Journal of Kyzylorda Scholarly Review

P-ISSN:3006-0265 | E-ISSN:3007-4681. Published by the Open University of Kyzylorda
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PREVALENCE OF EPSTEIN–BARR VIRUS INFECTION AMONG PATIENTS WITH CHRONIC TONSILLITIS

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Abstract. One of the most significant axes that links physiological latency with chronic inflammatory tissue remodeling is related to Epstein–Barr virus (EBV) colonization of the palatine tonsils. This cross-sectional study estimated the proportion of EBV-infected patients in severe chronic tonsillitis (n = 110) compared to non-inflammatory hypertrophic tonsils (n = 50) and normal asymptomatic controls (n = 80), and studied the viral load and cellular tropism of EBV. The palatine tonsils were dissected, digested to single-cell suspensions and sorted by fluorescence-activated cell sorting into the $CD3^+$ T-cell and $CD19^+$ B-cell subsets. The prevalence of EBV DNA found in the tissues of the tonsillitis patients (59.1%) was significantly higher than that in the controls (23.8%) by quantitative real-time PCR targeting the conserved BALF5 locus. The median EBV viral load in the intratonsillar tissue was 4.8×10^4 copies/μg DNA with the presence of EBV type 1 being more prevalent than EBV type 2. Amplification of viral DNA was detectable in the sorted $CD19^+$ B lymphocytes in all 35 sorted fractions; however, unambiguous amplification was found in 25.7% of the positive samples in the sorted $CD3^+$ T cells. The abundance of the intracellular genome did not show a linear relationship with patient chronological age, suggesting that there is only localized dysregulation of cells, not cumulative systemic exposure. The data described here indicate that inflamed tonsillar parenchyma is an active anatomical site for viral reactivation.

Keywords: Epstein–Barr virus, Chronic tonsillitis, Real-time PCR, Viral load, Tonsillar mononuclear cells.

1. Introduction

Epstein–Barr virus or human gammaherpesvirus 4 (EBV) is a persistent lymphotropic virus that infects 90% of the world population (Wong et al., 2022). Virions are transmitted via saliva, are able to cross the mucosal barrier, and contact primary targets of the host in the oral cavity (Womack & Jimenez, 2015). In the pharynx, the lymphoid aggregate called Waldeyer's ring plays a role as a portal for local viral colonization (Thorley-Lawson et al., 2013). The tonsillar crypts are deep invaginations that allow easy penetration of viral particles into naïve and memory B lymphocytes, where the virus alters the gene expression pattern of the host cell, thus affecting its survival (Babcock et al., 1998). Once the virus enters, and the typical germinal center (GC) model is followed, the latent membrane proteins and nuclear antigens of EBV direct B-cell differentiation towards a resting memory phenotype (Thorley-Lawson & Gross, 2004). While they circulate in the blood, these memory cells travel between the blood and lymphoid microenvironments every so often (Chaganti et al., 2009). It has been suggested that shifts in viral latency programs in lymphocytes affect whether they are "silent" or produce infectious progeny (Miyashita et al., 1997). Under these conditions, tonsillar stroma becomes an immunologic battlefield, and cycles of immune surveillance and immune evasion develop in tonsils (Tugizov et al., 2013).

Chronic tonsillitis is a persistent inflammation which does not resolve after repeated infections and causes changes in the architecture of lymphoid follicles (Bathala & Eccles, 2013). The etiology is still debatable: although a bacterial cause has been much more prevalent in clinical thinking in the past, it is increasingly becoming clear that viruses act as underlying provokers (Silvoniemi et al., 2020). Chronic antigenic challenge causes lymphocytic hyperplasia, obstruction of crypts and tissue fibrosis (Önal et al., 2015). Uncontrolled herpesvirus proliferation in the tonsil parenchyma compromises cytokine orchestration, which can further worsen tonsillar damage and sustain recurring tonsillar symptoms (Dias et al., 2009).

Although extensively studied, the molecular behaviour of EBV in inflamed vs hypertrophic and/or healthy tonsillar niches has not been clearly defined. When studying the tonsillar tissue, quantitative distinction between intra-epithelial viral genomes and cell-associated lymphoid burdens is often not made and chronic non-malignant inflammation is not distinguished from malignant head and neck disease. There is a lack of systematic verification of the precise lineage distribution of viral persistence, whether it is confined to the resident tonsillar B lymphocytes, or extends to local tonsillar T lymphocytes in chronic inflammation. The purpose of this work is to quantify the EBV DNA positivity rate in fresh palatine tonsil tissues from patients with medically refractory chronic tonsillitis, identify the relative distribution of EBV genotypes (EBV-1 versus EBV-2) and to establish the main EBV-infected cellular reservoir in the sorted subpopulations of tonsillar mononuclear cells.

2. Materials and Methods

2.1. Patient Cohort and Clinical Selection Criteria

From January 2021 to October 2023, consecutive pediatric and adult patients with medically resistant chronic tonsillitis who underwent bilateral tonsillectomy were included in the study at the tertiary level otorhinolaryngology (ORL) surgical unit (Gürler et al., 2025). Chronic tonsillitis was diagnosed clinically by having recurrent acute tonsillitis at least five times within a calendar year and persistent sore throat for over 3 months with clinical evidence of tonsillar follicular enlargement, distortion of tonsillar crypts and regional cervical lymphadenopathy (Strzelczyk et al., 2022). Patients with acute respiratory infections at surgery, systemic autoimmune disease, previous or concurrent cancer or congenital immune deficiencies were excluded (Wong Chung et al., 2018). A control group of patients with non-inflammatory tonsillar hypertrophy (non-inflammatory based on the absence of infectious history, mechanical upper airway obstruction and Brodsky grade ≥3) was also recruited at the same time (Kalantari et al., 2024). Superficial mucosal scrapings and saliva were collected from healthy volunteers, who had indications for minor oral surgical procedures, as healthy controls (Kalantari et al., 2024; Gürler et al., 2025).

2.2. Tissue Procurement and Cellular Processing

Surgery of the tonsils was performed under general anesthesia by either cold-knife dissection or bipolar electrocautery to avoid parenchymal damage. Immediately after excision, removal of superficial oral contaminants was accomplished by rinsing tonsil tissue three times with sterile ice-cold 1× phosphate-buffered saline (PBS) containing 100 U/mL penicillin and 100 µg/mL streptomycin. The tissue blocks were divided with one block fixed in 10% neutral-buffered formalin, embedded in paraffin and then sent for histopathological verification, another block was snap-frozen in liquid nitrogen and stored at −80°C for total genomic extraction while the last piece, which was fresh, was processed within 2 h for cellular isolation (Johnston et al., 2009). Tonsillar tissues were mechanically chopped into 2–3 mm^3 size pieces, and then digested with 0.5 mg/mL collagenase type IV and 0.05 mg/mL DNase I in RPMI-1640 medium at 37°C for 30 minutes to obtain single-cell suspensions. The resulting digest was passed through a nylon filter (70 µm) and placed on top of Ficoll-Paque PLUS density gradient medium and centrifuged for 25 minutes at 400 × g with no brake and tonsillar mononuclear cells (TMCs) were isolated (Johnston et al., 2009; Koech et al., 2025).

2.3. Fluorescence-Activated Cell Sorting (FACS)

Only preparations with >90% cell viability in the trypan blue exclusion assay were considered viable and used to evaluate the TMCs (Koech et al., 2025). Cells were then stained at a concentration of 1.0×10^7 cells/mL in staining buffer (PBS containing 1 mM ethylenediaminetetraacetic acid and 2% fetal bovine serum) with a human Fc-receptor blocking reagent for 15 minutes at 4°C (Johnston et al., 2009). The suspension was then incubated in the dark with the following fluorochrome-conjugated monoclonal antibodies: phycoerythrin (PE)-conjugated anti-human CD19 (clone HIB19) to isolate B lymphocytes and Pacific Blue-conjugated anti-human CD3 (clone UCHT1) to delineate T lymphocytes (Wohlford et al., 2018; Koech et al., 2025). Zombie Aqua fixable dye was used to perform viability discrimination. Cells were sorted using high-speed flow cytometry on a BD FACSAria III with an 85 µm nozzle using a sheath pressure of 45 psi. All cellular fractions collected for $CD19^+$ and $CD3^+$ were found to be greater than 98.5% purity after post-sort re-analysis (Koech et al., 2025).

2.4. DNA Extraction Protocols

The homogenized whole tonsil tissue (25 mg wet weight), total TMCs (5.0×10^6 cells), sorted $CD19^+$ and $CD3^+$ cellular fractions (at least 2.0×10^5 cells), and mucosal control scrapings were all used for the extraction of genomic DNA. Lysis of tissues and cell pellets was performed in the presence of proteinase K, at 56°C for 1 hour. DNA was extracted following the manufacturer's instructions for the QIAamp DNA Mini Kit, eluting the purified DNA in 50 µL of sterile nuclease-free buffer AE (Johnston et al., 2009; Strzelczyk et al., 2022). The GeneMATRIX Swab-Extract DNA Purification Kit was used as follows for mucosal swabs (Strzelczyk et al., 2022). Yield and optical purity of DNA were spectrophotometrically measured using the NanoDrop 2000, which resulted in A_{260}/A_{280} ratios between 1.80 and 1.95 that were acceptable (Johnston et al., 2009; Strzelczyk et al., 2022).

2.5. Quantitative Real-Time PCR and Viral Genotyping

The EBV genome was amplified and quantified by using an Applied Biosystems 7500 Real-Time PCR System. Primer pairs and a dual-labeled fluorogenic TaqMan probe were designed to target the single-copy BALF5 gene encoding the viral DNA polymerase, while parallel reactions were targeted to the human β-globin or β-actin locus to be used as a normalization factor for the amount of absolute cellular input (Strzelczyk et al., 2022; Koech et al., 2025). Reaction mixtures (total volume 20 µL) included 10 µL of 2× TaqMan Universal Master Mix II, 300 nM of forward primer, 300 nM of reverse primer, 200 nM of FAM-labeled probe and 5 µL of template DNA. Ten-fold serial dilutions of a linearized plasmid containing the BALF5 target sequence (from 1.0×10^1 copies/reaction to 1.0×10^7 copies/reaction) were used to create the standard calibration curves. The thermal cycling program used consisted of initial denaturation at 95°C for 10 minutes, followed by 45 cycles of denaturation at 95°C for 15 seconds, followed by annealing/extension at 60°C for 60 seconds (Strzelczyk et al., 2022; Koech et al., 2025). The lower limit of detection was determined to be 10 viral copies/reaction (50 copies/µg of host DNA).

All specimens positive for EBV were genotyped using a nested real-time PCR that discriminates between the sequence differences in the EBNA3C region (Coleman et al., 2017; Koech et al., 2025). The divergent 246-bp region characteristic of type 1 and the 162-bp region distinctive of type 2 were amplified by specific primers (Koech et al., 2025). Namalwa (ATCC CRL-1432) and Jijoye (ATCC CCL-87), representing EBV-1 and EBV-2 prototype cell lines, respectively, were used as reference control DNA. Negative water blanks were used throughout the runs to help exclude the possibility of cross-contamination (Johnston et al., 2009; Coleman et al., 2017).

2.6. Statistical Evaluation

SPSS Statistics (Version 27.0, IBM Corp.) and GraphPad Prism (Version 9.5) were used for data analysis. The Shapiro–Wilk test was used to evaluate the distributional normality of continuous parameters. Medians with interquartile ranges (IQR) are shown for variables that are not normally distributed such as viral copy numbers. The two-sided Pearson Chi-square (χ^2) test

or Fisher's exact test was used for the analysis of categorical frequencies and prevalence rates. Comparisons in viral load rank of independent groups were carried out using the Mann–Whitney U test or the Kruskal–Wallis test with multiple comparisons by Dunn's post hoc correction. Agreement between sorted viral loads of paired cellular lineages was analyzed by the Wilcoxon matched-pairs signed-rank test and agreement between tissue and swab PCR was determined in terms of Cohen's kappa coefficient, with the tissue PCR considered the reference method; and swab sensitivity was calculated against the reference tissue PCR. Spearman's rank correlation coefficient (r_s) was used to quantify the correlation between two non-parametric continuous variables. A p-value < 0.05 (two-tailed) was considered to be statistically significant.

3. Results

3.1. Demographic Profiles and Clinical Stratification

A cohort of 110 patients with chronic tonsillitis (median age 24.5 years, IQR 14.0–34.0), 50 patients with non-inflammatory tonsillar hypertrophy who underwent tonsillectomy, and 80 healthy individuals who provided mucosal controls (median age 28.0 years, IQR 20.0–38.5) was evaluated. Sex distribution showed a good balance of males and females with no significant difference between the sexes among groups. Patients' clinical and demographic data are shown in Table 1.

Table 1. Demographic and Clinical Characteristics of the Study Population.

Parameter	Chronic Tonsillitis (n = 110)	Hypertrophy (n = 50)	Control (n = 80)	*p*-value
Age (years), Median [IQR]	24.5 [14.0–34.0]	7.5 [4.0–11.0]	28.0 [20.0–38.5]	<0.001
Age Subsets, n (%)				
< 10 years	16 (14.5%)	35 (70.0%)	4 (5.0%)	<0.001
10–18 years	21 (19.1%)	11 (22.0%)	12 (15.0%)	0.812
> 18 years	73 (66.4%)	4 (8.0%)	64 (80.0%)	<0.001
Sex, n (%)				
Male	53 (48.2%)	28 (56.0%)	39 (48.8%)	0.624
Female	57 (51.8%)	22 (44.0%)	41 (51.2%)	0.624
Symptom Duration (months), Median [IQR]	18.0 [8.0–36.0]	12.0 [6.0–18.0]	N/A	0.008
Recurrent Tonsillitis/Year, Median [IQR]	6.0 [5.0–8.0]	0 [0.0–1.0]	N/A	<0.001
Brodsky Score (Tonsil Size), n (%)				
Grade 1–2	48 (43.6%)	0 (0.0%)	N/A	<0.001
Grade 3–4	62 (56.4%)	50 (100.0%)	N/A	<0.001
Smoking History, n (%)	28 (25.5%)	0 (0.0%)	21 (26.3%)	<0.001

Note. Data are expressed as median [interquartile range, IQR] for continuous variables and frequency, n (%) for categorical variables. *p*-values were calculated using the Kruskal–Wallis test for continuous variables or Pearson's Chi-square test for categorical proportions. N/A: not applicable.

3.2. Prevalence of Tonsillar EBV DNA and Quantitative Viral Burdens

EBV DNA was detected in 65 of 110 patients with chronic tonsillitis (59.1%), representing a prevalence significantly higher than the 23.8% (19/80) identified within control oral mucosal specimens (p < 0.001). Patients with tonsillar hypertrophy exhibited an intermediate positivity

Journal of Kyzylorda Scholarly Review
P-ISSN:3006-0265 | E-ISSN:3007-4681
https://bulletin.ouk.kz/index.php/bulletin/index.php

rate of 52.0% (26/50), which did not differ statistically from the chronic tonsillitis group (p = 0.395), confirming that tonsillar lymphoid tissue per se serves as a hyper-concentrated niche for viral persistence compared to peripheral mucosal scrapings.

Within the EBV-positive subgroups, tonsillar viral load quantification revealed substantial disparity. Patients with chronic tonsillitis yielded a median viral burden of 4.8×10^4 copies/µg DNA (IQR 8.2×10^3 to 2.9×10^5), whereas the hypertrophy group exhibited a median of 1.1×10^4 copies/µg DNA (IQR 2.4×10^3 to 8.6×10^4; p = 0.009). The control oral mucosal cohort displayed nominal copy numbers, with a median of 4.2×10^2 copies/µg DNA (IQR 1.1×10^2 to 1.8×10^3). Comparative data are compiled in Table 2.

Table 2. EBV DNA Prevalence and Intratonsillar Viral Loads Across Cohorts.

Cohort	Total (n)	EBV DNA Positive (%)	Median Viral Load (copies/µg DNA) [IQR]	p-value
Chronic Tonsillitis	110	65 (59.1%)	4.8×10^4 [8.2×10^3 – 2.9×10^5]	Reference
Tonsillar Hypertrophy	50	26 (52.0%)	1.1×10^4 [2.4×10^3 – 8.6×10^4]	0.009
Asymptomatic Controls	80	19 (23.8%)	4.2×10^2 [1.1×10^2 – 1.8×10^3]	<0.001

p-values reflect comparisons of viral loads against Chronic Tonsillitis using Mann–Whitney U tests.

The distribution of quantitative viral copy numbers across clinical classifications showed distinct clustering, with tissue compartments exhibiting logarithmic expansion over surface mucosal samples.

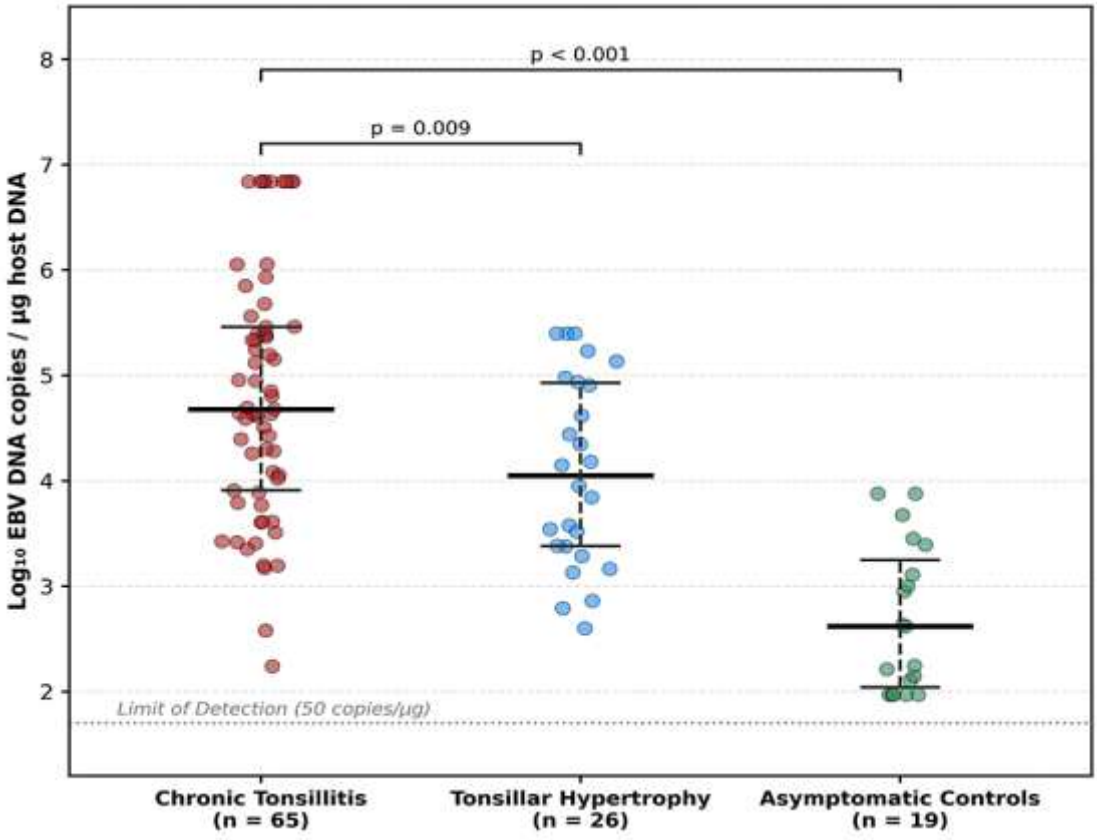

Figure 1. Intratonsillar and Mucosal Viral Load Profiles Across Patient Cohorts. Log_{10} EBV DNA copies/µg host DNA are plotted for all positive specimens: Chronic Tonsillitis (n=65), Tonsillar Hypertrophy (n=26), and Asymptomatic Controls (n=19). Group median viral loads are reported in Table 2. Horizontal bars represent

the group median and interquartile ranges. Significant pairwise differences confirmed: Chronic Tonsillitis vs. Hypertrophy ($p = 0.009$); Chronic Tonsillitis vs. Controls ($p < 0.001$).

3.3. Viral Genotype Distribution

Among the 65 EBV-positive chronic tonsillitis cases, EBNA3C genotyping classified 44 specimens (67.7%) as EBV-1, 16 specimens (24.6%) as EBV-2, and 5 specimens (7.7%) as co-infections displaying concurrent amplification of both alleles. In the tonsillar hypertrophy cohort (n = 26), EBV-1 accounted for 19 cases (73.1%), EBV-2 for 6 cases (23.1%), and dual infection was detected in a single case (3.8%). Genotypic patterns exerted a marked impact on viral copy abundance. In chronic tonsillitis, EBV-2-infected tissues manifested a median viral load of 1.4×10^5 copies/µg DNA, whereas EBV-1 tissues exhibited 2.8×10^4 copies/µg DNA ($p = 0.016$). Table 3 provides the full breakdown.

Table 3. EBV Genotype Distribution and Corresponding Viral Loads in Tonsillar Diseases.

Clinical Group	Genotype	Specimens, n (%)	Median Load (copies/µg DNA) [IQR]	p-value
Chronic Tonsillitis (n=65)	EBV-1	44 (67.7%)	2.8×10^4 [5.6×10^3 – 1.2×10^5]	0.016*
	EBV-2	16 (24.6%)	1.4×10^5 [3.1×10^4 – 6.8×10^5]	
	Co-inf.	5 (7.7%)	8.6×10^4 [1.8×10^4 – 4.2×10^5]	
Tonsillar Hypertrophy (n=26)	EBV-1	19 (73.1%)	9.1×10^3 [2.1×10^3 – 4.8×10^4]	0.082
	EBV-2	6 (23.1%)	3.4×10^4 [8.5×10^3 – 1.9×10^5]	
	Co-inf.	1 (3.8%)	2.2×10^4 [N/A]	

*Statistically significant elevation of viral load in EBV-2 compared to EBV-1 within the chronic tonsillitis cohort. Co-inf.: co-infection.

3.4. Cellular Compartmentalization: B-cell versus T-cell Tropism

Fluorescence-activated cell sorting partitioned viable tonsillar mononuclear cells from 35 EBV-positive chronic tonsillitis patients into pure $CD19^+$ B lymphocytes and $CD3^+$ T lymphocytes. All 35 sorted $CD19^+$ fractions yielded detectable BALF5 DNA (100%), carrying a median load of 8.9×10^4 copies/µg DNA (IQR 1.9×10^4 to 4.1×10^5). Unexpectedly, $CD3^+$ T-cell fractions from 9 of these 35 patients (25.7%) also harbored viral DNA, demonstrating a median load of 1.2×10^3 copies/µg DNA (IQR 3.1×10^2 to 4.7×10^3). Genomic detection within T cells was confined to specimens demonstrating aggregate whole-tissue viral burdens exceeding 1.0×10^5 copies/µg DNA. The data regarding lineage partitioning are detailed in Table 4.

Table 4. EBV DNA Distribution Between Sorted Tonsillar B and T Lymphocytes (n=35).

Lymphocyte Subset	Positivity Rate, n (%)	Median Load (copies/µg DNA) [IQR]	p-value
$CD19^+$ B Lymphocytes	35 / 35 (100.0%)	8.9×10^4 [1.9×10^4 – 4.1×10^5]	<0.001
$CD3^+$ T Lymphocytes	9 / 35 (25.7%)	1.2×10^3 [3.1×10^2 – 4.7×10^3]	

Pairwise analysis reflects Wilcoxon matched-pairs signed-rank test comparing viral burdens between paired sorted cellular lineages.

The sorting purity validation and absolute copy number divergence between the isolated

Journal of Kyzylorda Scholarly Review
P-ISSN:3006-0265 | E-ISSN:3007-4681
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immune subsets emphasize that viral retention spans multiple immune cell lineages within the tonsillar bed.

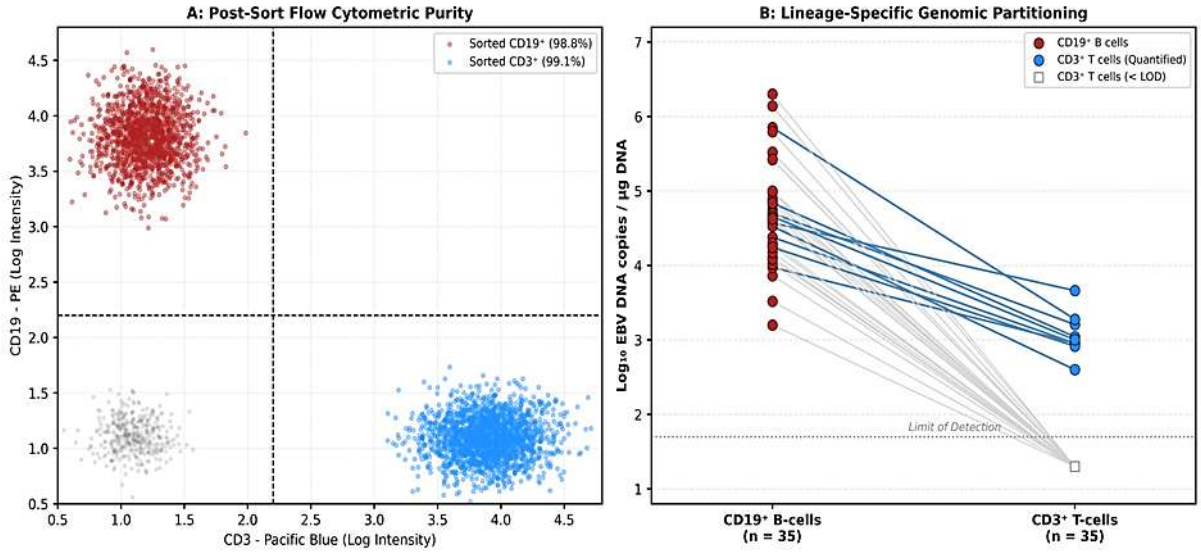

Figure 2. Cellular Partitioning of Viral Genomes in Sorted Tonsillar Lymphocytes. (A) Flow cytometric gate verification confirming >98.5% post-sort purity for CD19⁺ PE and CD3⁺ Pacific Blue single-positive populations. (B) Paired dot-plot comparing log_{10} EBV DNA copies/µg DNA between purified CD19⁺ B-cell fractions (median 4.95 log_{10}) and CD3⁺ T-cell fractions (median 3.08 log_{10} for positive subsets; 26 samples negative or below analytical threshold). Dashed connector lines indicate paired isolates from the same donor.

3.5. Influence of Age, Symptom Chronicity, and Tonsillar Morphology

Analysis of the chronic tonsillitis cohort revealed that patient age did not maintain a statistically significant linear correlation with intratonsillar viral burden ($r_s = -0.091$, $p = 0.472$). Similarly, tonsil size scored by the Brodsky system did not dictate viral copy numbers: patients with grade 1–2 tonsils exhibited a median viral load of 4.2×10^4 copies/µg DNA (IQR 7.8×10^3 to 2.1×10^5), whereas those with grade 3–4 tonsils demonstrated 5.4×10^4 copies/µg DNA (IQR 9.1×10^3 to 3.4×10^5; $p = 0.536$). In contrast, the frequency of annual acute exacerbations correlated positively with tonsillar viral load ($r_s = 0.384$, $p = 0.002$). Results are shown in Table 5.

Table 5. Correlation Matrix of Clinicopathological Variables Against Tonsillar Viral Load.

Clinical Variable	Stratification / Range	Median Load [IQR]	Correlation / p
Age Groups	< 10 years (n=19)	5.1×10^4 [9.4×10^3–2.7×10^5]	$r_s = -0.091$ $p = 0.472$
	10–18 years (n=13)	3.8×10^4 [6.1×10^3–1.9×10^5]	
	> 18 years (n=33)	4.9×10^4 [8.5×10^3–3.2×10^5]	
Brodsky Score	Grade 1–2 (n=27)	4.2×10^4 [7.8×10^3–2.1×10^5]	Mann–Whitney $p = 0.536$
	Grade 3–4 (n=38)	5.4×10^4 [9.1×10^3–3.4×10^5]	
Annual Exacerbations	≤ 5 episodes (n=24)	1.6×10^4 [4.2×10^3–6.8×10^4]	$r_s = 0.384$ $p = 0.002$

Clinical Variable	Stratification / Range	Median Load [IQR]	Correlation / p
	> 5 episodes (n=41)	8.2×10^4 [2.4×10^4–4.9×10^5]	
Smoking Status	Smoker (n=17)	5.8×10^4 [1.1×10^4–3.9×10^5]	Mann–Whitney p = 0.418
	Non-smoker (n=48)	4.4×10^4 [7.5×10^3–2.4×10^5]	

Correlations computed via Spearman rank analysis (r_s). Categorical comparisons via Mann–Whitney U test.

3.6. Diagnostic Concordance: Parenchymal Tissue versus Mucosal Swabs

In a paired sub-study of 45 patients with chronic tonsillitis, pre-operative oral swabs were tested alongside surgically excised parenchyma. Concordant positivity occurred in 22 cases (48.9%), whereas 13 cases (28.9%) were positive exclusively in core tonsillar tissue, failing detection via superficial swabbing. Zero cases exhibited swab-positive, tissue-negative discordance. The diagnostic sensitivity of superficial mucosal sampling relative to tissue biopsy was 62.9% (95% CI: 45.0%–78.0%). Data are displayed in Table 6.

Table 6. Diagnostic Concordance Between Core Tissue Biopsy and Superficial Mucosal Swabbing (n=45).

Tissue Core PCR Status	Mucosal Swab PCR Positive	Mucosal Swab PCR Negative	Total
EBV DNA Positive	22 (48.9%)	13 (28.9%)	35 (77.8%)
EBV DNA Negative	0 (0.0%)	10 (22.2%)	10 (22.2%)
Total	22 (48.9%)	23 (51.1%)	45 (100.0%)

Cohen's Kappa coefficient $\kappa = 0.429$ (Moderate agreement). Diagnostic Sensitivity of swab = 62.9%.

4. Discussion

The palatine tonsils are an important anatomic nexus where the aerodigestive tract meets secondary lymphoid surveillance. Based on the data obtained during this investigation, it is concluded that in people who have chronic tonsillitis, EBV DNA can be found in 59.1% of the patients in the parenchyma. This is very similar to the recent presence of EBV found in Mexican patients with chronic tonsillitis (60.0%) (González-Lucano et al., 2019). Notably, the yields obtained in studies that used superficial swabs or throat gargles were generally low (18%–25%), with a higher diagnostic sensitivity for superficial mucosal swabbing observed in our study (62.9%) (Ikuta et al., 2000). Compared with mucosal scrapings from healthy individuals, intratonsillar tissue contains a significant amount of latent viral genomes, suggesting that this organ is more of a viral nursery rather than a bystander (Hug et al., 2010).

Whole-tissue tonsillar viral burdens were higher in tonsillitis (4.8×10^4 copies/µg host DNA) than the levels that were found in non-inflammatory hypertrophic tissue. Previous quantitative appraisals of tonsillar hypertrophy in children showed median loads ranging from 1.3×10^4 to 6.6×10^4 copies/g tissue, indicating that the profile of the child's infection was mostly characterised by restricted viral latency but without any obvious cytopathic lysis (Nadal et al., 2002). In the chronic tonsillitis group, continuous antigenic stimulation and immunological challenge might induce frequent cellular turnover, which could lead to lytic cycle reactivation and to an increase of the total pool of viral genomes in the memory B cells and in the surrounding epithelial crypt cells (Endo et al., 2001). The correlation of high viral load in patients with a high annual number of acute exacerbations (>5/year) supports the immunological imbalance model which postulates that periodic activation (breaking of latency) occurs as a result of local inflammatory bursts (Dogan et al., 2010).

One important finding of this study is that there was a significant difference in the viral load measured between viral genotypes. Although EBV-1 was found to be the predominant group of EBV in prevalence (67.7%), as seen in other non-African populations, tissues infected with EBV-2 had viral loads ~0.7 $\log_{10}$ higher than those with EBV-1 (Coleman et al., 2018). The difference is due to naturally occurring molecular differences between the two strains. EBV-2 has a higher tendency to enter the spontaneous lytic cycle and engage more NFATc1 pathways than EBV-1 due to sequence variations in the immediate-early promoter (IEP) Zp and the nuclear antigens EBNA2 and EBNA3C (Romero-Masters et al., 2020). The presence of EBV-2 in most cases is associated with a higher production of virions (both cell-free and cell-associated) from continuous lytic cycles that lead to increased local viral loads in the lymphoid architecture (Bristol et al., 2018).

Cellular fractionation showed that the most predominant host lineage that contained the most copies of the virus was $CD19^+$ B lymphocytes, but more than a quarter of sorted EBV^+ cases were also $CD3^+$ T lymphocytes. Traditionally, EBV was thought to be a B-lymphotropic virus, but more recent phenotypic studies have shown that low-density CD21 expression in human T lymphocytes occurs at specific maturation stages, allowing viral entry (Barros et al., 2019; Coleman et al., 2018). Activated resident tonsillar T cells might be vulnerable to EBV entry and/or uptake in a chronic inflammatory microenvironment characterized by an abnormal secretion of cytokines, such as increased levels of interleukin-2 and tumor necrosis factor-alpha (TNF-α) (Romero-Masters et al., 2020). Limited carriage of the virus, with copy numbers not being high enough to indicate complete oncogenic transformation, is indicated by the low copy numbers in T-cell fractions. However, this viral presence within tonsillar T cells suggests an alternative viral reservoir in T cells and more complex intra-tissue viral dynamics in chronic inflammatory conditions.

The absence of an age association with tonsillar EBV viral load supports the idea that after EBV colonization of the palatine tonsil, the load of EBV persists in a steady state determined by local immune control and not by chronological time. Early childhood seroconversion in developing settings results in high basal viral setpoints, while in adults, memory B cells maintain latency (Nadal et al., 2002; Coleman et al., 2018). In chronically inflamed tonsillar tissue, however, aberrations in the structure of the follicular dendritic cell network and disruption of age-related immune maturation by dysregulated cytotoxic $CD8^+$ T-cell surveillance override age-dependent effects (Dogan et al., 2010). As a result, the intra-parenchymal viral titers of adults with chronic tonsillitis can be as high as in young children.

The results could offer an explanation for the ineffectiveness of standard approaches to the treatment of chronic tonsillitis, like repeated courses of oral antibiotic therapy, in this group of patients. The infectious role of bacterial pathogens like Streptococcus pyogenes or Staphylococcus aureus is important, causing acute purulent flares; however, chronic baseline inflammation, recurrent crypt architecture collapse and mucosal fragility may be further perpetuated by chronic persistence of EBV in the tonsillar crypts (Endo et al., 2001; González-Lucano et al., 2019). The complete physical elimination of this viral reservoir with tonsillectomy eliminates the local viral "epicenter", and might therefore have a beneficial effect on the symptoms by eliminating the bacterial substrate and the major reservoir of chronic viral replication.

5. Conclusion

Epstein–Barr virus persistence in palatine tonsils is an important factor in the pathogenesis of chronic tonsillitis as far as clinical and biological effects are concerned. We found viral DNA in almost 60% of patients who had tonsillitis refractory to medical treatment and were undergoing tonsillectomy, as observed in our cohort. The high prevalence is much higher than what has been reported in healthy populations and shows that the palatine tonsil serves not only as a convenient site for the passage of virions from the oral cavity, but also as a primary site for long-term persistence of virions. The microenvironment of chronic tonsillitis is an

environment with a significant amount of viral genomes, up to 4.8 × 10^4 copies/μg of host DNA. This high load is tightly linked with the rate of acute exacerbations, and indicates that continued local tissue irritation and local immune control fluctuations provide conditions for local maintenance of the virus and low-grade reactivation.

In addition, molecular testing showed that EBV type 1 is the predominant genotype of the virus in our population, but in tissues with EBV type 2, the amount of the virus is increased. The elevation was also proportional to differences in immediate-early promoter control and lytic capacity, suggesting that these differences in viral genotype determine the local burden of virus in host lymphoid niches. Although the viral genome was most frequently detected in sorted $CD19^+$ B cells, the detection of viral genomes in sorted $CD3^+$ tonsillar T cells in 25.7% of evaluated specimens suggests an expanded cellular tropism within severely inflamed lymphoid microenvironments, indicating greater intra-tissue exchange than classical paradigms of EBV latency.

These findings have clear clinical implications. Superficial diagnostic swabbing is not very sensitive to diagnose intra-tissue infections, with more than one-third (37.1%) of the infections being missed by it. Therefore, surface swabs are not a good way to accurately detect the actual viral colonization of deep lymphoid parenchyma. Maintaining high EBV loads within the tonsils could contribute to the chronic inflammatory infiltration and chronic disease cycle, accounting for why standard antibiotic regimens against bacteria only address acute disease. Complete surgical removal (tonsillectomy) may therefore be effective, in part, because of the elimination of this focal, immunologically protected reservoir of viral latency and persistent immune stimulation.

References:

Babcock, G. J., Decker, L. L., Volk, M., & Thorley-Lawson, D. A. (1998). EBV persistence in memory B cells in vivo. *Immunity*, *9*(3), 395-404.

Barros, M. H. M., Vera-Lozada, G., Segges, P., Hassan, R., & Niedobitek, G. (2019). Revisiting the tissue microenvironment of infectious mononucleosis: identification of EBV infection in T cells and deep characterization of immune profiles. *Frontiers in immunology*, *10*, 146.

Bathala, S., & Eccles, R. (2013). A review on the mechanism of sore throat in tonsillitis. *The Journal of Laryngology & Otology*, *127*(3), 227-232.

Bristol, J. A., Djavadian, R., Albright, E. R., Coleman, C. B., Ohashi, M., Hayes, M., ... & Kenney, S. C. (2018). A cancer-associated Epstein-Barr virus BZLF1 promoter variant enhances lytic infection. *PLoS pathogens*, *14*(7), e1007179.

Chaganti, S., Heath, E. M., Bergler, W., Kuo, M., Buettner, M., Niedobitek, G., ... & Bell, A. I. (2009). Epstein-Barr virus colonization of tonsillar and peripheral blood B-cell subsets in primary infection and persistence. *Blood, The Journal of the American Society of Hematology*, *113*(25), 6372-6381.

Coleman, C. B., Daud, I. I., Ogolla, S. O., Ritchie, J. A., Smith, N. A., Sumba, P. O., ... & Rochford, R. (2017). Epstein-Barr virus type 2 infects T cells in healthy Kenyan children. *The Journal of infectious diseases*, *216*(6), 670-677.

Coleman, C. B., Lang, J., Sweet, L. A., Smith, N. A., Freed, B. M., Pan, Z., ... & Rochford, R. (2018). Epstein-Barr virus type 2 infects T cells and induces B cell lymphomagenesis in humanized mice. *Journal of virology*, *92*(21), 10-1128.

Dias, E. P., Rocha, M. L. D., Carvalho, M. O. D. O., & Amorim, L. M. D. F. D. (2009). Detection of Epstein-Barr virus in recurrent tonsillitis. *Revista Brasileira de Otorrinolaringologia*, *75*, 30-34.

Dogan, B., Rota, S., Gurbuzler, L., Bozdayi, G., Ceyhan, M. N., & Inal, E. (2010). The correlation between EBV viral load in the palatine tonsils of patients with recurrent tonsillitis and concurrent serum titers of VCA–IgG. *European archives of oto-rhino-laryngology*, *267*(1), 143-148.

Endo, L. H., Ferreira, D., Montenegro, M. C. S., Pinto, G. A., Altemani, A., Bortoleto Jr, A. E.,

& Vassallo, J. (2001). Detection of Epstein-Barr virus in tonsillar tissue of children and the relationship with recurrent tonsillitis. *International journal of pediatric otorhinolaryngology*, *58*(1), 9-15.

González-Lucano, L. R., Vasquez-Armenta, G. V., Pereira-Suárez, A. L., Ramírez-de Arellano, A., Ramirez-de Los Santos, S., & Lopez-Pulido, E. I. (2019). Prevalence of Epstein-Barr virus DNA in tonsillar tissue from patients with chronic tonsillitis in Mexican population. *The Journal of Infection in Developing Countries*, *13*(08), 764-767.

Gürler, M., Gōkcan, M. K., Yüksel, S., & Karahan, Z. C. (2025). Association between human herpesviruses and head and neck squamous cell carcinoma: a molecular perspective. *Annals of Saudi Medicine*, *45*(2), 104-111.

Hug, M., Dorner, M., Zucol Fröhlich, F., Gysin, C., Neuhaus, D., Nadal, D., & Berger, C. (2010). Pediatric Epstein-Barr virus carriers with or without tonsillar enlargement may substantially contribute to spreading of the virus. *The Journal of infectious diseases*, *202*(8), 1192-1199.

Ikuta, K., Satoh, Y., Hoshikawa, Y., & Sairenji, T. (2000). Detection of Epstein-Barr virus in salivas and throat washings in healthy adults and children. *Microbes and infection*, *2*(2), 115-120.

Johnston, A., Sigurdardottir, S. L., & Ryon, J. J. (2009). Isolation of mononuclear cells from tonsillar tissue. *Current protocols in immunology*, *86*(1), 7-8.

Kalantari, S., Zadheidar, S., Heydarifard, Z., Nejati, A., Sadeghi, K., Malekshahi, S. S., ... & Shafiei-Jandaghi, N. Z. (2024). Epstein-Barr virus in tonsillar tissue of Iranian children with tonsillar hypertrophy: Quantitative measurement by real-time PCR. *World Journal of Otorhinolaryngology-Head and Neck Surgery*, *10*(04), 270-274.

Koech, E., Ogolla, S., Amolo, A. S., Waomba, K., Onditi, I., Sabourin, K. R., ... & Samayoa-Reyes, G. (2025). Epstein-Barr virus type 2 infection is associated with higher viral loads in pediatric tonsils from western Kenya. *Microbiology Spectrum*, *13*(8), e00740-25.

Miyashita, E. M., Yang, B., Babcock, G. J., & Thorley-Lawson, D. A. (1997). Identification of the site of Epstein-Barr virus persistence in vivo as a resting B cell. *Journal of virology*, *71*(7), 4882-4891.

Nadal, D., Blasius, M., Niggli, F. K., Meier, G., & Berger, C. (2002). Epstein-Barr virus (EBV) DNA levels in palatine tonsils and autologous serum from EBV carriers. *Journal of medical virology*, *67*(1), 54-58.

Önal, M., Yılmaz, T., Bilgiç, E., Müftüoğlu, S. F., Kuşçu, O., & Günaydın, R. Ö. (2015). Apoptosis in chronic tonsillitis and tonsillar hypertrophy. *International journal of pediatric otorhinolaryngology*, *79*(2), 191-195.

Romero-Masters, J. C., Huebner, S. M., Ohashi, M., Bristol, J. A., Benner, B. E., Barlow, E. A., ... & Kenney, S. C. (2020). B cells infected with Type 2 Epstein-Barr virus (EBV) have increased NFATc1/NFATc2 activity and enhanced lytic gene expression in comparison to Type 1 EBV infection. *PLoS pathogens*, *16*(2), e1008365.

Silvoniemi, A., Mikola, E., Ivaska, L., Jeskanen, M., Löyttyniemi, E., Puhakka, T., ... & Jartti, T. (2020). Intratonsillar detection of 27 distinct viruses: a cross-sectional study. *Journal of Medical Virology*, *92*(12), 3830-3838.

Strzelczyk, J. K., Świętek, A., Biernacki, K., Gołąbek, K., Gaździcka, J., Miśkiewicz-Orczyk, K., ... & Misiołek, M. (2022). PCR Detection of Epstein-Barr Virus (EBV) DNA in Patients with Head and Neck Squamous Cell Carcinoma, in Patients with Chronic Tonsillitis, and in Healthy Individuals. *BioMed Research International*, *2022*(1), 8506242.

Thorley-Lawson, D. A., & Gross, A. (2004). Persistence of the Epstein–Barr virus and the origins of associated lymphomas. *New England Journal of Medicine*, *350*(13), 1328-1337.

Thorley-Lawson, D. A., Hawkins, J. B., Tracy, S. I., & Shapiro, M. (2013). The pathogenesis of Epstein–Barr virus persistent infection. *Current opinion in virology*, *3*(3), 227-232.

Tugizov, S. M., Herrera, R., & Palefsky, J. M. (2013). Epstein-Barr virus transcytosis through polarized oral epithelial cells. *Journal of virology*, *87*(14), 8179-8194.



Wohlford, E. M., Baresel, P. C., Wilmore, J. R., Mortelliti, A. J., Coleman, C. B., & Rochford, R. (2018). Changes in tonsil B cell phenotypes and EBV receptor expression in children under 5-years-old. *Cytometry Part B: Clinical Cytometry*, *94*(2), 291-301.

Womack, J., & Jimenez, M. (2015). Common questions about infectious mononucleosis. *American family physician*, *91*(6), 372-376.

Wong, Y., Meehan, M. T., Burrows, S. R., Doolan, D. L., & Miles, J. J. (2022). Estimating the global burden of Epstein–Barr virus-related cancers. *Journal of Cancer Research and Clinical Oncology*, *148*(1), 31-46.

Wong Chung, J. E., van Benthem, P. P. G., & Blom, H. M. (2018). Tonsillotomy versus tonsillectomy in adults suffering from tonsil-related afflictions: a systematic review. *Acta Oto-Laryngologica*, *138*(5), 492-501.