The role of Human papilloma virus (HPV) genotyping in recurrent respiratory papillomatosis in Rasoul Akram Hospital

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Abstract
Background: The most common laryngeal mass in children is recurrent respiratory papillomatosis (RRP). Studies have attempted to correlate viral typing and its aggressiveness.

Method: 29 patients with histologically confirmed RRP enrolled in adjuvant therapies. Patients underwent several surgical interventions.

Results: HPV genotyping demonstrated 45% HPV-6 and 55% HPV-11. The mean age at the first surgical intervention was 52.39 months (SD=102.28) (range from 4 months to 426 months). The mean number of surgical intervention was 10.39 (SD=7.76) (range from 2 to 30). The mean time of surgical intervals was 4.63 months (SD=4.02) (range from 2 to 24 months). In fourteen patients (48%) tracheotomy was done. All patients who had tracheotomy received alpha-interferon. One of our cases was a male who had pulmonary extension with HPV-6.

Conclusion: A review of patients with RRP was regarding to HPV genotyping and need for adjuvant therapy and tracheotomy. Mean number of surgical procedure was 10/40 and nearly fourteen patients (48%) need to tracheotomy. The clinical differences between HPV6 and HPV11 disease may not be accurately predictable. Patients with less age and with HPV-11 seemed to have more severe problems, but these differences were not statistically significant which needs much more investigations for reasonable starting point of evaluation for these differences.

Keywords: Respiratory tract diseases, Papillomatosis, Laryngeal neoplasms, Human papillomavirus, Human papillomavirus

Introduction
Recurrent respiratory papillomatosis (RRP) is the most common benign neoplasms. It has potentially morbid consequence due to involvement of the airway and risk of malignant conversion (1). It is highly variable in its severity and courses with spontaneous remission or aggressive papillomatosis re-growth which requires multiple surgical procedures over many years (2). Clinically it consists of a mild

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dysphonia that may progress to aphonia. It may be complicated by stridor, respiratory distress, chronic cough, recurrent pneumonia, failure to thrive, dyspnea, dysphagia and even death (3). The initial localization of juvenile onset RRP occurs to be in the anterior commissure and the anterior third of the vocal cords, and then can affect all parts of the larynx, spreading into the trachea and sometimes to bronchi and pulmonary parenchyma (4,5). With the establishment of HPV-6 and HPV-11 as the most common types identified in airway lesions, several studies attempted to correlate viral type with aggressive disease in different populations. We studied patients with RRP to explain distribution of HPV-types, surgical procedures frequencies and intervals and using of adjuvant therapies (6).

**Methods**

From 1377 to 1388 all patients with confirmed RRP pathology referred to Rasoul-e-Akram Hospital enrolled in the study. Relevant data such as age at the onset of the disease, signs and symptoms of patients, age at the first surgical intervention tracheotomy procedure, surgical intervals, adjuvant therapies (with alpha-interferon and indole-3-carbinol), existence of systemic disease and site of involvement were collected.

**Laboratory Methods:** All biopsy specimens were digested using digestion buffer containing proteinase K (200µg/ml), followed by extensive extraction with phenol/chloroform [7]. In case of paraffin embedded tissues, sections of 5–10 µm wide were prepared from each specimen, avoiding any cross-contamination between samples (using separate disposable items such as gloves, blades and tubes; most importantly the first section of each specimen plus gloves and blade were discarded and new blade and gloves were used for main sectioning). Sections were subsequently deparaffinized by xylene. The extracted DNA was stored at 4°C until tasted. DNA quality was evaluated by PCR using forward primer PCO3: 5'-ACACAACGTTGTTCACTAGC-3' and reverse primer PO4: 5'-CAAC TTCATCAGTTGCC-3' that amplify a 110 bp product from the human b-Globin gene (12). b-Globin positive samples were subjected to HPV nested PCR. MY09 (5'-CGTCCACAAGGGGATACTGAC-3') and MY11 (5'-GCACCAGGGATCTATAAC/ TAATGG-3') primers were used as outer primers. GP5+ (5'-TTTTGTTACTGTTGATAGACTACYAAATGC-3') and GP6+ (5'-AAATGAACAGTTGAATCATATGGC-3') were used as inner primers. GP5+/GP6+ primers amplify a 150 bp product from the HPV L1 ORF [7]. Extracted DNA from HeLa cell line was used as HPV positive control and no DNA was added for negative control. HPV positive PCR products were subjected for automated sequencing (Bioneer, Daejeon, Korea). Using BLAST in Gene Bank (http://blast.ncbi.nlm.nih.gov) sequences assessed and genotype determined.

**Surgical Methods:** Prior surgery, patients were intubated with the smallest tube size by an experienced anesthesiologist. Patients ventilated with mixture of 40% oxygen and air, anesthesia maintained with intravenous agents. In no case inhalational agents were used. Wet surgical cottonoid used for protecting tube cuff and subglottic area. Wet gauze used for safeguarding patients’ closed eyes and dental guard used in all cases. Laser emission to teeth, skin and mucosal membranes (except in larynx and trachea) was prohibited. In all patients direct laryngoscopy with appropriate sized laryngoscope was done. After suspension of the laryngoscope, CO₂ laser with 2-6 watt power in continuous mode used considering lesions severity. Defocused CO₂ laser and epinephrine soaked cottonoids used for hemostasis. In anterior commissure region special attention paid to preventing web formation. Smoke suctioning used in conjunction with surgical suction in all cases. Using appropriate suction, disseminating of viral particles in environment was minimized. There were no case of fire and dental, opthalmic and cutaneous trauma in our series. Most of the patients underwent surgery with 3 months
The role of Human papilloma virus (HPV) Genotyping

Table 1. Patients’ characteristics.

|                      | Frequency | Percent |
|----------------------|-----------|---------|
| Number of patients   | 29        | 100     |
| Male                 | 16        | 55      |
| Female               | 13        | 45      |
| HPV 6                | 13        | 45      |
| HPV 11               | 16        | 55      |
| Need for Tracheostomy| 14        | 48      |
| Adjuvant therapy with alph-interfron | 22        | 76      |
| Site of involvements |           |         |
| supraglottis         | 2         | 7       |
| glottis              | 5         | 17      |
| supraglottis and glottis | 7       | 24      |
| glottis and subglottis| 2        | 7       |
| supraglottis, glottis and subglottis | 13    | 45      |

intervals. Surgical intervals adjusted regarding clinical course of the disease and age of onset. However, timing of procedures were regulated to minimize the need for tracheostomy, and therefore, the patients experienced minimal respiratory and vocal discomfort. Close out-patient follow-up planned and patients with more aggressive disease underwent serial surgeries with fixed intervals.

Statistical Methods: Frequency percentages and mean [standard deviation (SD)] are used to describe qualitative and quantitative variables, respectively.

Results

From 1998 to 2009 totally 29 patients with histologically confirmed RRP included in the study (45% were female and 55% were male), the mean age of patients at the onset of disease was 52.39 months (SD=102.28) (range from 4 to 426 months). HPV genotyping demonstrated that thirteen patients (45%) had HPV-6 and sixteen patients (55%) had HPV-11. Among the patients, twenty-two (76%) had voice disorder and seven patients (24%) had breathing disorder as presenting symptoms. Considering sites of involvement; two patients (7%) had only supraglottic and five patients (17%) had only glottic involvement, respectively. However seven patients (24%) had both supraglottic and glottic involvement and two patients (7%) had both glottis and subglottic involvement. Interestingly thirteen patients (45%) had simultaneous supraglottic, glottic and subglottic involvement, and none of them had merely subglottic involvement (Table 1). All patients underwent several surgical procedures (range: 2-30, mean: 4.63), with intervals ranged from 2 months to 24 months (SD=4.021 months). They were subsequently treated with indole-3-carbinol (I-3-C) preparations and omeprazole. Nearly twenty-two (76%) of patients were treated with alpha-interferon. The mean age at the first surgical intervention was 52.39 months (SD=102.28) (range from 4 months to 426 months). The mean number of surgical intervention was 10.39 (SD=7.76) (range from 2 to 30). The mean time of surgical intervals was 4.63 months (SD=4.02) (range from 2 to 24 months). In fourteen patients (48%) tracheotomy was done. All patients who had tracheotomy received alpha-interferon. One of our cases was male who had pulmonary extension with HPV-6.

Discussion

Basic and clinical researches in the realm of RRP have been increased in the last 2 decades and identifying prognostic factors has been the focus of these studies. Age at onset of the RRP is a well-established factor in predicting aggressiveness and severity of the disease. Epidemiologic studies in US showed that patients, whose disease manifests before the age of 5 years, require more surgical interventions, undergo adjuvant
therapy, tracheotomy and higher rate of tracheal and pulmonary extension (8, 9). In this study, it seems that the disease need more surgical interventions, shorter surgical intervals, more frequently received adjuvant therapy and underwent tracheotomy more than older age while manifested at lower age. More cases were required to do tracheotomy in this subgroup of patients. HPV genotyping has been the focus of interest in evaluating the aggressiveness of RRP. In this regard, Wiatrak and colleagues demonstrated that who were infected with HPV-11 had more aggressive disease than who infected with HPV-6. It was demonstrated by considering higher disease severity scores, need for more frequent surgical interventions, greater requirement for adjuvant therapy, higher incidence of tracheal and pulmonary disease, and greater need for tracheotomy (9, 10). The patients who were infected with HPV-11 had more frequent surgical intervention, shorter surgical intervals and greater requirement for adjuvant therapy in comparison with HPV-6. However aforementioned differences did not reach statistical significance. One case had pulmonary extension and required tracheotomy that was infected with HPV-6. There was no case of malignant transformation in the study. Considering low incidence of disease, multi-centric studies with long term follow-up is necessary to determine the factors which are responsible in the prognosis and remission of this disease (6). Patients with less age and HPV-11 infection had more severe problems. Although the clinical differences between HPV-6 and HPV-11 infections were not accurately predictable, further investigation is recommended to realize the source and reason of these differences.

**Conclusion**

An 11-year retrospective review of patients with RRP in Rasoul-e-Akram Hospital from 1377 to 1387 was carried out regarding to HPV genotyping. Twenty nine cases were identified, 45% with HPV-6 and 55% with HPV-11 infection. Patients with HPV-11 had more severe problems. Furthermore we showed that patients with less age had more severe problems, but these differences did not reach statistical significance which needs much more investigations for more reasonable evaluation for these differences.

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