High-risk human papillomavirus prevalence among patients with cervical cancer at Moewardi General Hospital in Surakarta, Indonesia

Brian Wasita, Suyatmi, Riza Noviarta Pesik, Ratih Dewi Yudhani, Nanang Wiyono, Rachmi Fauziah Rahayu, Kristanto Yuli Yarso

ABSTRACT

Introduction: In Indonesia, cervical cancer is the second most prevalent type of cancer among the general population. Cervical cancer has the second-highest mortality rate of all cancers. Infection with the Human Papilloma Virus (HPV) is one of the causes that starts the growing phase of cervical cancer. The purpose of this study, therefore, is to determine the prevalence of HPV infection in patients with cervical cancer who were examined at Dr. Moewardi Hospital and lived in the ex-Surakarta Residency.

Methods: Cervical tissue biopsy at the Anatomical Pathology Installation of Dr. Moewardi General Hospital Surakarta which had been diagnosed as cervical cancer was examined by the detection of E6 protein from HPV 16 and HPV 18 using immunohistochemical staining techniques. The data obtained would be analyzed based on the characteristics of the patient's age and the degree of histopathological malignancy with a p-value < 0.05 deemed significant.

Results: Overall, the prevalence of high-risk HP infection was 77.8% (35/45), with women aged between 41-55 years having the highest prevalence of HPV infection (45.7, 16/35), followed by the well-differentiated group (34.3%) and the moderately differentiated group (11.4%). The results of statistical tests showed that no significant relationship was found between HPV infection and age (p = 0.428). High-risk HPV infection was most prevalent in the poorly differentiated group (54.3%), followed by the well-differentiated group (34.3%) and the moderately differentiated group (11.4%). The results of statistical tests revealed a significant association between HPV infection and tumor grade (p = 0.027).

Conclusion: To lower patient morbidity and mortality, it is crucial to receive a prophylactic HPV vaccine due to the incidence of HPV 16 and 18 infections in patients with cervical cancer at the Regional Hospital Dr. Moewardi Surakarta, with a prevalence of 77.8%.

Keywords: Cervical cancer, HPV, ex-Surakarta Residency.


INTRODUCTION

The second-ranking cancer is cervical cancer, which causes the most deaths. Public health issues are well-known to exist with this type of cancer, particularly in developing nations like Indonesia. In Indonesia, there are reported 32,469 instances of cervical cancer annually. Ages 15 to 44 or productive age had the highest incidence of cases. Even the death rate from this cancer reached 9.3%. The cervical cancer incidence in Indonesia currently ranks second after breast cancer.

One of the main causes of cervical cancer is the human papillomavirus (HPV). Infection from this virus can be single or multiple. In addition, the oncoproteins E6 and E7 from HPV direct cells toward malignancy as well as become markers for cells that are already malignant.

In Ghana, there were 78.15% of people with high-risk HPV genotypes (HPV 18, HPV 59, HPV 45, and HPV 16); in addition, high-risk HPV genotypes (HPV 16, HPV 18, HPV 45, HPV 31, HPV 32, HPV 52) were detected in 86.2% of the cervical cancer patient in Sweden.

In 83% of Indonesian cases, high-risk HPV is involved. Both are believed to be strong causes for the cases that occurred in West Java and Bali; 76.4% of cervical adenoscarcinomas and adenosquamous carcinomas had high-risk HPV infection in Surabaya, East Java. Also, a total of 72.1% of cervical cancer cases had high-risk HPV infection in Riau Province. However, the data from Central Java, especially ex-Surakarta Residency, has not been identified. This study aims to scrutinize the prevalence of high-risk HPV infection in patients diagnosed with cervical cancer in the ex-Surakarta Residency including the genotype of the virus.
METHODS

Study Subjects
This case-control study used thirty paraffin blocks from the biopsy of patients with carcinoma diagnosis and thirty paraffin blocks of cervical tissue with a diagnosis of benign lesions at Dr. Moewardi General Hospital in 2019. Sample inclusion criteria were the paraffin block which is in good condition and sufficient to make slides for examination anti-HPV immunohistochemistry and paraffin block which has been diagnosed histopathologically as cervical cancer type squamous cell carcinoma, whereas the exclusion criteria were the paraffin block with borderline lesion diagnosis. The patient data regarding age, tumor grade, and tumor stage were collected from the medical record.

Immunohistochemistry Staining
Detection of HPV infection was performed using immunohistochemistry staining. HPV16 E6 and HPV18 E6 Cocktail Polyclonal Antibody were used to detect E6 protein from HPV virus subtypes HPV 16 and HPV 18, classified as HPV viruses with high risk. In addition, 4-5 m thick tissue sections were deparaffinized in xylene and rehydrated for immunohistochemical examination. The slides were submerged in citrate buffer solution (pH 6.0, Biocare MedicalCA, USA) at 95 °C for 20 minutes to perform epitope retrieval. Besides, Peroxidase Blocking Solution blocked endogenous peroxidase activity (Biocare Medical, CA, USA); the slides were then exposed to protein blocks for 30 minutes (Biocare Medical, CA, USA). Next, HPV16 E6 + HPV18 E6 Polyclonal Antibody was incubated for an hour at room temperature as the primary antibody (1:200 dilution) (Bioss Inc, Boston, MA, USA). The sections were incubated with poly-HRP Goat Anti-Rabbit IgG for an hour at 37°C, and a DAB working solution was used to incubate them until the expected stain intensity manifests. The sections were then rinsed in tap water, counter-stained, cleared, and mounted. The slides were washed for 3 minutes in PBST buffer between the steps. The stain was considered positive when there was nuclear staining in the nuclei of the tumor tissue.

RESULTS

Table 1 displays the primary characteristics of the study subjects. The age mean was 56.4 years, ranging between 28–74 years. Age between 56–70 years was the most frequent (45.7). Infection with HPV subtypes 16 and 18 was demonstrated by positive staining on immunohistochemical examination (Figure 1).

Overall, the high-risk HPV prevalence was 77.8 % (35/45). Women aged between 41–55 years had the highest HPV prevalence (45.7, 16/35), followed by women with age 56–70 (42.9, 15/35), women the age more than 70 years (8.6%, 3/35), and women with the age under 40 (2.9%, 1/35), respectively. Age and HPV infection were not shown to be significantly correlated (p = 0.428). All the study subjects were diagnosed with squamous cell carcinoma with different grades of histological differentiation. Most of the subjects had poor differentiation (48.9%, 22/45). The high-risk HPV prevalence was the highest in the poor differentiation group (54.3%, 19/35), followed by the good differentiation group (34.3%, 12/35) and the moderate differentiation group (11.4%, 4/35), respectively. Tumor grade and HPV infection were significantly correlated (p = 0.027).

DISCUSSION
The Papillomaviridae family of human papillomaviruses has been proven to have a high correlation with cervical cancer. High-risk HPVs are linked to invasive cervical cancer types, while low-risk...
HPVs are mostly responsible for genital warts.14 The majority of cases of cervical cancer are caused by the high-risk HPV strains 16 and 18, which account for about 90% of all occurrences. Compared to women infected with low-risk HPV strains, those with high-risk types 16 and 18 had a higher risk of developing invasive cancer.15 Although HPV infection alone cannot cause cervical carcinogenesis, the initial development and subsequent progression of this type of cancer are entirely dependent on the expression of two major oncogenes, E6 and E7, which are expressed constitutively and cause tumorigenesis in the process of replicating the viral genome. These oncogenes can cause all the characteristics of a cancer cell, including angiogenesis, uncontrolled cellular proliferation invasion, metastasis, and unrestricted telomerase activity, as well as the avoidance of apoptosis and growth suppressor activity.4,13,5

Our results revealed that 77.8% of people had high-risk HPV overall, which was greater than the results of Tobing et al., who conducted a study at Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. They found that HPV-16 was present in the majority of cervical cancer patients, accounting for 62.5% of the analyzed samples contaminated by multiple HPVs (90%).16 The prevalence of our study was lower than the finding of Schellekens et al. They found that in Jakarta, Indonesia, 96% of cervical carcinomas included HPV DNA from 12 different HPV strains.17 The difference in the HPV infection’s prevalence is probably because of the number of samples and the methods used to detect HPV infection. Our result showed that the age of the subject has no significant association with HPV infection; however, ages 41 to 55 had the highest frequency of HPV infection, similar to the result of Vet et al. who carried out population-based research on the human papillomavirus prevalence in three Indonesian regions.18 The primary cause of cervical cancer in later life is HPV infections inflicted in infancy.19,20

In our study, we discovered a strong correlation between tumor grade and HPV infection. The high-risk HPV’s highest prevalence was owned by the poor differentiation group (54.3%, 19/35). The HPV infection connected to the emergence of a high-grade precursor lesion or “precancer”, and invasion are phases in cervical carcinogenesis.19,21 Our result showed that the high-risk HPV infection led to developing a higher grade of cervical cancer.

Our study utilized the immunohistochemistry method which is relatively cheaper and easier compared to more expensive genotyping methods; thus, this method can be used as a screening test in the population and support the HPV vaccination program in Indonesia. The HPV vaccination program demonstrates a cost-effective approach, and Indonesia can afford the funding needed to implement this policy.22

This study has some limitations that must be noted. Our study was not population-based, but it was performed in a single hospital in Surakarta. Thus, the result could not be generalized at the population level. We only detected HPV 16 and HPV 18, so the infection possibility with other types of high-risk HPV could not be identified.

CONCLUSION

In Dr. Moewardi General Hospital Surakarta, the prevalence of HPV 16 and 18 infections among cervical cancer patients is 77.8 % and has an association with high-grade cervical cancer. Therefore, the prophylactic HPV vaccine is crucial to lowering patient mortality and morbidity.

ETHICAL CLEARANCE

The Moewardi General Hospital’s Health Research Ethics Committee granted ethics approval for the study with the number 1.028/VIII/HREC/2020.

FUNDING

This research was funded by the 2020 Universitas Sebelas Maret Research Group Research Grant.

CONFLICT OF INTEREST

No author has disclosed any conflicts of interest.

AUTHOR CONTRIBUTION

All authors contributed equally to this study.

REFERENCES


This work is licensed under a Creative Commons Attribution