exons encoding 633 amino acids. There are 3 SNPs that occur in *XRCC1*, the genetic variant (C>T) in exon 6 which converts Arg to Trp (Arg194Trp), genetic variant

(G>A) in exon 9 which converts Arg to His (Arg280His) and genetic variant (G>A) in exon 10 which converts Arg to Gln (Arg399Gln). All of SNPs can decrease DNA repair activity.[14] This study focused on the genetic variation Arg 399 Trp in an exon 10.

Single nucleotide polymorphism (SNPs) is the most common polymorphisms in humans with a frequency≥ 1% of the population. SNPs that occur in DNA repair genes result in decreased DNA repair ability, increased mutation rate and cancer risk.[15] SNPs that occur in the *XRCC1* exon 10 gene produced 2 allele variants, namely 399 Arg and 399Gln. 399Gln alleles can be a risk factor for head and neck cancer, lung adenocarcinoma,[16] breast cancer in African-Americans,[17] stomachs, colon and esophagus.[18,19] The aim of the study was to examine the genotype frequencies of SNPs of *XRCC1* exon 10 among medical radiation workers.

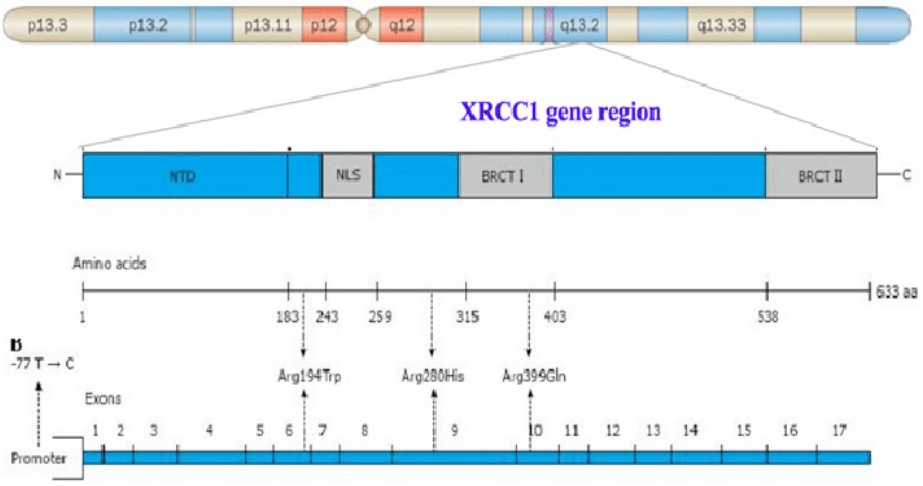

FIGURE 1. The schematic diagram shows the regions of XRCC1 gene and shows the structure of XRCC1 with the locations of single nucleotide polymorphisms: Arg194Trp, Arg280His and Arg399Gin[12]

MATERIALS AND METHODS

Blood samples processing

Blood samples were obtained from 77 (39 male and 38female, mean age 37.44 ± 11.65 years) radiation workers at several hospitals in Indonesia. Genomic DNA was purified from lymphocytesextracted from whole blood using the QIAamp DNA Kit (Qiagen) according to the manufacturer's instructions. The obtained DNA was stored at -20°C until analysis. An informed consent was obtained from each subject at the start of this study.

PCR-RFLP genotyping assays

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.*[20] with the forward primer was 5'- AGTAGTCTGCTGGCTCTGG-3' and the reverse primer5'-TCTCCCTTGGTCTCCAACCT-3'.The PCR reactions were carried out with denaturation of 95°C for 3 min, followed by 30 cycles of 15 sec at 95°C, 15 sec at 60°C and 15 sec at 72°C and final 1 min at 72°C. 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).

Statistical analysis

The statistical analysis of the data were conducted with SPSS version 16.0 for windows. Data were expressed asmean ±SD (standard deviation). Characteristics of the subjects were analyzed by the Mann-Whitney U test. Genotype and allele frequencies were shown on frequencies distribution table. A p value < 0.05 was considered to be significant.

RESULTS

In this study, a SNPs of the *XRCC1* gene Arg399Gln was investigated. The genotype analysis of these SNPs of the *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.

TABLE 1. Characteristics of subjects

Character	Subjects	p
Gender		
• Male	39	0.909
• Female	38	
Age (years)	37.44 ± 11.65	0.527

This study was used 77 samples which consisted of 39 male and 38 female. Statistically, the characteristics of age and gender did not show any differences among the subjects.

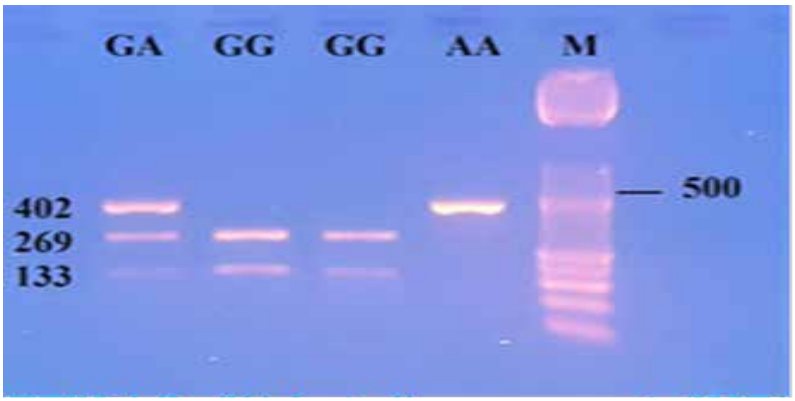

FIGURE 2. Results of genotyping for Arg399Gln polymorphism of *XRCC1* gene on 3% electrophorese gel. GG (wildtype), GA (mutant heterozygous), AA (mutant homozygous), M (DNA ladder 100 bp)

Based on FIGURE 2, GG genotype (wild type) was shown with 269+133 bp fragment length, GA genotype with 402+269+133bp, AA genotype with 402bp. Overall, the variant allele frequencies were 0.61 for *XRCC1* 399Arg (G allele) and 0.39 for *XRCC1* 399Gln (A allele). The genotype frequencies of XRCC1 gene polymorphism showed in TABLE 2.

TABLE 2. Genotype frequencies of *XRCC1* gene polymorphism (n = 77)

Genotype	n	Frequency (%)
Genotype codominant		
• GG	31	40.26
• GA	32	41.56
• AA	14	18.18
Dominant		
• GG	31	40.26
• GA+AA	46	59.74
Recessive		
• GG+GA	63	81.82
• AA	14	18.18
Allele		
• G	94	61.04
• A	60	38.96

DISCUSSION

All the genotypes distributions were in Hardy-Weinberg equilibrium. Several reports indicate that the variant alleles of the repair polymorphisms examined may truly affect DNA repair function. The SNPs in the *XRCC1* exon 10 gene resulted in the substitution of a base nitrogen number 28152, guanine (G) to adenine (A) at codon number 399, is resulting in amino acid conversion fromarginine (CGG) into glutamine (CAG). This polymorphism is in the BRCT domain which is very important to bind PARP-1. PARP-1 will quickly bind the SSBs and will be activated soon. Activation of PARP-1 will increase DNA protein accumulation in damaged DNA. This polymorphism given will decrease the interaction of PARP-1, so DNA repair proteins can not work properly.[21]

Several previous studies have shown different results. Norjmaa *et al.*[22] reported that patients with Arg/Gln (mutant heterozygous) and Gln/Gln (mutant homozygous) genotypes are at greater risk of myelodysplastic syndrome (MDS) than patients with Arg/Arg (normal) genotypes. Arg399Gln genotype carrier variants have higher levels of DNA adducts,[23] greater risk of ionizing sensitivity[13] and DNA damage associated with smoking habits.[24-26] A meta-analysis study in the Asian population shows that patients with 399Gln alleles have a higher risk of breast cancer (OR = 1.54; 95% CI: 1.18-2.01).[27]

The radiation workers in this study, such as radiographers, X-ray machine operators and radiologists worked in installations using low-dose ionizing radiation sources. These results in susceptibility to DNA damage due to exposure to ionizing radiation, especially DNA repair gene. Ionizing radiation is the one that has high energy and is able to release ions that can break covalent bonds, which in turn can damage the structure of human DNA. Another result is the emergence of ROS that can oxidize proteins and lipids, and induce the formation of SSBs. All such damage can cause cell death.[28] Ionizing radiation includes X-rays, gamma rays, alpha, beta and neutron particles. The radiation dose is measured by the amount of radiation absorbed by 1 kg of tissue in Gray (Gy).[29]

The nuclear energy regulatory agency of Indonesia arranges the average effective dose limit value for radiation workers is 20 mSv per year averaged over 5 years. It means that doses accumulated for 5 years should not exceed 100 mSv. The average equivalent dose foreyepieces is 20 mSv per year, the average equivalent dose forthe skin is 500 mSv per year and the equivalent dose for the hand or foot is 500 mSv per year.[30]

CONCLUSION

Individuals carrying a *XRCC1 399Gln* (A allele) havea lower frequency than *399Arg*(0.39 vs. 0.61). Our results suggested that 39% of medical radiation workers have a risk of repair efficiency of DNA damage and might influence

an individual's risk of cancer. Ionizing radiation induces many types of damage to DNA, requiring multiple repair pathways to restore genomics integrity. Other important genes/pathways, especially those for DNA double-strand break repair, might also play a role and should be further investigated. Furthermore, polymorphisms leading to inefficient DNA repair might also be associated with late reactions to radiotherapy.

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REFERENCES

1. UNSCEAR, Ionizing Radiation: Sources and Biological Effects, United Nations Scientific Committee on Effects of Atomic Radiation, New York, 1982.
2. Brenner DJ, Doll R, Goodhead DT, Hall EJ, Land CE, Little JB, *et al*. Cancer risks attributable to low doses of ionizing radiation: assessing what we really know. Proc Natl Acad Sci USA 2003; 100(24):13761-6. https://doi.org/10.1073/pnas.2235592100
3. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001; 411(6835):366-74. https://doi.org/10.1038/35077232
4. Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. J Natl Cancer Inst 2000; 92(11):874-97. https://doi.org/10.1093/jnci/92.11.874
5. Friedberg EC. DNA damage and repair. Nature 2003; 421(6921):436-40. https://doi.org/10.1038/nature01408
6. Wood RD, Mitchell M, Sgouros J and Lindahl T. Human DNA repair genes. Science 2001; 291(5507):1284-9. https://doi.org/10.1126/science.1056154
7. Hu Z, Ma H, Chen F, Wei Q, Shen H. XRCC1 polymorphisms and cancer risk: a meta-analysis of 38 case-control studies. Cancer Epidemiol Biomarkers Prev 2005; 14(7):1810-8. https://doi.org/10.1158/1055-9965.EPI-04-0793
8. Schneider J, Classen V, Helmig S. XRCC1 polymorphism and lung cancer risk. Expert Rev Mol Diagn 2008; 8(6):761-80. https://doi.org/10.1586/14737159.8.6.761
9. Norjmaa B, Tulgaa K, Saitoh T. Base excision repair pathway and polymorphisms of XRCC1 gene. J Mol Pathol Epidemiol 2016; 1:1-4.
10. Parsons JL, Dianov GL. Co-ordination of base excision repair and genome stability. DNA Repair 2013; 12(5):326-33. https://doi.org/10.1016/j.dnarep.2013.02.001
11. Audebert M, Salles B, Calsou P. Involvement of poly (ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. J Biol Chem 2004; 279(53):55117-26. https://doi.org/10.1074/jbc.M404524200
12. Thompson LH, West MG. XRCC1 keeps DNA from getting stranded. Mutat Res 2000; 459(1):11-8. https://doi.org/10.1016/S0921-8777(99)00058-0
13. Hu JJ, Smith TR, Miller MS, Mohrenweiser HW, Golden A, Case LD. Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. Carcinogenesis 2001; 22(6):917-22. https://doi.org/10.1093/carcin/22.6.917
14. Sterpone S, Cozzi R. Influence of XRCC1 genetic polymorphisms on ionizing radiation-induced DNA damage and repair. J Nucleic Acids 2010; 2010:780369. https://doi.org/10.4061/2010/780369
15. Ochiai H. Single-base pair genome editing in human cells by using site-

specific endonucleases. Int J Mol Sci 2015; 16(9):21128-37. https://doi.org/10.3390/ijms160921128
16. Divine KK, Gilliland FD, Crowell RE, Stidley CA, Bocklage TJ, Cook DL, *et al*. The XRCC1 399 glutamine allele is a risk factor for adenocarcinoma of the lung. Mutat Res 2001; 461(4):273-8. https://doi.org/10.1016/S0921-8777(00)00059-8
17. Duell EJ, Millikan RC, Pittman GS, Winkel S, Lunn RM, Tse CK, *et al*. Polymorphisms in the DNA repair gene XRCC1 and breast cancer. Cancer Epidemiol Biomarkers Prev 2001;10(3):217-22.
18. Mateuca RA, Roelants M, Iarmarcovai G, Aka PV, Godderis L, Tremp A, *et al*. hOGG1(326), XRCC1(399) and XRCC3(241) polymorphisms influence micronucleus frequencies in human lyphocytes in vivo. Mutagenesis 2008; 23(1):35-41. https://doi.org/10.1093/mutage/gem040
19. Weng H, Weng Z, Lu Y, Nakayama K, Maromoto K. Effects of cigarette smoking, XRCC1 genetic polymorphisms, and age on basal DNA damage in human blood mononuclear cells. Mutat Res 2009; 679(1-2):59-64. https://doi.org/10.1016/j.mrgentox.2009.07.005
20. Andreassi MG, Foffa I, Manfredi S, Botto N, Cioppa A, Picano E. Genetic polymorphisms in XRCC1, OGG1, APE1 and XRCC3 DNA repair genes, ionizing radiation exposure and chromosomal DNA damage in interventional cardiologists. Mutat Res 2009; 666(1-2):57-63. https://doi.org/10.1016/j.mrfmmm.2009.04.003
21. Seibold P, Schmezer P, Behrens S, Michailidou K, Bolla MK, Wang Q, *et al*. A polymorphism in the base excision repair gene PARP2 is associated with differential prognosis by chemotherapy among postmenopausal breast cancer patients. BMC Cancer 2015;15:978. https://doi.org/10.1186/s12885-015-1957-7
22. Norjmaa B, Saitoh T, Kasamatsu T, Minato Y, Murakami H. XRCC1 Arg194Trp and XRCC1 Arg399Gln polymorphisms affect clinical features and prognosis of myelodysplastic syndromes. Kitakanto Med J 2015; 65(1):11-9. https://doi.org/10.2974/kmj.65.11
23. Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res 1999; 59(11):2557-61.
24. Duell EJ, Wiencke JK, Cheng TJ, Varkonyi A, Zuo ZF, Ashok TD, *et al*. Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis 2000; 21(5):965-71. https://doi.org/10.1093/carcin/21.5.965
25. Abdel-Rahman SZ, El Zein RA. The 399Gln polymorphism in the DNA repair gene XRCC1 modulates the genotoxic response induced in human lymphocytes by the tobacco-specific nitrosamine NNK. Cancer Lett 2000; 159(1):63-71. https://doi.org/10.1016/S0304-3835(00)00532-2
26. Lei YC, Hwang SJ, Chang CC, Kuo HW, Luo JC, Chang MJ, *et al*. Effects on sister chromatid exchange frequency of polymorphisms in DNA repair gene XRCC1 in smokers. Mutat Res 2002; 519(1-2):93-101. https://doi.org/10.1016/S1383-5718(02)00127-4
27. Saadat M. Haplotype analysis of XRCC1 (at codons 194 and 399) and susceptibility to breast cancer, a meta-analysis of the literatures. Breast Cancer Res Treat 2010; 124(3):785-91. https://doi.org/10.1007/s10549-010-0895-y
28. Borrego-Soto G, Ortiz-López R, Rojas-Martínez A. Ionizing radiation-induced

DNA injury and damage detection in patients with breast cancer. Genet Mol Biol 2015; 38(4):420-32. https://doi.org/10.1590/S1415-475738420150019
29. Dunne-Daly CF. Principles of radiotherapy and radiobiology. Semin Oncol Nurs 1999; 15(4):250-9. https://doi.org/10.1016/S0749-2081(99)80054-0
30. BAPETEN. Regulation of Head of Bapeten 4/2013