Original Article

Human Papillomavirus DNA in Paraffin-Embedded Retinoblastoma

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Background: Human papillomavirus [HPV] has been shown to cause several cancers including cervical, anal, and oropharyngeal by interfering with the tumor suppressor protein function resulting in unregulated cell proliferation. The virus can replicate only in stratified epithelium by direct contact to the basal cell layer. For retinoblastoma, the most common primary intraocular malignancy in children, the tumor derives from immature retinal cells originating from the neuroepithelium. Controversy remains regarding the detection of HPV-DNA in retinoblastoma.

Objective: To investigate the presence of HPV genomic sequence in retinoblastoma tissues.

Materials and Methods: One hundred seventeen paraffin-embedded retinoblastoma samples between January 2000 and December 2013 were included in the study. Real-time polymerase chain reaction was used to identify HPV genomic sequence from the samples. We used 21 paraffin-embedded ocular tissues, which enucleated from other causes as a control group. We used the same tissue preparation and process in both groups.

Results: After removing 37 (31.6%) invalid data from 117 retinoblastoma samples, we did not identify HPV-DNA in any of 80 (68.4%) valid data samples. In control group, HPV-DNA was not found in 12 (57.1%) valid data samples. Nine (42.9%) samples were invalid.

Conclusion: Our results confirm the negative correlation between the HPV and retinoblastoma, which can be explained by the virulence mechanisms of this oncogenic virus.

Keywords: Human papillomavirus, Retinoblastoma, Oncogenic virus, Polymerase chain reaction, Health promotion and prevention

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Human papillomavirus [HPV] has been proven to be involved in developmental process of several cancers including cervical, rectal, and upper respiratory tract(1). More than 150 types of HPV have been identified, 40 of them can be easily transmitted from one person to another by direct skin-to-skin or skin-to-mucosa contact, especially during sexual contact. This makes HPV one of the most common sexually transmitted infections. At least 13 types of HPV were identified as high-risk group due to their ability to develop cancers in infected tissues.

Retinoblastoma is the most common primary intraocular malignancy in children. Development of retinoblastoma is known to be the result of retinoblastoma gene [RB1] defect. Biallelic inactivation of this gene results in the lack of retinoblastoma protein [pRb] production, which leads to tumor development. Although retinoblastoma was an inheritable cancer, less than 10% of cases presented with positive family history. This suggests that more than 90% of cases had sporadically developed a mutation in the RB1 gene. The causes of sporadic RB1 gene mutation have not been described. During the past decade, six studies demonstrated HPV genomic DNA in retinoblastoma fresh tissues or paraffin-embedded slides via polymerase chain reaction [PCR] and/or immunohistochemistry and HPV-DNAs were detected in 4.6% to 69.7%(6-11). This raises the possibility of HPV as an alternative mechanism of retinoblastoma development. However, studies from various institutes could not identify HPV in retinoblastoma samples(12,13). The number of retinoblastoma samples among those studies varied from 39 to 154.

Therefore, we aimed to answer this controversial issue by performing a realtime-PCR to identify HPV-DNAs in a large number of retinoblastoma samples.

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Materials and Methods

Study populations

We enrolled all enucleated retinoblastoma samples between January 2000 and December 2013 at Siriraj Hospital, Bangkok, Thailand. One hundred seventeen eyes of 111 patients were included. All paraffin-embedded slides were reviewed, and the diagnoses were confirmed by the experienced ocular pathologist. Twenty-one paraffin-embedded blocks from other conditions including Coats disease, endophthalmitis, and congenital glaucoma were enrolled as controls.

Tissue preparation

The best represented retinoblastoma tumor blocks from each eye were chosen for DNA extraction and PCR analysis. Under proper tissue handling technique, all chosen paraffin-embedded blocks were sliced with microtome blade into 5 microns thickness. A new sterile microtome blade was used for each sample to avoid contamination between cases. A paraffin slice was rolled and placed in a centrifuge tube for washing process using 200 microliters of xylene solution. The DNA was extracted using the Nuclisens easymag (bioMérieux) automated RNA/DNA extraction system. Extracted DNA was submitted to realtime-PCR machine for HPV detection (Anyplex II HPV28 (seegene)). This model of realtime-PCR machine can identify 19 types of high risk HPV (16,18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82) and nine types of low risk HPV (6, 11, 40, 42, 43, 44, 54, 61, 70). Realtime-PCR was repeated using three slices of 5 microns paraffin-embedded section for those samples showing invalid results due to artifacts in tissue samples.

Results

One hundred seventeen retinoblastoma samples from 111 patients were analyzed. Of the 111 patients, 56 (50.5%) were male and 55 (49.5%) were female. There were 70 (63.1%) unilateral and 41 (36.9%) bilateral cases. The mean age of presenting symptoms was 23 months in unilateral group and seven months in bilateral group. The presenting symptoms included leukocoria (87.2%), red eye (6.8%), strabismus (5.1%), and cloudy cornea (0.9%). The mean age at diagnosis was 28 months in unilateral group and 13 months in bilateral group. No family history of retinoblastoma was identified. According to the International Classification of Retinoblastoma, seven (6%) eyes were group D, 100 (85.5%) eyes were group E, and ten eyes (8.5%) had extraocular extension detected by pathological examination.

In 21 control samples, the pathological diagnoses included Coats disease (6), congenital glaucoma (3), endophthalmitis (3), phthisis bulbi (3), granulomatous inflammation (1), retinal dysplasia (1), immature teratoma of the orbit (1), neurofibromatosis type1 (1), rhabdomyosarcoma (1), and traumatic ruptured globe (1).

HPV-DNA was not detected in 68 samples. For those 49 invalid samples, repeated PCR with increasing amount of paraffin-embedded tissue were negative for HPV-DNA in 12 samples and invalid in 37 samples. In control group, HPV-DNA was not found in 12 (57.1%) samples, and nine (42.9%) samples of HPV-DNA were invalid.

Discussion

Chintu et al reported the incidence of retinoblastoma before and after HIV epidemic era in Zambia[14]. They found a significant increase of retinoblastoma incidence after the epidemic of HIV infection. In the study of 609 retinoblastoma cases, Heck et al found the association between maternal sexual transmitted diseases [STD] infection in pregnancy and bilateral retinoblastoma (OR = 3.59, 95% CI 1.58 to 8.15)[15]. This evidence showed that the increased incidence of retinoblastoma might be related to STDs and HIV infection.

Orjuela et al was the first group to report the presence of HPV genomic sequence in retinoblastoma tissue in 2000[6]. They found HPV-DNA in 14 (36%) of 39 unilateral sporadic retinoblastoma samples. Based on their results, they concluded that HPV might play a role in retinoblastoma development. Studies from Brazil and India have reported the detection of HPV sequences in their retinoblastoma samples with the range between 4.6% and 69.7%[7-11]. Their results supported the result of Orjuela et al's study[6]. These findings are against the normal virology of HPV and still controversial. Theoretically, HPV infects only in basal cell of stratified epithelium. Retinoblastoma is a malignant tumor of retina, which is a neurosensory tissue. HPV infection and replication should not occur in the retina.

In the study of 40 fresh-frozen retinoblastomas in 2007, Gillison et al investigated the evidence of HPV-DNA sequences by using realtime PCR[12]. All the tumors were negative for 37 HPV types, 51 adenovirus (HadV) types, and polyomavirus (BKV and JCV) genomic sequences. In 2013, Ryoo et al did a study of 54 paraffin-embedded retinoblastoma samples[13]. HPV-DNA was not detected in any of their specimens. They reported that HPV infection might not have a causal relation to retinoblastoma in Korean population.

The results of these two studies were different from the previous-mentioned studies, which have positive results for HPV genetic sequences.

Our results confirmed the negative correlation between retinoblastoma and HPV. We suggest that any related conditions and risk factors of retinoblastoma should be explored. Knowing more about the cause, we could plan our health promotion and prevention strategy to reduce the incidence of this deadly cancer in the future.

What is already known on this topic?

HPV has been proven to cause cancer in many tissues but not yet proven for retinoblastoma. Reports showed relationship between HPV and retinoblastoma, but other studies did not find the relations. To understand the risk of retinoblastoma development, this contradiction needs to be proved. This information would help plan a disease prevention program.

What this study adds?

Our results confirm the negative correlation between the HPV and retinoblastoma in Thai children.

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Potential conflicts of interest

The authors declare no conflict of interest.

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