Relationship of estrogen beta (ERβ) receptor genes polymorphism with epithelial ovarian cancer

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Abstract. Ovarian cancer is the leading cause of death among all uterine diseases. Ovarian cancer is affected by exogenous and endogenous estrogen factors. Beta estrogen receptors are predominant estrogen receptors in the normal ovary. Polymorphisms in the beta estrogen receptor gene (ERβ) can affect the risk of epithelial ovarian cancer through regulation of cell proliferation and apoptosis. The purpose of this study was to determine the relationship of beta estrogen receptor gene polymorphism (ERβ) with epithelial ovarian cancer incidence. Method: a case-control study, there were 60 samples. A single nucleotide polymorphism in the beta estrogen receptor (RsaI/rs1256049) is selected. Genotyping using PCR-RFLP. Data were analyzed by Chi-square, considered significant if the p-value is less than 0.05. Results: there is no significant difference in the characteristics of respondents. Genotypic frequency in the case group was 56.7% GG, 43.3% GA/AA and control group was 23.2% GG, 76.7% GA/AA. The frequency of alleles in the case group was 78.3% G, 21.7% A and control which was 50% G, 50% A allele. ERβ polymorphism is associated with epithelial ovarian cancer (p 0.018). Allele A in RsaI was associated with epithelial ovarian cancer (p 0.002). Polymorphism of the beta estrogen receptor gene associated with epithelial ovarian cancer.

Introduction

Cancer is a process of cell division (proliferation) that does not follow the standard rules of proliferation found in the body (abnormal proliferation) [1]. Ovarian cancer is a malignant tumor in the ovary (ovary). More than 90% of ovarian cancers are classified as "epithelial cancer" and are believed to originate in ovarian epithelial tissue [2]. In developed countries, such as the United States, the incidence of ovarian cancer looks higher, namely 25% of all uterine cancer and about 47% of deaths from cancer are caused by ovarian cancer. The highest incidence occurred in Sweden, namely 21/100,000 women. Whereas in other countries, such as Norway 16/100,000, America 15/100,000, Britain 14/100,000, Africa 4/100,000, and Japan 3/100,000. In Indonesia, uterine cancer is one of the main causes of death among all uterine diseases. Ovarian cancer ranks third after cervical cancer and breast cancer [3]. Data obtained at RSUP Dr. Mohammad Hoesin Palembang showed that the incidence of ovarian cancer in 2006 amounted to 12% of all gynecological cancers, and in 2007 decreased to 7%, while in 2008 it reached 10% third in all cancer incidence.

The cause of ovarian cancer is not yet clear, but environmental and hormonal factors play an important role in its pathogenesis. However, recent research shows that fallopian tubes can also be a

source of several ovarian cancers. This is because the ovaries and fallopian tubes have similar types of cells, so the hypothesis arises that fallopian tube cells can develop into cancer cells like ovarian cancer. Apart from epithelial tissue, cancer can also originate from egg cells (germ cell tumors) or supporting cells (sex cord/stroma) [4]. The role of estrogen in human physiology is very broad, so it was found that estrogen is also implicated in the development and progression of various diseases, including various types of cancer, namely breast cancer, ovarian cancer, colorectal cancer, prostate cancer, endometrial cancer, and osteoporosis, neurodegenerative, cardiovascular diseases, insulin resistance, lupus erythematosus, endometriosis, and obesity. It is known that most epithelial ovarian cancers develop from the epithelial surface of the ovary (ovarian surface epithelial), where on the surface of the ovarian epithelium there are receptors that are responsive to estrogen [5].

The effect of estrogen is mediated by two estrogen receptors, the alpha estrogen receptor (ERα) and the beta estrogen receptor (ERβ). Beta estrogen receptors are predominant estrogen receptors in the normal ovary. Polymorphism in the beta estrogen receptor gene (ERβ) can affect the ovarian epithelium through regulation of cell proliferation and apoptosis, so this can affect ovarian cancer [6]. The beta estrogen receptor (ERβ) is located on chromosome 14q22-24 and has 8 exons [7]. Beta estrogen receptor protein (ERβ) consists of 530 amino acids [8].

A study in the Chinese female population examined the role of ERβ rs 1256049 gene polymorphisms and rs 4986938 in menstrual abnormalities [9]. Other studies suggest that ERβ rs 1256049 and rs 4986938 gene polymorphisms are associated with ovulation dysfunction. Polymorphisms on introns or exons can affect the cutting of introns or exons (splicing), changes in position or even disappear, or often a reduction in GT or addition of AG. This causes a lot of heterogeneity of protein products. Polymorphisms in introns or exons can also play a role in malignancy [10]. Based on the problems that exist in the background, the formulation of the problem of this study is how is the relationship of beta (ERβ) RsaI gene (rs 1256049) receptor polymorphism with epithelial ovarian cancer.

2. Methods

This research is an observational analytic study. Implementation of research with laboratory examination with a case-control approach. This research was conducted at the Palembang Institute of Health Laboratory (BBLK) in South Sumatra Province in collaboration with the Department of Obstetrics and Gynecology RSUP Dr. Mohammad Hoesin Palembang. The study began in March 2011 to June 2011.

The study population was patients who came to the gynecology polyclinic and were treated at the Obstetrics Inpatient Installation and Gynecology RSUP Dr. Mohammad Hoesin Palembang. Researchers determined the number of samples of case and control groups was 30 people respectively. Samples were taken by consecutive sampling technique, ie all patients who met the inclusion criteria would be taken as samples. Inclusion criteria in the case group were patients diagnosed with epithelial ovarian cancer as evidenced by histopathological features, willing to take blood (based on diagnosed days), and willing to sign Informed Consent forms. While the inclusion criteria in the control group were patients who were not diagnosed with epithelial ovarian cancer, were willing to take blood (after the case group was diagnosed based on PA results), and were willing to sign the Informed Consent form. While the exclusion criteria for both groups were patients who were diagnosed with more than one malignancy (neoplasm) and patients who were not willing or refused to participate in the study.

The research materials used include blood sampling materials, DNA extraction materials, PCR-RFLP materials, and electrophoresis materials. Blood sampling procedure is a blood sample taken through 3 ml antecubital vein puncture and inserted into a test tube containing 3 ml of ethylene diamine tetra-acetic (EDTA) then stored at a maximum temperature of 40 Celsius until PCR examination is carried out.

The DNA extraction procedure was carried out using Chelex-100 DNA extraction method using Phosphate Buffer Saline (PBS) pH 7.4, Saponin 0.5% in PBS, and Chelex 20% in ddH2O pH 10.5. As for the allele analysis using PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) method. Then electrophoresis and visualization using Gel-Doc equipment were connected to a computer. Furthermore, the results of visualization were analyzed with a computer using the Quantity One program.

All data were analyzed using the SPSS 15.0 for Windows program to assess the distribution, genotypic frequency and RsaI gene alleles (rs 1256049) of estrogen beta receptors in the case group and control group. To determine the relationship of ERβ gene polymorphism with epithelial ovarian cancer using the X2 test (Chi-square). Odds ratio (OR) with a 95% confidence limit and $\alpha = 0.05$.

3. Results

3.1. Characteristics of research subjects

In this study, the characteristics of the respondents studied were age, marital status, menopausal status, family history of cancer, and history of contraception.

Table 1. Characteristics respondents.

Characteristics Respondents		Group		*p*
		Case (%)	Control (%)	
Age	≤ 50 years	18 (60)	23 (76,7)	0,267[a]
	> 50 years	12 (40)	7 (23,3)	
Marital status	Marriage	22 (73,3)	27 (90)	0,181[b]
	Not married	8 (26,7)	3 (10)	
Menopause status	Menopause	13 (43,3)	7 (23,3)	0,171[a]
	Not menopause	17 (56,7)	23 (76,7)	
Family history of cancer	Yes	5 (16,7)	1 (3,3)	0,195[b]
	No	25 (83,3)	29 (96,7)	
Contraception history	Hormonal	14 (46,7)	12 (40)	0,794[a]
	Non hormonal	16 (53,3)	18 (60)	

[a] *X^2 test*
[b] *Fisher's Exact Test*

From table 1. it can be seen that of the 60 respondents, most were ≤50 years old. In the case group, there were 18 respondents (60%) while in the control group 23 respondents (76.7%). The youngest age group is 18 years and the oldest is 70 years, while in the youngest age control group is 21 years and the oldest is 65 years. Respondents aged >50 years were larger in the case group (40%) than the control group (23.3%).

Chi-square test results showed no significant relationship between age with epithelial ovarian cancer incidence, p-value 0.267> α 0.05. WHO (2009) states that deaths from ovarian cancer, more than half of which occur in women aged between 55-74 years, and a quarter of these deaths occur in women aged between 35-54 years. Older women are at the highest risk.

In this study, the results obtained in both groups were mostly married, with 22 respondents (73.3%) in the case group and 27 respondents (90%) in the control group. Based on the bivariate analysis showed no significant relationship between marital status with the incidence of epithelial ovarian cancer, p-value 0.181> α 0.05.

Based on menopausal status, respondents who had experienced menopause in the case group were 13 respondents (43.3%) greater than the control group, namely 7 respondents (23.3%). The proportion of respondents who did not experience menopause in the case group were 17 respondents (56.7%) and the control group 23 (76.7%). From the chi-square test results showed no significant relationship between menopausal status with epithelial ovarian cancer incidence, p-value 0.171> α 0.05.

Respondents who had a family history of more cancer in the case group were 5 respondents (16.7%) than the control group 1 respondent (3.3%). While the proportion who did not have a family history of cancer in the case group 25 (83.3%) and control group 29 (96.7%). Fisher's Exact test results showed no significant relationship between family history of cancer with epithelial ovarian cancer incidence, p-value 0.195> α 0.05.

From the study, it was found that the proportion of ovarian cancer incidence was greater in respondents who had a history of non-hormonal contraception, namely 16 respondents (53.3%). While

the chi-square test results showed no significant relationship between contraceptive history with epithelial ovarian cancer incidence, p-value 0.794> α 0.05.

3.2. Estrogen Beta (ERβ) Receptor Genes Polymorphism

Table 2. Genotype distribution of beta estrogen receptors.

Genotype Gen ERβ	Group		OR (95%CI)	*p*
	Case (%)	Control (%)		
***RsaI* (rs 1256049)**				
GA[a]/AA[b]	13 (43,3)	23 (76,7)	0,233	0,018[d]
GG[c]	17 (56,7)	7 (23,3)	(0,077-0,708)	

[a] GA: heterozygote.
[b] AA: homozygous (mutant).
[c] GG: wild-type (normal).
[d] Chi-Square Test (α = 0,05)

RsaI polymorphism (rs 1256049) in epithelial ovarian cancer patients obtained genotypic frequency, namely GG 17 respondents (56.7%), GA / AA 13 respondents (43.3%). Whereas in the control group, the GG 7 genotype frequency (23.2%), GA / AA 23 respondents (76.7%) were obtained. The chi-square test results showed a significant association of RsaI polymorphism with epithelial ovarian cancer incidence with a p-value of 0.018.

Table 3. Distribution of estrogen beta (ERβ) receptor alel genes.

Alel Gen ERβ	Group		OR (95%CI)	*p*
	Case (%)	Control (%)		
***RsaI* (rs 1256049)**				
A[a]	13 (21,7)	30 (50)	0,277	0,002[c]
G[b]	47 (78,3)	30 (50)	(0,125-0,613)	

[a] A: RsaI-positive.
[b] G: RsaI-negative.
[c] Chi-Square Test (α = 0,05).

The number of alleles shown in table 3 is doubled from the number of samples, which is 60 for each group. This is due to the genotype being a "double strain" so that one genotype has 2 alleles.

The analysis of alleles obtained results, namely the G allele frequency in epithelial ovarian cancer patients is 78.3% greater than the control group, which is 50%. While the frequency of allele A in the case group (epithelial ovarian cancer) 21.7% and the control group 50%. Chi-square test results showed a significant relationship between RsaI allele and epithelial ovarian cancer incidence with a p-value of 0.002.

4. Discussions

4.1. Characteristics of research subjects

When viewed based on the incidence of epithelial ovarian cancer, more occurred in respondents aged <50 years, as many as 18 respondents (60%). This result is supported by the results of Galina Lurie et al. [8] which states that the prevalence of epithelial ovarian cancer varies according to age. In the age group <45 years 22%, 45-54 years 29%, 55-64 years 24%, and the age group> 64 years 25%. So, the risk of epithelial ovarian cancer is more experienced by women between the ages of 45-54 years. WHO states that deaths from ovarian cancer, more than half of which occur in women aged between 55-74 years, and a quarter of these deaths occur in women aged between 35-54 years. Older women are at the highest risk.

The prevalence of ovarian cancer was greater in the non-menopausal group, namely 17 respondents (56.7%) as stated by Faisal Yatim [3] that the risk of ovarian cancer is higher when giving birth in old age and the late arrival of menopause. That is, non-menopausal individuals, increase the risk of ovarian

cancer. But this is contrary to the results of Galina Lurie et al. [8] that the risk of ovarian cancer is greater in the menopause group, which is 65%.

Women with a family history of ovarian cancer in the first lineage are relatively at risk for ovarian cancer. In contrast to the results of this study, respondents who experienced epithelial ovarian cancer were even more devoid of a family history of cancer, namely 25 respondents (83.3%). Likewise, the results of Galina Lurie et al. [8] namely the prevalence of epithelial ovarian cancer is greater in the group without a family history of ovarian cancer (95%). Although genetic factors play an important role, it cannot be denied that environmental and hormonal factors play an important role in their pathogenesis. In industrialized countries, it is estimated that the incidence of ovarian cancer is associated with consumption of foods that are high in fat [3]. Genkinger et al. [11] and La Vecchi et al. [12] added that relatively high alcohol consumption can also increase the risk of epithelial ovarian cancer.

From the study, it was found that the proportion of ovarian cancer incidence was greater in respondents who had a history of non-hormonal contraception, namely 16 respondents (53.3%). The same is mentioned by Galina Lurie et al. [8] that the association of estrogen beta receptor genes (rs1271572 and rs1256030) with ovarian cancer risk was stronger in women who had never used hormonal contraception than users of hormonal contraception ($p = 0.04$). While the chi-square test results showed no significant relationship between contraceptive history with epithelial ovarian cancer incidence, p-value $0.794 > \alpha\ 0.05$.

There are a number of ways to reduce or eliminate the risk of ovarian cancer, one of which is the use of oral contraceptives (birth control pills) for five years or more [13]. Beral et al. [14] mentioned in a prospective study that there was an association between the use of oral contraceptives and the incidence of ovarian cancer. Women who use oral contraceptives for 10 years only have about 60% reduction in the risk of ovarian cancer.

4.2. Estrogen Beta (ERβ) Receptor Polymorphism

Polymorphism is a variation of two or more phenotypes that are genetically caused by differences in alleles. In the RsaI gene polymorphism (rs 1256049) the change of G to A in 1082 nucleotide in exon 5 (RsaI restriction site is also known as G1082A). RsaI enzyme is an enzyme used to cut PCR products. Individuals without polymorphism (wild type) in the RsaI gene (rs 1256049) do not have sequences with the G1082A site, whereas in individuals who have polymorphisms, at this point the mutation can have a restriction site that causes the PCR product to be truncated.

PCR products in normal (wild-type) individuals will produce one DNA band with a size of 700 bp. If mutations (homozygotes) occur in individuals, the PCR product will be cut into two DNA bands with a size of 375 bp and 325 bp. And if the individual has normal and mutant alleles (heterozygotes), DNA bands will be obtained with a size of 700 bp, 375 bp, and 325 bp.

Research on the beta estrogen receptor gene has been carried out, but research on the relationship of the RsaI gene (rs 1256049) to epithelial ovarian cancer has not been reported. However, studies have been reported on epithelial ovarian cancer at other sites of the beta estrogen receptor gene, a study conducted by Galina Lurie et al [8] in Hawaii, involving 313 women with epithelial ovarian cancer and 574 controls. The selected genes are 2 SNPs on the intron (rs1256030 at intron 2 and rs1256031 on intron 3) and 2 SNPs in area 5 '(rs3020450 and rs1271572). The results obtained rs1256030 (OR = 1.67), rs1271572 (OR = 1.79) and women with haplotypes, including alleles rs1271572, rs1256030, and rs1256031, the risk for ovarian cancer increased significantly.

As shown in table 2 that the RsaI gene polymorphism (rs 1256049) has a significant relationship with the incidence of epithelial ovarian cancer with a p-value of 0.018. This shows that the hypothesis states that there is a relationship between RsaI gene polymorphism (rs 1256049) with epithelial ovarian cancer incidence, proven. The role of these single nucleotide polymorphisms (SNPs) in relation to the occurrence of a disease is focused on the tendency of these polymorphisms to influence genes, whether through modification of their expression or the protein itself. SNPs come from errors in replication or repair of DNA [9].

Koehler et al. [15] stated that the expression of beta estrogen receptor (ERβ) is abundant in various tissues such as the uterus, breast, prostate, central nervous system, brain, immune system, cardiovascular

system. This was confirmed by Herynk and Fuqua [16] that beta estrogen receptors on exons 2, 3, 4, 5 and 6 were found in ovarian tissue.

The results of the chi-square test on alleles showed a significant relationship between RsaI alleles and epithelial ovarian cancer incidence with a p-value of 0.002. However, if judged by the Odds ratio = 0.277 it means that the RsaI allele frequency functions as a protective factor. Bardin et al. explained that the increase in ERα / ERβ mRNA ratio observed in ovarian cancer showed a selective decrease in ERβ mRNA expression without significant variation in ERα levels. The fall in the expression of beta estrogen receptor (ERβ) is important in ovarian cancer, although the mechanism is still being studied.

The RsaI gene (rs1256049) has many genetic variants. Another study of the RsaI gene (rs1256049) as was done by Nott et al. [9] in the Chinese female population, there was a role for ERβ rs 1256049 gene polymorphism in menstrual abnormalities. Another study, conducted by Britt and Findlay [10] stated that ERβ rs 1256049 gene polymorphism is associated with ovulation dysfunction.

The same is done by Chitra Sundarrajan et al. [17] that the RsaI gene may be associated with ovulation dysfunction. Although RsaI in the beta estrogen receptor gene does not cause changes in amino acids in the beta estrogen receptor protein, this is likely linkage polymorphism with other sequence variations that might affect expression or function.

It is increasingly clear that SNPs (single nucleotide polymorphism) may be useful as a marker for the identification of genetic factors associated with complex disease features. In addition, it has been reported that genes containing SNPs can cause different mRNA structural folds. This mRNA variant may have different biological functions that interact with other cellular components.

5. Conclusions

Based on the results of this study it can be concluded that the beta estrogen receptor polymorphism (rs1256049) of beta estrogen receptor is associated with epithelial ovarian cancer. It is suggested that genetic counseling is needed for early detection and preventive measures for individuals at risk of ovarian cancer and further research is needed using larger samples to adequately represent the Indonesian population and use more varied characteristics so that factors can be found influence the incidence of epithelial ovarian cancer. Finally, it is necessary to conduct research on other mutation points so that genetic variants can be found in epithelial ovarian cancer.

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