

## **Identification of the type of Human Pappilomavirus (HPV) in patients with cervical cancer in Makassar City.**

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# **Identification of the type of Human Papillomavirus (HPV) in patients with cervical cancer in Makassar City.**

## **Abstract**

**Background:** The most common cause of cervical cancer is infection with the Human Papillomavirus. HPV can be grouped based on the level of malignancy generated in host cells according to the genotype of the virus. This study aims to determine the genotype of HPV in cervical cancer patients in Indonesia, especially in the Makassar city.

**Methods:** The research sample at Hasanuddin University Teaching Hospital and Wahidin Sudirohusodo Central Hospital found 30 people who met the inclusion criteria and had carried out an examination of the HPV genotype through the PCR (Polymerase Chain Reaction) method. Women with stage IIB cervical cancer confirm with pathological anatomy examination and based on physical examination and radiological investigations.

**Results:** Only 5 types of HPV genotypes were found in all samples, namely Types 18, 45, 51, 52, and 59, out of 15 possible genotypes to be detected using PCR and hybridization methods.

**Conclusions:** The results of examination of the HPV genotype in stage IIB cervical cancer patients in Makassar city were most infected with HPV type 18, namely 10 people or 33%..

*Keywords: cervical cancer, human papillomavirus, polymerase chain reaction*

## **Introduction**

Cervical cancer is the second most common type of cancer that affects women worldwide after breast cancer (1). In Indonesia, cervical cancer is still a serious health problem for women due to its high incidence and mortality (2). The most common cause of cervical cancer is infection with the Human Papillomavirus (HPV). This virus is mainly transmitted through sexual contact and can also be transmitted through non-sexual contacts, such as transmission from mother to baby and the use of contaminated tools (3).

HPV can be grouped based on the level of malignancy generated in host cells according to the genotype of the virus. There are three groups of HPV based on their level of malignancy, namely: Low-Risk HPV (LR-HPV), Potential high-Risk HPV (pHR-HPV), and High-Risk HPV (HR-HPV) (4). HPV infection occurs when the virus enters the epithelial cells and then infects the young keratinocyte cells in the basal layer of the epithelium. Viral proteins consist of early proteins (E1-E8) and late proteins (L1 and L2). E1 and E2 proteins are the main proteins to start the viral replication process. The E4 protein is involved in the reorganization of the components of the cell cytoskeleton, while the E6 and E7 proteins are proteins found in HPV that are oncogenic and function in the host cell transformation process. These high levels of E6 and E7 proteins will affect the function of the tumor suppressor (p53 protein) and retinoblastoma protein (pRb), so that they can inhibit the process of cell apoptosis which can ultimately lead to the formation of tumor cells or cancer cells (5).

It is known that serotypes 16 and 18 are the most common causes of cervical cancer (6). In the development of cervical cancer, molecular detection of HPV is an important factor in addition to examination of abnormal cervical epithelial cells by the Pap smear method. To detect

cervical cancer early it is better to do a combination of Pap smear examination to see lesions on the cervix and detection of HPV DNA to determine the type of HPV. The amplification method (Polymerase Chain Reaction/PCR) is a sensitive method and can detect HPV types, especially high risk HPV (7).

Previous studies examining the prevalence of HPV genotypes in the Southeast Asian region were dominated by HPV types 16, 18, 52, 58 and 66 (8). Another study conducted by Munagala et al. found that cervical cancer with a single HPV infection was 87% and 42.8% with multiple HPV infections which were always accompanied by one of the components of HPV 16/18 (9). Other studies have also shown that the distribution of HPV genotypes varies geographically. Meanwhile, in South Sulawesi, especially Makassar City, there is no detailed data regarding the genotype of HPV in cervical cancer patients. This study aims to determine the genotype of HPV in cervical cancer patients in Indonesia, especially in the Makassar city.

## **Materials and methods**

### *Subjects*

We performed a cross-sectional study at the affiliated hospitals for Obstetrics and Gynecology Department, Faculty of Medicine, Hasanuddin University in Makassar Indonesia from June 2021 to January 2022. This study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University. Informed consent was obtained from all subjects prior to the study.

Women with stage IIB cervical cancer confirm with pathological anatomy examination and based on physical examination and radiological investigations (USG, CT Scan or MRI) were eligible for this study. The exclusion criteria were subjects undergoing treatment (chemotherapy or radiotherapy) for cervical cancer, other oncological diseases, and cervical mass is a metastatic malignancy of another organ or oncological diseases were excluded.

### *Laboratory measurement*

Cervical carcinoma tissues from research subjects were obtained during the disease staging procedure. The patient was subjected to cervical swab sampling using Thinprepcytobroom and put into a vial preservative solution which would then be sent to the laboratory for DNA HPV Genotyping using PCR and Hybridization methods..

### *Statistical analyzes*

Data were analyzed using the SPSS program (SPSS program v24.0, SPSS Inc., Chicago, IL). Data are shown as mean  $\pm$  standard deviation (SD), median, minimum and maximum.

## Results

In the results of taking research samples at Hasanuddin University Teaching Hospital and Wahidin Sudirohusodo Central Hospital, Makassar City, it was found that 30 people met the inclusion criteria. After examination of the HPV genotypes through the PCR (Polymerase Chain Reaction) method and hybridization based on the results of cervical swabs in stage IIB cervical cancer patients, only 5 types of HPV genotypes were found in all samples, namely Types 18, 45, 51, 52, and 59, out of 15 possible genotypes to be detected using PCR and hybridization methods (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68). In addition, there were 7 samples where the HPV genotype was not detected.

Based on table 1, the distribution of the Human Papillomavirus (HPV) genotype in stage IIB cervical cancer patients showed that the most results were typed 18, 10 people or 33%, type 45, 7 people, or 23%, type 52, 4 people, or 13%, and the most slightly type 51 and 59 each as much as 1 person or 3%. In addition, there were 7 people, or 23% who had no detectable HPV genotype.

The risk factors for stage IIB cervical cancer patients analyzed in this study included age at first intercourse or coitus (Coitarche) and parity. Based on table 2, it was found that more people had sexual intercourse for the first time when they were > 20 years old, namely 24 people or 80% compared to those aged  $\leq$  20 years, only 6 people or 20%. In this study, the most multiparas were 25 people or 83%, then primiparas were 4 people or 13%, and the least nulliparas were 1 person or 3%.

## Discussion

All of the HPV genotypes in this study were included in the high-risk HPV group. This is consistent with the theory that high-risk HPV is the most common cause of cervical cancer. The genotypes of the HPV virus are called HPV oncogenes. This is because HPV infection is a risk factor for the entry of carcinogens E6 and E7, where both proteins are carcinogens for cervical cancer (6,7). E6 acts as an oncogene by stimulating the growth and transformation of host cells by inhibiting p53 which is called an onco suppressor protein. E7 acts as an oncogene by inducing cell proliferation by inhibiting the pRb, p107, and p130 proteins (10).

In addition, there were 7 people, or 23% who did not detect the HPV genotype, this could have been because the virus type was not included in the 15 HPV genotypes. Although the results of this study were slightly different from data from previous studies that examined the prevalence of HPV genotypes in the Southeast Asian region, which were dominated by HPV types 16, 18, 52, 58, and 66 (8).

In this study, no genotype of HPV type 16 was found. This could be due to the insufficient number of patients studied in this study. In addition, this study did not find multiple infections in stage IIB cervical cancer patients. In the literature, it is said that cervical cancer is caused by infection with oncogenic genotypes of HPV, especially by HPV types 16 and 18. Other studies have also shown that the spread of HPV genotypes varies geographically. Namely worldwide HPV 16 in 50% of cases and HPV 18 in 14% of cases of cervical cancer. Only Indonesia has a higher incidence of HPV 18 genotype (49%) than HPV 16 (32%) (11).

It was also found that stage IIB cervical cancer patients in this study had more sexual intercourse for the first time when they were > 20 years old, namely 24 people, while only 6 people aged  $\leq$  20 years. According to the results of Noor's research, HM (2010) reported that the age of marriage less than 20 years has a risk of 6-7 times experiencing cervical cancer compared to those aged more than 20 years. The same thing was reported there was a relationship between the age at first sexual intercourse with the incidence of cervical cancer. Sexual intercourse that is carried out at an early age is at risk of cervical cancer because at that age the sexual organs are not ready and the mucosal cells in the cervix are immature, so they are still vulnerable to stimulation. Therefore, sperm containing histone complement can react with cervical cell DNA. Sperm that are alkaline can cause hyperplasia and neoplasia of cervical cells (12).

One of the risk factors associated with the incidence of cervical cancer is the number of parity . Women who give birth to many children are a risk factor for cervical cancer (13). Women with parity > 2 are at risk of 4.55 times cervical cancer compared to women with parity  $\leq$  than 2. In this study, it was found that the most stage IIB cervical cancer patients were multiparas, namely 25 people or 83%, while primiparas were only 4 people. or 13%, and the least number of nulliparas is 1 person or 3%. This is related to the eversion of the cervical columnar epithelium during pregnancy which causes new dynamics of immature metaplastic epithelium which can increase the risk of cell transformation and trauma to the cervix it facilitates HPV infection (14).

#### Disclosure statement

The present article is not under consideration for publication elsewhere. The authors declare that this research is conducted in the absence of any commercial or financial relationship that can be a potential conflict of interest.

#### Funding

The author(s) reported there is no funding associated with the work featured in this article.

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Table 1. Distribution of HPV genotypes in stage IIB cervical cancer patients in Makassar City

HPV genotype (N = 30)	n (%)
18	10 (33)
45	7 (23)
51	1 (3)
52	4 (13)
59	1 (3)
Not detected	7 (23)
Total	30 (100)

Table 2. The risk factors for stage IIB cervical cancer in Makassar City

Risk Factors (N = 30)	n (%)
<b>Coitarche</b>	
> 20 years old	24 (80)
≤ 20 years old	6 (20)
<b>Parity</b>	
Nulliparity	1 (3)
Primiparity	4 (13)
Multiparity	25 (83)