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Association of *XRCC1* genetic polymorphism with the risk of thyroid cancer in Indonesia

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ABSTRACT

One of the important DNA repair pathways is base excision repair (BER), which plays in the human body to maintain DNA integrity, prevent cancer and DNA damage. X-ray repair cross-complementing group 1 (*XRCC1*) is the most important DNA repair protein in base excision repair (BER) pathways and has been reported to have a relationship with the risk of developing various cancers. The study was purposed to assess the genetic polymorphism of *XRCC1* exon 6 and 10 as a risk factor for thyroid cancer. A total of 90 participants were enrolled in this study, consisted of 30 thyroid cancer patients as a case group and 60 non-cancer patients as a control group. Examination of *XRCC1* genotypes was carried out by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method and statistical analysis using a Chi-square test. In this study, we reported the frequency of *XRCC1* genetic polymorphism was not significantly different between cancer patients and control groups both in exon 6 and 10, and we predict that these polymorphisms were not a risk factor for thyroid cancer (P-value>0.05). In conclusion, both mutant variants of XRCC1 exon 6 and 10 are not risk factors for thyroid cancer. In further studies, it is necessary to assess genetic polymorphisms in populations with controlled non-genetic factors, such as diet, lifestyle, and environmental factors.

Keywords: Base excision repair, PCR-RFLP, Thyroid cancer, XRCC1 exon 6 and 10

Introduction

The World Health Organization (WHO) estimates that there were 18.1 million new cancer cases and 9.6 million deaths that occurred in 2018. The increasing number of cancer sufferers has made WHO predict cancer will become the number one cause of death in the world. Ministry of Health (Kemkes)) stated that the prevalence of cancer has increased in the last five years. According to the Basic Health Research (Riskesdas) in 2018, the prevalence of cancer in Indonesia reached 1.79 per 1000 population, an increase from 2013 which was 1.4 per 1000 population (WHO, 2018; GLOBOCAN, 2018).

Cancer is defined as a cell that grows continuously uncontrollably, is not limited, and is not normal (abnormal). Cancer cell growth is uncontrolled due to DNA damage, causing mutations in vital genes that control cell division (Cooper, 2000). Thyroid cancer is cancer that occurs in the thyroid gland and is ranked ninth in the incidence of cancer in Indonesia, but among other endocrine glands, thyroid cancer is one of the most common types of cancer. The cause of thyroid cancer is unknown, but the number of people suffering from it continues to increase and is increasing

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from year to year. One of the risk factors for thyroid cancer is exposure to ionizing radiation (IR), especially during childhood (Nguyen et al., 2015; Pellegriti et al., 2013).

Base Excision Repair (BER) is very important in rebuilding DNA integrity after irradiation. The BER pathway is the primary pathway responsible for recognizing, cutting, and repairing changes in a single base (oxidative DNA damage) caused by endogenously generated free radicals or upon exposure to exogenous agents such as IR. In short, BER is a multistep process that involves several proteins, one of which is X-ray repair cross-complementing group 1 (*XRCC1*) (Krokan & Bjoras, 2013; Singh & Purohit, 2016; Pizzino et al., 2017).

XRCC1 is a protein that is important for repairing DNA damage through the BER pathway. XRCC1 is located on chromosome 19q13.2 and consists of 17 exons that code for 633 amino acids. XRCC1 has three common SNPs, namely exon 6 (C>T) produces Arginine/Tryptophan substitution at codon 194, exon 9 (G>A) changes amino acids Arginine / Histidine at codon 280 and exon 10 (G>A) changes amino acids Arginine/Glutamine at codon 399. This polymorphism was reported to increase the risk of hepatocellular carcinoma in the Egyptian population (Surniyantoro et al., 2019; Surniyantoro et al., 2018; Naguib et al., 2020).

A previous study by Surniyantoro et al (2018) indicates that the genetic polymorphism of the XRCC1 gene exon 6 with the mutant heterozygous/cytosine-thymine (CT) mutant variant shows an association with the level of DNA damage in hospital radiation officers in this study. In addition, the XRCC1 genetic polymorphisms are thought to have associations with various types of cancer, such as breast, colorectal, gastric, and papillary thyroid cancer (Krivokuca et al., 2016; Nissar et al., 2013; Qiao et al., 2013; Zhu et al., 2018).

In contrast, Pu et al (2020) identified that the XRCC1 399 Gln/Gln genotype was associated with a significant reduction in ESCC risk before trend matching and is not associated with the risk of gastric cancer in the Kashmiri population and is not involve in head and neck susceptibility (Pu et al., 2020; Nissar et al., 2018; Lou et al., 2013). To our best knowledge, the research about the investigation of single nucleotide polymorphism of the XRCC1 DNA repair gene to the susceptibility of thyroid cancer has not been done in Indonesia. This present study was purposed to assess the genetic polymorphism of *XRCC1* exon 6 and 10 as a risk factor for thyroid cancer.

Material and Methods

Study population

This study has received ethical permission from the National Commission for Health Research Ethics, Ministry of Health of the Republic of Indonesia, No. LB.02.01 / 5.2.KE.079 / 2017. Peripheral blood samples of thyroid cancer patients (30 samples) were obtained from Dharmais Hospital, Jakarta, Indonesia, and control samples (60 samples) were obtained from healthy subjects. Blood samples were taken using a disposable syringe, about 5 mL of peripheral blood was taken from the research subject and then put into a vacutainer containing EDTA in it which functions as an anticoagulant so that the blood sample does not clot and is not damaged. The genomic DNA was isolated from whole blood using the Genomic DNA Purification kit (Geneaid). Furthermore, the obtained genomic DNA was stored at -20 °C and was continued by PCR-RFLP and electrophoresis assay.

PCR-RFLP assay

Detection of single nucleotide polymorphism in the XRCC1 gene exon 6 and 10 using the PCR-RFLP method. The samples were amplified by using the Kapa Biosystems® PCR master mix (containing buffers, $MgCl_2$, dNTPs, DNA Taq polymerase). The primers used for each SNP are as follows:

Table 1. Forward and reverse primers in each SNP

SNP location	Forward Primer	Reverse Primer	Reference
XRCC1 exon 6	5'- GCC CCG TCC CAG GTA -3'	5'-AGC CCC AAG ACC CTT TCA CT -3'	Ryu et al, 2011
XRCC1 exon 10	5'-CAAGTACAGCCAGGTCCTAG-3'	5'- CCTTCCCTCATCTGGAG-TAC -3'	Andreasi et al, 2009

The PCR reactions were performed with an initial denaturation at 95 °C for 2 min, followed by 35 cycles at 94 °C

Statistical analysis

Data analyses were displayed on the average frequency of genotype, allele, heterozygosity, homozygous, and Hardy-Weinberg equilibrium following the equation and presented in table form. The Hardy-Weinberg equilibrium and Odds ratio was calculated by using the Chi-square test. The SPSS version 25 was used in this study. The significance threshold in the present study was set at P-value< 0.05.

Results and Discussion

The genotype distribution in this study was consistent with the Hardy-Weinberg equilibrium for all the SNPs studied, both inpatients and controls. The frequencies of XRCC1 exon 6 and 10 genotypes and the Odds ratio calculation are shown in Table 2.

Table 2. The frequencies of XRCC1 exon 6 and 10 genotypes, and the Odds ratio calculation

Genotypes XRCC1 exon 6	Controls (60)	Patients (30)	Odds ratio	P-value
CC	30 (50%)	18 (60 %)	1	
CT	19 (31.67%)	5 (16.67%)	0.44	0.15
TT	11 (18.33%)	7 (23.33%)	1.06	0.92
Alleles				
C	79 (0.66)	41 (0.68)	1	
T	41 (0.34)	19 (0.32)	0.89	0.74
Genotypes XRCC1 exon 10	Controls (60)	Patients (30)	Odds ratio	P-value
GG	30 (50%)	20 (66.67%)	1	
GA	20 (33.33%)	8 (26.67%)	0.6	0.31
AA	10 (16.67%)	2 (6.67%)	0.3	0.13
Alleles				
G	80 (0.67)	48 (0.8)	1	
A	40 (0.33)	12 (0.2)	0.5	0.06

The detection of genotypes, alleles, and frequencies of the SNP selected for the XRCC1 exon 6 and 10 genes were listed in Table 2 and were calculated using the Chi-square test. Genetic polymorphisms in the XRCC1 exon 6 gene will form 2 types of mutant genotypes, namely heterozygous mutants (CT) and homozygous mutants (TT), whereas in XRCC1 exon 10 gene will form 2 types of mutant genotypes, namely heterozygous mutants (GA) and homozygous mutants (AA). The two types of mutants have different effects on developing thyroid cancer. In this study, we calculated the risk (Odds ratio) for each of these mutant genotypes. In XRCC1 exon 6, individuals with heterozygous mutants (CT) had 0.44 times lower of developing thyroid cancer than individuals with normal genotype (CC). Individuals with homozygous mutants (TT) have 1.06 times higher of developing thyroid cancer than individuals with the normal genotype (CC). In XRCC1 exon 10, individuals with heterozygous mutants (GA) had 0.6 times lower of developing thyroid cancer than individuals with normal genotype (GG). Individuals with homozygous mutants (AA) have 0.3 times lower of developing thyroid cancer than individuals with the normal genotype (GG). Although all of these risks were not statistically significant ($P>0.05$).

The results showed that the polymorphism in DNA Repair XRCC1 exon 6 genes reduced the risk of cancer, although not significantly. This is due to the limited number of samples so that it is not relevant in this study, the number of samples was not taken randomly, patient information such as smoking habits, age, and gender was not used as research parameters, and were not statistically tested. In addition, only polymorphisms in genotypes and alleles were calculated for their risk factors for cancer incidence. And research conducted by Li et al. (2013) showed that the presence of polymorphisms in the XRCC1 R399Q gene could increase the risk of bladder cancer among smokers, and the XRCC1 R194W and R280H gene polymorphisms both increased the risk of bladder cancer among Asians (Li et al., 2013). One research by Ratki et al. (2016) found that most patients with thyroid cancer have a histopathological type of Papillary Thyroid Carcinoma (n = 388, 89.54%). Based on research at the Sanglah Central General Hospital, All cases of thyroid cancer were found to be well-differentiated cases of thyroid cancer as much as 92.3% of which 80% were papillary type thyroid cancer and 15% were follicular type. Thyroid nodules are the most common symptom of thyroid carcinoma well differentiated. Surgery is the treatment of choice in carcinoma well differentiated. In papillary carcinoma of the thyroid, total thyroidectomy is recommended by some surgeons as the treatment of choice (Ratki et al., 2016).

In the case of breast cancer, XRCC1 R399Q polymorphism has shown an association with an increased risk of breast cancer in Serbia, especially in the form of hereditary disease and in young breast cancer patients. The dominant allele of the combined genotypes RAD51, TP53, and XRCC1 shows a strong protective role against hereditary breast cancer (Krivokuca et al., 2016). According to Saeb and Al-Naqeb (2016), the Hardy-Weinberg equilibrium is one very important principle in population genetics. The Hardy-Weinberg equilibrium confirms the existence of natural selection, mutation, non-random mating, migration, gene flow, random genetic storage, the genotype frequency and allele frequency of a population remain constant of generation after generation. Genetic balance is an ideal condition to serve as a baseline for measuring genetic change. The relatively small sample size must be considered a limitation of the study, and thus further research is needed in different populations with larger sample sizes to clarify the role of the XRCC1 exon 6 and 10 variants in the DNA damage response.

Conclusion

In this study, we reported the frequency of *XRCC1* genetic polymorphism was not significantly different between cancer patients and control groups both in exon 6 and 10. We conclude these polymorphisms were not a risk factor for thyroid cancer. In further studies, it is necessary to assess genetic polymorphisms in populations with controlled non-genetic factors, such as diet, lifestyle, and environmental factors.

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