The Role of Human Papilloma Virus Type 16 in Retinoblastoma

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ABSTRACT

The purpose of this study is to observe the role of HPV type 16 in retinoblastoma. This study is a case-control design. The target populations were retinoblastoma patients attending to dr. Yap Eye Hospital and dr. Sardjito General Hospital in Yogyakarta. The population of concern were patients with unilateral retinoblastoma, bilateral retinoblastoma and patients with non-neoplastic eye diseases. Examinations were performed with Polymerase Chain Reaction (PCR) technique to 17 retinoblastoma preparations and 17 non-neoplastic retinal preparations embedded on paraffin blocks. The primary DNA used for this study was SiHa cell line as a positive control. All of the examination toward HPV 16 showed negative result (100%) to both of retinoblastoma group and control group. Factor that might influence the results was that the retinoblastoma specimens were not a fresh tissue. HPV 16 had no role in retinoblastoma in this study. We suggested to do the examination toward DNA of another HPV type in a fresh tissue specimen.

Key words: human papilloma virus 16, retinoblastoma, polymerase chain reaction

INTRODUCTION

Retinoblastoma is a malignant tumor of the retina that affects babies and children. It emerges as a result of loss or inactivation of Rb protein (pRb), an RB1 gene product in chromosome 13. Retinoblastoma protein, pRb, has an important role in controlling cell activation and stopping several processes which are able to cause cancers in human, including retinoblastoma.¹

Retinoblastoma emergence can be explained as retinoblastoma cell has lost its pRb function. pRb binds and inactivates transcription factors, such as E2F group that regulates cell cycle progression. Mutation in retinoblastoma gene (RB1) causes loss of pRb function and leads to the increase of E2F protein released. Most of retinoblastoma cases showed a mutation on its RB1, and it was reported that 17%–80% of non-hereditary retinoblastoma cases have an intact RB1 gene.¹

In Palazzi et al. (2003) study, it was reported that there were Human Papilloma Virus (HPV) oncogenic DNA in retinoblastoma specimens. It is well known that HPV is the cause of cervical cancer and it is also known that it has a correlation with ocular lesion. HPV is seen as a papilloma, dysplasia and conjunctival carcinoma and also can be found in normal mucosa. Presence of HPV DNA could be detected within paraffin-embedded tumor tissue of unilateral retinoblastoma with Polymerase Chain Reaction (PCR) technique.

HPV 16 poses high risk factors for malignant progression of human primary cell. Genetic analysis toward HPV 16 showed that E7 protein is an virus encode from oncoprotein.² In malignancy-causing HPV, the leading role protein are E6 and E7. The main mechanism of E6 and E7 HPV protein in cancer progression process is trough their interactions with p53 protein and retinoblastoma (pRb) protein. E6 protein binds to p53, a tumor suppressor gene, causing the cell to lose its ability to do apoptosis. In the other hand, E7 protein binds to pRb, another tumor suppressor gene, so that the cell totally lose its proliferation controlling system. E6 and E7 protein in high risk HPV have higher affinity to both of p53 and pRb than those in low risk HPV.³
HPV alone is actually not a carcinogenic agent according to the epidemiology and clinical data. This explains that the malignant HPV transformation in an infected cell is caused by genetic changes. Ability of E6 and E7 protein to do transformation depends on the kind of tumor suppressor gene (tumor suppressor gene protein: p53 and pRb) that binds to those proteins. E7 binds several cellular proteins including pRb, p107, p130 and cyclin A. E7 and pRb binding complex causes the release of E2F1 and pRb transcription factors.\(^4\) It clarifies why HPV is able to form retinoblastoma.

The purpose of this study is to know whether HPV 16 has a role in retinoblastoma.

**MATERIAL AND METHOD**

Our present study employed a case control design. Target population of this study were patients with retinoblastoma who visited dr. Yap Eye Hospital and Dr. Sardjito general Hospital, both located in Yogyakarta, Indonesia. Population available for this study were patients with unilateral retinoblastoma, bilateral retinoblastoma and also those with non-neoplastic eye disease. Inclusion criteria for this study were patients with unilateral retinoblastoma and bilateral retinoblastoma who underwent enucleation or eccentrication, and patients with non-neoplastic retina who underwent evisceration or enucleation. Exclusion criteria was set on patients with unilateral and bilateral retinoblastoma and those with non-neoplastic retina whose specimen could not be examined because of destructed or were failed in the PCR. We also examined the quality of the DNA from HPV 16 with SiHa (primary) cell line as positive control.

**RESULTS**

This study took place in the Eye Clinic of Dr. Sardjito General Hospital and dr. Yap Eye Hospital. There were 34 subjects eligible for this study. Those 34 subjects were divided into two different groups named retinoblastoma group (17 sample) and control group (17 sample).

In this study, we found a difference in the age distribution in the two groups. The mean age of the subjects in the retinoblastoma group was 21 ± 14.7 months and in the control group was 48.3 ± 17.6 years. There was a statistically significant difference in the age of the two groups (\(p = 0.001\)). Retinoblastoma is an intraocular malignancy that most commonly found in children.\(^5\) We required a normal retinal specimen for the control group, but this group had a distribution in the adult age. Diagnosis for the control group were: 2 cases of corneal staphyloma, 1 cases of bulb rupture, 2 cases of endophthalmitis, 7 cases of prolapsed bulb’s viscera, 4 cases of panophthalmitis and 2 cases of hemophthalmos. All those cases were indicated for evisceration and then the patients underwent a histopathologial examination to exclude tumor and were then examined with PCR.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Retinoblastoma group</th>
<th>Control group</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>21 ± 14.7 months</td>
<td>48.3 ± 17.6 years</td>
<td>0.001</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>10 (58.82%)</td>
<td>12 (70.58%)</td>
<td>0.473</td>
</tr>
<tr>
<td>Female (%)</td>
<td>7 (41.17%)</td>
<td>5 (29.41%)</td>
<td></td>
</tr>
<tr>
<td>Genetic history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exist</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>17 (100%)</td>
<td>17 (100%)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraocular</td>
<td>9 (52.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraocular</td>
<td>8 (47.1%)</td>
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</tbody>
</table>

In this study, we found that all of the specimens were HPV 16 negative (100%), both for the two groups. This result indicated that there was no difference in the result of the examination for HPV 16 in the retinoblastoma and control group.

**DISCUSSION**

HPV is presumed to be one of the risk factors in retinoblastoma emergence. Role of HPV in retinoblastoma emergence is suspiciously caused by infectious agent exposure or another environmental factors which are leading to \textit{in utero} mutation. It happened as a result of loss or inactivation of Rb protein (pRb), an RB1 gene product in chromosome 13.\(^1\)

Human Papilloma Virus (HPV) is DNA virus which has capability to cause genetic alteration. This virus has epitheliotropic characteristic which dominantly infects skin and mucosa through epithel proliferation at the site of infection.\(^7\) The way of HPV to invade and go into neuroectodermal layer at the time of retina formation is still in ongoing study. Role of HPV in retinoblastoma emergence

**Table 2.** Results of HPV 16 examination

<table>
<thead>
<tr>
<th></th>
<th>Retinoblastoma</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 16 positive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HPV 16 negative</td>
<td>17 (100%)</td>
<td>17 (100%)</td>
</tr>
</tbody>
</table>

In this study, we found that all of the specimens were HPV 16 negative (100%), both for the two groups. This result indicated that there was no difference in the result of the examination for HPV 16 in the retinoblastoma and control group.
is suspiciously caused by infectious agent exposure or another environmental factors leads to in utero mutation. According to Orjuela et al. (2000), case of retinoblastoma increases along with HIV (Human Immunodeficiency Virus) spread. HIV is suspected as an infectious agent. An advanced study is still needed to reveal the role of HPV in retinoblastoma emergence.

In our study, HPV examination to both of retinoblastoma group and nonneoplasma retina group showed negative result in all samples. The former study we did, HPV examination toward retinoblastoma samples with immunohistochemistry technique, that was with HPV antibody staining, did not specifically define the DNA in conformity with HPV type. Positive result of immunohistochemistry examination did not point to HPV type. It was probably not an HPV 16 on the staining samples.

Another influential factor for this negative result is no DNA of HPV 16 found in DNA chain examination. Polymerase Chain Reaction (PCR) is an in vitro method for DNA amplification, a kind enzymatic reaction. The working principle of PCR is by increasing DNA specific segment which is initiated by dNTP adhesion in thermal reaction. When the examining DNA specific segment does not contain HPV 16, then the result will be negative.

The former studies found the DNA of HPV 16 and 18 in United States of America,8 DNA of HPV 16 and HPV 35 in Brazil and DNA of HPV 16 in India. Those findings clarify that the difference of HPV distribution could be influenced by geographical aspect. In the case of cervical cancer, the HPV type involved is different one and another; HPV 16 often to be found in Europe, while HPV 18 is more common in Asia. In Asia, there is also another HPV type, those are HPV 58 (5.8%) and HPV 52 (4.4%), and they are more common than HPV 45, 31 and 33. For retinoblastoma, however, there is still no report yet about HPV distribution according to geographical site. Mohan et al. (2009) has done an initial study to detect DNA of HPV 16 and HPV 18 in fresh tissue retinoblastoma samples by PCR technique. DNA of HPV 16 were positively detected in 12 samples from 21 retinoblastoma samples (57%), while no DNA of HPV 18 were detected in all retinoblastoma samples (100%). They still do the continuing study about how does the role of HPV in retinoblastoma emergence.

Some study could show the DNA of HPV 16 in their positive result in retinoblastoma samples. Study about the definite mechanism in retinoblastoma emergence caused by HPV still in continuity. Hope an advance studies could reveal retinoblastoma emergence mechanism with HPV as one of its risk factor.

CONCLUSION

We concluded that there was no role of HPV 16 in retinoblastoma in our study. We suggested to do the examination toward DNA of another HPV type and to a use fresh tissue specimen.

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