Sys Rev Pharm 2020;11(9):163-168
A multifaceted review journal in the field of pharmacy

Correlation between P53 and KI67 with Aggressiveness Factor in Recurrent Respiratory Papillomatosis

Rizka Fathoni Perdana[1*], Sri Herawati[1], Muhtarum Yusuf[1], Irwan Kristyono[1], Puguh Setya Nugroho[2]

[1]Department of Otolaryngology-Head and Neck Surgery, Faculty of Medicine Universitas Airlangga/ Dr. Soetomo Teaching Hospital, Surabaya Indonesia
[2]Department of Otolaryngology-Head and Neck Surgery, Universitas Airlangga Academic Hospital, Surabaya Indonesia

***Corresponding author:** Rizka Fathoni Perdana
Department of Otolaryngology-Head and Neck Surgery, Faculty of Medicine Universitas Airlangga/ Dr. Soetomo Teaching Hospital, Jl. Mayjen Prof. Dr. Moestopo No.47, Surabaya, Jawa Timur 60132
Email: rizka-f-p@fk.unair.ac.id

ABSTRACT

Background: Recurrent respiratory papillomatosis (RRP) is caused by HPV types 6 and 11 and has a substantial papilloma growth in the airway and is recurrent. The activity of HPV neoplasms is related to the cell cycle, a complex series of processes that cause cells to grow and replicate. The p53 and Ki67 proteins, as one of the tumor gene controllers, have the function of controlling the cell cycle and assisting the apoptotic process of damage to DNA.
Objective: This study aims to analyze the relationship between RRP aggressiveness and p53 and Ki67 protein expression as one of the predictive factors for aggressive disease progression.
Methods: This study was an observational analytic study with a cross-sectional form. The p53 and Ki67 protein examination results were obtained from immunohistochemical examination by staining monoclonal antibody rabbit Anti-Human p53 and Ki67 clone 318-6-11. Data analysis used binary logistic regression to determine the relationship between the independent and dependent variables with a significant p <0.05.
Results: From 19 samples, the distribution of the sex distribution of male patients (42.10%), while for women of eleven patients (57.90%). The type of RRP that was served the most by the participants was RPP for 13 children (68.40%). Positive p53 protein expression was found in 8 (42.10%) patients, while 11 (57.90%) patients had negative p53 protein expression. Moreover, on positive Ki67 protein expression, there were 5 (26.30%) patients and 14 (73.70%) patients with negative Ki67 protein expression.
Discussion: High p53 protein expression indicates that the cell cycle is not controlled, so it is regulated by the p53 protein to control the cycle through the process of apoptosis or capture. The uncontrolled cell cycle causes tumors to grow very fast. Meanwhile, high Ki67 protein expression indicates an uncontrolled cell cycle because most of them are not resting.
Conclusion: There is no relationship between the aggressiveness of recurrent tract papillomas with p53 and Ki67 protein expression.

Keywords: Recurrent Respiratory Papillomatosis; p53; Ki67; Aggressiveness Factor; Microscopic Laryngeal Surgery

Correspondence:
Rizka Fathoni Perdana
Department of Otolaryngology-Head and Neck Surgery, Faculty of Medicine Universitas Airlangga/ Dr. Soetomo Teaching Hospital, Jl. Mayjen Prof. Dr. Moestopo No.47, Surabaya, Jawa Timur 60132
Email: rizka-f-p@fk.unair.ac.id

INTRODUCTION

Recurrent respiratory papillomatosis (RRP) is caused by HPV types 6 and 11 and has manifestations of papilloma growth in the airway and is recurrent. RRP type consists of pediatric-onset or juvenile type (juvenile-onset RRP) and adult-onset (adult-onset RRP). The juvenile type has a peak incidence at 2-4 years of age and is aggressive, while the adult type has a peak incidence at the age of 20-40 years[1]. The prevalence of JoRRP in the Free State province of South Africa to be 3.88 / 100000 population and 0.8 / 100000 population in European studies. A recent study estimated the prevalence of RRP in the general UK population to be 1.42 per 100 000 population[2–4]. The number of RRP cases in Indonesia was 34 cases, with details of 28 patients (82%) being JoRRP and six patients (18%).) is AoRRP[5].

The disease can be non-aggressive and experience remission. Besides that, it can grow more aggressively so that it needs to be done repeatedly[1]. The aggressive disease is characterized by the recurrence or growth of papillomas in more than one location. The most common sites for papilloma growth are the oral cavity, trachea, and bronchi. Another criterion is the number of operations required to achieve disease-free[6]. Human papillomavirus (HPV) is the causative factor agent RRP[1], which consists of 2 groups: low risk and high-risk HPV[7]. A study was conducted to identify the HPV type in 15 juvenile RRP patients at RSUD Dr. Soetomo, with the results obtained in six positive patients with HPV-6 and nine positive patients with HPV-11[8]. HPV neoplasm activity is related to the cell cycle, a complex series of processes that cause cells to grow and replicate[9–11]. The HPV viral genome consists of regions that produce neoplastic proteins (E6 and E7), viral protein products (E1, E2, and E5), and viral capsid proteins (L1 and L2)[7]. The integration of HPV DNA (deoxyribose nucleic acid) causes the E6 and E7 genes to remain active. The E6 and E7 genes encode oncoproteins that cause transformation in the host cell[12].

The p53 protein, known as a gene suppressor tumor, has the function of controlling the cell cycle and regulating the apoptosis process in the event of damage to DNA. HPV through E6 protein can damage and degrade the p53 protein, leading to the inactivation of the p53 protein[13]. Meanwhile, the E7 gene binds to pRb so that E2F that binds to pRb will be released and cause cells to undergo the cell cycle. The E6 HPV gene can induce p53 degradation so that cells cannot undergo arrest. The

result of these two processes is that the cells experience an uncontrolled cell cycle. These oncogenic activities are the basis of tumorigenesis caused by HPV, resulting in uncontrolled cell division[13]. It is suspected that RRP aggressiveness is due to a high prophylactic activity so that tumor growth is so fast with the consequent clinical symptoms. Identifying factors that can predict RRP's aggressiveness, especially the juvenile type, is an actual disease management effort. Identification of these factors in high-risk sufferers can improve patient management[14]. This study aims to analyze the relationship between RRP aggressiveness and p53 and Ki67 protein expression as one of the predictive factors for aggressive disease progression.

METHOD

This study was an observational analytic study with a cross-sectional form. The study population was RRP patients treated at SMF ORL-NHS RSUD Dr. Soetomo for the period 2012 - 2014. The study sample obtained 19 patients who met the criteria for acceptance of the sample and were examined for p53 protein expression and proliferation index on laryngeal specimens from microscopic laryngeal surgery stored in the Pathology Anatomy Installation of RSUD Dr. Soetomo in slide form. Inclusion criteria included: recurrent respiratory papilloma patients of all ages, patient medical records during complete treatment, and anatomical pathology slides were well available and sufficient to examine the expression of p53 and Ki67 proteins, as well as the proliferation index of both. Meanwhile, the criteria for rejection of exclusion included: the patient had undergone adjuvant therapy less than 30 days before the study.

The p53 and Ki67 protein examination results were obtained from immunohistochemical examination by staining monoclonal antibody rabbit Anti-Human p53 and Ki67 clone 318-6-11 (Dako Denmark A / S Produktionsvej 42, DK-2600 Glostrup Denmark). The p53 and Ki67 protein expressions will be divided into four levels of expression, namely; Score 0: if no brown granules are seen in the cell nucleus; score one if brown granules are seen in the cell nucleus amounting to less than 10% of the entire field of view at 400x magnification; score two if brown granules are seen in the nucleus of between 10% and 50% of the entire field of view at 400x magnification; score three if brown granules are seen in the cell nucleus amounting to more than 50% of the entire field of view at 400x magnification. The scoring results are then interpreted as unfavorable (-) if the scores are 0 and 1, and positive (+) if the scores are 2 or 3. (Yamashita et al., 2004).

Data analysis used binary logistic regression to determine the relationship between independent and dependent variables with a significant $p < 0.05$, which means if the statistical test shows a value of $p < 0.05$, then there is a significant relationship between independent and dependent variables. The data ware was analyzed using the statistical software program Statistical Package for the Social Science (SPSS) version 16.0. (SPSS.Inc., Chicago, IL).

RESULT

Basic Data

The primary data analyzed in this study included gender and disease aggressiveness criteria for RRP sufferers. In this study, the distribution of sex was male is eight patients (42.10%), while for women, as many as eleven patients (57.90%). The types of RRP who participated included RPP for children and adults, where the type of child was the most common type with 13 patients (68.40%). The adult type was obtained by several six patients (31.6%). The study participants' number of operations was six times, and the least was once. Obtained mean 2.42 ± 1.43 times and median three times. Distal distribution of papillomas was found in 7 patients (36.80%), and 12 patients (63.20%) had no history of distal laryngeal papilloma. Meanwhile, a history of tracheotomy was obtained for 13 patients (68.40%), and six patients (31.60%) had no history of tracheotomy. The average number of patients with microscopic laryngeal surgery who received nine patients (47.40%) received microscopic laryngeal surgery, and ten patients (52.60) had a history of surgery less than three times per year.

Table 1: Characteristics of respondents

Characteristics	Number	Percentages
Sex		
- Male	8	42.10
- Female	11	57.90
RRP types		
- Pediatric type	13	68.40
- Adult type	6	31.60
Distal laryngeal papilloma		
- Yes	7	36.80
- No	12	63.60
Tracheotomy history		
- Yes	13	68.40
- No	6	31.60
Microscopic Laryngeal Surgery		
- ≥ 3	9	47.40
- ≤ 3	10	52.60
Aggressiveness		
- Aggressive	12	63.20
- Not aggressive	7	36.80
The highest number of operations in a year		
Mean ± SD	Median	Range
2.42 ± 1.43	3	1-6

Distribution of p53 and Ki67 Protein Expressions

In the study, positive p53 protein expression was found in 8 (42.10%) patients, while 11 (57.90%) p53 protein expression was negative. Furthermore, on positive Ki67 protein expression, there were 5 (26.30%) patients and 14 (73.70%) patients with negative Ki67 protein expression.

Table 2: Distribution of p53 and ki67 protein expressions

Protein expressions	Number	Percentages
P53		
- Positive	8	42,10
- Negative	11	57,90
Ki67		
- Positive	5	26.30
- Negative	14	73.70

Analysis of the Relationship of Recurrent Respiratory Papillomas

Relationship with p53 Protein Expression

A history of BLM <3x was obtained in five patients (50%) in both positive and negative p53 protein expression, while a history of BLM ≥ 3x found three patients (33.33%) with positive p53 protein expression and six samples (66.67%).)) with negative p53 protein expression. The distal laryngeal papilloma history was obtained in 4 patients (57.10%) with positive p53 protein expression and three patients (42.90%) with negative expression. Patients without a history of papilloma distal larynx consisted of 4 patients (33.33%) with positive p53 protein expression and eight patients (66.67%) with negative expression. Tracheotomy history was found in 4 patients (30.80%) on positive p53 protein expression and nine patients (69.20%) on negative expression. Patients without a history of tracheotomy found many patients (66.67%) with positive p53 protein expression and two patients (33.33%) with negative protein expression. Recurrent respiratory papilloma consisting of 4 patients with positive expression (30.80%) and nine patients with negative expression (69.20%). Respiratory papilloma was not aggressive from 4 patients with positive expression (66.67%) and two patients (33.33%) with negative expression. Fisher's test results obtained a p-value of 0.166. This shows that PSPB aggressiveness and p53 protein expression had no significant relationship (p> 0.05).

Table 3: Distribution of the relationship between yearly microscopic laryngeal surgery history, distal laryngeal papilloma, and tracheotomy history with p53 and Ki67 protein expression

	Protein expressions p53		Protein expressions Ki67	
	Positive	Negative	Positive	Negative
BLM history per year				
< 3x	5 (50%)	5 (50%)	3 (30%)	7 (70%)
≥ 3x	3 (33,33%)	6 (66,67%)	2 (22,20%)	7 (77,80%)
Distal laryngeal papilloma				
Yes	4 (57,10%)	3 (42,90%)	5 (71,40%)	2 (28,60%)
No	4 (33,33%)	8 (66,67%)	3 (25%)	9 (75%)
Tracheotomy history				
Yes	4 (30,80%)	9 (69,20%)	3 (23,10%)	10 (76,90%)
No	4 (66,67%)	2 (33,33%)	2 (33,30%)	4 (66,70%)

Ki67 Protein Expression

A history of BLM <3, there were three patients (30%) with positive Ki67 protein expression and seven negative Ki67 patients, while a history of BLM ≥ 3x was obtained in two patients (22.20%) with positive Ki67 protein expression and seven patients (77, 80 %) with negative Ki67 protein expression. Papilloma history was found in five patients (71.40%) with positive Ki67 protein expression and two patients (28.60%) with negative Ki67. Patients without a history of distal laryngeal papilloma consisted of three patients (25%) with positive Ki67 protein expression and nine patients (75%) with negative Ki67. Tracheotomy history was found in three patients (23.10%) with positive Ki67 protein expression and ten patients (76.90%) on negative Ki67. Patients without a history of tracheotomy found two patients (33.30%) with positive Ki67 protein expression and four patients (66.70%) with negative Ki67 expression. Aggressive respiratory papillomas consisted of 3 patients with positive Ki67 expression (23.10%) and ten patients with negative Ki67 expression (76.90%). Respiratory papilloma was not aggressive from 2 patients with positive Ki67 protein expression (33.30%) and four patients (66.70%) with negative Ki67. Fisher's test results obtained a p-value of 0.520. This showed that PSPB aggressiveness and Ki67 protein expression had no significant relationship (p> 0.05).

Table 4: Relationship of RRP aggressiveness with p53 and Ki67 protein expression

	P53 protein expression		Ki67 protein expression	
	Positive	Negative	Positive	Negative
RRP aggressiveness - Aggressive - No	4 (30.80%) 4 (66.67%)	9 (69,20%) 2 (33,33%)	3 (23,10%) 2 (33,30%)	10 (76,90%) 4 (66,70%)
Fisher	0.166		0.520	

DISCUSSION

The annual BLM history was grouped into two categories: a history of BLM more than or equal to 3x (≥ 3x) in one year and a history of BLM less than 3x in one year, provided that a history of BLM ≥ 3x was an aggressive type of RRP category. The study obtained 35 RRP samples for p53 and Ki67 proteins and several other indicators of aggressiveness. The results showed that high enough p53 and Ki67 protein expression was found in aggressive RRP, so it can be concluded that the p53 and Ki67 protein expression examination can be used to predict the aggressiveness of the RRP course, especially the type of child[15].

This study's hypothesis was rejected because the Fisher test showed no relationship between the aggressiveness of recurrent respiratory papillomas and the expression of p53 and Ki67 proteins. The discussion in terms of sampling is associated with the small sample size in this study. Data with a sample size of 19 patients is the optimal number that can be collected, but these data do not describe a normal distribution. Data with abnormal distribution has a considerable enough variation so that if the analysis test is carried out, it will give results with a reasonably considerable variation. The life cycle and carcinogenesis of HPV are closely related to the differentiation process of infected host cells, epidermal, and mucosal cells. HPV infects the basal layer and enters host cells and expresses E6 and E7 to deactivate pRb and p53, thereby affecting cell division[16]. Cell cycle and division are influenced by the E6 and E7 proteins, which are derived from HPV. Ki67 protein is a picture of the state of the cycle or cell division. The p53 protein plays a role in resting cells or cells in the G0 phase of the DNA repair process if the damage is found. The E7 protein deactivates pRb so that the cell cycle occurs without a resting phase. This causes the p53 protein expression to be higher so that it inhibits the cell cycle through apoptosis or stopping.

High p53 protein expression indicates an uncontrolled cell cycle so that there is a regulatory mechanism by p53 protein to control the cell cycle through apoptosis and arrest processes. The uncontrolled cell cycle causes tumors to grow very fast. Meanwhile, the high Ki67 protein expression indicates an uncontrolled cell cycle because most cells are not resting. The uncontrolled cell cycle causes tumors to grow very fast. The clinical manifestation of rapid cell division is that the papilloma grows faster and has various consequences. High cell division causes the tumor to grow back quickly after being cleared with BLM. This results in shorter BLM operating intervals so that the BLM operating frequency becomes more frequent.

Papillomas overgrow with the potential to spread to a more distal location of the airway and grow in a location more distal to the larynx. So, this indicates an aggressive disease. The mass of papillomas that overgrows causes the tumor to fill the airway resulting in airway obstruction. As a result, the patient required a tracheotomy to create an artificial airway because BLM surgery could not be performed. Patients undergoing tracheotomy are an indicator that PSPB disease is aggressive. This study's hypothesis is also not proven because there is no relationship between RRP aggressiveness and p53 and Ki67 protein expression. This can be caused by the degradation of the p53 protein and the high rate of Ki67 cell division caused by the E6 protein derived from HPV. The E6 protein produced by HPV will bind and cause degradation of the p53 protein. This is the synergy of the virus's attempts to control the cycle and cell division.

Naturally, the p53 protein can cause cells to stop or apoptosis, thus inhibiting abnormal cell growth. However, HPV will express E6 protein so that it binds to p53, and degradation occurs. Protein E6 affinity for p53 varies and depends on the type of HPV. High-risk HPV will have a much stronger affinity for binding to the p53 protein and cause more significant degradation than low-risk HPV. The low expression of p53 and Ki67 proteins in this study needs to be investigated further because it depends on the E6 protein's affinity for binding and degrading p53 protein. The affinity of E6 protein with p53 protein can also vary between low-risk HPVs because it is influenced by the HPV itself's mutation pattern. If the mutation in HPV is in the codon that makes up the E6 protein, it will affect its performance.

The ability of E6 protein affinity to the p53 protein can differ between HPV types. This study did not detect this type of virus, so it is not known the distribution of the types of HPV that infect patients with PSPB. The difference in base pairs in E6 can be 10% different, impacting different affinity for binding and degrading p53 protein. Disruption of the DNA base sequence disrupts the product of the gene. Genetic polymorphisms within an HPV type are related to various phenotypes, such as pathology and clinical. This genetic diversity will cause variations in disease aggressiveness[17]. Protein expression can also be affected by mutations, insertions, and duplications. It is suspected that only site-specific changes influence disease expression and aggressiveness[2].

Diseases caused by HPV, with mutations in E6 / E7 that are up-regulating, will cause aggressive disease due to uncontrolled cell division. Mutations that are down-regulating in E6 / E7 will result in less aggressive diseases. Single point mutations are not known to cause aggressive disease or not, but variants of HPV are linked to the virus's biological potential in isolates from the same geographic area[2]. Another study found insertion in all patients with HPV-11. The mutation reflects the geographic location of the origin of the HPV-11 isolates. This specific polymorphism is not directly related to aggressiveness. This is because the point mutation occurs

in all HPV-11 of the same geographic and ethnic origin[17]. If the polymorphism that occurs in E6/E7 is a silent mutation, it does not affect the disease's aggressiveness. Changes in URR are thought to cause changes in the E6/E7 gene protein expression, thereby affecting disease aggressiveness. The weakness of the study was the number of patients analyzed[17].

Analysis of the relationship between polymorphism and HPV variants to aggressiveness is still being studied because it requires many sufferers. Further studies on the analysis of virus coinfection, molecular genetic disorders, DNA ploidy, and cell proliferation in PSPB as predictors of aggressiveness have yielded varying results[17]. The proliferation of the antigen cell nucleus and the replication of the host induced by the viral gene activates the cellular DNA replication engine to support viral replication. It is suspected that HPV inhibits the cell cycle and inhibits tumor suppressor genes, thus preventing its function as a regulator of cell growth. Flow cytometry results show increased cell proliferation in aggressive PSPB. The aggressive disease also exhibits a high S-phase fraction so that it can be used as a predictive factor for disease aggressiveness[18].

Factors other than HPV that contribute to aggressiveness are host factors, particularly the immune response. One sufferer can suffer from an aggressive disease while the other is mild. The susceptibility of the host to viral infection is related to polymorphisms in genes regulating the immune response. Effective immune responses to viral infections include natural and adaptive immune responses, with a balance between Th1 and Th2 and the chemokines and cytokines. This immune response can even control and predict disease susceptibility and aggressiveness[19,20]. HPV infection can cause an inadequate immune response so that HPV replication occurs. These sufferers have a low tolerance to HPV-6 infection and an inadequate response to the virus's proteins. The local immune response to HPV that causes the infection is permissive[20,21].

CONCLUSION

There was no relationship between the aggressiveness of recurrent respiratory papillomas with p53 and Ki67 protein expression.

ETHICAL CLEARANCE

Taken from Ethical Committee Faculty of Medicine, Universitas Airlangga.

SOURCE OF FUNDING

The research was funded by the authors.

CONFLICT OF INTEREST

None

REFERENCES

1. Larson DA, Derkay CS. Epidemiology of recurrent respiratory papillomatosis. Apmis. 2010;118(6-7):450–4.
2. Seedat RY, Schall R. Age of diagnosis, incidence and prevalence of recurrent respiratory papillomatosis—A South African perspective. Clin Otolaryngol. 2018;43(2):533–7.
3. Buchinsky FJ, Valentino WL, Ruszkay N, Powell E, Derkay CS, Seedat RY, et al. Age at diagnosis, but not HPV type, is strongly associated with clinical course in recurrent respiratory papillomatosis. PLoS One. 2019;14(6):e0216697.
4. Saswita E, Asyari A, Novialdi N, Fitri F. Diagnosis dan Penatalaksanaan Papiloma Laring Berulang pada Dewasa. J Kesehat Andalas. 2018;7:85–91.
5. Perdana RF. Transnasal Esophagoscopy Examination in Outpatient Unit, Department of Otorhinolaryngology Head and Neck Surgery, Dr. Soetomo Hospital, Surabaya. Folia Medica Indones. 2020;56(1):75–81.
6. Donne AJ, Clarke R. Recurrent respiratory papillomatosis: an uncommon but potentially devastating effect of human papillomavirus in children. Int J STD AIDS. 2010;21(6):381–5.
7. Dunne EF, Markowitz LE. Genital human papillomavirus infection. Clin Infect Dis. 2006;43(5):624–9.
8. Perdana RF, Herawati S, Surarso B, Aksono EB. Correlation of Aggressivity Papilloma Recurrent Respiratory Tract With Human Papillomavirus Types 6 And 11. Med Leg Updat. 2020;20(1):471–6.
9. Ma DJ, Price K, Moore EJ, Patel SH, Hinni ML, Chintakuntlawar A V, et al. Two-year results for MC1273, a phase 2 evaluation of aggressive dose de-escalation for adjuvant chemoradiation in HPV+ oropharynx squamous cell carcinoma (OPSCC). Int J Radiat Oncol Biol Phys. 2017;99(5):1320.
10. Seiwert T, Melotek JM, Foster CC, Blair EA, Karrison TG, Agrawal N, et al. OPTIMA—a phase 2 trial of induction chemotherapy response-stratified radiation therapy dose and volume de-escalation for HPV+ oropharyngeal cancer: efficacy, toxicity, and HPV subtype analysis. Int J Radiat Oncol Biol Phys. 2018;100(5):1309.
11. Foster I. Cancer: A cell cycle defect. Radiography. 2008;14(2):144–9.
12. Ganguly N, Parihar SP. Human papillomavirus E6 and E7 oncoproteins as risk factors for tumorigenesis. J Biosci. 2009;34(1):113–23.
13. Ghittoni R, Accardi R, Hasan U, Gheit T, Sylla B, Tommasino M. The biological properties of E6 and E7 oncoproteins from human papillomaviruses. Virus Genes. 2010;40(1):1–13.
14. Wiatrak BJ, Wiatrak DW, Broker TR, Lewis L. Recurrent respiratory papillomatosis: a longitudinal study comparing severity associated with human papilloma viral types 6 and 11 and other risk factors in a large pediatric population. Laryngoscope. 2004;114(S104):1–23.
15. Rehman SU, Mueller T, Januzzi JL. Characteristics of the novel interleukin family biomarker ST2 in patients with acute heart failure. J Am Coll Cardiol. 2008;52(18):1458–65.
16. Castillo A. HPV infection and carcinogenesis in the upper aero-digestive tract. Colomb Med. 2011;42(2):233–42.
17. Pou AM, Weems J, Nason R, Deskin RW, Payne DA. Molecular characterization of mutations in patients with benign and aggressive recurrent respiratory papillomatosis: a preliminary study. Ann Otol Rhinol Laryngol. 2004;113(3):180–6.
18. Stern Y, Mueller K, Willging JP, Myer CM, Cotton RT. S-phase fraction as a predictor of prognosis in juvenile respiratory papillomatosis. Arch Otolaryngol Neck Surg. 1998;124(5):541–4.
19. Gelder CM, Williams OM, Hart KW, Wall S, Williams G, Ingrams D, et al. HLA class II polymorphisms and susceptibility to recurrent respiratory papillomatosis. J Virol. 2003;77(3):1927–39.

20. Bonagura VR, Vambutas A, DeVoti JA, Rosenthal DW, Steinberg BM, Abramson AL, et al. HLA alleles, IFN-γ responses to HPV-11 E6, and disease severity in patients with recurrent respiratory papillomatosis. Hum Immunol. 2004;65(8):773–82.
21. DeVoti JA, Steinberg BM, Rosenthal DW, Hatam L, Vambutas A, Abramson AL, et al. Failure of gamma interferon but not interleukin-10 expression in response to human papillomavirus type 11 E6 protein in respiratory papillomatosis. Clin Diagn Lab Immunol. 2004;11(3):538–47.