

## Research Article

**The Effect of Polymorphisms of Estrogen Receptor  $\beta$ RS1271572 to the Incidence of Epithelial Ovarian Carcinoma*****Pengaruh Polimorfisme Reseptor Estrogen  $\beta$ RS1271572 Terhadap Kejadian Karsinoma Epitel Ovarium*****Dwi S. Indrasari, Rizal Sanif, Yusuf Effendi, Irsan Saleh***Department of Obstetrics and Gynecology  
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Palembang***Abstract****Objective:** To determine the effect of polymorphism of estrogen receptor  $\beta$  to the risk of epithelial ovarian carcinoma at Dr. Mohammad Hoesin Hospital Palembang**Methods:** This population-based case control study included 40 women with epithelial ovarian carcinoma and 40 controls, from January 2010 until December 2011. Data analysis was performed by Chi Square test.**Results:** Distribution of estrogen receptor beta genotypes, both GT and TT (wild type-mutant and mutant), among case subjects was significantly higher than control (24 subjects {60%} vs. 5 subjects {12.5%}). The genotype effect was statistically significant for rs1271572 (OR=2.636; p=0.039) with Chi Square analysis. Whereas the allotype effect was also statistically significant (OR=1.949; p=0.047).**Conclusion:** Polymorphism of estrogen receptor  $\beta$  may play a role in the risk of epithelial ovarian carcinoma at Dr. Mohammad Hoesin Hospital Palembang.

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**Keywords:** epithelial ovarian carcinoma, polymorphism of estrogen receptor  $\beta$ , risk factor of ovarian carcinoma**Abstrak****Tujuan:** Mengetahui adanya pengaruh polimorfisme rs1271572 gen reseptor estrogen  $\beta$  pada kejadian karsinoma ovarium epitel di Rumah Sakit Dr. Mohammad Hoesin Palembang.**Metode:** Penelitian ini merupakan desain studi kasus control dengan subjek 40 kasus dan 40 kontrol. Penelitian dimulai Januari 2010 sampai Desember 2011. Analisis data menggunakan uji Chi Square.**Hasil:** Dari hasil penelitian didapatkan distribusi genotip polimorfisme rs1271572 gen reseptor estrogen  $\beta$ , di mana genotip GT dan TT (wild type-mutan dan mutan) lebih banyak terdapat pada kelompok kasus yaitu 24 kasus (60%) dan 5 kasus (12,5%), sedangkan pada kelompok kontrol, yang terbanyak adalah genotip GG (wild type) dengan 20 subjek (50%). Pengaruh polimorfisme gen reseptor estrogen  $\beta$  pada kejadian karsinoma ovarium epitel berdasarkan genotipnya dari analisis statistik dengan uji Chi Square, didapatkan nilai p=0,039 dan Odd Ratio 2,636. Sedangkan dari analisis statistik terhadap pengaruh polimorfisme reseptor estrogen  $\beta$  berdasarkan allotipenya didapatkan nilai p=0,047 dan Odd Ratio=1,949.**Kesimpulan:** Polimorfisme gen reseptor estrogen  $\beta$  mempengaruhi terjadinya karsinoma ovarium epitel di Rumah Sakit Dr. Mohammad Hoesin Palembang.

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**Kata Kunci:** faktor risiko karsinoma ovarium, karsinoma ovarium epitel, polimorfisme gen reseptor estrogen  $\beta$ 

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**INTRODUCTION**

Ovarian cancer is the most common gynecological malignancy, with over 5,000 new cases diagnosed every year in the UK and 22,000 in United States. Malignant neoplasm of the ovaries occur at all ages and the incidence rates increase dramatically with age. Starting from women aged 40-44 years, which has a rate of 15.7 per 100,000, and increased to a rate of 54 per 100,000 in women 75-79 years old. The rate increase to more than doubles after the age of 50 years, while one third of the case was found in women over 65 years old.<sup>1-6</sup>

The highest incidence was found in Norwegia (15.3/100,000), while the lowest incidence was found in Japan (3.2/100,000). In the Southeast Asia region, including Indonesia, the incidence rate is 6.6%. Data from 2006-2008 in Department of Pathology Medical Faculty University of Sriwijaya Palembang demonstrated that ovarian cancer accounts for 12% of all gynecological malignancy, while in 2007 the incidence decreased until 7% and in 2008, ovarian cancer accounts for 10% of all gynecological malignancies.<sup>7,8</sup>

Epithelial ovarian tumor consist of serous, mucinous, endometrioid, clear cell, and mixed epithelial type. Epithelial type is the most common type found in ovarian cancer, which accounts for 85%-90%.<sup>6-8</sup> Based on the theory, etiology of ovarian cancer consist of incessant ovulation hypothesis, gonadotropin hypothesis, androgen hypothesis, progesteron hypothesis, parity, pill contraception, tubal ligation, menopausal hormonal replacemant therapy, and hereditary factors. One of these that also affect the incidence of ovarian cancer is involvement of estrogen receptor, both the alpha and beta receptor. Thus, polymorphism of estrogen receptor gene may play a role in the development of ovarian cancer.<sup>6,9,10</sup>

Endogenous and exogenous estrogen play a role in ovarian carcinogenesis. Estrogen effects are mediated by two estrogen receptors, estrogen receptor alpha ( $ER\alpha$ ) and estrogen receptor beta ( $ER\beta$ ). Both ER subtypes are present in ovarian surface epithelial cells, and reponsive to the estrogen.<sup>9,10</sup>

Ovarian carcinoma is the most complex form of all of malignancy, both hystopathologically and immuno hystochemically. Many researchers try to determine the factors that may play a role in ovarian carcinogenesis. One of those is polymorphism of estrogen receptor gene. Lurie et al (2008) demonstrated the effect of polymorphism of estrogen receptor gene to the risk of epithelial ovarian cancer and found that there is effect polymorphism of estrogen receptor  $\beta$  to the risk of epithelial ovarian carcinoma.<sup>10,11</sup>

$ER\beta$  is the predominant type in the normal ovary. It has been shown that it plays a role incellular proliferation and apoptosis. This polymorphism of  $ER\beta$  will altered the function of  $ER\beta$  in cellular regulation. A loss of  $ER\beta$  expression could thus constitute a crucial step in ovarian carcinogenesis, although the precise mechanism of  $ER\beta$  role in ovarian carcinogenesis remains to be undetermined.<sup>5,6</sup>

Lurie et al found that there is an association between polymorphism of  $ER\beta$  rs1271572 and the risk of developing invasive ovarian carcinoma.<sup>10-12</sup> That, combined with the fact that there is no previous finding about polymorphism of  $ER\beta$  in Dr. Mohammad Hoesin Hospital Palembang, caused the researcher to be interested in this finding.

## METHODS

This study was a case control study, held in Obstetrics and Gynecology Department at Dr. Mohammad

Hoesin Hospital, Palembang, in collaboration with Palembang Distric Laboratory, South Sumatera, Indonesia, from January 2010 until December 2011. Subject of this study was women diagnosed with epithelial ovarian carcinoma admitted to the obstetric and gynecology ward in Dr. Mohammad Hoesin hospital, Palembang.

Inclusion criterias were women diagnosed with epithelial ovarian carcinoma, proven by histopatolology examination, agree for blood examination and signing the informed consent form. The control group consists of patients diagnosed with no neoplasm, who were willing to be the subject of the study and signing the informed consent form.

Patient meeting the inclusion criteria received counseling and a questionnaire. The blood sample was taken and placed in reaction glass which was already filled with 3 cc ethylene diamine tetraacetic (EDTA). Then, the glass was placed at maximum temperature 40° Celcius until the PCR was done, followed by DNA extraction.

To determine the polymorphism of estrogen receptor  $\beta$ , the allele was analyzed with PCR method followed with sequencing. This study determined the polymorphism of estrogen  $\beta$  gene receptor at 5' region (rs1271572) using a pair of primary nucleotide forward TGCCAGCGACACTCT and reverse AGGCCTTTCGCGTTAGATCA resulting 700 bp. Amplification with PCR method was done at thermal cycle DNA branded ICycler BIO-RAD Laboratories GB programmed for two step cycle based on modified Yaich method at a temperature of 95° Celcius denaturation for 5 minutes, followed by 30 cycles of denaturation at a temperature of 94° Celcius for 60 seconds, annealing at a temperature of 66° Celcius for 60 seconds and extension at a temperature of 72° Celcius for 60 seconds. Recently performed final extension cycle for 6 minutes at a temperature of 72° Celcius.

DNA sequencing was performed to confirm the presence of allele polymorphism. DNA sequencing was performed in the laboratory of molecular biology Eijkman Jakarta. All data were analyzed using SPSS 16.0 for Windows.

## RESULT

From January 2010 until December 2011, we had 80 subjects, divided into 40 subjects as case group and 40 subjects as control. Age was divided into 2 groups based on the value of mean  $43.13\pm 10.74$

(43 years). We found that 67.5% of the subjects in the cases group are in  $\geq 43$  year age range. Largest proportion of parity in the case is nulliparous of 37.5%. History of hormonal contraceptive use in the case of 40.0% where as in the control group by 52.5%. There is 62.5% subjects with menopausal status at the case group which is the largest percentage, while there were 55.0% subject in the control group not menopause yet. In the case group, found that 2.5% of the subjects had a family history of ovarian cancer while in the control group it was 10% of the subject.

To assess the polymorphism of  $\beta$  estrogen receptor, alleles analyzed by PCR (Polymerase Chain Reaction) and followed by DNA sequencing to confirm the presence of allele polymorphism. From the DNA sequencing, individuals with no polymorphisms in  $\beta$  estrogen receptor gene showed with wild-type allele with GG genotype. The existence of polymorphisms in  $\beta$  estrogen receptor gene is indicated by changes in G to T. Subject who have a homozygous mutant allele with the TT genotype while in individuals with wild-type and mutant alleles (heterozygous) with GT genotype. At electropherogram of DNA sequencing, interpretation is peaks consist of four colors representing each nucleotide that is green for adenine (A), red for thymine (T), blue for cytosine (C), and black for guanine (G).

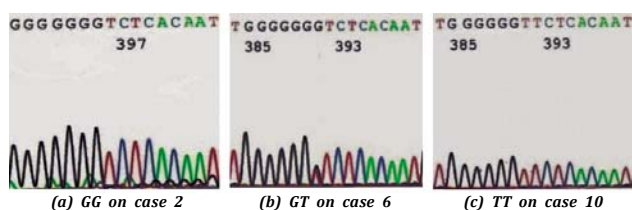


Figure 1. DNA sequencing.

There were 60% subject at case group with GT genotype while there were 45% at the control group. Case group had 12.5% with TT genotype while control group had 5% of it.

Table 1. Distribution of genotype polymorphism rs1271572 at 5'area receptor  $\beta$  estrogen gene

Genotype polymorphism rs1271572 at 5' area	Group			
	Case	%	Control	%
GG (Wild type)	11	27.5	20	50.0
GT (Wild type/mutant)	24	60.0	18	45.0
TT (Mutant)	5	12.5	2	5.0
Total	40	100.0	40	100.0

Statistical analysis Chi Square test found a significant effect of genotype polymorphism rs1271572 in the area of 5' estrogen receptor  $\beta$  gene on the incidence of epithelial ovarian carcinomas ( $p=0.039$ ) with the odds ratio of 2.63.

Table 2. Effect of genotype polymorphism rs1271572 on 5' area estrogen receptor  $\beta$  gene to the incidence of epithelial ovarian carcinomas.

Genotype polymorphism rs1271572 on 5' area	Group			
	Case	%	Control	%
TT + GT	29	72.5	20	50.0
GG	11	27.5	20	50.0
Total	40	100.0	40	100.0

Chi Square test,  $p = 0.039$ ; OR = 2.636; 95% CI (1.04-6.68)

T allotype (mutant) is higher in case group (42.5%) while in control group (27.5%).

Table 3. The effect of allotype polymorphism rs1271572 in 5' area estrogen receptor  $\beta$  gene to the incidence of epithelial ovarian carcinoma.

Allotype polymorphism rs1271572 in 5' area	Group			
	Case	%	Control	%
T (Mutant)	34	42.5	22	27.5
G (Wild type)	46	57.5	58	72.5
Total	80	100.0	80	100.0

Chi Square test,  $p = 0.047$ ; OR = 1.949; 95% CI (1.01-3.77)

There are 16 subjects in case group who use hormonal contraceptive and there are 21 subjects in control group. From 16 subjects in case group, majority of women has T allotype, it's about 46.9% while G allotype is 71.4% found in control group.

Table 4. The effect of allotype polymorphism rs1271572 estrogen receptor  $\beta$  gene in 5' area to the incidence of epithelial ovarian carcinoma based on hormonal contraceptive use

Allotype polymorphism rs1271572 in 5' area	Group			
	Case	%	Control	%
T (Mutant)	15	46.9	12	28.6
G (Wild type)	17	53.1	30	71.4
Total	32	100.0	42	100.0

Chi Square test,  $p = 0.105$ ; OR = 2.206; 95% CI (0.84-5.78)

## DISCUSSION

The incidence rate of epithelial ovarian carcinoma increase dramatically with age. The age group 40-44 years, which has a rate of 15.7 cases per 100,000, and the rate more than doubles after the age of 50 years to about 35 cases per 100,000.

The case subjects in this finding, majority of women is nulliparous. McGowan et al reported in his study that 197 women with ovarian cancer found the nulliparous had a 2.45 times increased risk of malignant ovarian tumor compared with the parous women (with parity 3 or more).

Based on history of hormonal contraceptive use, percentage of contraceptive use is higher in control group, it's about 21 subjects (52%) than case group, it's about 16 subjects (40%). Many of studies report that pill contraceptive use can decrease risk of ovarian carcinoma.

Majority of case subjects in this research is postmenopausal women, it's about 25 subjects (62.5%) Based on the theory more than 80% epithelial ovarian carcinoma is found in postmenopause. While in premenopausal women, malignant ovarian tumor is found only 7%. Based on the theory, menopause itself didn't affect ovarian cancer, but the study report that there is association between use of hormonal replacement therapy during menopause in long period (more than 10 years) with the increase of ovarian cancer risk. Although in this study, all of menopausal subjects, there is no subject who use hormonal replacement therapy. History of familial ovarian carcinoma is found only 2.5% in case group, while the higher percentage is 10% found in control group. The majority of hereditary ovarian cancer is caused by mutation of BRCA<sub>1</sub> and BRCA<sub>2</sub> genes, also in breast cancer.

Result of allele analysis with PCR method (Polymerase Chain Reaction) followed with DNA sequence, found polymorphism of estrogen receptor  $\beta$  rs1271572 which is shown there is transition allele G $\rightarrow$ T. Where as the reference of nucleotide sequence is taken based on data from National Center for Biotechnology Information (NCBI) for SNPs rs1271572: TTAGGCACAGATGTGACACTGGGGGG[G/T] TCTACAATGGCCTGTGGGTCACAT. From this sequence found three genotype such as GG (wild type), GT (wild type-mutant), and TT (mutant).

Based on the genotype distribution from the result of allele analysis through DNA sequence found 60% subjects in case group with GT genotype,

while control group is 45%. For TT genotype, there is 12.5% in epithelial ovarian carcinoma group and 5% in control group. While the control group has higher proportion for GG genotype (wild type).

In epithelial ovarian carcinoma group found that there is significantly differentiation in percentage between GT and TT (wild type-mutant and mutant) compared to GG (wild type), it's about 72.5% for genotype polymorphism, while only 27.5% for normal genotype. From the result of Chi Square test statistically found that there is effect of genotype polymorphism ER $\beta$  rs1271572 significantly to the risk of epithelial ovarian carcinoma with p value = 0.039 and OR=2.636. Result of analysis statistically based on allotype to determine the effect to the risk of epithelial ovarian carcinoma that also show the significantly result with p value = 0.047 dan OR = 1.949.

The effect of rs1271572 polymorphism suggest the result of previous finding which also suggest the similar result. From 10 case control studies found that there is association TT genotype rs1271572 with risk of ovarian cancer. Estrogen receptor  $\beta$  is the predominant type in the normal ovary. Estrogen interact with their receptors to mediate various signaling pathways and biologic activity of estrogen play a role to affect the growth and cell differentiation as well as function of reproductive tissue. The role of estrogen receptor  $\beta$  itself is in regulation of cancer cells that play a role of regulation cellular proliferation (antiproliferative), cell motility, and as cell apoptosis. However, with there is this polymorphism may alter the role of regulation in cellular proliferation which will affect transcription process become abnormal and then as antiapoptotic cell.

Based on hormonal contraceptive use, the effect of allotype polymorphism rs1271572 itself to the risk of epithelial ovarian carcinoma found statistic analysis with Chi Square test show that there is no effect significantly with p value=0,105. In the study by Lurie et al found there is association between polymorphism rs1271572 to the risk of ovarian cancer in women who never use hormonal contraceptive with p value = 0.04.

## CONCLUSION

In this study found there is significantly effect between polymorphism rs1271572 in 5' region estrogen receptor  $\beta$  to the incidence of epithelial ovarian cancer (p=0.039).

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