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Polymorphism of p53 Codon 72 Gene on Cervical Cancer Incidence in Malay Population

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ABSTRACT

In Indonesia, the cases of cervical cancer are estimated at around 50 per 100.000 people. It was estimatedthattherearemore than 1 millionwomenworldwidewho have cervical cancer, andmostof themhave not been diagnosed yet or do not have access to screening and medical treatment. P53 codon 72 polymorphism can affect the risk of cervical cancer through the regulation of proliferationandcellapoptosis.The purpose of this research was to investigate the association between p53 codon 72 polymorphism and cases of cervical cancer. This research was observational analytic research. The research was done by examining in the laboratory of Molecular Biology, Faculty of Medicine, Universitas Sriwijaya by using a case-control study approach.Thesampleoftheresearchconsistedof 70 subjects,and they were divided into two groups:35in case group and 35 in the control group. The determination of genotype and allotype was done by using PCR-RFLP technics. The results of the research showed there was a significant difference between p53 codon 72 polymorphism between the case group and control group. The results of genotypes of p53 codon 72 polymorphism in the case group were seven respondents (20.0%) with Pro/Pro genotype, five respondents (14.3%) with Arg/Arg genotype, and 23 respondents (65.7%) with Pro/Arg. While in control group, there were 28 respondents (80.0%) with Pro/Pro genotype, 0 respondent (0.0%) with Arg/Arg genotype, and 7 respondents (20%) with Pro/Arg. The frequency of the Prolin allele in case group was 37 (52.9%), and the Arginin allele was 33 (47.1%), while the frequency of the Prolin allele in the control group was 63 (90%), and the Arginin allele was 7 (10%). The Chi-Square of genotype was valued 0.000, OR 1,304 and CI 95% 1,071-5,891, while the allele was valued 0.000, OR 8.027 and CI 95% 3.228-19.962. There was an association between genotype and allele of p53 codon 72 polymorphism and cases of cervical cancer.

1. Introduction

Cervical cancer is a malignant tumour that grows in the cervical area (cervix).[1] In 2010, there were 43,470 new cases diagnosed and 7950 deaths in the United States.[2,3] In Asia, the incidence of cervical cancer is 20-30 / 100.000 women deaths 5 - 10 / 100.000 women with cervical cancer are mainly found at the age of 45 - 50 years.[4] In Indonesia, the incidence of cervical cancer is estimated at around 50 per 100.000 population.5 It is estimated that there are more than 1 million women worldwide with cervical cancer and most of them have not been diagnosed or do not have access to screening and treatment.[6]

The high mortality rate is due to late treatment, about 70% of which come with an advanced stage.[1,7] Based on the patient's age group, the incidence of cervical cancer is low at <20 years of age, and increases rapidly and persists at the age of 50 years, while carcinoma in situ starting at a younger / earlier age and peaking at the age of 30-34 years, whereas dysplasia peaks at the age of 20-29 years and decreases to the age of 50-59 years and increases again at the older age.

The cause of cervical cancer is the Human Papilloma Virus (HPV), which is transmitted through sexual contact or skin contact with the genital area

with HPV.[8,9] The most common cause of death in women is the HPV viruses types 16 and 18.[10] HPV infection in the long term is likely to occur. cervical cancer, but genetic variations that affect the immune response in the host / host determine the results of high-risk HPV infection. Several risk factors for cervical cancer are sexual intercourse at the age of less than 16 years, changing sexual partners, smoking, race, parity. high, and low socioeconomic.[9]

HPV, which is an initiating factor for cervical cancer, which causes cervical cell disorders. Oncoproteins E6 and E7 derived from HPV are the cause of malignant degeneration. The integration of viral DNA with the body's cell genome is the beginning of the process that leads to transformation. Viral DNA integration starts in the E1-E2 region. Integration causes E2 to malfunction, E2 malfunction causes stimulation of E6 and E7 which inhibits p53 and pRb. The second inhibition of TSG (Tumor Supressor Gene) causes the cell cycle to go out of control, DNA repair does not occur, and apoptosis does not occur. E6 will bind to p53 so that the p53 Tumor Suppressor Gene (TSG) will lose its function, namely to stop the cell cycle in the G1 phase. The termination of the cycle in the G1 phase by p53 aims to provide opportunities for cells to repair the damage that occurs. After the repair is complete, the cells will enter the S phase. The p53 gene stops the cell cycle by inhibiting the CDK-cyclin complex. If cell termination in the G1 phase does not occur, and the repair does not occur, the cells will continue to enter the S phase without any repair. These abnormal cells will continue to divide and grow without control. In addition, p53 also functions as a stimulant for apoptosis, which is a cell death process that begins with intracellular gene destruction. The loss of p53 function causes the apoptosis process to not run. [10-12]

Two genes that can trigger apoptosis are the tumor suppressor p53 gene and the prototooncogen, c-myc. These genes will interact with genes that regulate apoptosis to determine whether cells will respond to apoptotic stimuli. In normal cell replication, this gene is inactive. It will be active if there is DNA damage. If the damage is too severe, then p53 stimulates programmed apoptosis as a last resort. If the p53 tumor suppressor gene is still deactivated by a mutation even though there is DNA damage, then the main defense against cell propagation by damaging DNA will be lost, so that cells with damaged and mutated DNA will enter the next phase to carry out cell division. This cell division will take place uncontrollably, forming a collection that causes cancer in an organ.[13]

To date, only five polymorphisms have been reported in the coding region in exon 4 in codon 34, 36, 47, and 72, and one in exon 6 in codon 21. However, polymorphisms of the p53 gene were also found in the intronic region, two in the intron. 1 (19, 20), one on intron 2 (21), one on intron 3 (22), two on intron 6 (23, 24), five on intron 7 (25,26), and one on intron 9.14

In a study conducted on a population in Brazil, polymorphism in p53 occurred as a result of proline to arginine in codon 72 exon 4 and in a population study in Sweden stated that there was no relationship between Squamous Cell Carsinoma (SCC), Cervical Intraepitheal (CIN) and p53 codon 72. Whereas in China, research on the role of p53 and p21 gene polymorphisms on the risk of cervical cancer obtained an Odd Ratio of 2.25 with a Confident Interval (CI) of 1.1 - 4.54 for thep53.15 gene.

In another study, the p53 codon 72 perapolimorphism has been investigated which causes another mutation in the p53 gene. In lung cancer cells, the mutated p53 codon 72 pro allele was associated with an increased risk of tumor development and less chance of patient survival. Thus, codon 72 p53 polymorphisms may contribute to differences between individuals or racial groups in disease susceptibility and severity.

The population of the Malay race in South Sumatra is related to the race in China where the Odd Ratio of the p53 codon 72 gene polymorphism for cervical cancer is quite large so it is likely that the population in South Sumatra is also quite large due to racial kinship and the number of marriages between Chinese and Malay races in South Sumatra. Therefore it is

necessary to do further research on the relationship of the p53 codon 72 gene polymorphism with the incidence of cervical cancer in South Sumatra, especially Palembang.

2. Reaserch Methods

This research is an analytic observational study with a Case-Control design where it is an analytical study concerning how risk factors are studied using a retrospective approach. Case Control is used to find out how far the risk factors influence the occurrence of disease.16 In this study, the role of the p53 codon 72 gene polymorphism with the incidence of cervical cancer is 1:1.

The population in this study were patients who had come to the Obstetrics and Gynecology polyclinic and patients in the Obstetrics Gynecology Department of the Hospital Dr. Mohammad Hoesin Palembang and who was diagnosed with cervical cancer as evidenced by histopathology and patient blood had become a collection of samples stored in a freezer at a temperature of -20oC in the Molecular Biology laboratory of the Faculty of Medicine, Unsri Palembang.

The test used in this analysis is the chi-square test with a confidence level of 95% and a significance level (α) of 5% if the p value is ≤ 0.05, conversely if the p value is> 0.05, then there is no relationship between the two research variables.

3. Results and Discussion

Univariate Analysis

From this study, the characteristics of cervical cancer cases were more prevalent in the case group aged ≥ 50 years (57.1%), with a family history of cervical cancer (62.9%), age at first birth <25 years. (88.6%) and parity ≥2 children (62.9%)

Bivariate Analysis

The polymorphism of the p53 codon 72 gene will be visualized using ultraviolet light, it can be seen that 3 variations of the genotype of the BstUI retractile enzyme cut on DNA extraction / isolation products, namely: the Arg-Arg (GG) Wild Type genotype shows 2 bands, namely 113 bp and 86 bp means that there is no truncation in both alleles. genotype Pro-Arg (GC) mutant heterozygote shows a 3-band picture, namely 86 bp, 113 bp, and 199 bp, which means that there is a cut in one allele and there is no cutting in the allele partner. The homozygote mutant Pro-Pro (CC) genotype shows a 1-band image of 199 bp, which means that there is no cutting in one allele.

In this study, a statistical analysis was carried out with the Chi-Square test to determine the relationship between the p53 codon 72 gene polymorphism and cervical cancer. Statistically, there was a significant relationship between the genotype distribution of the p53 codon 72 gene between the case and control groups with ρ value = 0.000 (OR 1.304; 95% CI 1.071 - 5.891). This study is in line with the previous study17 in 2010 which found a significant relationship between the p53 codon 72 gene polymorphism and cervical cancer ρ value = 0.026 (OR 2.15; 95% CI 1.34-3.78).

In addition, this study is also in line with previous research conducted by Jee et al. In 2003, it was found that there was a significant relationship between the polymorphism of the p53 codon 72 gene and cervical cancer ρ value 0.002 (OR 1.50; 95% CI 1.2- 2.0). As stated by Zhu et al, 200718 in their research, it was found that the genotype of the p53 codon 72 gene polymorphism has the potential as a genetic risk factor for cervical cancer where the statistical results were obtained with a ρ value of 0.005 (OR 1.19; 95% CI 1.00-1.41).

From the results of this study and the various studies above, it can be seen that the genotypic relationship of Arg72 Pro in the p53 tumor suppressor gene is very important for cell cycle regulation related to cell proliferation. Decreased activity of p53 has become the main cause of cervical cancer. The p53 gene mutant type exhibits polymorphism at codon 72 in exon 4, with one nucleotide change. The p53 codon 72 gene polymorphisms are located in proline-rich regions of the p53.19 Protein p53 gene has the ability to induce

apoptosis and protect cells from neoplastic development that is better than the p53 gene.17 According to Storey, in vivo, E6 oncogenes from HPV type 11 (low risk) do not cause p53 degradation, however oncogenes from HPV Type 11 will trigger degradation. p53Arg, although not as efficient as degradation by the E6 HPV type 16 and 18 oncogens which have a high risk of cervical cancer incidence, this suggests that the p53 gene polymorphise increases the susceptibility to degradation of the p53 gene by the E6 oncogenes regardless of whether the infection is low or high risk HPV. The proline form p53 is more susceptible to degradation by the HPV E6 protein than the Arg72 form, and patients with the proline form have a seven-fold higher risk of developing cervical cancer compared to the arginine form.

In this study, a statistical analysis was performed using the Chi-Square test to determine the relationship between the allele Pro gene p53 codon 72 and cervical cancer (Table 3). It was found that there was a significant relationship between the Pro allele in the p53 gene codon 72 and the incidence of cervical cancer ρ value = 0,000 (OR 8,027; 95% CI 3,228 –19,962).

This study is in line with research previously conducted by Xin Zhou et al in 20122 which found a significant relationship between the Pro72 mutant allele and the risk of developing cervical cancer with a ρ value of 0.005 (OR 1.25; 95% CI 1.02-1, 53). In line with the results of the prognostic research on the impact of the p53 codon 72 gene polymorphism conducted by Katkoori et al14 in 2009, it was concluded that individuals with Pro72 allele frequency had a higher risk of cervical cancer during their lifetime than individuals with the Arg72 allele had the potential to perform better apoptosis in responds to cellular stress, so that it is protected from cancer risk with ρ value = 0.005 (OR 2.15; 95% CI 1.02- 4.53).

The frequency of the Pro72 allele was significantly greater than the frequency of the Arg72 allele, this was found in certain races in cases of cervical cancer, in addition to that because polymorphism is also influenced by lifestyle, diet, and environmental exposure which varies according to race and ethnicity, research about the p53 codon 72 gene polymorphism which is located in the proline-rich region is very important because functionally it plays a role in suppressing cancer cell growth and apoptosis.

Limitations of the study: the subjects in this study were categorized as few and there were also no tissue samples for HPV typing, although it is known that HPV infection is a pathogenic factor in the development of cervical cancer, in this study only linked HPV as a causative factor for cervical cancer by causing a cell proliferation disorder reaction. This study only examined the relationship of p53 codon 72 gene polymorphisms with the incidence of cervical cancer.

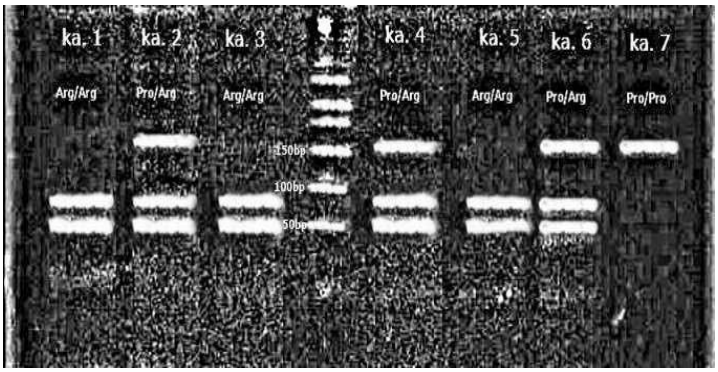

Figure 1. RFLP results with the BstU1 enzyme p53 codon 72 gene

Notes: M: marker ladder 50 bp; genotype GG (113bp and 86bp) of subjects serial number: 1,3,5; genotype GC (86bp, 113bp and 199bp) subject serial number: 2,4,6; genotype CC (199bp) subject serial number: 7.

4. Conclusion

The genotype distribution of the p53 codon 72 gene for the Pro / Pro case group was 20%, Pro / Arg 65.7%, Arg / Arg 14.3%, the Prolin allele 52.9% allele distribution and 47.1% arginine allele. The genotype distribution of the p53 codon 72 gene for the control group Pro / Pro 80%, Pro / Arg 20%, and for Arg / Arg was not found, the distribution of the Prolin allele 90% and the arginine allele 10%. There is a correlation between genotype polymorphisms and allele of the p53 codon 72 gene with the incidence of cervical cancer.

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