ORIGINAL ARTICLE

The role of human papillomavirus deoxyribonucleic acid for distinguishing between cervical adenocarcinoma and endometrial adenocarcinoma

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ABSTRACT

Objectives: To analyze the role of HPV DNA for distinguishing between uterine cervical adenocarcinoma and endometrial adenocarcinoma.
Materials and Methods: This was a case control study using paraffin block samples from uterine cervix adenocarcinoma and endometrial adenocarcinoma operation at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. Each group was tested for HPV DNA using PCR method. Sample size was 18 in each group.
Results: A total of 36 samples fulfilled the inclusion criteria in this study. Each group comprised 18 samples. There were 83.3% of uterine cervical adenocarcinoma and 11.1% of endometrial adenocarcinoma that revealed high risk HPV. Chi-Square test result found significant correlation between high risk HPV and uterine cervical adenocarcinoma ($p<0.05$) with Odds Ratio (OR) 40.00 (CI 95%).
Conclusion: There was a significant correlation between high risk HPV and uterine cervix adenocarcinoma. High-risk HPV infected patients had a risk to suffer from uterine cervical adenocarcinoma compared to those with endometrial adenocarcinoma. HPV DNA test had a role for distinguishing between uterine cervical adenocarcinoma and endometrial adenocarcinoma.

Keywords: Uterine cervical adenocarcinoma; endometrial adenocarcinoma; human papillomavirus; polymerase chain reaction; cancer; maternal health

ABSTRAK

Tujuan: Menganalisis peran pemeriksaan DNA HPV dalam membedakan adenokarsinoma serviks dan adenokarsinoma endometrium.
Bahan dan Metode: Penelitian ini adalah penelitian case control dengan sampel berupa blok parafin hasil operasi adenokarsinoma serviks uteri dan adenokarsinoma endometrium di RSUD dr. Soetomo, Surabaya, Indonesia. Masing-masing diperiksa DNA HPV menggunakan metode PCR. Besar sampel 18 pada masing-masing kelompok.
Hasil: Sebanyak 36 sampel sesuai kriteria inklusi pada penelitian ini. Setiap kelompok terdiri atas 18 sampel. Sebanyak 83,3% adenokarsinoma serviks dan 11,1% adenokarsinoma endometrium menunjukkan HPV risiko tinggi. Hasil uji Chi Square menunjukkan hubungan bermakna antara HPV risiko tinggi dengan adenokarsinoma serviks uteri ($p<0,05$) dan Odds Ratio (OR) sebesar 40,00 (CI 95%).
Simpulan: Terdapat hubungan bermakna antara HPV risiko tinggi dengan adenokarsinoma serviks uteri. Penderita yang terinfeksi HPV risiko tinggi mempunyai risiko menjadi adenokarsinoma serviks uteri dibandingkan adenokarsinoma endometrium. Pemeriksaan DNA HPV dapat berperan dalam membedakan adenokarsinoma serviks uteri dan adenokarsinoma endometrium.

Kata kunci: Adenokarsinoma; serviks uteri; adenokarsinoma endometrium; human papillomavirus; polymerase chain reaction; kanker; kesehatan ibu

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INTRODUCTION

Uterine cervical cancer is still the problem of the women's health worldwide. World Health Organization in 2018 has reported that uterine cervical cancer is the fourth most common malignancy in women after breast, colorectal, and lung cancer. It was estimated there were 569.847 new cases and 311.365 deaths caused by cervical cancer in that year. Approximately 80% of those new cases are found in developing countries. Cervical cancer is the second majority in 2018 after breast cancer in Indonesia, followed by ovarian cancer and colorectal cancer. It is estimated there are 32.469 new cases and 18.279 mortalities.[1]

Determining primary tumor between uterine cervical adenocarcinoma and endometrial adenocarcinoma sometimes is more complicated. This can happen due to tiny part of the biopsy or curettage specimen and overlapped histology morphological spectrum.[2] The preoperative distinction is clinically important, because the treatment of endometrial and endocervical adenocarcinoma differs. Uterine cervical adeno-carcinoma and endometrial adenocarcinoma can have similar histopathological description.[3] Immunohisto-chemistry result such vimentin, estrogen reseptor (ER), progesteron reseptor (PR), carcinoembryonic antigen (CEA), and p16 can distinguish tumor origin. Uterine adenocarcinoma has had positive result for p16 and CEA, but negative for ER, PR, and vimentin with accuration of 65-80%.[4] In other researches, there are contradictory result, in which CEA has statistically significant for endometrial carcinoma but not significant for cervical adenocarcinoma, especially for mucinous differentiation.[5] Magnetic Resonance Imaging (MRI) can determine primary tumor by means of considering morphological characteristic and perfusion. MRI can provide information about tumor spreading. MRI accuration to determine endometrial tumor originis quite high (up to 85%) although using limited data. Several researchers found MRI limitation to determine tumor origin with lower sensitivity (21%) especially for determining stage II endometrial cancer.[6,7]

High risk Humanpapilloma virus (HPV) is a main cause of cervical transformation to become malignant, however it does not contribute of endometrial carcinogenesis process.[8] The aim of this research was to find the role of HPV DNA for distinguishing between uterine cervical adenocarcinoma and endometrial adenocarcinoma using case control approach which was conducted in March 2019 at Gynecologic Oncology Outpatient Clinic and Anatomic Pathology Laboratory at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. Research samples were paraffin blocks from surgery results, as many as 18 from cervical adenocarcinoma patients and 18 from endometrial adenocarcinoma patients.

MATERIALS AND METHODS

This study used a case control approach using paraffin block samples from uterine cervix adenocarcinoma and endometrial adenocarcinoma surgery result at Gynecologic Oncology Outpatient Clinic and Anatomic Pathology Laboratory Dr. Soetomo General Academic Hospital,, Surabaya, that fulfilled inclusion and exclusion criteria. Each group was tested for HPV DNA using PCR method. Sample size was 18 from each group. The samples comprised surgery specimens that were surgically removed in 2014–2018. All tumor specimens were fixed in 10% buffered formalin, processed routinely and embedded in paraffin. Therefore, we had 18 paraffin blocks containing cervical adenocarcinoma and 18 blocks containing endometrial adenocarcinoma to investigate the presence of HPV DNA by PCR method. This study was approved by Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (approval No. 1036/KEPK/III/2019).

Paraffin blocks were taken randomly for DNA extraction. The paraffin blocks were cut to pieces of 25 mg. Deparaffinization was done by xylene and rehydration with ethanol. DNA was extracted by using QIAamp DNA Mini Kit according to its manual and used as a template for polymerase chain reaction (PCR) experiments. The heating scheme for the PCR experiments was as follows: activation of the DNA-polymerase for 10 min at 95ºC, followed by 50 cycles of a 30-sec denaturation at 95ºC, a 30-sec annealing at 50oC, and a 30-sec elongation at 72ºC. The PCR experiments were ended with a 5-min final step at 72oC.[9-11] Genotyping of HPV was conducted with Ampliquality HPV-Type Express (AB Analitica) that can detect HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 87, 89, 90.[12] The type of HPV was reported in the form of band on a blot membrane corresponding to the genotype of HPV.

RESULTS AND DISCUSSION

This study was performed on 36 paraffin block samples of 18 cervical adenocarcinoma tissue derived from patients with the median age 47.5 years old (range from 35 to 75) and 18 endometrial adenocarcinoma tissue derived from patients with the median age of 57.5 years old (range from 34 to 64). The patients with cervical

adenocarcinoma were younger than endometrial adenocarcinoma.

Table 1. Characteristics of the patients

	Cervical adenocarcinoma	Endometrial adenocarcinoma	p
Age (year)	47.5 (35 - 75)	57.5 (34 - 64)	0.087
30-39	3	2	
40-49	8	3	
50-59	4	8	
60-69	2	5	
70-79	1	0	

Tumors were evaluated for staging and grading in accordance with the International Federation of Gynaecologic Oncology (FIGO), 2009.[13] Among the cervical adenocarcinomas, eight (44.4%) cases were stage IB1, 3 (16.7%) stage IB2, 2 (11.1%) stage IIA1, 1 (5.6%) stage IIA2, and 4 (22.4%) stage IIB (Table 2). The stage of endometrial adenocarcinoma samples varied from IA to IIIC, with the first majority was stage IB in 5 (27.8%) cases (Table 3).

There were 15 cervical adenocarcinoma patients who had high-risk HPV (HPV 16, 18, 31, 33, 45, dan 66). We found HPV 18 in 11 patients, HPV 16 (2 patients), HPV 66 (2 patients), HPV 31, 33, and 45 in 1 patient respectively. There were 3 patients who had HPV coinfection (HPV 18 and 66, 18 and 33, 45 and 66). There were also low-risk HPV found in this study (HPV 6,11, and 40), although there was no association between low-risk HPV and cervical carcinogenesis process. Of 18 endometrial adenocarcinoma patients, there were 2 patients contained high-risk HPV (HPV 66) and low-risk HPV (HPV 6, 11, and 40) (Table 4).

Table 2. The stages of cervical adenocarcinoma patients

Stage	n	%
IB1	8	44.4
IB2	3	16.7
IIA1	2	11.1
IIA2	1	5.6
IIB	4	22.2

Study result showed that 83.3% of uterine cervical adeno-carcinoma had high-risk HPV, while only 11.1% of endometrial adenocarcinoma had high-risk HPV. Chi-square test result found that there was statistically significant difference in the presence of HPV-DNA in uterine cervical adenocarcinoma and endometrial adenocarcinoma (p<0,0001). Analysis using OR (Odds Ratio) revealed 40.00 (5.85-273.62), meaning that high-risk HPV infected patients had probable risk of 40 times to have uterine cervical adenocarcinoma compared to endometrial adenocarcinoma. These data and the results of the present study strongly indicated a causal relationship between HPV infection and the development of primary cervical adenocarcinoma, and that HPV types 18 had a particular predilection for cervical adenocarcinoma, but not endometrial tissues. However, there have been a few reports in which the authors found HPV DNA in endometrial tissues as well. According to a study by Fedrizzi et al, HPV DNA was demonstrated by PCR in 8% of samples.[14] In another study by Giatromanolaki et al, six of 25 endometrial adenocarcinomas were HPV 16-positive (24%), and 5 of 25 (20%) were HPV 18-positive. But, none of the positive cases in the series demonstrated cytological evidence of HPV infection.[15] It appeared that the presence of HPV in the endometrium, as detected by PCR, did not play any role in the initiation or prognosis of endometrial adenocarcinoma.

Table 3. The stages of endometrial adenocarcinoma patients

Stage	n	%
IAG1	3	16.7
IBG1	5	27.8
IBG2	2	11.1
IBG3	2	11.1
II	2	11.1
IIG1	1	5.6
IIIA	1	5.6
IIIB	1	5.6
IIIC	1	5.6

Table 4. HPV analysis among the Patients

High-risk HPV	Cervical adenocarcinoma	Endometrial adenocarcinoma	p	OR (CI95%)
+	15 (83.3%)	2 (11.1%)	<0.0001	40.00 (5.85 – 273.62)
-	3 (16.7%)	16 (88.9%)		

CONCLUSIONS

There was a statistically significant difference in the presence of HPV-DNA in uterine cervical adenocarcinoma and endometrial adenocarcinoma. High risk HPV infected patients have probable risk to have uterine cervical adenocarcinoma compared to endometrial adenocarcinoma. HPV DNA test has a role for distinguishing between uterine cervical adenocarcinoma and endometrial adenocarcinoma.

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