Human Papillomatosis Genotyping and Severity in Patients with Recurrent Respiratory Papillomatosis

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Background: *Recurrent respiratory papillomatosis (RRP) is caused by human papilloma virus (HPV) and is the most common benign laryngeal neoplasm in children. Although RRP is rarely fatal, the disease requires prolonged, extensive medical and surgical treatment, leading to physical and emotional suffering in affected children and their families. Previous studies show HPV type 11 and type 6 in the lesion of Thai subjects with RRP. There is currently no published data available from Thailand that compares staging and severity with RRP genotypes.*
Objective: *To identify the genotypes of human papilloma virus (HPV) isolated from patients with RRP and compare the staging and severity with human papilloma virus genotype.*
Material and Method: *This prospective study was designed to assess population characteristics and follow the clinical course of RRP from January 2011 to January 2013. At each endoscopic evaluation or debridement, all surgeons to stage each patient's disease severity used a consistent scoring system. The samples were analyzed for HPV genotype.*
Results: *Fifteen Thai children (7 female, 8 male) with respiratory papillomatosis were enrolled. Results showed that HPV type 6 and HPV type 11 caused RRP in 6 (40%) and 9 (60%) of the children, respectively. No co-infection between HPV type 6 and type 11 was found. Overall mean age at diagnosis of patients with RRP was 2.65±0.82 years. The age at diagnosis was significantly different between both HPV genotypes (p = 0.008). The mean disease severity score for HPV type 6 infection was 13.83±9.94, and that of HPV type 11 infection was 27.44±8.24. The mean disease severity score of HPV type 11 infection was significantly higher than that of HPV type 6 infection (p = 0.013).*
Conclusion: *HPV type 6 and type 11 caused RRP in Thai children. RRP attributable to infection with HPV type 11 is more aggressive in disease severity and has a shorter life time than HPV type 6 at time of first diagnosis. RRP is an incurable disease that requires long-term medical management. Improved awareness and understanding amongst the Thai population should be promoted to limit the spread of the disease. In addition, further research on the treatment of RRP would benefit patients.*

Keywords: *Recurrent respiratory papillomatosis, Human papilloma virus, Human papilloma virus genotype*

J Med Assoc Thai 2014; 97 (Suppl. 6): S136-S141
Full text. e-Journal: *http://www.jmatonline.com*

Recurrent respiratory papillomatosis (RRP) is caused by human papilloma virus (HPV) and is the most common benign laryngeal neoplasm in children. Although RRP is rarely fatal, the disease often requires extensive medical and surgical treatment over a long period of time, which, in turn, can cause physical and emotional suffering in affected children and their families. The disease typically presents with progressive hoarseness and stridor as a result of exophytic lesions that grow on the larynx and the trachea. Papillomas tend to grow primarily on the larynx, but other sites along the respiratory tract, such as the trachea, esophagus, lungs, oropharynx, oral cavity, and nasal cavity can also be involved[1,2]. The natural history of RRP in children varies. Children who develop RRP before 3 years of age typically have frequent recurrent lesions that require multiple surgical debridements[3,4].

The incidence of RRP amongst children in the United States is approximately 4.3 per 100,000[5] and the infection rates between genders are nearly equal[6,7]. A study in Thai children reported the presence of HPV DNA in RRP lesions. In that study, HPV-11 and HPV-6 were isolated from 84% and 4% of the children (n = 25), respectively[8]. To date, no published data exist in

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Thailand that compare staging and severity of HPV genotypes in children with RRP.

Material and Method

The present study was designed to assess the characteristics of children under the age of 16 years with RRP and to follow the clinical course of the disease. The authors interviewed the parents/guardians of all participants to obtain basic demographic information and the medical history of the participants. Ear-nose-and-throat specialists at the participating hospitals collected papillomatous tissue samples during endoscopic biopsies. Coltrera-Derkay staging of disease severity at the time of each endoscopic debridement was evaluated (Fig. 1). The samples were then analyzed for HPV genotype at the Unit of Virology and Molecular Pathology, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Thailand. The INNO-LIPA HPV genotyping Extra Amp kit was used to amplify part of the L1 region, the most conserved region in the HPV genome between genotypes, by the polymerase chain reaction (PCR). Several consensus PCR primer sets have been described in this region.

The authors used Fisher's exact tests and Student's t-tests to assess relationships between discrete variables and continuous variables, respectively. The *p*-value of less than 0.05 was considered statistically significant.

A. Clinical score
1. Describe the patient's voice today:
normal___(0), abnormal___(1), aphonic___(2)
2. Describe the patient's stridor today:
absent___(0), present with activity___(1), present at rest___(2)
3. Describe the urgency of today's intervention:
scheduled___(0), elective____ (1), urgent___(2), emergent___(3)
4. Describe today's level of respiratory distress:
none___(0), mild___(1), moderate___(2), severe___(3), extreme___(4)
Total Clinical Score (Questions 1 through 4) = _____
B. Anatomical score
For each site, score as: 0 = none, 1 = surface lesion, 2 = raised lesion, 3=bulky lesion
LARYNX:
Epiglottis: Lingual surface___ Laryngeal surface___
Aryepiglottic folds: Right___ Left___
False vocal cords: Right___ Left___
True vocal cords Right___ Left___
Arytenoids: Right___ Left___
Anterior commissure_____
Posterior commissure_____
Subglottis_____
TRACHEA:
Upper one-third_____
Middle one-third_____
Lower one-third_____
Bronchi: Right___ Left___
Tracheotomy stoma____
OTHER:
Nose_____
Palate_____
Pharynx_____
Esophagus___
Lungs_____

Fig. 1 Coltrera-Derkay staging of disease severity.

Results

Patient demographics

From January 2011 to January 2013, 15 Thai children (8 female, 7 male) with respiratory papillomatosis were enrolled. Eleven of the children received treatment at Queen Sirikit National Institute of Child Health (QSNICH) and 4 received treatment at the Maharaj Nakorn Chiang Mai Hospital. There was one patient whose mother died before enrollment. Three of 14 children had maternal genital condylomata. One of these mothers was diagnosed during pregnancy, whereas the other two were diagnosed after the childbirth. All 15 patients with RRP were delivered via vaginal delivery. Eleven of the children were first-born children and the other 4 children were second-born children (Table 1).

Overall mean age at diagnosis was 2.65±0.82 years. The patients with HPV6 had mean age at diagnosis of 3.29±0.71 years. For patients infected with HPV11, the mean age at diagnosis was 2.22±0.60 years. The age at diagnosis was significantly different between both genotype ($p = 0.008$) (Table 3).

Clinical course

The most common sign and symptom of RRP amongst the children was hoarseness (n = 12, 80%). The second and third most common signs and symptoms were stridor condition and respiratory distress, reported in 10 (66.7%) and 9 patients (60%), respectively (Table 2). In one of the children with HPV type 11 infection, RRP had spread to the lungs. Two of the children with HPV type 11, underwent a tracheostomy (Table 3).

HPV type and severity

HPV type 6 and HPV type 11 caused RRP in 6 (40%) and 9 (60%) of the children, respectively. No co-infection between HPV type 6 and type 11 was found. The mean of disease severity for HPV type 6 infection was 13.83±9.94, and that of disease severity for HPV type 11 infection was 27.44±8.24. The mean disease severity of HPV type 11 infection was significantly higher than that of HPV type 6 infection ($p = 0.013$) (Table 3).

Ehtical consideration

The present study protocol was approved by the Institutional Review Board of the Queen Sirikit National Institute of Child Health and Maharaj Nakorn Chiang Mai Hospital and conducted in accordance with principles outlined in the Declaration of Helsinki. Informed consent was obtained from the parents/

Table 1. The relationship between demographic characteristics, environmental factors, and HPV genotype in patients with recurrent respiratory papillomatosis

	HPV6		HPV 11		*p*-value
	Number	%	Number	%	
Gender					
Male	4	57.1	3	42.9	0.32
Female	2	25	6	75	
Maternal history of genital wart					
Yes	1	45.5	2	54.5	0.65
No	5	33.7	6	66.7	
Unknown*					
Order of birth					
Firstborn child	4	36.4	7	63.6	1.00
Second child and up	2	50	2	50	
Tracheostomy					
Yes	0	0	2	100	0.49
No	6	46.2	7	53.8	
Lung involvement					
Yes	0	0	1	100	1.00
No	6	42.9	8	57.1	

* One mother died of HIV before patient enrolled

guardians of the participants prior to enrollment in the present study.

Discussion

Previous studies reported the mean age at diagnosis ranged from 2 to 4 years[3,4].

In the present study, the mean age at diagnosis was 2.65±0.82 years. Earlier age at diagnosis was significantly associated with progression of the disease[9]. HPV 11 is more closely associated with a younger age at diagnosis[16]. In the present study, age at diagnosis was significantly related to the HPV genotype (p = 0.008).

Thus far, several studies have investigated the relationship between the type of HPV infections and the severity of disease. Some of these studies reported HPV type 11 to have a greater severity than HPV type 6[10,11,12], whereas others found HPV type 6 to have greater severity than HPV type 11[12]. In the present study, HPV type 11 was found to have greater severity than HPV type 6 (p = 0.013).

Retrospective and recent prospective studies have confirmed that HPV may be caused by vertical transmission from mother to child[14,15]. Silverberg et al showed that children born from mothers with active condylomata had a 231-fold increased risk of developing RRP when compared to children born from disease-free mothers[16]. In addition, they showed that children born from mothers with active condylomata had a two-fold increased risk of developing RRP if labor lasted more than 10 hours. Kashima et al found that RRP patients were more likely to be first born and vaginally delivered than control patients of similar age[7]. In the present study, maternal history of genital warts, first-born status, and the incidence of vaginal delivery were not significantly different between patients with HPV type 6 and HPV type 11 diseases. All mothers had delivered RRP babies vaginally. Although one of the mothers was diagnosed with vaginal papillomas during pregnancy, vaginal delivery rather than a caesarean section was performed.

The presenting symptoms of hoarseness (80% of children) and stridor (66.7% of children) were similar to the presenting symptoms reported in other studies[17]. Previous studies reported that in 3.3% of patients, RRP had spread to the lungs and that 16% of these patients develop cancerous lung tumors[18]. In the present study, 1 patient with HPV type 11 disease showed involvement of the lungs. Some patients with RRP received tracheotomy to demonstrate the severity of the disease spreading to the trachea[19-21]. In the present study, two patients, who required tracheostomy, were found to have HPV type 11.

Study limitations included the relatively small number of patients; therefore, additional studies should be conducted to augment the significance of the research.

Table 2. Initial signs and symptoms of patients with recurrent respiratory papillomatosis

Signs and symptoms	Number	%
Hoarseness	12	80
Stridor	10	66.7
Respiratory distress	9	60
Shortness of breath	6	40
Snoring	6	40
Noisy breath	2	13.3

Conclusion

The present study enrolled 15 patients with RRP. Children with HPV type 11 diseases were significantly younger than children with HPV type 6 disease at time of the first diagnosis. Furthermore, disease severity was significantly higher in children with HPV type 11 than in those with HPV type 6 diseases. RRP is an incurable disease that requires long-term medical management. Improved awareness and understanding amongst the Thai population should be promoted to limit the spread of the disease. In addition, further research on the treatment of RRP would benefit patients.

Table 3. The relationship between disease severity, age at diagnosis and HPV genotype

	HPV-6	HPV-11	p-value
Derkay score	13.83±9.94	27.44±8.24	0.013
Age at diagnosis(yr)	3.29±0.71	2.22±0.60	0.008

Data presented by mean ± SD

Potential conflicts of interest

None.

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การศึกษาจำแนกสายพันธุ์เนื้องอกหูดในระบบทางเดินหายใจและความสัมพันธ์ระหว่างสายพันธุ์และความรุนแรงของโรคในผู้ป่วยเด็กในประเทศไทย

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ภูมิหลัง: เนื้อหูดในระบบทางเดินหายใจ เป็นโรคเนื้องอกชนิดไม่ร้ายแรงที่พบบ่อยที่สุดในเด็ก มีลักษณะพยาธิสภาพเป็นชนิดไม่ร้ายแรงแต่ก็จะมีภาวะการกลับเป็นซ้ำและมีภาวะคุกคามต่อชีวิต ปัจจุบันทั่วโลกมีการศึกษาถึง การจำแนกสายพันธุ์เนื้องอกหูด ซึ่งเป็นสาเหตุที่ทำให้เกิดโรคเนื้องอกหูดในกล่องเสียงโดยพบว่า HPV type 11 และ type 6 เป็นสาเหตุสำคัญของการเกิดโรคในประเทศไทยเคยมีการศึกษาสายพันธุ์ของ HPV แต่ไม่มีการศึกษาถึงระดับความรุนแรงของโรคเทียบกับสายพันธุ์

วัตถุประสงค์: เพื่อจำแนกหาสายพันธุ์ของไวรัสที่เป็นสาเหตุของโรคหูดในระบบทางเดินหายใจในผู้ป่วยเด็กที่มารับการรักษาและหาความสัมพันธ์ ระหว่างความรุนแรงของโรคเปรียบเทียบกับสายพันธุ์

วัสดุและวิธีการ: การศึกษาแบบไปข้างหน้าในผู้ป่วยเนื้องอกหูดในระบบทางเดินหายใจตั้งแต่ เดือนมกราคม พ.ศ. 2554 ถึง เดือนมกราคม พ.ศ. 2556 โดยเก็บข้อมูลประชากรศาสตร์ ระดับความรุนแรงของโรคและเก็บชิ้นเนื้องอกหูด ในระบบทางเดินหายใจโดยการส่องกล้องและนำตัวอย่างชิ้นเนื้อจะนำส่งไปห้องปฏิบัติการเพื่อทำการวิเคราะห์แยกสายพันธุ์ของเนื้องอกหูด

ผลการศึกษา: ผู้ป่วยเป็นโรคหูดในระบบทางเดินหายใจที่เข้าร่วมโครงการวิจัยทั้งสิ้น 15 ราย เพศชาย 8 ราย เพศหญิง 7 ราย โดยค่าเฉลี่ยอายุที่ได้รับการวินิจฉัยเท่ากับ 2.67 ค่าเบี่ยงเบนมาตรฐานเท่ากับ 0.049 และพบว่าช่วงอายุที่ได้รับการวินิจฉัยมีความสัมพันธ์กับชนิดของเชื้อไวรัส HPV อย่างมีนัยสำคัญทางสถิติ ($p = 0.008$) สายพันธุ์ของไวรัสที่เป็นสาเหตุภาวะหูดในระบบทางเดินหายใจพบว่าจำแนกเป็น HPV type 6 จำนวน 6 ราย (ร้อยละ 40) HPV type 11 จำนวน 9 ราย (ร้อยละ 60) ซึ่งการศึกษานี้ไม่พบการติดเชื้อร่วมกันระหว่าง HPV type 6 และ HPV type 11 ค่าเฉลี่ยของความรุนแรงของเชื้อ HPV type 6 มีค่า 13.83 ค่าเบี่ยงเบนมาตรฐาน 9.94 และค่าเฉลี่ยของความรุนแรงของ HPV type 11 มีค่า 27.44 ค่าเบี่ยงเบนมาตรฐาน 8.24 โดยค่าเฉลี่ยของความรุนแรง HPV type 11 มีค่าสูงกว่า HPV type 6 และพบความสัมพันธ์อย่างมีนัยสำคัญทางสถิติระหว่างความรุนแรงของโรคกับสายพันธุ์ไวรัสหูด ($p = 0.013$)

สรุป: ผู้ป่วยเนื้องอกในระบบทางเดินหายใจในการศึกษานี้ตรวจพบสายพันธุ์ 11 และ 6 ที่เป็นสาเหตุของโรคโดยพบว่าผู้ป่วยโรคหูดทางระบบทางเดินหายใจที่ตรวจพบว่าติดเชื้อ HPV type 11 จะพบว่ามีช่วงอายุที่น้อยกว่าและมีความรุนแรงของโรคมากกว่าผู้ป่วยที่ติดเชื้อ HPV type 6 อย่างมีนัยสำคัญทางสถิติ ผู้ป่วยโรคหูดทางระบบทางเดินหายใจเป็นโรคที่กลับเป็นซ้ำและยังไม่มีการรักษาใดที่ทำให้โรคดังกล่าวหายขาด ดังนั้นในอนาคตควรมีการส่งเสริมความรู้ความเข้าใจแก่ประชาชนเพื่อป้องไม่ให้เกิดโรค รวมทั้งควรมีการวิจัยในการรักษาโรคหูดทางระบบทางเดินหายใจมากขึ้นเพื่อผลประโยชน์แก่ผู้ป่วยโรคหูดในระบบทางเดินหายใจ