Research Article

BRCA1 Gene Q356R (1186A→G) Polymorphism and Epithelial Ovarian Cancer Incidence

Polimorfisme Gen BRCA1 Q356R (1186A→G) dan Insidensi Kanker Ovarium Epitelial

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Abstract

Objective: To determine the association between BRCA1 gene Q356R (1186A→G) polymorphism and epithelial ovarian cancer incidence.

Methods: This study is an observational analytic study with case-control study design. All patients diagnosed with epithelial ovarian cancer that were treated in the outpatient clinic and inpatient ward of the Department of Obstetrics and Gynecology, Dr. Mohammad Hoesin Hospital, Palembang who met the inclusion criteria were included in this study. DNA extraction was performed on blood samples, followed by PCR-RFLP process.

Results: We obtained the genotype distribution of BRCA1 Q356R (1186A→G) polymorphisms to be QQ genotype (wild-type) on all 50 subjects in the case group (100%) and 50 control subjects (100%). Similarly, all BRCA1 alleles have the Q allele. The results of this study found no polymorphism of the BRCA1 Q356R (1186A→G) in the ovarian cancer and control groups.

Conclusion: Polymorphism of BRCA1 gene Q356R (1186A→G) was not significantly associated with epithelial ovarian cancer incidence.

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Keywords: BRCA1 gene, epithelial ovarian cancer, polymorphism

Abstrak

Tujuan: *Untuk mengetahui adanya hubungan polimorfisme gen BRCA1 Q356R (1186A→G) dengan kejadian karsinoma ovarium epitelial.*

Metode: *Penelitian ini merupakan studi observasional analitik dengan desain studi kasus-kontrol. Semua pasien yang didiagnosis karsinoma ovarium epitel yang datang ke Poliklinik Ginekologi dan yang dirawat di Instalasi Rawat Inap Obstetri dan Ginekologi RSMH Palembang yang memenuhi kriteria inklusi masuk ke dalam penelitian ini. Dilakukan ekstraksi DNA pada sampel darah dilanjutkan dengan proses PCR-RFLP.*

Hasil: *Dari hasil penelitian ini didapatkan distribusi genotip polimorfisme gen BRCA1 Q356R (1186A→G) yaitu genotip QQ (wild type) pada semua subjek dalam kelompok kasus (100%) dan 50 subjek kontrol (100%). Begitu pula dengan alel BRCA1 yang semuanya memiliki alel Q. Hasil penelitian ini tidak menemukan polimorfisme gen BRCA1 Q356R (1186A→G) pada kelompok karsinoma ovarium dan kontrol.*

Kesimpulan: *Polimorfisme gen BRCA1 Q356R (1186A→G) tidak berhubungan secara bermakna dengan kejadian karsinoma ovarium epitelial.*

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Kata kunci: *gen BRCA1, karsinoma ovarium jenis epitel, polimorfisme*

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INTRODUCTION

Ovarian cancer has the highest mortality rate among all the gynecologic cancers, being the 7th most common cancer in the United States, representing 3% of cancer malignancy to date, and contributing to 6% of the mortality rate due to cancer in women. Epithelial ovarian cancer represents 90-95% of all ovarian cancer. They occur in all ages, but have a higher incidence in older patients. Ovarian cancer incidence in women aged 40-44 years old is 15.7 every 100,000, inclining to 54 every 100,000 in women aged 75-79 years old.[1-3]

In South East Asia, including Indonesia, ovarian cancer incidence is 5.2%. Record of new cases of ovarian cancer for 3 years at the Histopathology Unit of Sriwijaya University at Dr. Mohammad Hoesin Hospital Palembang stated that ovarian cancer incidence in 2006 constitutes 12% of all cancers, with a decline to 7%, and rebounding to 10% in 2008, which represents a third of all malignancies.[2-5]

The important risk factor for ovarian cancer is family history of breast or ovarian cancer, where about 5-10% of patients have a predisposing genetic factor.[2] Hereditary Breast and Ovarian Cancer

Syndrome (HBOC) is an inherited cancer prone syndrome. The hallmark of this syndrome is more than one family member with breast or ovarian cancer, or both, one family member having both, and one family member having breast cancer in a very young age. Germline mutation of BRCA1 and BRCA2 is the culprit and main cause of most HBOC, even though an individual with one defective allele in BRCA1 or BRCA2 from maternal or paternal origin had a second functional allele, and even when this second allele becomes non-functional, cancer can still develop through accumulation of other mutations.[6]

Some studies have stated that polymorphism in other genes can change the penetration of BRCA1 and BRCA2 for ovarian cancer.[3] The risk of ovarian cancer is higher in BRCA1 carriers. Therefore, the functional polymorphism in BRCA1 is considered to be more likely to influence the occurrence of ovarian cancer compared to BRCA2. There are 10 polymorphisms in BRCA1 with allele frequency >5% in caucasians. Nevertheless, only 5 polymorphisms provoke the substitution of amino acid, which are Q356R (1186A➙G), P781L (2731T➙C), E1038G (3232A➙G), K1183R (3667A➙G) and S1613G (4956A➙G). These polymorphisms, with the exception of Q356R (1186A➙G), are in significant linkage disequilibrium, and the effects of all of these on ovarian cancer can be ascertained by considering only the Q356R (1186A➙G).[7,8]

Anton-Culver et al carried out a population based study about the variants of BRCA1 sequence, and concluded that there is a significant correlation between BRCA1 mutation with cancer patients having a family history of ovarian cancer. Janezic et al described the distribution of 91 polymorphism cases in Caucasians, a rare form of Q356R polymorphism (1186A➙G) where the rare 1186G allele of Q356R (1186A➙G) polymorphism has a higher frequency, and associated with family history of ovarian cancer (p=0.003). It shows that this polymorphism affects the occurrence of ovarian cancer. Wenham RM et al reported BRCA1 Q356R gene polymorphism provides the odd ratio of 1.9 to the occurence of ovarian cancer.[7-9]

Most studies on BRCA1 focused only on Caucasian populations. However, allele frequency from the gene with higher penetration to ovarian cancer in the Asian population is possibly higher than Caucasian population. Asian immigrant women, especially from South Asia who lived in western countries showed a higher incidence of ovarian and breast cancer. Risch et al reported a higher mutation frequency of ovarian cancer in Indo-Pakistani descent than North-West European or British descent who lived in Ontario, Canada.[10]

The contribution of BRCA1 mutation towards incidence of epithelial ovarian cancer in Asian population, especially in Indonesia has not been elucidated yet. Based on this reason, this study needs to be done. This study focuses on polymorphism of BRCA1 gene at the Q356R (1186➙G) position.

METHODS

This study is an observational-analytic study with case-control study design which was done from September 2013 until March 2014 at Dr. Mohammad Hoesin Hospital in Palembang.

The subjects were divided into two groups, a case group with patients who have been diagnosed with epithelial ovarian cancer based on surgical and histopathologic findings (n=50), and a control group with patients who have not been diagnosed with malignancy (n=50). Subjects who met the criteria was given explanation about the study procedures and the study benefit. Subjects who agreed, signed the informed consent and had blood samples drawn.

The extraction method of DNA Chelex-100 used Phosphate Buffer Saline (PBS) pH 7.4; Safonin 0.5% on PBS; and Chelex 20% on dd H_2O pH 10.5. PCR was performed using the forward primer 5'-GGA CTC CCA GCA CAG AAA AA-3' and reverse primer 5'-TCC CCA TCA TGT GAG TCA TC-3'. The reaction was conducted in a final volume of 15 μl containing 0.5 ng/μl genomic DNA, 0.5 nmol/l forward primer, 0.5 nmol/l reverse primer, 0.2 mmol/l deoxynucleotide triphosphate (dNTP), 1.5 mmol/l MgCl2. PCR buffer, and 0.025 units/μl Taq DNA polymerase. PCR conditions consisted of an initial denaturation step at 95°C for 3 minutes, 30 cycles of 94°C for 45 seconds, 57°C for 45 seconds, and 72°C for 1 minute; an extension step at 72°C for 10 minutes, then at 4°C until digested. A digest of the amplicon was performed by combining 15 μl of the PCR amplification product, 2 μl Buffer and 10 units of AluNI enzyme in a final volume of 20 μl. Samples were incubated at 37°C for 4.5 hours, and analyzed immediately on a 2% agarose gel. The undigested arginine (R) allele can be seen as a band at 211 bp, whereas the glutamine (Q) allele

is represented by the digestion products at 134 and 77 bp.

Electrophoresis and visualisation was done on agarose gel made using 2 grams of agarose in Erlenmeyer glass with 40 ml buffer TAE added in. The mixture is mixed and heated until boiling point and then 4 μl of ethidium bromide was added. Solution was chilled in a mold for 30 minutes. Five μl PCR product was mixed with long buffer and was added to an electrophoresis device, which was set on 75 mV voltage, 350 A for 30 minutes. Visualization was performed using ultraviolet light with Gel-Doc made by BIO-RAD Laboratories USA, connected to a computer, and the visualisation result was analyzed using Quantity one software programme.

RESULTS

There were 50 women with epithelial ovarian cancer in the case group and 50 normal women or non-malignancy diagnosis in the control group. Subject characteristics can be seen in Table 1.

Table 1. Characteristics of Study Subjects

Characteristic	Case (n=50)		Control (n=50)		p
	n	%	n	%	
Age					
10 - 20	1	2.0	4	8.0	
21 - 30	5	10.0	23	46.0	
31 - 40	10	20.0	16	32.0	0.001
41 - 50	17	34.0	5	10.0	
51 - 60	16	32.0	1	2.0	
>60	1	2.0	1	2.0	
Ethnic group					
Sumateran	49	98.0	47	94.0	0.617
Javanese	1	2.0	3	6.0	
Education					
Elementary	13	26.0	7	14.0	
Junior High School	5	10.0	2	4.0	0.144
Senior High School	26	32.0	37	64.0	
Graduate	6	12.0	4	8.0	
Job					
Midwife	43	86.0	45	90.0	
Merchant	0	4.0	1	2.0	
Student	1	2.0	0	0	0.532
Farmer	1	2.0	0	0	
Civil servant	5	10.0	4	8.0	
History of contraception					
Hormonal	17	34.0	25	50.0	
Non-hormonal	0	0	1	2.0	0.139
None	33	66.0	24	48.0	
Family history of ovarian cancer					
Yes	17	34.0	9	18.0	0.068
No	33	66.0	41	82.0	

Table 1 demonstrated that there was no significant difference in terms of subject characteristics based on ethnic group, education, job, history of hormonal contraception, and family history of ovarian cancer between case group and control group, with p value of 0.617, 0.144, 0.532, 0.139 and 0.068, respectively. Only age showed a significant difference between the case and control group with p=0.001.

Based on surgical and histopathology result in the case group, the most common stage of epithelial ovarian cancer was stage IIIC and IIIB which constitutes 36% and 18% of the cases. For the remaining cases, the proportion were 2-10%. The complete result of epithelial ovarian cancer staging is presented in Table 2.

Table 2. Ephitelial Ovarian Cancer Staging Distribution in Case Group

Epithelial ovarian cancer staging	Case	
	n	%
Inadequate staging	6	12.0
stadium I A	4	8.0
stadium I C	5	10.0
stadium II A	2	4.0
stadium II C	1	2.0
stadium III A	3	6.0
stadium III B	9	18.0
stadium III C	18	36.0
stadium IV	2	4.0
Total	50	100.0

BRCA1 Q356R (1186A⇢G) Genotype

Examination result with PCR-RFLP (*Polymerase Chain Reaction - Restriction Fragment Length Polymorphism*) method showed all of the subjects in both groups have a wild type-normal genotype (QQ) of BRCA1 Q356R (1186A⇢G). There was no heterozygote genotype (QR) of BRCA1 Q356R (1186A⇢G) or mutant-homozygote (RR). Thus, there was no significant difference in the genotype of BRCA1 Q356R (1186A⇢G) between the case group and control group (p=0.999).

BRCA1 Q356R (1186A⇢G) allele on both case group and control group is Q. Thus, there was no significant difference in regards to BRCA1 Q356R (1186A⇢G) allele between case group and control group (p=0.999).

BRCA1 Polymorphism Relationship with Ephitelial Ovarian Cancer Incidence

To assess the polymorphism, we performed allele analysis with PCR-RFLP method. The results of this study showed no polymorphism discovery in the case group and the control group, indicating the absence of changes in codon 356 of glutamine (Q) amino acid to arginine (R) amino acid. In this study, the case or control groups amplicon digested on 211 bp, which means that the nucleotide was a wild type-normal or glutamine amino acid (Q) on codon 356. Polymorphism of BRCA1 (1186A⇢G) was not found in this study, which can be seen as that there was no digested amplicon on 134 bp and 77 bp or arginine amino acid (R) on codon 356. The PCR-RFLP result is shown on picture 1, for case sample number 1 to 4 (KS1 - KS4) and control number 1 to 4 (KT1 - KT4).

This proves that there was no variation of more than one phenotype that was genetically caused by the allele differences. Based on Fisher's exact test, there was no significant correlation between polymorphism of BRCA1 and ephitelial ovarian cancer incidence (p=0.999).

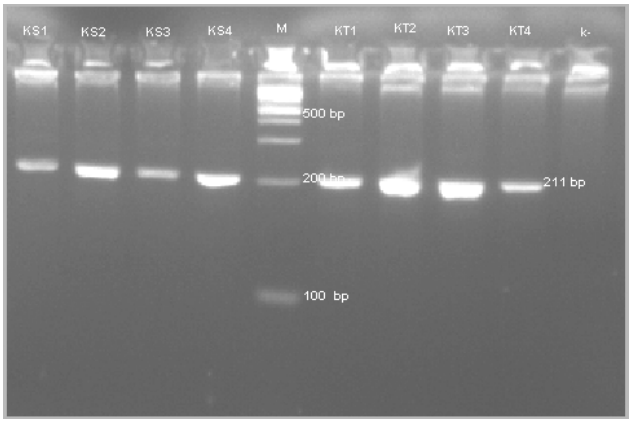

Figure 1. PCR-RFLP Result for Sample Case no 1-4 (KS1-KS4) and Control Case no 1-4 (KT1- KT4)

DISCUSSION

BRCA1 is a tumor supressor gene that will inhibit cellular function process and will lose its function when it is mutated. Growth factor causes cell growth, and if the growth noted by the body is adequate, then the growth suppression factor will be activated until homeostasis is achieved. If a muta-

tion occurred to this gene, the suppression process can not be done, and genomic instability will occur, which will ultimately lead to cancer.

The highest age distribution of ephitelial ovarian cancer patients in this study was 41-50 years old (34%) and the most common parity status was multipara (36%). This number was similar to a study by Wenham RM et al, which reported epithelial ovarian cancer to be encountered mostly in the age group of 20-50 years old (50%), and in the multiparous group (51%).[7] Several former studies have stated that ovarian cancer could occur in all age groups and that the incidence increases with age.[1-3]

Based on ethnicity, the Sumateran ethnic group makes up the largest proportion in both groups in our study, which is 98% in the case group and 94% in the control group. This is because the study was held in South Sumatera province, specifically in Palembang.

History of ovarian cancer in the family in the case group was 34%, which was higher than in the control group (18%). Menopause was more commonly found in the case group, which was 36%, compared to 4% in the control group. Schorge JO stated that risk factor of ovarian cancer that gave the most contribution to ovarian cancer incidence is family history of ovarian cancer, where 5-10% patients have an inherited genetic predisposition.[2] The most commonly used method of contraception was hormonal contraception in the case group (34%) and also in the control group (50%). Center of Disease Control (CDC) have stated that there is a decreased risk of ovarian cancer as much as 40% in women aged 20-24 years old who used oral contraceptives, with a relative risk of 0.6.[7] Other studies reported that oral contraceptive use for one year, decreases the risk by 11%. Meanwhile, the use of oral contraceptives for five years decreases the risk until 50%. The decreasing risk gets more significant with longer use.[11-13]

Ovarian cancer diagnosis in our study showed that many patients with epithelial ovarian cancer was diagnosed in the late stage, which is stage IIIC (36%) and IIIB (18%). This is caused by the fact that ovarian cancer is often asymptomatic and there is no effective screening method for ovarian cancer, so that 70% of cases are found in the late stage, and metastatic tumor have extended oustide the ovary.[13]

Our study showed that there was no significant relation of BRCA1 Q356 R (1186A→G) polymorphism with the incidence of epithelial ovarian cancer ($p>0.05$). The examination with PCR-RFLP showed that there was no variation of more than one phenotype that was genetically caused by the allele differences. This was proven by the absence of polymorphism at base pair 1186 from adenine nucleotide (A) into guanine (G); or glutamine (Q) on amino acid position 356 which is digested on 211 bp into arginine (R) which will be digested on 134 bp and 77 bp. Finally, there was no QR (heterozygote) genotype and RR (mutant-homozygote) genotype on BRCA1 Q356R (1186A→G) gene.

This result was concordant with a study by Wenham et al, who reported that BRCA1 Q356R gene has no correlation with risk of ovarian cancer. Their study stated that the frequency of heterozygote and homozygote from R allele is 9% and <1% in ovarian cancer cases, and in controls being 10% and <1%, respectively.[7]

Thus, the occurence of ovarian cancer in our study did not occur on DNA level, but it could occur due to epigenetic process. Epigenetic is a phenotype status change which is not based on the genotype changes, in other words there are gene expression changes that are not caused by changes in the DNA sequence. DNA mutations lead to changes in DNA sequence and irreversible gene expression. This epigenetic process is potentially reversible, but remains stable during cell differentiation so that this epigenetic changing can be inherited to children at cell differentiation.[14] According to Kwon, besides mutation the inactivation of BRCA1 as a tumor suppressor gene is related to hypermethylation of DNA on CpG islands. Transcriptional inactivation of tumor suppressor genes by DNA promoter hypermethylation on BRCA1 CpG island is one of the mechanism leading to development of tumor on ovarian cancer cells.[15]

Anton-Culver et al summarized that there was a significant correlation between the BRCA1 mutation with ovarian cancer cases with family history of ovarian cancer. Janezic et al found that a common polymorphism was determined in 91 Caucasian cancer cases. A rare form of polymorphism, Q356R (1186G), was significantly associated with a family history of ovarian cancer ($p=0.03$), suggesting that this polymorphism affects the occurence of ovarian cancer. In our study, there is no evidence of family history of ovarian

cancer being related to epithelial ovarian cancer incidence (p=0.068). This was thought to be caused by the difference of race, where in our study we involved the Indo-China race; meanwhile the Anton-Culver study was involving non-Hispanic white population, and Janezic et al involved Caucasians.

On this study, DNA sequencing was not done. It was based on PCR-RFLP result using *AluN1* enzyme, that showed no polymorphism on both case and control groups. *AluN1* enzyme specifically digests the nucleotide adenine (A) and guanine (G) on glutamine amino acid (Q) and arginine (R), allowing for nucleotides A or G to be identified precisely.

CONCLUSION

We can conclude that BRCA1 Q356R (1186A➙G) genotype on the epithelial ovarian cancer cases and controls was QQ (wild type-normal). Meanwhile, the allele of gene BRCA1 q356R (1186A➙G) in epithelial ovarian cancer patients as well as in controls was Q. There was no significant association between BRCA1 polymorphism with epithelial ovarian cancer incidence.

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