Laryngoscope
Investigative Otolaryngology

ORIGINAL RESEARCH OPEN ACCESS

Evaluation of Lytic and Persistent Human Adenovirus Infections in Tonsil Tissue of Children With Tonsillar Hypertrophy: A Matched Case–Control Study

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Received: 20 November 2024 | **Revised:** 29 January 2025 | **Accepted:** 20 February 2025

Keywords: human adenoviruses (HAdVs) | lytic infection | persistent infection | quantitative real-time PCR | tonsillar hypertrophy | tonsils

ABSTRACT

Background: Tonsillar hypertrophy is a common condition in children, and human adenoviruses (HAdVs) may contribute to its development. However, the mechanisms underlying HAdVs' persistence in tonsils remain unclear. This study investigates the role of HAdVs in tonsillar hypertrophy.

Methods: In a case–control study, oropharyngeal swabs and tonsillar tissues were collected from 50 children with and without tonsillar hypertrophy. HAdV viral load, mRNA expression, and virus shedding were considered to differentiate lytic and persistent infections.

Results: HAdV genomes were detected in 32% of hypertrophic tissues, exclusively HAdV-C, with no presence in controls. Viral loads varied, peaking in children under 5 years old, ranging from 4×10^3 to 1.9×10^6 copies/g, with a median of 2.6×10^4 copies/g. Predominantly, infections were persistent (81%), with a smaller number of lytic or reactive cases.

Conclusion: The study supports and extends previous findings regarding HAdV-C persistence in tonsillar tissues and its potential contribution to hypertrophy, with viral loads tending to decrease with age. These findings contribute to the growing body of evidence on HAdVs' role in tonsillar hypertrophy, reinforcing the importance of persistent infections in the tonsils.

1 | Introduction

Tonsillar hypertrophy is common in children and primarily treated with tonsillectomy, the most frequent pediatric surgery. The exact mechanism of hypertrophy remains unclear [1]. Factors such as genetics, smoking, air pollution, and viral and bacterial infections are under investigation [1, 2]. Human adenoviruses (HAdVs) have been associated with the recurrent episodes of tonsillitis and tonsillar hypertrophy due to the inflammatory response they elicit [3, 4]. The adenovirus genome, 26–46 kilobases long, encompasses 23–46 protein-coding genes, organized into transcription units needed for viral replication and host interaction [5]. HAdV are divided into seven species (A–G) with over 100 identified types [6]. Tonsils are the primary site for HAdV replication during acute infections, and the virus can persist in these tissues even after the acute phase [7]. While the mechanisms of HAdV persistence are not fully understood [8], latent infections can be reactivated due to the various stimulations, leading to renewed inflammation [9]. The persistent infections can lead to continuous immune responses in the tonsils, correlating with conditions such as sleep apnea, signifying a role in chronic adenotonsillar diseases, including tonsillar hypertrophy [10]. Additionally, the tonsillar microbiota may influence adenovirus reactivation and the inflammatory response. Studies

have indicated that certain microbial metabolites could trigger adenoviral reactivation in lymphocytes within the tonsils, exacerbating inflammation and contributing to hypertrophy [11]. While specific antiviral treatments for adenovirus are limited, understanding infection persistence can help manage chronic cases [12]. During lytic infection, the E1A gene is expressed early and acts as a transcriptional activator, inducing other viral mRNAs. Interferon alpha/beta signaling inhibits E1A transcription, facilitating the transition to the persistent phase [13–15]. Studies indicate that the E3 gene of HAdV encodes proteins counteracting the host's antiviral mechanisms. For example, E3 14.7K prevents TNF-induced apoptosis, and E3-19K, a vital glycoprotein, functions in both lytic and persistent phases. It activates IRE1α nuclease, increasing E1A transcription, E3-19K levels, and lytic infection. E3-19K also sustains persistent infection by regulating a feedforward loop with interferon [16]. Additionally, E3-19K inhibits the transfer of MHC-I-associated antigen complexes on infected cells, protecting them from CTL-induced cell death, thus helping the virus evade the host immune system during persistent infection [17]. Research on lymphocyte cell lines shows that HAdV virus-associated RNAs (VARNAs) are processed into VA RNA-derived small RNAs (mivaRNAs) through cellular microRNA mechanisms. These mivaRNAs can influence cellular mRNA translation via the RNA-induced silencing complex (RISC) [18, 19]. One target of viral mivaRNAs is TIA-1 mRNA, which encodes a protein that activates apoptosis. By decreasing TIA-1 levels, HAdV VARNAs may inhibit apoptosis [14]. It is noteworthy that VARNAs are not essential for lytic infection [18, 20]. Despite limited knowledge of viral factors in HAdV persistence, it is widely accepted that HAdV copy numbers greater than a specific threshold in tissues and stool indicate active or lytic infection, while lower numbers suggest persistent infections [10, 21]. Viral transcripts like E1A, a transcriptional activator, and hexon, a major capsid protein, are markers of the lytic phase. Other transcripts, expressed during both phases, are evaluated for their role in HAdV pathogenesis [16]. The mechanisms of HAdV persistence and its link to adenotonsillar disease remain poorly understood. This study investigated the presence and viral load of HAdV in children with hypertrophic tonsils compared to healthy controls in a matched case–control study. It examines both lytic and persistent HAdV infections in tonsillar tissue, aiming to enhance understanding of HAdV pathogenesis in hypertrophic tonsils.

2 | Methods

2.1 | Study Design and Samples Preparation

All samples included in this case–control study were collected between August 1 and October 30, 2019. The study comprised a total of 100 oropharyngeal specimens and 50 tonsillar tissue samples. The control group consisted of 50 oropharyngeal swabs obtained from children aged 15 and younger without tonsillar disease or respiratory symptoms. The case group included 50 oropharyngeal swabs and 50 tonsil tissues collected from patients undergoing tonsillectomies due to tonsillar hypertrophy at Marvasti Hospital in Tehran, Iran. Each case was individually matched to a control by age. Due to ethical considerations, tonsillar tissue samples could not be obtained from the control group.

Tissue samples were then divided into two microtubes for the assessment of DNA and RNA, separately. A detailed description of the sample collection was previously published by our research group [22]. This study was approved by the Ethics Committee of Tehran University of Medical Sciences with the approval code IR.TUMS.SPH.REC.1398.162.

2.2 | Isolation of DNA and RNA

Figure 1 shows an overview of our workflow. DNA was extracted from fresh tonsil tissues using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The High Pure Viral Nucleic Acid (Roche, Germany) was used for the isolation of DNA from throat swabs for the determination of virus shedding. RNeasy Mini Kit (Qiagen) was used for RNA extraction from samples that were submerged in RNA protect Tissue Reagent (RNA later, Qiagen). All nucleic acid extraction procedures were performed according to the manufacturer's instructions. RNase-Free DNase Set (Qiagen) was used for the removal of DNA contamination. After preparing the kit, 10 μL of DNase I and 70 μL RDD buffer were mixed and added to each column of samples during RNA extraction.

To assess the quantity of the extracted viral DNA and RNA, the absorbance at 260 nm of all extractions was measured by a NanoDrop Lite spectrophotometer. The absorbance ratio 260/280 for pure DNA and RNA should be 1.8–1.9 and 2, respectively.

The extracted DNA was amplified and qualified using real-time PCR for β-actin gene (ACTB) and real-time RT-PCR for RNaseP as a housekeeping mRNA was performed to assess the quality of RNA extraction. Primers P1 and P2 were used to amplify ACTB, which was detected with probe P3, and RNaseP mRNA was detected with primers P37 and P38 and probe P39 (Table S1). For ACTB real-time PCR, the thermal cycler program was as follows: a pre-denaturation step of 3 min at 95°C, followed by 40 cycles of 10 s at 95°C for denaturation, and 45 s at 60°C for annealing and extension. Also, for RNaseP, the thermal cycler program was as follows: a reverse transcription step of 10 min at 50°C, initial activation of 3 min at 95°C, followed by 40 cycles of 10 s at 95°C for denaturation, and 30 s at 60°C for annealing and extension.

Nested-PCR for adenovirus typing: Nested-PCR tests were carried out using specific primers to amplify the conserved region of the hexon gene (P4-P7) as described previously [22].

2.3 | Evaluation of Lytic and Persistent HAdV Infection

In our study, we aimed to differentiate between lytic and persistent HAdV infections in tonsil tissue of children with tonsillar hypertrophy by assessing various parameters. These parameters included an in-house quantitative real-time PCR to measure the viral load, detection of critical viral mRNA expression associated with each phase, evaluation of viral shedding in the oropharynx, and identification of VARNAs. A High viral load (> 10^6 copy/g) together with virus shedding in throat of young children and expression of hexon and E1A mRNA were considered

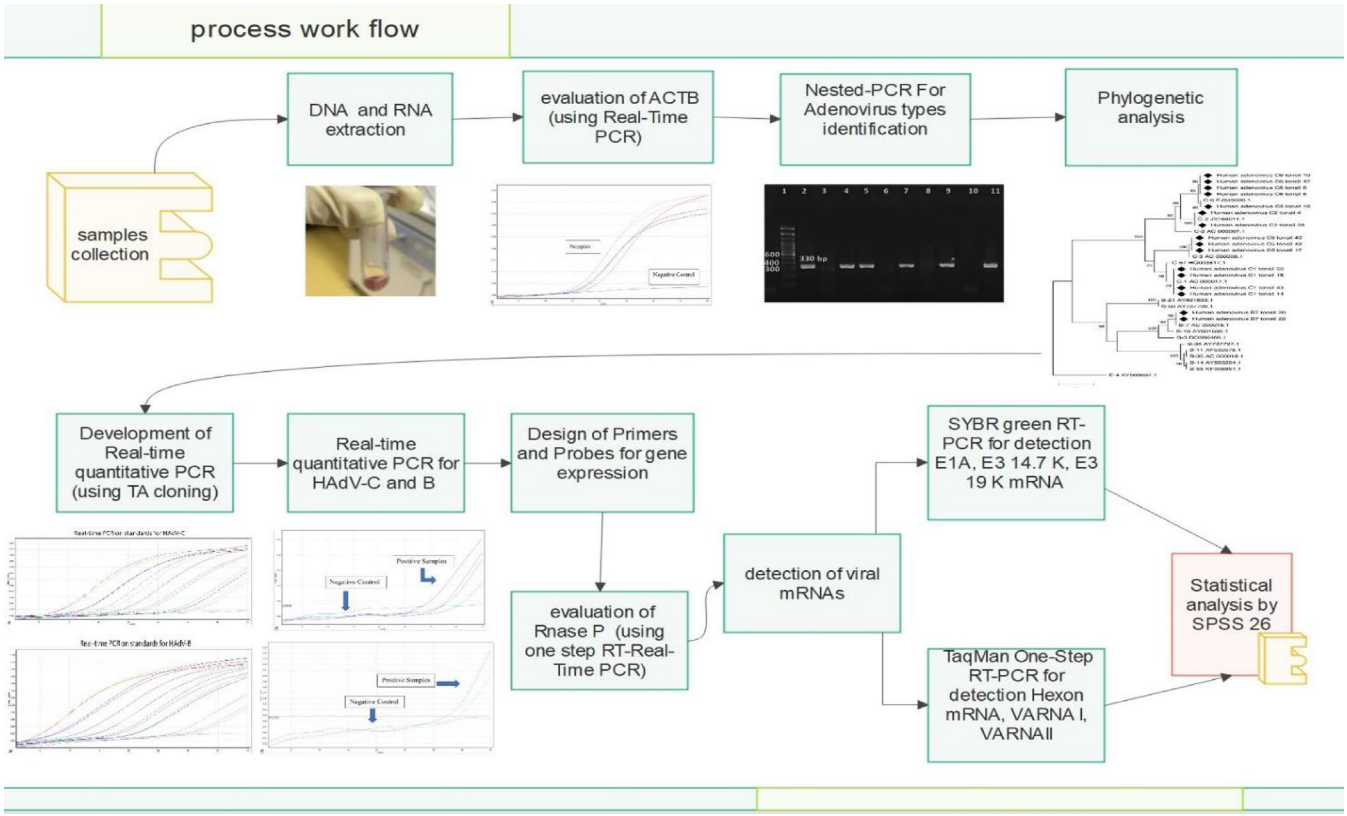

FIGURE 1 | The sequential stages from HAdV identification in tonsil tissues to distinguish lytic and persistent infections.

as indicator of a lytic infection. In contrast, a low viral load (< 10^6 copy/g), without virus shedding in throat, and no expression of hexon and E1A mRNA were considered as representatives of a persistent infection. Additionally, the expression of E3 19K, and E3 14.7K mRNA and VARNA I, II were considered as persistency related factors [10, 16, 23].

2.4 | Primers and TaqMan Probes Design

Initially, the nucleotide sequences of all HAdV-C and B types were obtained from GenBank and aligned using BioEdit software (BioEdit 7.2) to find conserved regions. Then, primers and probes were designed for conserved regions, utilizing Primer Blast (NCBI) and Oligo Analyzer 3.1 (Integrated DNA Technologies, Skokie, IL, USA). Finally, the efficacy and specificity assessments were performed for all designed primers and probes using in silico analysis by Oligo Analyzer and Primer Blast, respectively.

2.5 | Development of Quantitative Real-Time PCR

To assess the viral load, a quantitative real-time PCR assay was conducted using a standard curve derived from serial dilutions of a TA cloning product containing the cloned target DNA sequence. The TA cloning product was procured from the SinaClone TA cloning kit, manufactured in Iran. To prepare the standards, specific primers (P8, P9 for HAdV-C and P13, P14 for HAdV-B) were designed for the hexon region of the respective adenovirus types. The resulting PCR product was inserted into a TA cloning vector at a ratio of 1:3 (vector: insert) according to the manufacturer's instructions.

After ligation, the *E. coli* TOP10 bacteria were cultured in LB Broth medium in the absence of antibiotics. Subsequently, the bacteria underwent incubation in calcium chloride, followed by a heat shock process to introduce the plasmid into the bacterial cells. The transformed bacteria were transferred to LB Broth medium, incubated again, and eventually spread onto LB Agar plates supplemented with ampicillin. Individual colonies were selected and incubated in LB Broth solution. Plasmid extraction was conducted using the AddPrep Plasmid Extraction Kit (addbio), and further purification of the plasmid was performed using the QIAquick PCR Purification Kit (Qiagen). Serial dilutions of the purified plasmid were then prepared, encompassing a range from 10 copies/μL to 10^9 copy/μL. The limit of detection (LOD) was determined using a dilution series of known standards to generate the standard curve. To endorse the reproducibility of the results, the assays were performed in triplicate and coefficient of variation (CV) values was calculated for each assay. Finally, to check the reliability of the test, positive and negative controls were included in each run of Real time PCR performance and the results were normalized by amplification of the β-actin gene for clinical samples.

Quantitative Real-time PCR for HAdV-C: quantitative assessment of species Cadenovirus hexon DNA in tonsil and throat swab was performed usingreal-time PCR for 40cycles. Briefly, PCR amplification was carried out in 20μL reaction mixtures consisting of Capital qPCR Master MIX 1×, 0.5μM of each primer, and 0.3μM of TaqMan probe. Primers P10 and P11 were designed to amplify

a conserved region of the species C adenovirus hexon gene, which was detected with the internal TaqMan probe P12. Subsequently, amplifications were conducted under the following conditions: Hold step of 3 min at 95°C followed by 40 cycles of 10 s denaturation at 95°C, 30 s annealing and extension at 60°C.

Quantitative Real-time PCR for HAdV-B: A quantitative assay of species B in tonsil was assessed by real-time PCR. All protocols were the same as previously described for species C, except for designed primers P15, P16, and TaqMan Probe P17. The same thermal protocol was used as well.

2.6 | Detection of Viral mRNAs

Given the phylogenetic analysis of our previous study in which the majority of adenovirus species detected in our samples were HAdV-C, primers were exclusively designed for mRNA detection of different types of species C.

2.7 | Two- Step RT-PCR for Detection of E1A, E3 14.7K, and E3 19K mRNAs

The AddScript cDNA Synthesis Kit from addbio was used to synthesize complementary DNA (cDNA) following this approach: 0.5 micrograms of RNA were combined with 20 picomoles of Random Hexamer, according to the manufacturer's instructions. Then, SYBR green Real-Time PCR was performed using a 20 μL mixture containing Add SYBR Master from Addbio. An Applied Biosystems Real-Time PCR Instrument (USA) was used for this process, along with specific primers for E1A, E3 14.7, and E3 19K. Various primers for different types of HAdVs were identified in Table S1. The annealing temperature was determined based on the melting temperature of the primers. Melting-curve analysis was performed for each Real-Time PCR run to ensure the accuracy of the amplicons.

2.8 | One-Step Real-Time RT-PCR for Detection of Hexon mRNA, VARNAI, and VARNAII

Hexon gene mRNA was detected in tissue and throat samples using real-time reverse transcription polymerase chain reaction (RT-PCR), targeting the same region as HAdV-C quantitative PCR (qPCR). Specific primers and probes described in Table S1 were used for the detection of VARNAI and VARNAII. In brief, a total volume of 20 μL of RT reaction mixture containing three microliters of purified water was used, and 0.5 μg of purified RNA was directly utilized for the detection of hexon mRNA, VARNAI, and VARNAII. RNase P was included as an internal control for all the samples to ensure the integrity of extracted RNA and the reliability of the results.

2.9 | Statistical Analysis

Categorical analysis was performed by Chi-square or Fisher's exact test. Normal distribution for numerical variables was assessed by Kolmogorov–Smirnov and Shapiro–Wilk tests. Normally distributed numerical variables were presented as mean ± SD and compared using the one-way ANOVA test. In contrast, variables with skewed distributions were presented as median [interquartile range] and compared using the Kruskal–Wallis and Mann–Whitney *U* tests. All assays were carried out using the Statistical Package for the Social Sciences (SPSS) Windows version 22 (SPSS Inc., Chicago, IL), and a p value of ≤ 0.05 was adopted for significance for all statistical tests.

3 | Result

3.1 | Validation of Quantitative Real-Time PCR

The qPCR results with sigmoidal amplification curves and a Ct value of ≤ 40 were considered positive for HAdV genome detection. For viral load assessment, using the standard curves (Figure 2), the LOD was determined to be approximately 1 copy of the HAdV hexon gene. The intra-assay CV ranged from 0.25% to 2.92% across all standards, indicating high precision and reproducibility of the qPCR assay.

As mentioned for clinical samples, the results were normalized by amplification of the β-actin gene included in duplicate in all batches of tests separately. With this approach, viral loads were determined as the number of copies of HAdV DNA per gram (copy/g) for tissue specimens and per microliter (copy/μL) for throat swab samples.

3.2 | HAdV Detection and Genotyping in Two Groups of Children: Case Group With Tonsillar Hypertrophy and Control Group Without Tonsillar Hypertrophy

This study evaluated a total of 150 samples, consisting of 50 tonsil tissues and 50 throat swabs from children with tonsillar hypertrophy (case group) and 50 throat swabs from children without hypertrophy (healthy control group). The mean age of participants in the case and control groups was 8.07 ± 2.85 years. All samples were divided into three age groups, including 9 participants (18%) under 5 years, 31 (62%) between 5 and 10 years, and 10 (20%) between 11 and 15 years. Among the 50 samples from the case group, there were 14 (28%) females and 36 (72%) males, while the control group included 19 (38%) females and 31 (62%) males.

The nested-PCR assay detected the HAdV genome in 16 (32%) tissue specimens and 5 (10%) swab samples from the case group. However, no HAdV genome was detected in the control group using highly sensitive techniques such as nested PCR and real-time PCR, resulting in a borderline significant difference between the case and control groups ($p = 0.056$) (Table 1).

3.3 | HAdV-C and B Viral Load in Tonsil Tissue Specimens and Throat Swab Samples

The in-house real-time PCR determined the viral load in tonsil tissues from HAdV-C positive children with tonsillar hypertrophy, ranging between 4×10^3 and 1.9×10^6 copies/g with median of 2.6×10^4 copies/g. Out of the five positive throat swabs from the case group, four had < 10 copies/μL, and one had ≥ 10 copies/

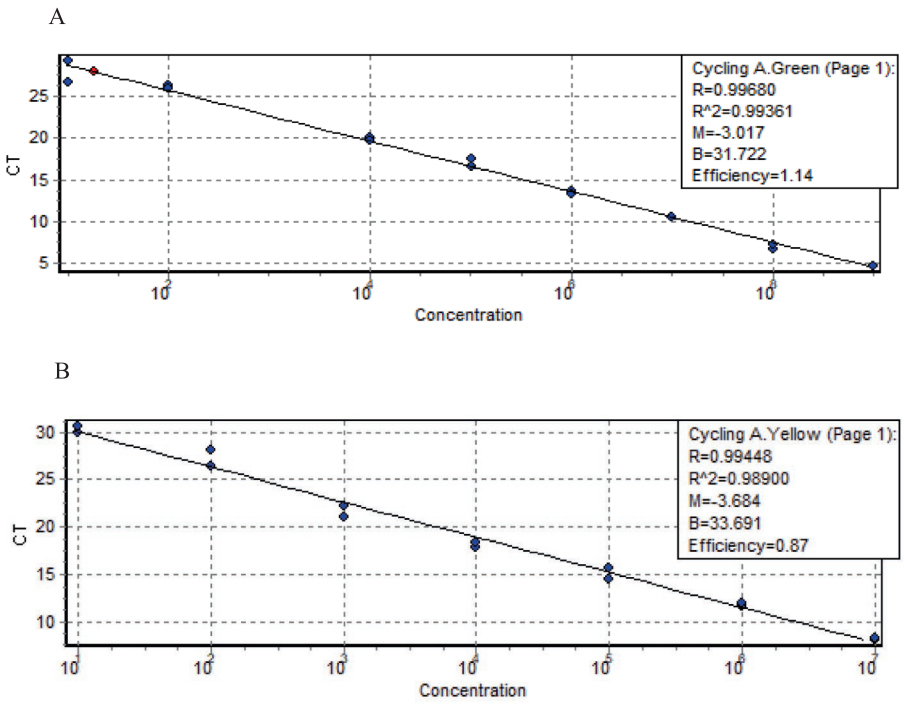

FIGURE 2 | Standard curves for quantitative real-time PCR detection of HAdV-B (A) and HAdV-C (B). Serial 10-fold dilutions of plasmid DNA ranging from 10^1 to 10^7 copies/μL were amplified.

TABLE 1 | Comparison of HAdV DNA detection rates and viral loads in throat swabs of children with tonsillar hypertrophy (case group) and children without tonsillar hypertrophy (control group).

Groups	HAdV-positive cases *N* (*n*%)		HAdV-negative cases *N* (*n*%)	Fisher's exact test[a]
	Load			
	Copy/μL < 10	Copy/μL ≥ 10		
Case	4 (8%)	1 (2%)	45 (90%)	*p* value* = 0.056
Control	0	0	50 (100%)	
Total	4 (8%)	1 (2%)	95 (95%)	

[a]Fisher's exact test was used to compare HAdV detection rates between the case and control groups.
*p Value < 0.05 was considered statistically significant.

μL. The median HAdV-C load in the tonsil tissues was quite similar in males and females ($p > 0.05$) (Table 2). The viral load significantly differed by age group, with the median HAdV-C loads being higher in children < 5 years of age than in older children ($p < 0.05$) (Table 2 and Figure 3). The median viral load of the two HAdV-B positive samples was1 $\times 10^4$ copy/g.

3.4 | HAdV Persistence Infection in Tonsil Tissues of Children With Tonsillar Hypertrophy

The analysis of 16 HAdV-C positive cases in this study showed E3 14.7K and E3 19K had the highest expression rate (4/16 cases, 25%). This was followed by hexon, E1A, VARNA II (3/16 cases, 18.7%), and VARNA I (2/16 cases, 12.5%). Most of the samples (13/16 cases, 81.25%) were considered to be in a persistent infection state due to the low viral load (range 4×10^3 to 8.5×10^5 copy/g) and expression of viral mRNA related to persistency. Furthermore, reactive infection (2/16 cases, 12.5%) was reported for patients who had an expression of lytic mRNAs including E1A, hexon, E3 14.7K, and E3 19K, with the mean viral load of 2.1×10^4 copy/g and with viral shedding in throat. Finally, only one case (1/16 case, 6.25%) with a high viral load (1.9×10^6 copy/g) and expression of mRNA related to active infection, was considered as a lytic form of infection (see Table 3).

4 | Discussion

The study aimed to evaluate adenovirus infection in hypertrophic tonsils of children to determine its association with hypertrophy. While previous studies have linked persistent adenovirus infection to various clinical issues, the causal relationship has not been definitively established [14, 24]. Reactivation of adenovirus has been identified as a cause of problems in both immunocompetent individuals and those with compromised immune systems or transplant recipients [25, 26]. These findings suggested a potential role of adenovirus infection in the development of hypertrophic tonsils and highlight the need for further investigation [27]. The adenovirus genome, surrounded by pseudo-histone structures, can be reactivated by epigenetic alterations induced by histone deacetylase inhibitors (HDACs) [28]. Stress can also lead to adenovirus reactivation by initiating cellular signaling and epigenetic changes [28]. Previous studies reported that the presence of HAdV in tonsil tissue of children with tonsillar hypertrophy ranging from 52.8% to 84% suggested a significant association between HAdV infection and tonsillar hypertrophy in younger populations [9, 10]. A case report

TABLE 2 | Comparison of HAdV load in children with tonsillar hypertrophy (case group) based on age and sex.

Characterization	Scattering parameters				
	Median	Interquartile range	*p* Value[a]	Effect size (r)[b]	95% CI
Age					
≤5	4.4×10^5	1.2×10^6	0.02*	0.83	$(2.52\times10^5, 6.28\times10^5)$
6–10	2.6×10^4	8.5×10^4		0.80	$(1.27\times10^4, 3.93\times10^4)$
11–15	4×10^3	4.3×10^3		—	$(3.32\times10^3, 4.68\times10^3)$
Sex			0.709		
Female	4×10^4	3.6×10^5		—	—
Male	2.6×10^4	4.7×10^5		—	—

[a]Due to the non-normal distribution of the samples, Non-parametric tests were used.
[b]Effect sizes were calculated using the Mann–Whitney *U* test between consecutive age groups.
*p Value of less than 0.05 were considered to be statistically.

suggested that hypertrophy might have been caused by the reactivation of persistent adenovirus in adenoid tissue as well [3]. Furthermore, adenovirus viremia was investigated in children post-bone marrow transplant, where high levels of HAdV in the blood or plasma were associated with increased mortality, emphasizing the importance of viral load monitoring [29]. Acute adenovirus infection can have severe outcomes and be life threatening in immunocompromised individuals, such as cancer patients undergoing therapy. In one study, adenovirus identified in cancer patients with acute pneumonia led to three out of four patients succumbing to disseminated infections [30]. Another case showed that a child with tetrasomy and bronchiolitis obliterans had a persistent adenovirus infection for 10 weeks, leading to chronic lung inflammation and obstructive bronchitis due to impaired interferon function [31]. Lastly, adenoviruses have been detected in brain tumor tissues of children with leukemia, lymphoma, and solid tumors, suggesting that persistent adenovirus infection in the central nervous system may play a role in the development of brain tumors [32].

In the presented study, adenovirus was detected in 10% of throat swab samples from children with tonsillar hypertrophy, significantly higher than in healthy controls, indicating a potential link between adenovirus infection and the condition that has required further investigation. Adenovirus loads were consistent with prior findings by Medona et al. [10] and higher in T lymphocytes than in B lymphocytes, as reported by Asadian et al. [9]. Herein, no gender differences were found. However, viral loads were higher in children under 5, supporting the notion that viral infections are primary in younger children and their incidence decreases by age, as noted in previous studies [9, 10]. In our study, adenovirus genomes and some of its mRNA expressions were evaluated in 16 tonsil tissue samples from children with hypertrophy. A majority of these samples (81.25%) exhibited signs of persistent infection—aligning closely with research conducted by Garnett et al. [23] (87.8%), Modena et al. [10] (91.8%), and Wang et al. [28] (87.6%), all reporting similarly high rates of persistent infections within their respective studies' samples. These previous studies assessed persistent HAdV infection by measuring viral mRNA expression levels—including hexon gene expression and E3 genes such as E3 14.7K and E3 19K—as well as overall viral load within tonsil tissues. Our findings suggested that adenovirus group C might contribute to tonsillar hypertrophy mechanisms such as inhibiting apoptosis via the expression of VARNA along with E3 19K and E3 14.7K during the period of persistent infection [33, 34]. A significant detection rate of adenovirus genomes was observed in hypertrophic tissues, with a significant decrease in viral load with increasing age, suggesting a probable link between persistent infection and hypertrophy. The findings underscore the necessity for additional investigation into the markers of lytic and persistent adenovirus infections to deepen the understanding of their role in tonsil hypertrophy.

It should be noted that recent studies have indicated that various respiratory viruses may be implicated in the development of tonsillar hypertrophy. For example, one study proposed that early exposure to respiratory syncytial virus (RSV) could lead to adenotonsillar hypertrophy via the upregulation of nerve growth factor (NGF) and neurokinin-1 (NK1) receptor-dependent pathways [35]. Another investigation identified Human Bocavirus 1 (HBoV1) in 32% of children with tonsillar hypertrophy, suggesting that the tonsils may act as reservoirs for HBoV1. This could contribute to chronic inflammation and hypertrophy as a result of persistent viral infection [36, 37]. Given the frequent detection of certain viruses within tonsillar tissues, monitoring viral load was recommended to elucidate the dynamics of viral infections associated with tonsillar hypertrophy [38]. Elevated viral loads might be correlated with clinical manifestations such as obstructive sleep apnea or recurrent infections [37]. For instance, higher salivary loads of Epstein–Barr virus (EBV) have been found to correlate with more extensive infection within the tonsillar crypts, potentially necessitating more aggressive therapeutic interventions [39].

Even considering the primary association of tonsillar hypertrophy development in children with infections and immune responses, confounding factors such as socioeconomic status (SES) and environmental elements could indirectly affect its occurrence and severity. Some studies showed that the lower SES was correlated with a higher prevalence of tonsillar hypertrophy and related conditions [40, 41]. Moreover, exposure to environmental toxicants was more prevalent in lower SES areas.

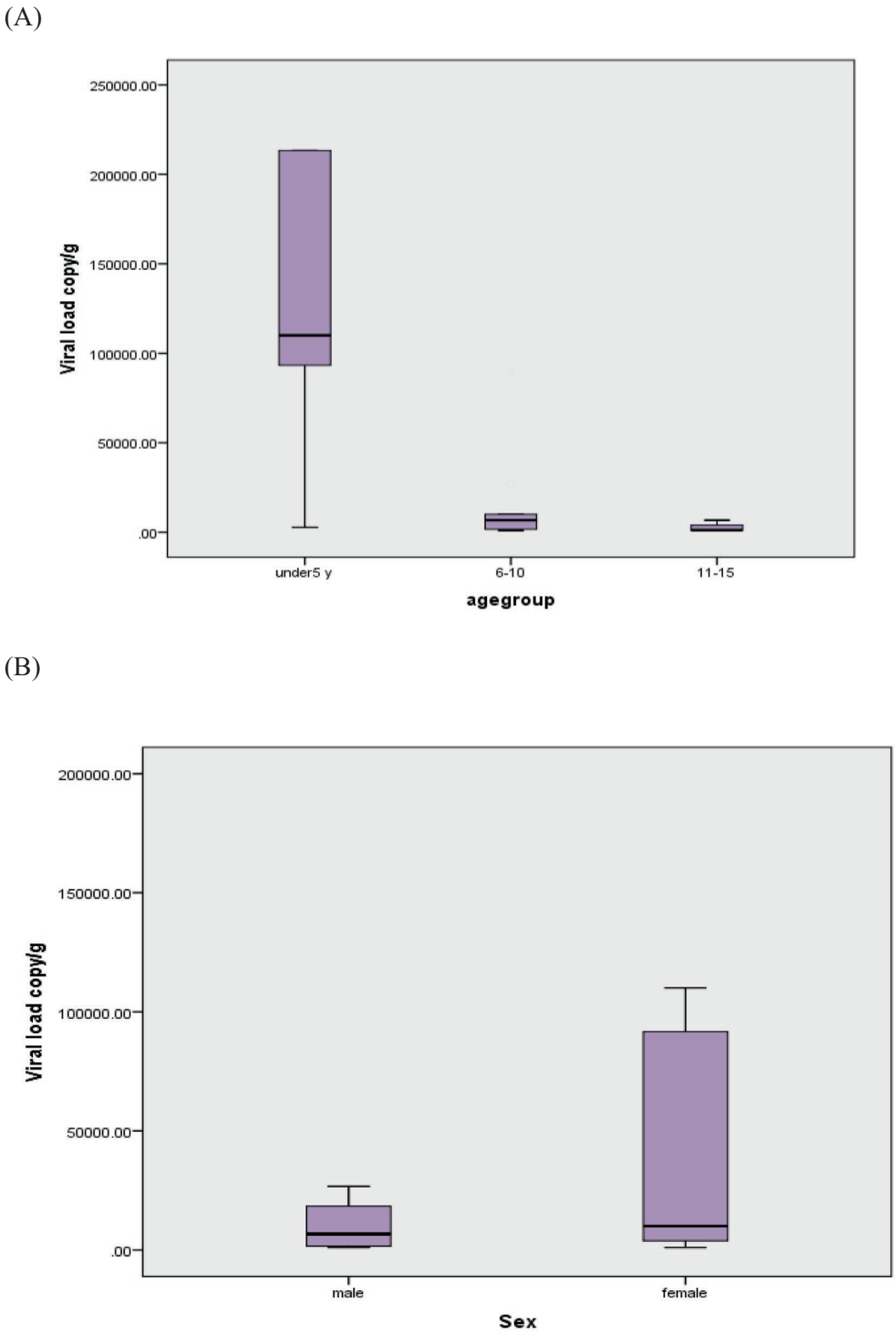

FIGURE 3 | Bar chart comparing viral load between age and gender groups. Error bars represent 95% confidence intervals.

Passive smoking indoors, another environmental risk factor associated with tonsillar disease, was more common in lower SES households as well [41]. Additionally, air pollution, specifically augmented exposure to NO_2 and particulate matter $\leq 2.5\mu m$ (PM2.5), has been associated with enlarged tonsils and sleep apnea [42]. Unfortunately, in the present study, we had no access to socioeconomic status and environmental elements affecting the patients.

This study had some limitations that should be acknowledged. In an ideal experiment, comparing HAdV infections in both hypertrophic and normal tonsil tissues would provide

TABLE 3 | Evaluation of lytic and persistent HAdV infection in 16 HAdV-positive children with tonsillar hypertrophy.

Sample	Hexon mRNA	E1A mRNA	E3 19K mRNA	E3 14.7K mRNA	VARNAII	VARNA1	High viral load, $>10^6$ copy/g	Viral load in tonsil (copy/g)	Virus shedding	Type of infection		
										Reactive	Persistent	Lytic
1	+	+	+	+	−	−	−	6.6×10^3	+	+	−	−
2	−	−	−	−	−	−	−	2.6×10^4	+	−	+	−
3	−	−	−	−	−	−	−	1×10^4	−	−	+	−
4	−	−	−	−	−	−	−	4×10^3	−	−	+	−
5	−	−	−	−	−	−	−	4×10^3	−	−	+	−
6	−	−	+	−	+	−	−	4×10^4	−	−	+	−
7	−	−	−	−	−	−	−	4×10^3	−	−	+	−
8	+	+	+	+	−	−	−	3.6×10^5	+	+	−	−
9	−	−	−	−	−	−	−	2.6×10^4	−	−	+	−
10	−	−	−	−	−	−	−	8.5×10^5	−	−	+	−
11	−	−	−	−	−	−	−	3.7×10^5	−	−	+	−
12	−	−	−	+	+	+	−	1×10^5	−	−	+	−
13	−	−	−	−	−	−	−	4.4×10^5	−	−	+	−
14	−	−	−	−	−	−	−	2×10^4	+	−	+	−
15	−	−	−	−	−	−	−	4×10^3	−	−	+	−
16	+	+	+	+	+	+	+	1.9×10^6	+	−	−	+

more comprehensive insights. However, the unavailability of normal tonsil samples necessitated the use of throat swabs for comparison between case and control groups. This substitution might reduce the reliability of the conclusions regarding the role of HAdV in inducing tonsillar hypertrophy. Additionally, the relatively small sample size might limit the generalizability and robustness of the findings related to this hypothesis.

In conclusion, our study was aligned with existing literature by highlighting the prevalence of persistent adenovirus infection in hypertrophic tonsil tissue. Through the evaluation of various parameters, we sought to gain a deeper understanding of HAdVs pathogenesis in hypertrophic tonsils, suggesting a probable association between persistent adenovirus infection and tonsil hypertrophy. Addressing the identified limitations, future research with access to normal tonsil tissue and larger sample sizes could yield more definitive insights into the pathogenesis and management of tonsil-related conditions.

Acknowledgments

This work was supported by Tehran University of Medical Sciences, Tehran, Iran (Grant No. 98-02-27-42957).

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki and national and institutional standards, and it was approved by the Ethics Committee of Tehran University of Medical Sciences with the approval code IR.TUMS.SPH.REC.1398.162.

Consent

The patient's parents signed written informed consent and accepted the publication of data for research.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.