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Recurrent respiratory papillomatosis: clinical characteristics and viral genotyping in a Brazilian population

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ABSTRACT

This study presents 25 cases of recurrent respiratory papillomatosis (RRP) that occurred in Sao Luis, Maranhao State, Northeast region, Brazil, between January 2007 and December 2018. Sociodemographic and clinical profile of patients as well as human papillomavirus (HPV) infection status were evaluated. Clinical and histopathological data were collected from the patients' medical records. For the HPV infection analysis, DNA was extracted and subjected to amplification by a nested polymerase chain reaction. Viral genotyping was performed by automated sequencing. The median age of patients was 12.40 ± 12.6. years, and the juvenile form of the disease (68%) was the predominant form of disease. Female participants were predominant (60%), and they were from cities located in the interior of the State (60%). The most common clinical manifestation was dysphonia; recurrence was observed in most cases (56%), and tracheostomy was necessary in seven patients (26.9%). When comparing the RRP forms, patients in the juvenile-RRP group had higher recurrence rates and need of tracheostomy than those in the adult-RRP group. The viral genotyping analysis revealed that 47.8% of patients had low-risk HPVs, whereas 13.1% had high-risk HPVs, and in 39.1% of patients the viral genotype was not obtained. HPV-6 was the most prevalent type and Juvenile-RRP was more prevalent in our population. HPV was present at a high rate, and HPV-6 was the predominant genotype. This study serves as the basis for further studies to be conducted in the Brazilian population. Our findings aid the better understanding of RRP, possibly suggesting some prognostic factors associated with the disease aggressiveness.

KEYWORDS: Recurrent respiratory papillomatosis. Human papillomavirus. Genotype. Larynx.

INTRODUCTION

Recurrent respiratory papillomatosis (RRP) occurs in children and adults. It is the most common benign neoplasm in children and has a high recurrence rate that negatively affects the patient's quality of life, leading to higher healthcare expenses[1].

RRP is characterized by the development of exophytic proliferative lesions of the connective tissue covered by epithelium, which occur in the airway mucosa. Although papillomas can be present anywhere along the respiratory tract, they occur mainly on the larynx[2,3].

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This condition is initiated by a human papillomavirus (HPV) infection, especially the ones caused by low-risk genotypes such as HPV-6 and HPV-11[2,4]. Highly oncogenic genotypes such as HPV-16 and HPV-18 are implicated in less than 5% of the cases[5,6]. In children, studies have shown that transmission may occur during the intrauterine life or during a vaginal delivery[5]. In adults, infection is likely to occur through oral sex[2].

According to the patient's age, RRP can be classified as juvenile-RRP when it occurs in individuals aged ≤12 years old and adult-RRP when it affects individuals aged >12 years[7,8]. Juvenile-RRP is generally more aggressive due to the rapid growth of multiple lesions with possible airway obstruction, recurrence and metastasis[2,9]. Adult-RRP is usually characterized by a non-aggressive solitary lesion with low recurrence rates[10,11].

The course of disease is unpredictable, ranging from spontaneous remission to aggressive disease progression, which can spread to the lungs, requiring multiple surgical interventions[9,10]. Patients with RRP usually present non-specific symptoms of airway involvement, including chronic cough, hoarseness, voice change and breathing difficulties[3,12]. Because of the unspecified clinical scenario, the disease can mimic other common laryngeal and respiratory diseases such as laryngitis, asthma, bronchitis and croup. Therefore, errors and delays in diagnosis and inadequate initial treatment are frequent[1].

Understanding the progressive nature of RRP and its potentially lethal consequences are critical to an effective intervention. Moreover, it is necessary to understand the characteristic symptoms that should raise an early suspicion of this condition, despite the relative rarity of the disease[13].

This study was performed in the Northeast region of Brazil, an area with a high poverty index and low social indices[14]. Knowledge about the viral genotype present in RRP patients is vital to formulate therapeutic and prophylactic measures for the disease, such as vaccination. Based on the clinical description of the cases, it is expected to provide, together with other Brazilian studies, an estimate of the incidence of RRP in Brazil and to raise the awareness on the importance of RRP across the world.

This study aimed to present cases of RRP that occurred at the University Hospital of Sao Luis city, Maranhao State, in Brazil, between January 2007 and December 2018. Molecular analysis was performed to detect and genotype HPV in addition to the patients' clinical description. Statistical analysis was also conducted to assess the presence of associations between variables of the disease aggressiveness and the form of presentation of the disease (juvenile × adult) in this specific population.

MATERIALS AND METHODS

Study period and design

A retrospective cross-sectional study was conducted in patients who had juvenile- and adult-RRP between January 2007 and December 2018.

Patients' data and biological samples

All details of RRP cases between 2007 and 2018 were collected from the histopathological records book of the Pathology Service. After surveying the number of cases, paraffin blocks containing the biological samples were retrieved from the pathology department. Data on the patients' sociodemography, clinical and histopathological features were obtained from the medical records of the hospital and inserted into a protocol form for organizing data and subsequent analyses.

The inclusion criteria were patients with RRP of any age org ender. The exclusion criteria included patients who had missing or incomplete medical records and/or paraffin blocks or those with paraffin blocks that were inappropriate to perform DNA extraction owing to poor quality.

DNA extraction from Formalin-Fixed Paraffin-Embedded (FFPE) tissue

Fifteen sections (approximately 5-µm thick) were prepared from blocks containing the surgical resection material and were used for DNA extraction. The sections were stored in 2.0-mL tubes at 4 °C until the next step, which corresponds to dewaxing.

The dewaxing process included the addition of 1 mL of Xylol P.A (Vetec, Rio de Janeiro, Brazil) to the sample, which was vigorously vortexed for approximately 10 s. The mixture was then subjected to centrifugation at 21,000 g for 4 min, and this step was repeated three or four times. In cases in which the paraffin remained after this process, the sample was heated to 55 ºC. The supernatant was removed and 1 mL of ethanol P.A (Vetec, Rio de Janeiro, Brazil) was added, followed by vortex homogenization and centrifugation at 21,000 g for 6 min. Again, 1 mL of ethanol was added to the sample, followed by vortex homogenization and centrifugation at 21,000 g for 6 min. The supernatant was removed and the sample was centrifuged again at 21,000 g for 1 min to completely remove the ethanol. The tubes were left open and were incubated at 90 ºC in a thermomixer for 1 min until the complete ethanol evaporation.

Total genomic DNA was extracted after the dewaxing process, as described in the QIAamp DNA FFPE Tissue

Kit protocol (QIAGENInc. Hilden, Germany). DNA purity and concentration were determined using the Nanovue spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

HPV detection

To identify HPV DNA in samples, a polymerase chain reaction (PCR)-nested amplification was performed using the primers PGMY09 and PGMY11 for the first round and GP5+ and GP6+ for the second round[15].

Briefly, the PGMY09/11 reaction consisted of an initial denaturation step for 2 min at 95 °C followed by 40 cycles of denaturation for 40 s at 95 °C, annealing for 40 s at 55 °C and extension for 40 s at 72 °C. The final reaction volume was 25 µL, containing 10 pmol of each primer, 2.5 µL of 10× buffer, 1.5 µL of 50 mM magnesium chloride, 10 mM dNTP and 0.2 µL of Platinum Taq Polymerase (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA)[15].

The second round of amplification was carried out with primers GP5 + / GP6 + with an initial denaturation step for 4 min at 95 °C, followed by 45 cycles of denaturation for 45 s at 95 °C for 1 min, annealing at 40 °C for 1 min and extension for 1 min at 72 °C. At the end of the reaction, the final reaction volume was 25 µL, containing 10 pmol of each primer, 2.5 µL of 10× buffer, 1.5 µL of 50 mM magnesium chloride, 10 mM dNTP and 0.3 µL of Platinum Taq Polymerase (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Both rounds were performed in a Veriti™ 96-Well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA).

The reaction products were visualized by electrophoresis in 1.5% agarose gels prepared in TBE buffer. A 5-µL aliquot of DNA was homogenized in a loading buffer solution (Sigma-Aldrich, USA) and 0.1% Gel Red dye, and the entire mixture was applied to the gel. Electrophoresis was performed for 30-50 min at 5 V/cm^2 in a horizontal electrophoresis apparatus (Life Technologies, USA). The amplified DNA fragments were visualized in an ultraviolet light transilluminator (BIO-RAD Laboratories, USA).

HPV genotyping

HPV-positive PCR products were purified by using the Genelute PCR Clean-up kit (Sigma-Aldrich, USA), according to the manufacturer's protocol. Automated sequencing was performed by the company ACTGene Molecular Analyses (UFRGS, Brazil) using the platform ABI 3500 (Thermo Fisher Scientific, Waltham, MA, USA). The purified samples were diluted using a 6-µL solution containing 30–60 ng of the purified amplification product, 5 pmol of primer and double-distilled water.

For the confirmation and identification of the HPV genotype, a comparison of the sequenced samples nucleotide sequences with GenBank prototypes by using the BLAST program.

Statistical analysis

Samples' data were submitted to the Shapiro-Wilk normality test, resulting in a normal distribution. The Student's t-test for independent samples was used to analyze continuous variables. Discrete variables were presented as proportions. A univariate analysis was performed using the Fisher's exact test to compare the proportions between the juvenile-RRP and adult-RRP groups.

To verify if independent variables could predict the presentation of juvenile RRP, a crude analysis and one adjusted analysis were performed through a binary logistic regression model, considering only the variables that presented statistical significance ($p < 0.05$). The Kaplan-Meier analysis was performed using the log-rank test to compare survival curves between the two groups. The dependent variable for the survival analysis was the period of follow-up going from the date of admission until the last date found in the medical record. The outcome of interest was the recurrent need for hospitalization. The time elapsed until the outcome was expressed in years. The survival time was analyzed using independent significant variables ($p<0.05$ in the univariate analysis). These analyses were performed using the SPSS® Statistics, version 24.0 (IBM Corp., Armonk, New York, USA).

Ethical approval

The study was approved by the Committee of Ethics and Research of the Federal University of Maranhao, document Nº 2935.566, in accordance with the Declaration of Helsinki for research in humans

RESULTS

Table 1 shows data on the social and clinical variables of the 25 patients included in the study, divided into the juvenile-RRP and adult-RRP groups. Most patients (68%) had the juvenile form of the disease, while 32% had the adult form. The mean age of the patients was 12.40 ± 12.6 years, with the sample composed mainly of female participants (60%), coming from cities in the interior of the State (60%).

The most common clinical manifestation was dysphonia, present in 80% of cases. Other manifestations such as

Table 1 - Comparison of social and clinical variables between patients with juvenile-RRP and adult-RRP.

	Total	Juvenile-RRP n=17 (%)	Adult-RRP n=8 (%)	p-value
Mean age (±SD)	12.40 (±12.6)	5.23 (±3.07)	27.6 (±11.86)	**0.000***
Gender				0.6
Male	10 (40%)	6 (35.3%)	4 (50%)	
Female	15 (60%)	11 (64.7%)	4 (50%)	
Origin				0.8
Capital	10 (40%)	7 (41.2%)	3 (37.5%)	
Interior	15 (60%)	10 (58.8%)	5 (62.5%)	
Dysphonia				0.1
Yes	20 (80%)	15 (88.2%)	5 (62.5%)	
No	5 (20%)	2 (11.8%)	3 (37.5%)	
Dyspnea				0.1
Yes	9 (36%)	8 (47.1%)	1 (12.5%)	
No	16 (64%)	9 (52.9%)	7 (87.5%)	
Dysphagia				0.08
Yes	4 (16%)	1 (5.9%)	3 (37.5%)	
No	21 (84%)	16 (94.1%)	5 (62.5%)	
Recurrence				**0.03***
1	11 (44%)	5 (29.4%)	6 (75%)	
≥ 2	14 (56%)	12 (70.6%)	2 (25%)	
Tracheostomy				**0.04***
Yes	7 (28%)	7 (41.2%)	0 (0%)	
No	18 (72%)	10 (58.8%)	8 (100%)	
HPV				0.8
Low risk	11 (45.8%)	8 (50%)	3 (43%)	
High risk	3 (16.7%)	2 (12.5%)	1 (14%)	
Indeterminate	9 (37.5%)	6 (37.5%)	3 (43%)	

Data are presented as mean values, standard deviations and proportions; the Student's t and the Fisher' s exact test were used to compare the differences between both groups; a significance level of 95% was adopted; *p <0.05; SD = standard deviation; RRP = recurrent respiratory papillomatosis; HPV = human papillomavirus.

dyspnea and dysphagia were less common (36% and 16%, respectively). Most patients (56%) had recurrence of lesions. Tracheostomy was performed in seven patients (26.92%).

The molecular evaluation showed that 92% (23/25) of the patients had HPV DNA.

When comparing the proportions between groups (juvenile-RRP X adult-RRP) through an univariate analysis, the variables age, recurrence of lesions and tracheostomy showed a 95% statistical significance level (Table 1). Patients in the juvenile-RRP group had higher recurrence rates and needed to undergo more tracheostomies than those in the adult-RRP group.

The univariate analysis performed using the Fisher's exact test showed that the variables age, recurrence of lesions and tracheostomy could be included as predictive variables in the regression model, as they presented with statistical significance ($p < 0.05$). However, the variable age was excluded, as it was used to divide the patients into the juvenile RRP and the adult-RRP groups.

Crude and adjusted analyses were performed using a binary logistic regression model. The variables analyzed for recurrent need of hospitalizations and tracheostomy were significant regarding the crude odds ratio; however, when adjusted for the variables in the binary logistic regression model, patients in the juvenile-RRP group did not show higher recurrence rates, but the need of tracheostomy was statistically higher in the juvenile RRP group when compared with the adult-RRP one (Table 2).

The variable tracheostomy was added as an independent variable to analyze the survival curve constructed using the Kaplan–Meier method. As it is a categorical variable, the dummy variable method was used assuming two values (0 or 1) to indicate the respective category, for tracheostomy, 0 (No) and 1 (Yes) were considered for this procedure.

When evaluating the survival curves during the follow-up (in years), according to the medical records and considering the recurrence of hospitalizations as an outcome of interest, the probabilities of patients who underwent tracheostomy and who did not undergo tracheostomy did

Table 2 - Risk assessment for the development of juvenile RRP infection.

	Juvenile-RRP			
	Raw odds ratio IC (95%)	*Adjusted odds ratio IC (95%)*	*Wald*	*P-value*
Recurrence	0.139 (0.021-0.938)	0.222 (0.029-1.709)	2.088	0.1
Tracheostomy	0.588 (0.395-0.876)	0.000	0.000	0.9

RRP = recurrent respiratory papillomatosis.

not differ in the log-rank test (p = 0.5). The curve has also shown that there is no proportionality of risk during the observation period. This Schoenfeld residue assessment test confirmed this proportionality. Thus, there was no relationship between performing tracheostomy and the number of recurrences of the disease.

When the juvenile-RRP group was assessed separately, the age at the time of diagnosis ranged between 1 and 12 years old, as shown in Table 3. Most patients (62.5%) (10) were diagnosed up to their fifth year of life, and 37.5% (7) were diagnosed after six years of age.

Table 3 - Age at the time of diagnosis in juvenile-RRP cases.

	N (17)	%
Age (years)		
1	02	11.76
2	02	11.76
3	01	05.8
4	02	11.7
5	03	17.6
6	02	11.7
7	01	05.8
8	01	05.8
9	02	11.7
12	01	05.8
Mean (SD)	3.2 (±1.6)	

SD = standard deviation; RRP = recurrent respiratory papillomatosis.

When we compared two groups of juvenile-RRP populations, namely, ≤ 5 years and > 5 years, we did not observe any statistically significant difference (Table 4).

Among the patients who had HPV DNA (92%), 47.8% had low-risk HPVs, whereas 13.1% had high-risk HPVs, and in 39.1% of the patients, it was not possible to determine the genotype by automated sequencing. The low-risk genotype, HPV-6, was predominant, followed by the low-risk genotype, HPV 11. The high-risk genotypes HPV-16, HPV-51 and HPV-18 were found in one patient each.

Table 4 - Comparison of social and clinical variables among juvenile-RRP patients who were older or younger than 5 years old.

Juvenile-RRP	≤ 5 years n = 10 (%)	> 5 years n = 7 (%)	P
Gender			0.15
Female	5 (50)	6 (85.71)	
Male	5 (50)	1 (14.29)	
Origin			0.26
Capital	3 (30)	4 (57.14)	
Interior	7 (70)	3 (42.86)	
Dysphonia			0.66
Yes	9 (90)	6 (85.71)	
No	1 (10)	1 (14.29)	
Dyspnea			0.33
Yes	6 (60)	2 (28.57)	
No	4 (40)	5 (71.43)	
Dysphagia			0.5
Yes	1 (10)	0 (0)	
No	9 (90)	7 (100)	
Recurrence			0.59
1	2 (20)	3 (42.85)	
≥5	8 (80)	4 (57.14)	
Tracheostomy			0.13
Yes	6 (60)	1 (14.28)	
No	4 (40)	6 (85.72)	
HPV			0.41
Positive	10 (100)	6 (85.71)	
Negative	0 (0)	1 (14.28)	

The Student's t and the Fisher's exact test were used to compare the differences between both subgroups; a significance level of 95% was adopted; *p <0.05; RRP = recurrent respiratory papillomatosis; HPV = human papillomavirus.

DISCUSSION

This study presents the clinical and sociodemographic characteristics of patients with juvenile-RRP and adult-RRP, as well as the detection and genotyping of HPV from papillomatous lesions. All the analyzed lesions were located in the laryngeal region.

The mean age of the patients at the time of diagnosis was 12.4 years (±12.6). This value is in accordance with that reported in the study of Seedat[16] who observed a similar result in a population with RRP in South Africa, where the

mean patient age was 13.3 years old. Two other African studies reported a lower mean age of 8.7 and 3 years of age, respectively[17,18]. In contrast, a Colombian study reported a mean age of 31.5 years[19].

Regarding the patients' gender, there is no consensus in the literature about the prevalence of RRP according to the gender. Some authors reported a higher occurrence in males than in females, while others reported a similar prevalence rate between men and women[17,20,21]. In this study, females (60%) were predominant, but without statistical significance.

Juvenile-RRP was prevalent in this study, as in a Brazilian study conducted by Figueiredo *et al.*[22] who have also observed that most lesions were located in the larynx. However, two other Brazilian studies conducted in Sao Paulo found a slight higher prevalence of adult-RRP with respect to juvenile-RRP, of 56% and 52.27%, respectively[20,23]. Chirilă and Bolboacă[21], in a study carried out with 31 Romanian patients, have also observed a predominance of adult-RRP (58.06%). However, studies conducted in African regions have reported a higher prevalence of juvenile-RRP. Seedat[24] observed that 84.7% of patients had juvenile-RRP in a population of South Africa. In 2004, Nwaorgu[17], studying a Nigerian population, found the highest incidence of RRP in children of 6 to 10 years old, 95.3%of whom belonged to the lower social classes.

Many studies have shown that the occurrence of RRP is higher in populations with low socioeconomic status and low educational levels[2]. Derkay and Wiatrak[25], Larson and Derkay[26] and Venkatesan *et al.*[27] observed that the prevalence of the disease is associated with a low socioeconomic level. Leung *et al.*[28] assessed the association between the severity of juvenile-RRP and the socioeconomic status of Canadian children and observed no statistically significant correlation; however, they observed that more impoverished families were at a higher risk of developing the disease.

According to Larson and Derkay[26], most juvenile-RRP patients are diagnosed by the age of five. In this study, most juvenile patients were up to 5 years old at the time of diagnosis. A similar result was observed by Hermann *et al.*[29], as five of nine patients with juvenile RRP (55.5%) were up to 5 years old. Buchinsky *et al.*[30] identified that the age at the time of diagnosis highlights the most aggressive or least aggressive clinical course. For these authors, the aggressiveness was high for children aged < 5 years old. However, in this study, when we associated the age with aggressiveness variables, we did not observe any significant difference when compared to children aged > 5 years old. However, the small sample size in the present study may have caused this difference.

Several authors referred to dysphonia as the most common symptom, and dyspnea was the second most reported symptom in the literature among patients with laryngeal papillomatosis[13,20]. This is consistent with our findings, as dysphonia was the most common symptom among our patients, and dyspnea, was the second one. Ximenes *et al.*[20] observed that dyspnea was more prevalent in the juvenile-RRP form than in adult-RRP. In this study, the prevalence was also higher in the youth population; however, this difference did not reach statistical significance.

RRP aggressiveness can be assessed by the number of recurrences, age < 5 years, need of tracheostomy and the presence of HPV-11[16,18,31]. The recurrence rate found in our population was more prevalent in the juvenile-RRP group than in the adult-RRP one (p=0.03) when applying the Student's t test and the Fisher's exact tests to compare the differences between both groups. These data are in agreement with those reported in literature. Ximenes Filho *et al.*[20] observed that the general recurrence rate was 66%: 76.2% in the juvenile form and 56.5% in the adult form. Garcia-Romero *et al.*[10] have also reported that the recurrence rate of individuals with the adult-RRP form was 40%. However, when we performed more robust statistics, the recurrence variable lost its statistical significance.

The incidence of tracheostomy in patients with RRP as reported in the literature, ranges from 1.8% to 64%[13,18]. In the present study, it was observed that all the patients who required tracheostomy belonged to the juvenile-RRP group, corroborating data reported in the literature that found a greater aggressiveness of the disease in patients with juvenile-RRP[32]. The Student's t and the Fisher's exact tests were used to compare the differences between both groups (p = 0.04). However, as in the case of the recurrence variable, when a more robust statistical analysis was applied, tracheostomy has also lost its statistical significance.

HPV-DNA was found in almost all laryngeal papillomatosis lesions, similar to the results observed by Sanchez[19] who reported a 95% prevalence of HPV-DNA in papillomatous lesions. The findings reported in other studies have also corroborated our results, confirming that HPV is the etiological agent of laryngeal papillomatosis[10,20,33].

Other studies have found that low-risk HPV genotypes, such as HPV-6 and HPV-11, are present in approximately 90% of lesions, and only 10% of patients have high-risk genoypes[1,10,26]. In this study, low-risk oncogenic genotypes of HPV were prevalent in both juvenile-RRP and adult-RRP. HPV-6 was the most prevalent genotype in our population, corroborating the findings of other studies[17,18,22,34]. High-risk oncogenic genotypes were observed in 16.7% of our patients.

Matos *et al.*[23] observed the presence of HPV-6 (72%) with respect to HPV-11 (28%) in 25 patients with laryngeal papillomatosis, in a Brazilian population. Eftekhaar *et al.*[33] observed a prevalence of 75% of HPV-6 in relation to HPV-11 (16.7%) in 12 Iranian individuals. In a Swedish study of 55 patients, Hocevar *et al.*[35] found that 76% of patients were infected with HPV-6 and 24% with HPV-11. Garcia-Romero *et al.*[10] showed the presence of HPV-6 in 80% of patients and found that only 8% had HPV-11. We have also observed a prevalence of HPV-6 (63.6%) with respect to HPV-11 (36.4%) among RRP patients.

There are no studies evaluating the incidence of RRP in Brazil, but there are studies conducted in other countries, such as South Africa, Canada, United States, and Norway[1,11,24,26]. The cohort studies reported in the literature had relatively small sample sizes of RRP patients. Ximenes Filho *et al.*[20] conducted a ten-year retrospective cohort study with 44 patients, while Matos *et al.*[23] have also evaluated 25 patients with RRP, and in a third study, Silva *et al.*[36] performed a ten-year retrospective study with 21 patients. These three studies were carried out in Sao Paulo State. Recently, Mercuri *et al.*[37] have estimated the incidence and prevalence of laryngeal papillomatosis in Sao Paulo State, Brazil. The results varied between 1.87-11.46 and 6.86-22.92 per 1,000,000 inhabitants, respectively, for a total population of 11,871,852 inhabitants.

The Brazilian laryngeal papillomatosis studies are limited to Sao Paulo State. The present research is a 11-year retrospective cohort with a similar restricted sample size; however, it was conducted in the Northeast region of Brazil, which presents a lower human development index[14].

The automated sequencing technique was not effective for genotyping HPV in 39.1% of patients, which is a limitation of the study. This may be due to the presence of coinfections in these samples, which could have interfered with the virus detection, as described by Gharizadeh *et al.*[37] and Verteramo *et al.*[38].

CONCLUSION

The present study was the first in Brazil to evaluate RRP in juvenile and adult patients living in the Northeast region of Brazil. In a total of 25 patients, the median age was 12.40 ± 12.6. years, the juvenile form of the disease (68%) was predominant, as was the female gender (60%) and patients coming from cities in the interior of Maranhao State (60%). Recurrence was observed in most cases (56%), and was more frequent in the juvenile RRP group. Tracheostomy was necessary in seven patients (26.9%) and was also more frequent in the juvenile RRP group. Viral genotyping showed that 47.8% of patients had low-risk HPVs, whereas 13.1% had high-risk HPVs. The low-risk HPV-6 was the predominant genotype. We hope that the information obtained in this research will enrich the Brazilian and the world literature on recurrent respiratory papillomatosis.

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AUTHORS' CONTRIBUTIONS

AMASC, DSB, APAC, PMM and MBF collected data and performed their analyses; DSFRA and JOBP collected data and contributed to their analyses; MSA contributed to the analyses and wrote de paper; FCBV conceived and designed the analyses, wrote the paper and was responsible for the funding; NSF wrote the paper and gwas also responsible for the funding.

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