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The Role Of "Il 10 (-2 1082 A/G)" Promoter Polymorphism In Ovarian Epithelial Cancer Pathogenesis

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Article Info	ABSTRACT
	Ovarian cancer (ovarian cancer) is the abnormal development of cells in one or two ovaries. Many cytokine functions are related to the development of immunological and inflammatory responses that play essential roles in cancer pathogenesis. Among the cytokines, interleukin 10 (IL-10) is an anti-inflammatory cytokine involved in the downregulation of cytotoxic and inflammatory cell-mediated responses. This study aims to determine the relationship between the Gene Promoter Polymorphism Interleukin 10 (-1082 A/G) with the incidence of ovarian epithelial cancer. This research is an observational analytical study conducted at the Molecular Biology Laboratory in Palembang City in October-December 2021. The number of samples was 60 respondents consisting of 30 case groups and 30 control groups. Data were obtained by genotype and allele using PCR-RFLP, which was then analyzed by Chi-Square test to determine whether there was a relationship. The results showed no significant relationship between the promoter of the IL 10 gene polymorphism (-1082 A/G) and the incidence of ovarian epithelial cancer.
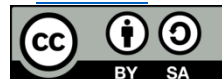
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1. INTRODUCTION

According to data, cancer is still the most significant health problem in Indonesia, with 400 thousand new cases and 230 thousand deaths (WHO 2020). This multifactorial disease results from a complex interaction between genetic and environmental factors (1–4). In Indonesia, the maternal mortality rate caused by gynecological diseases is ovarian cancer, which ranks third after cervical cancer and breast cancer (5).

Cancer is a disease with multifactorial causes that develop over a long period and progress through different stages (6–9). Nutritional factors are one of the most critical and complex aspects associated with the pathological process of cancer. In general, total intake of various fats may be related to an increased incidence of several primary cancers such as breast, colon, prostate, ovarian, endometrial, and pancreatic cancers (10). The status of excess nutrition called obesity has been proven in many studies as a risk factor for cancer, and physical activity is a factor in reducing obesity because it is the primary determinant in energy expenditure so that it can reduce the risk of cancer caused by obesity (11-14). That is stated in a study related to risk factors that predict cancer cell growth: smoking, diet, alcohol consumption, reproduction (pregnancy, breastfeeding, age at first menstruation, menopause), obesity, and lack of physical activity (8,10,15,16).).

Worldwide as many as 204,000 women per annum are diagnosed with ovarian cancer, and 125,000 women die from it (2). Data obtained from 90-95% of cases of ovarian cancer are asymptomatic epithelial types so that more than two-thirds of ovarian cancer patients are diagnosed in an advanced stage (17). The incidence of ovarian cancer in a woman aged 40-44 years was 157 out of 100,000 and increased to 54 out of 100,000 people at the age of 75-79 years (18,19). Cancer occurs at all ages, and the incidence increases with age (20).

In Indonesia 2020, ovarian cancer will be the fifth leading cause of death, and it is estimated that the death rate from this cancer is 5%, with 22,280 new cases, with a death rate of 14,240. (1) This cancer mainly occurs in older women; more than half of women diagnosed with ovarian cancer aged 63 years(18). Based on data from Dharmais

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Cancer Hospital from 2010-2013, ovarian cancer was ranked fourth after breast, cervical, and lung cancer, with 134 new cases in 2013 and 34(21)(22) deaths.

Risk factors for the development of ovarian cancer include environmental and genetic, one of which is a family history of breast cancer or ovarian cancer. This history contributes 5-10% to the incidence of ovarian cancer in subsequent genetics (17,18,23). Women with cancer have a defense against apoptosis that interferes with the work of specific genes so that there is continuous cell proliferation which is coded for by these genes. In ovarian cancer, the activity of the defense program occurs in the interleukin ten gene group (IL-\10)(24). Many cytokine functions related to the development of immunological and inflammatory responses play an essential role in the pathogenesis of cancer, including the cytokine interleukin 10 (IL-10), an anti-inflammatory cytokine involved in the downregulation cytotoxic and inflammatory cell-mediated responses.

Cytokines play an essential role in modulating the immune response, produced by immune stimulation of cells by binding to receptors; specific cytokines can activate the regulation, proliferation, and differentiation of target cells, respectively. Cytokines will regulate immune reactions and inhibit cell growth; cytokines also play a role in stimulating and inhibiting the production of other cytokines. Cytokines are divided into two groups based on their anti-inflammatory and pro-inflammatory functions; cytokines rapidly paralyze the part of the immune system (8). Many studies have concluded that the expression of cytokines at different levels is also determined by polymorphisms of the genes encoding these cytokines (20).

Interleukin 10 (IL-10) is an essential immunoregulatory pleiotropic cytokine mainly secreted by macrophages, T helper one and T helper two lymphocytes, dendritic cells, B lymphocytes, and trans cells. Several studies have shown that interleukin 10 (IL-10) can be produced in human cancer cell line states; IL-10 activity is mediated via IL- 10, which is remembered and from the class II cytokine receptor family. Interleukin 10 (IL-10) inhibits the capacity of monocytes and macrophages to antigen. Current to T cells via inhibitory effects on the expression of major histocompatibility complex (MHC) class II, stimulation of molecules such as CD 80 and CD 86, therefore downregulation of IL-1, IL-6, IL-8, IL-12 and tumor necrotic factor-alpha in B cells. Interleukin 10 (IL10) prevents apoptosis, increasing cell proliferation. Interleukin 10 (IL-10) is located on chromosome 1 at IQ 31-32, spanning 4.7 kb and containing four introns and five exons. Many genetic variations exist in the interleukin ten genes (IL- 10)(10,25).

Among the cytokine regulation seen in human cancer, interleukin 10 (IL-10) and transforming growth factor B (TGF-B) appear to be the two best immunosuppressive cytokine characters, changing growth factor-B (TGF-B) is a multifunctional protein with a wide range from the activity of biological cells from different lineages. Secretion of transforming growth factor-B (TGF-B) has been detected in the standard and neoplastic human ovarian epithelium; as in other tumors, the main effect of changing growth factor-B (TGF-B) in normal cells in vitro is reported to be inhibition of proliferation. . However, it has now been shown that transforming growth factor-B (TGF-B) strong suppresses T lymphocyte proliferation and antibody production by B cells and may also suppress the cytolytic activity of natural killer cells (26–28).

Research on the IL-10 gene polymorphism (-1082A/G) has been carried out in several countries, one of which by (10) in populations in Asia showed that it resulted in an increased risk of cancer. In another study conducted by (29), the expression of IL-10 and IL-6 was investigated in 50 patients with a proven diagnosis of colorectal cancer and 25 patients without malignancy. The result is that there was a significant relationship between IL-10 levels surgical outcomes.

2.   RESEARCH METHOD

This research is an analytic observational study. The research was conducted by laboratory examination with a case-control study approach (Case-Control. The population in this study was all patients diagnosed with ovarian epithelial cancer who came to the polyclinic and were treated at the Hospital in Palembang City. The patient's blood sample had become a sample collection from the laboratory Medical Biology, Faculty of Sriwijaya University, Palembang. The research sample amounted to 30, which was taken using the Consecutive Sampling technique.

DNA Extraction: Take 200 l of blood put into a sterile 1.5 ml tube, wash with 1000 L of PBS pH 7.4, then centrifuge at 5,000 rpm for 5 minutes, discard the supernatant. This stage is repeated 2-3 times. The supernatant was discarded, then 500 L of 0.5% saponins were added mixed well using a vortex. Incubation for 24 hours in a refrigerator at -20oC. Next, vortex back to let it melt immediately, then centrifuge at 12,000 rpm for 10 minutes. The supernatant was discarded, added PBS 1000 L, centrifuged at 5000 rpm for 10 minutes, discarded the supernatant, repeated two times until the supernatant was clear. The supernatant was discarded, added 50 l Chelex and 100 l ddH2O, incubated/boiled in boiling water (using a heat-lock device) for 5 minutes, and then vortexed. Centrifuge at

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1000 rpm for 1 minute, Incubated in boiling water for 10 minutes, Centrifuge at 12000 rpm for 10 minutes. DNA will be in the supernatant (DNA containing water). Then this part is transferred in a sterile tube and stored at -200C.

Polymerase Chain Reaction (PCR): The working principle of PCR goes through 3 stages, namely denaturation, annealing, and extension. In this study, the IL 10(-1082A/G) gene polymorphism was assessed with forwarding primer: 5'CTCGCTGCAACCCAACTGGC-3′ and reverse primer 5'TCTTACCTACCTATCCCTACTTCC-3'. For the PCR step, mix ddH2O ddH2O 9 l, Green goes Taq 10 l, Primer Forward 0.5 l, Primer Reverse 0.5 l, DNA 5 l. Amplification by PCR method was carried out on DNA Thermal Cycle brand Icycler BIO-RAD Laboratories GB programmed for two steps of denaturation at an initial temperature of 94oC for 5 minutes, followed by 35 denaturation cycles at 94oC for 45 seconds, annealing at 60oC for 45 seconds, and extension at 72oC for 45 seconds. The last cycle was carried out with a final extension for 7 minutes at a temperature of 72oC.

RFLP: The IL 10 gene polymorphism (-1082 A/G) was determined by PCR-RFLP analysis using the MnII enzyme. The RFLP process used a mixture of 3.5 l ddH2O, 1 l Buffer, 0.5 MnII Enzyme, 8 l Amplicons. Then, it was vortexed for a few seconds and incubated in a water bath at 37°C for 2 hours. After digestion by the MnII enzyme, the product was electrophoresed on 2% agarose gel and viewed with Ethidium Bromide (EtBr) staining.

Electrophoresis: A total of 2 grams of agarose was weighed and put in an Erlenmeyer glass. Add 100 ml of TAE buffer. Mixed and heated in the microwave for 1 1 minute 30 seconds. Then add 3.5 l of ethidium bromide to cool in the mold for 30 minutes. 4 l loading dye and 0.7 l DNA leader were mixed and used as markers. The 15 l PCR product and tag were inserted into the agarose well and then inserted into the electrophoresis apparatus. The device is set at 100mV, 400 amperes, for 25 minutes and then visualized using Gel-Doc equipment made by BIO-RAD Laboratories USA, connected to a computer using Quantity One.

3. RESULTS AND ANALYSIS

Figure 1. Results of RFLP examination of the IL-10 gene (-1082/G) inAA (wild type) at 139 bp, AG genotype (heterozygous) 3 bands (139.106.33 BP) GG homozygous mutant two bands (106.33 bp)
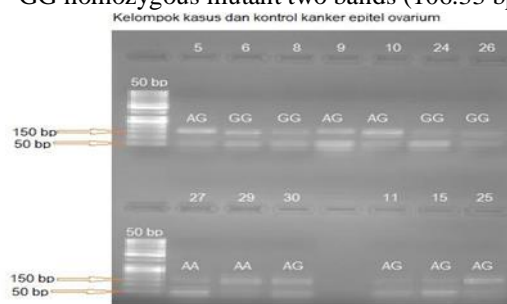

Table 1. Frequency Distribution of Genotype 10 (-1082 A/G)

Genotype	Epithelial Cancer Ovaries	No Epithelial Cancer Ovaries
	n (%)	n%
AA	9 (30,0)	11 (36,7)
AG	9 (30,0)	3(10,0)
GG	12 (40,0)	16(53,3)
Total	35 (100)	35(100)

The largest number of respondents found in the case and control groups were 12 respondents (40.0%) and 16 respondents (53.3%). The AG genotypes were 9 (30.0%) and 3 respondents (10.0 %). While the AA genotype, there were nine respondents in the case group (30.0%) and 11 respondents (36.7%) in the control group.

Interleukin 10(-1082A/G) gene promoter polymorphism relationship with the incidence of ovarian epithelial cancer

The interleukin 10 (-1082A/G) gene promoter polymorphisms in the ovarian epithelial cancer group and non-ovarian epithelial cancer can be seen in Table 4.3. In the ovarian epithelial cancer group that had

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polymorphisms with AG/GG genotype, 21 (70.0%) of the 30 subjects were examined. In the non-ovarian epithelial cancer group that had polymorphisms with AG/GG genotype, 19 (63.3%) of the 30 subjects were studied. Based on statistical analysis using the Chi-Square test, p-value = 0.075, it can be concluded that there is no statistically significant relationship between the interleukin ten gene promoter polymorphism (-1082 A/G) and the incidence of ovarian epithelial cancer (p>0.05).

Table 2. The relationship between the promoter polymorphism of the Interleukin10
gene (-1082A/G) with the incidence of ovarian epithelial cancer

		Group				p-Value
		Epithelial Cancer Ovaries		No Epithelial Cancer Ovaries		
		n	%	n	%	
polymorphism	There's Mutant (AG/GG)	21	70,0	19	63,3	0,075
	There's no Mutant (AA)	9	30,0	11	36,7	
Total		30	100,0	30	100,0	

Table 3. Allotypes of Interleukin 10 Genes (-1082A/G)

Allele Genes	Epithelial Cancer Ovaries	No Epithelial Cancer Ovaries
	%	%
A	30 (50)	38(63,3)
G	30(50)	22(36,7)
Total	60(100	60(100)

Allotype A in the case group has as many as 30 respondents (50%), while 38 respondents (63.3%) in the control group. For allotype G, the most cases were 30 respondents (50.0%), while the control group was 22 respondents (36.7%).

In this study, the promoter of the IL 10 gene (-1082 A/G) was examined. The results of this study were found in the case group that had 9 (30.0%) AA (wild type) genotypes, 12 AG (heterozygous mutants) genotypes(40.0%), GG genotype (homozygous mutants) was 9 (30.0%). From the results of this study, it was found that the frequency of individuals carrying the GG genotype (homozygous) was 9 (30.0%) higher in the ovarian epithelial cancer group compared to the control group of ovarian epithelial cancer as many as three respondents (10.3%). From the chi-square test results, there was no relationship between the IL 10 gene promoter polymorphism (-1082 A/G) and the incidence of ovarian epithelial cancer, p-value = 0.075. This study is in line with the proposed results (29,30) showing the ethnic distribution of the SNP. The European population showed more frequent GG genotypes (50%), whereas Asians were present with this haplotype in only a few cases (less than 5%).

However, these results are not in line with Stanczuk (2001) in Asia, who found that IL-10 expression in vitro appears to be determined mainly by the polymorphism at the -1082 position. Studies show an association of -1082 alleles A and G with low (AA), moderate (AG), high (GG)(31). The study conducted by Galizia et al. on the expression of IL-10 and IL-6 was determined in 50 patients with histologically proven colorectal cancer and 25 patients without malignancy (29). Blood was also collected before and after surgery. The results showed a significant association between IL-10 and surgical outcomes (p = 0.0005)

4. **CONCLUSION**
The interleukin ten gene polymorphism (-1082 A/G) does not support the development of ovarian epithelial cancer in women. A larger sample and comparison with other genes are needed to objectively see the effect of the interleukin gene on ovarian cancer.

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