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Association between Human Papilloma Virus (HPV) genotype and mutant protein 53 (p53) expression in cervical cancer

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ABSTRACT

Background: Human Papilloma Virus (HPV) infection is the main cause of cervical cancer, a primary malignant tumor developing from squamous epithelial cells. HPV expresses oncoproteins E6 and E7, known to inactivate tumor suppressor proteins, one of which is protein 53 (p53). A promising biomarker for diagnosing and prognosis malignancies brought on by the HPV genotype is the identification of p53. This study investigates the association between p53 expression and HPV genotype in cervical cancer patients.

Method: This observational cross-sectional study involved patients diagnosed with cervical cancer on histopathological examination who met the requirements. The polymerase chain reaction (PCR) method was used to determine the HPV genotype, and an immunohistochemistry analysis was used to assess the level of p53 expression. A Chi-Square test is utilized to evaluate the association between the two variables.

Results: Of the 49 patients, there were 7 (14.3%) patients with HPV type 16, 13 (26.5%) with HPV type 18, 14 (26.5%) other types of HPV and 15 (30.6%) negatives for HPV. Examination of p53 expression showed that 17 (34.6%) samples had <10% expression, 17 (34.6%) had 10-50% expression, and 15 (30.8%) samples had >50% expression. There was no correlation between p53 expression and HPV genotype ($p = 0.071$). However, an association between p53 expression and cervical cancer's clinical stage was identified ($p = 0.028$).

Conclusion: Increased cervical cancer stage can be associated with increased p53 expression. Thus, p53 can be used as a predictor of cervical cancer stage. However, in cervical cancer, p53 expression cannot be associated with the HPV genotype.

Keywords: Cervical Cancer, Genotype, Human Papilloma Virus, Neoplasm, p53.
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INTRODUCTION

Worldwide, there are a projected 569,847 new cases of cervical cancer and 311,365 fatalities in 2018. Based on age-standardized rates (ASRs), cervical cancer is the fourth most prevalent malignancy worldwide, with 13 cases per 100,000 people and a fatality rate of 6.9 per 100,000 people.[1]

The main cause of cervical cancer is the human papillomavirus (HPV). The two classifications of HPV are categorized as low-risk and high-risk. Condyloma acuminata is caused by low-risk HPV, but 15 genotypes of high-risk HPV, including sub-types 16 and 18, are carcinogenic and can result in precancerous lesions. The lesion may become malignant when it is not treated appropriately.[2]

A transcription factor known as protein 53 (p53) regulates genes involved in the cell cycle, apoptosis induction, DNA repair, genome stability, and gene mutations. In more than 50% of instances of malignancy, expression of p53 is the most prevalent molecular aberration, particularly in cervical, colorectal, and lung cancers.[3]

Most p53 mutations in cancer are missense mutations, which change the amino acids in wild-type proteins. These mutations generate mutant proteins that can increase cellular stability but are functionally defective. The p553 mutant accumulates in cells, reaching levels up to 10 to 100 times higher than the wild-type protein.[4]

Expression of p53 can also be used as a biological marker for cervical cancer for prediction and prognosis after treatment. The expression of p53 affects the degree of differentiation of cervical carcinoma and, as a histopathological characteristic, also has significant prognostic value and therapeutic relevance. Research conducted in Lithuania

also found significant differences in the expression of p53 in cervical cancer patients and healthy women.[5]

Various studies on the role of genetics have been developed to understand the etiology and pathophysiology of cervical cancer, either through direct examination of gene mutations or indirectly through the abnormal expression of proteins produced by mutated genes. As a tumor suppressor gene, this research is expected to find a relationship between p53 and the HPV genotype in cervical cancer.[5,6]

Regarding the problems above, the authors were interested in researching the distribution of HPV genotypes that cause cervical cancer in Makassar and the expression of 53 mutant proteins as biological markers for cancer staging. The results may be used to determine the prognosis and response to treatment of cervical cancer.

METHODS

The association between p53 expression and various HPV genotypes and clinical stages was examined in this observational study with a cross-sectional approach. The research was conducted at the obstetrics and gynecology polyclinic at the Wahidin Sudirohusodo Hospital and network hospitals from 09 May 2022 to 30 November 2022. This study's population was all women screened for cervical cancer through a biopsy examination at the study site. All patients diagnosed with cervical cancer based on screening results and willing to be involved in the study were included. Exclusion criteria in this study were patients who had undergone radiotherapy or chemotherapy, had relapsed tumors, had secondary tumors originating from primary cancer metastases from other organs, and had clinically infectious diseases. The Health Research Ethics Committee, Faculty of Medicine, Universitas Hasanuddin (No: 201/UN4.6.4.5.31/PP36/2022) reviewed and approved all of the study's protocols. Each subject was given an adequate explanation of the research procedure to be followed and the right to be willing or not to be involved in the research, to withdraw for some reason during the research, and to keep confidentiality.

The consecutive random sampling technique calculated several samples based on the Lemeshow formula. Alpha and beta values are 5% and 20%, respectively. The mean value of p53 used in this study took data from previous studies, namely 17.50 for positive HPV and 29.74 for negative HPV.[7] A minimum of 40 samples must be included in this study to obtain sufficient external validity values.

Samples from cervical cancer tissue were taken using a biopsy to assess p53 expression. Assessment of p53 expression was evaluated through a series of immunohistochemical examinations according to standard procedures. An epitope at the NHe-terminus between amino acids 35 and 45 of the mutant p53 protein type was identified by the mutant anti-mouse monoclonal p53 monoclonal antibody, DO7 (Dako Corporation Denmark). Deparaffinized tissue samples (3/lm) from the tumor were treated with 0.6% H202 in methanol to inhibit endogenous peroxidases. The slides were heated in a microwave for 10 minutes after being prepared in 10 mM citrate buffer (pH 6.0). The primary p53 monoclonal antibody was applied to the sections after they had been gently cooled, and it was left on them for 45 minutes at room temperature. The sections were first washed three times in PBS for five seconds, then incubated for 30 minutes with biotinylated rabbit anti-mouse immunoglobulin (DAKO 1:500), rinsed, and then set with strep-avidin-biotin complex HRP label (DAKO 1:1000). A diaminobenzidine compound H202 with 0.01 M imidazole (Merck) was used to observe the immunoreaction after the final wash with PBS. Sections from carcinomas previously demonstrated by this method as immunoreactive for p53 served as the positive control. Immunoreactive tumor cells for the p53 were investigated in numerous representative locations of each tumor.[8]

Immunohistochemical assessment of p53 protein using qualitatively and quantitatively monoclonal antibodies by a single anatomical pathologist without a known clinical diagnosis. Therefore, this study did not use a reliability test for interobserver evaluation. The p53 expression measurement results were categorized into low, moderate, and overexpression. Certain cut-offs used to classify mutant p53 are low if the expression of mutant p53 is less than 10% of tumor cells that are positive, moderate if the expression of mutant p53 is 10% to 50% of positive tumor cells and overexpression if p53 expression is detected more than 50% of cells positive.

The genotype of HPV was obtained based on the results of cervical swab sampling through PCR (Polymerase Chain Reaction) Amplification and Hybridization molecular biology tests (HPV XpressMatrix Genotype, Kalgen, Japan) to detect HPV viruses belonging to the high-risk types, namely types 16, 17, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 82. In addition, low-risk HPV types were detected, namely HPV 6, 11, 42, 43, 44, and 81.

The histological type of the tumor and its clinical stage were additional variables evaluated in the present study. The results of a traditional histopathological examination are used to determine the cell and tissue morphologies that can be seen under a microscope to classify a tumor as one of the following: squamous cell carcinoma, adenocarcinoma, clear cell, serous, adenosquamous, glassy cell, adenoid cystic, adenoid basal, small cell, or undifferentiated carcinoma.

The cervical carcinoma stage was assessed based on the Federation Internationale de Gynecolgie et d'Obstetrique (FIGO) classification (revised version in 2018) after clinical examinations, ultrasonography, and abdominal CT scans.[9] Patients with symptoms or signs typical of cervical cancer, such as leucorrhoea or watery discharge from the vagina, ong and smelly discharge from the vagina, bleeding after intercourse (postcoital bleeding), which then progresses to abnormal bleeding, postmenopausal bleeding and pain in the pelvic area or the lower abdominal area is examined if a mass is found in the mouth of the uterus with suspicion Cervical cancer is staged clinically with the FIGO classification 2018, pathological examination of cervical tissue anatomy is carried out and an abdominal CT scan is carried out to determine the spread of cervical tumors to avoid under-diagnosis of cervical cancer.

The statistical software for the social sciences (SPSS), version 23.0 (IBM, USA), was used to process the collected data. Data are expressed using frequency and percentage distributions on a categorical scale. The association between the HPV genotype and p53 expression was examined using the Chi-Square test. If the conditions for the Chi-Square test cannot be met, the Fisher Exact test is an alternative. A p-value of 0.05 or lower was considered significant.

RESULTS

This study initially enrolled 70 women who met the inclusion criteria during the study period. A total of 6 samples were excluded because no cervical carcinoma cells were found in the biopsy sample tissue, so 64 samples were examined for the HPV genotype. Based on the histopathological examination results, 56 samples met the study criteria and were randomized using the Random Sample Generator to receive 49 data followed by immunohistochemical examination. Characteristics of study participants are presented in Table 1. Most participants were over 30 years old, multiparous, coitarche at less than 19 years old, had a single sexual partner, did not smoke, and did not use oral contraceptives. Most participants had tumor sizes > 4 cm and were at an advanced stage. The dominant genotype in the participants was the negative type, followed by the other types and type 18. The expression of p53 was generally in categories 0 and +1.

Characteristics of study participants based on HPV genotype are presented in Table 2. According to patient characteristics, including age, parity, coitarche, use of oral contraceptives, smoking history, tumor size, clinical stage, and histopathological appearance, there were no significant differences based on HPV genotype. However, there are significant differences in the characteristics of sexual partners based on the HPV genotype.

Table 3 reveals the characteristics of study participants based on p53 expression. According to patient characteristics, including age, parity, coitarche, sexual partners, history of smoking, use of oral contraceptives, and tumor size, there were no significant differences based on p53 expression. However, the clinical staging characteristics and histopathological features significantly differed according to the p53 expression.

Table 4 shows the analysis of the association between p53 expression and HPV genotype. Patients with genotypes of HPV type 16, type 18, and other negative types mostly expressed p53 at moderate and high levels. The p53 expression and HPV genotype were not significantly associated (p=0.071).

Table 5 indicates the association between p53 expression and the clinical

Table 1. Characteristics of study participants

Variables	Frequencies (N=49)	Mean ±SD	Percentage (%)
Age (Years)		50.82±11.78	
≤ 30	1		2.0
> 30	48		98.0
Parity			
Primiparous	7		14.3
Multiparous	42		85.7
Coitarche (Years)		18.96±2.95	
≤ 19	28		57.1
>19	21		42.9
Sexual partner			
1	30		61.2
>1	19		38.8
Smoking history			
Yes	7		14.3
No	42		85.7
Oral Contraceptive			
Yes	12		24.5
No	37		75.5
Tumor Size			
≤ 4 cm	15		30.6
> 4 cm	34		69.4
Histopathology Results			
Squamous cell carcinoma	44		89.8
Adenocarcinoma	3		6.1
Adenosquamous	1		2.0
Clearcell carcinoma	1		2.0
Stage			
Early	5		10.2
Advanced	44		89.8
HPV Genotype			
Type 16	7		14.3
Type 18	13		26.5
Other Type	14		26.5
Type 33	4		8.2
Type 39	1		2.0
Type 45	5		10.2
Type 51	2		4.1
Type 58	1		2.0
Type 59	1		2.0
Negative	15		30.6
P53 Expression			
<10% (0)	17		34.6
10-50% (+1)	17		34.6
>50% (+2)	15		30.8

Table 2. Characteristics of study participants based on HPV genotype.

Characteristics	HPV Genotype (N=49)				P
	Type 16 n (%)	Type 18 n (%)	Other Types n (%)	Negative n (%)	
Age (Years)					
≤ 30	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0.419
> 30	7 (14.6)	12 (25.0)	13 (27.1)	16 (33.3)	
Parity					
Primiparous	0 (0.0)	4 (57.1)	1 (14.3)	2 (28.6)	0.207
Multiparous	7 (16.7)	9 (21.4)	12 (28.6)	14 (33.3)	
Coitarche (Years)					
≤ 19	5 (17.9)	8 (28.6)	8 (28.6)	7 (25.0)	0.581
>19	2 (9.5)	5 (23.8)	5 (23.8)	9 (42.9)	
Sexual Partner					
1	6 (20.0)	4 (13.3)	7 (23.3)	13 (43.3)	0.020*
> 1	1 (5.3)	9 (47.4)	6 (31.6)	3 (15.8)	
Oral Contraceptive					
No	7 (18.9)	8 (21.6)	11 (29.7)	11 (29.7)	0.202
Yes	0 (0.0)	5 (41.7)	2 (16.7)	5 (41.7)	
Smoking History					
No	6 (14.3)	11 (26.2)	11 (26.2)	14 (33.3)	0.995
Yes	1 (14.3)	2 (28.6)	2 (28.6)	2 (28.6)	
Tumor Size (cm)					
≤ 4	2 (13.3)	5 (33.3)	4 (26.7)	4 (26.7)	0.890
> 4	5 (14.7)	8 (23.5)	9 (26.5)	12 (35.3)	
Stage					
Early	0 (0.0)	3 (60.0)	2 (40.0)	0 (0.0)	0.148
Advanced	7 (15.9)	10 (22.7)	11 (25.0)	16 (36.4)	
Histopathology Result					
Non keratinizing SCC	7 (17.9)	8 (20.5)	10 (25.6)	14 (35.9)	0.422
Keratinizing SCC	0 (0.0)	3 (60.0)	1 (20.0)	1 (20.0)	
Adenocarcinoma	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	
Adenosquamous	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	
Clear cell carcinoma	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	

SCC: Squamous cell carcinoma; Chi-Square; *Statistically significant if p-value less than 0.05

stage of cervical cancer. There is a significant association between the clinical stage of cervical cancer and p53 expression (p=0.028).

DISCUSSION

This study found characteristics of cervical cancer patients commonly found in previous studies. Based on a case-control study in India, most patients with cervical cancer were 51-60 years old, averaging 54.3 years. Younger marriage age (10-20 years) and the number of sexual partners positively correlate with cervical cancer. Another study showed the mean age of patients with cervical cancer without recurrence was 40.8 years and with recurrences (within five years) 41.1 years.[10,11] Most patients had tumor size below 4 cm, 57% in stage II, and 80% in the SCC tumor subtype. Another study showed that 69.4% of patients with tumor sizes > 4 cm and the histopathological type of tissue found in 89.8% were squamous cell carcinoma.[11] Studies in Finland showed an increased incidence of cervical cancer and cervical intraepithelial neoplasia (CIN3) in multiparous patients with births of ≥ 5 children.[12] Multiparity is a risk factor for cervical cancer, with a relative risk of 3.8-5.1.48. Meta-analysis studies show that the number of sexual partners is correlated with non-malignant cervical abnormalities and invasive cervical cancer, with odds ratios of 1.82 and 1.77. 85.7% Long-term use of oral contraceptives had a significant association with the incidence of cervical cancer (p-value = 0.0005, OR = 12.4).[13] A meta-analysis study showed that individuals who smoke have a 2.03-

fold risk of developing cervical cancer compared to control populations who do not smoke.[14] Epidemiological data in India showed that 35.03% of patients came with stage II and 43.53% with stage III.[15] The genotypes of HPV that were most frequently found in samples of cervical cancer patients in previous studies also varied. The prevalence of HPV in the population in China is type 16 (30.8%) and combinations (22.6%) which are commonly found, namely 59, 31, 16, and 51.[16] The prevalence of HPV in the population in China is type 16 (30.8%) and combinations (22.6%).

Based on the HPV genotype, patient characteristics differed significantly only in sexual partners in this study. In multi-partner patients with a history of more than one partner as a risk factor for cervical cancer, there was an elevated incidence of cervical cancer and cervical intraepithelial neoplasia (CIN3), with a relative risk of 3.8-5.1. The carcinogenic nature of HPV depends on the affinity of the E6 protein in binding to the p53 gene and the E7 protein in binding to the Rb gene. The p53 gene stimulates cell cycle arrest, resulting in DNA repair by apoptosis, which aims to prevent the propagation of cells with DNA damage.[17] A study in India showed that 80% of cases of cervical cancer have p53 gene expression.

Based on p53 expression, the characteristics of the patients differed significantly only in clinical stage and histopathological appearance in this study. The special structure of the E6-protein in HPV 16 and 18 inhibits the control of cell proliferation by deactivating the p53 gene (a negative regulator of the cell cycle).[18] Significantly increased levels of E6 staining correlated with advanced (tumor stage-associated) T stage and FIGO classification.[19] Many genes are involved in cervical carcinogenesis. In addition to p53, the expression of other genes, such as MDM2 and gal-3, are positively correlated in cervical cancer.[20] The proportions of p53, Rb, and c-myc protein expression based on the immunohistochemical method were 40%, 30.8%, and 50.1%.[19,20]

The present study did not find an association between p53 expression and HPV genotype. However, the p53 expression was associated with the clinical stage of cervical cancer. The p53 expression variable is significantly related to the patient's clinical stage. The tumor suppressor p53 is crucial in preventing the progression of cancer. In healthy cells, the ubiquitin ligase Mdm2, whose gene expression is triggered by p53, maintains low levels of p53 by degrading it. A 50% mutation in the TP53 gene is found in human malignancies. Together with the

Table 3. Characteristics of study participants based on p53 expression

Characteristics	p53 Expression (N=49)			p
	Low	Medium	High	
	n (%)	n (%)	n (%)	
Age (Years)				
≤ 30	-	1 (100.0)	-	0.588
> 30	10 (20.8)	23 (47.9)	15 (31.3)	
Parity				
Primiparous	2 (28.6)	3 (42.9)	2 (28.6)	0.844
Multiparous	8 (19.0)	21 (50.0)	13 (31.0)	
Coitarche (Years)				
≤ 19	5 (17.9)	16 (57.1)	7 (25.0)	0.413
>19	5 (23.8)	8 (38.1)	8 (38.1)	
Sexual Partner				
1	8 (26.7)	15 (50.0)	7 (23.3)	0.242
> 1	2 (10.5)	9 (47.4)	8 (42.1)	
Oral Contraceptive				
No	9 (24.3)	19 (45.2)	14 (33.3)	0.412
Yes	1 (14.3)	5 (71.4)	1 (14.3)	
Smoking History				
No	9 (21.4)	19 (45.2)	14 (33.3)	0.427
Yes	1 (14.3)	5 (71.4)	1 (14.3)	
Tumor Size (cm)				
≤ 4	5 (33.3)	5 (33.3)	5 (33.3)	0.234
> 4	5 (14.7)	19 (55.9)	10 (29.4)	
Stage				
Early	3 (60.0)	-	2 (40.0)	0.028*
Advanced	7 (15.9)	24 (54.5)	13 (29.5)	
Histopathology Result				
Non keratinizing SCC	6 (15.4)	21 (53.8)	12 (30.8)	
Keratinizing SCC	-	3 (60.0)	2 (40.0)	
Adenocarcinoma	3 (100.0)	-	-	0.011*
Adenosquamous	1 (100.0)	-	-	
Clear cell carcinoma	-	-	1 (100.0)	

SCC: Squamous Cell Carcinoma; Chi-Square; *Statistically significant if p-value less than 0.05

Table 4. The association between p53 expression and HPV genotype in cervical cancer

p53 Expression	HPV Genotype (N=49)				p
	Type 16 n (%)	Type 18 n (%)	Other Type n (%)	Negative n (%)	
Low	0 (0.0)	2 (15.4)	5 (38.5)	3 (18.8)	0.071
Moderate	6 (85.7)	4 (30.8)	4 (30.8)	10 (62.5)	
High	1 (14.3)	7 (53.8)	4 (30.8)	3 (18.8)	

Chi-Square; *Statistically significant if p-value less than 0.05

Table 5. The association between the clinical stage of cervical cancer and p53 expression

p53 Expression	Stage (N=49)		p
	Early n (%)	Advanced n (%)	
Low	3 (60.0)	7 (15.9)	0.028*
Moderate	0 (0.0)	24 (54.5)	
High	2 (40.0)	13 (29.5)	

Chi-Square; *Statistically significant if p-value less than 0.05

cellular E3 ubiquitin ligases p53 and E6AP, HPV forms a tertiary complex. This combination results in the cellular levels of p53 being degraded by ubiquitin in a subsequent process.[21]

Studies show that changes in the p53 profile, such as mutations and changes in the epigenetic profile of p53, are associated with the pathogenesis of cervical cancer. p53 downregulation is associated with disease progression and variation in downregulation based on p53 polymorphisms.[18] Four point mutations in exons 5-8 of the p53 gene were discovered in 122 cervical cancer tissue samples from a study conducted in Brazil involving HPV-positive tissue samples. According to this, the development of cervical cancer may be influenced by independent pathways other than P53 inactivation.[22] The p53 mutation and oncogenic HPV infection cooperate in forming malignant cells based on samples with these risk factors.[23-27]

The most common HPV genotypes in cervical cancer in this study were HPV-negative genotypes. This may be due to the limitations of the reagent, which can only detect 21 of 118 genotypes of HPV. In addition, the accuracy of reading p53 expression using semiquantitative categories can be improved by measuring based on the intensity of the staining signal. This approach may get numerical data that might affect the results of the statistical tests to be carried out. Further research is suggested on a larger scale. Then, a systematic study is carried out to investigate how p53 expression is related to normal cervical tissue, pre-cancerous lesions, and cervical cancer so that the expression of this protein can be used as a prognosis for cervical cancer. In addition, relatively more tissue specimens can be taken to examine the degree of differentiation and involvement of LVSI. Examining HPV with more genotype types is also considered by using standardized sampling.

CONCLUSION

In cervical cancer, p53 expression is not related to the HPV genotype. Regardless, the clinical stage of cervical cancer is associated with higher p53 expression.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS CONSIDERATION

The Ethics Committee for Health Research, Faculty of Medicine, Hasanuddin University (No: 201/UN4.6.4.5.31/PP36/2022) has examined and approved all research designs.

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AUTHOR CONTRIBUTIONS

Samuel conceived and designed the analysis, collected the data, contributed data or analysis tools, and wrote the original paper. Sharvianty Arifuddin and David Lotisna conceived and designed the analysis, contributed data or analysis tools, and validated the final paper. M. Husni Cangara conceived and designed the analysis. Firdaus Hamid contributed to data or analysis tools and performed the analysis.

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