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ORIGINAL ARTICLE

Oral shedding of human herpesviruses and cytokine profiles in children and adolescents undergoing chemotherapy

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Abstract:

Objective: To characterize oral shedding of human herpesviruses (HHVs) and their association with salivary inflammatory cytokines in children and adolescents undergoing chemotherapy. **Methods:** This cross-sectional study included 65 participants: 39 in the study group (SG) and 26 in the control group (CG). Unstimulated saliva was analyzed by PCR for viral detection and quantification, and cytokines levels ((IL-4, IL-6, IL-8, IL-10, IFN-γ, and TNF-α) were measured using the Luminex® platform. Descriptive, comparative, and correlation analyses were performed ($p<0.05$). **Results:** In the SG, the most frequently detected viruses were HHV-7, HHV-6, EBV, and CMV. The HHV-7 viral load was significantly greater in the SG than in the CG (6.5 vs. 5.8 cp/mL; $p = 0.0288$). Only IL-4 differed significantly, with higher levels in the SG (8.4 vs. 7.2 pg/mL; $p=0.0374$). Weak correlations were found between CMV and IL-6 ($r=0.34$; $p=0.0095$) and between HHV-6 and TNF-α ($r=-0.33$; $p=0.0125$). **Conclusion:** Chemotherapy was associated with increased HHV-7 viral load and IL-4 levels, suggesting possible alterations in the oral immune environment, even in the absence of clinical manifestations. Overall, viral load had minimal influence on salivary inflammatory cytokines.

Keywords: Cytokines; Saliva; Cancer survivors; Oral shedding; Human herpesviruses.

INTRODUCTION

Malignant neoplasms in childhood and adolescence represent a significant global public health challenge, with chemotherapy constituting one of the main therapeutic approaches. Despite its effectiveness, chemotherapy can lead to substantial immunosuppression, increasing susceptibility to opportunistic infections and alterations in the oral microenvironment. In this context, human herpesviruses (HHVs) have emerged as pathogens of particular interest, as they can remain latent and be reactivated under conditions of immunosuppression, such as those induced by chemotherapy[1–6].

Viral reactivation in immunocompromised patients may occur asymptomatically, yet still contribute to persistent inflammatory stimulation and the development of oral complications, including mucositis and secondary infections. Several factors commonly observed in oncology patients, such as systemic stress, nutritional deficiencies, mucosal trauma, and pharmacologically induced immunosuppression, may act as triggers for viral reactivation[7]. A prolonged pattern of viral shedding or a high viral load may stimulate the release of salivary inflammatory cytokines as part of the host's attempt to control viral replication.

Statement of Clinical Significance
Increased salivary HHV-7 load and IL-4 levels in pediatric oncology patients may indicate early oral immune dysregulation, even without clinical signs. Saliva emerges as a noninvasive tool to monitor the oral microenvironment and identify patients at risk for treatment-related complications, supporting more timely preventive care strategies.

Among the eight known HHVs, HHV-7 is frequently detected in saliva, even in healthy individuals. However, its clinical significance in pediatric patients undergoing chemotherapy remains poorly understood[8,9]. In parallel, the local immune response in the oral cavity is mediated by cytokines, including IL-4, IL-6, IL-8, IL-10,

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IFN-γ, and TNF-α, which play key roles in regulating inflammation and host–pathogen interactions. Alterations in salivary cytokine profiles have been associated with oral dysbiosis, opportunistic infections, and increased susceptibility to oral lesions.

Saliva has emerged as a valuable, noninvasive biological fluid for monitoring inflammatory and infectious processes, particularly in vulnerable populations such as pediatric oncology patients[10–12]. Salivary biomarkers may reflect both local and systemic immune responses and offer a practical approach for longitudinal monitoring of the oral microenvironment.

Most studies investigating herpesvirus shedding and oral inflammatory markers have focused on adult populations or patients undergoing hematopoietic stem cell transplantation[1,4,5]. Evidence regarding pediatric patients receiving conventional chemotherapy remains limited, particularly studies integrating viral detection with inflammatory profiling in saliva.

Therefore, this study aimed to evaluate the frequency and patterns of oral shedding of human herpesviruses (HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7, and HHV-8) and to investigate their association with salivary inflammatory cytokine levels (IL-4, IL-6, IL-8, IL-10, IFN-γ, and TNF-α) in children and adolescents undergoing chemotherapy compared with healthy controls.

METHODS

This cross-sectional observational study was conducted in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines and was approved by the Research Ethics Committees of the School of Dentistry, University of São Paulo (approval no. 5.278.459), and the Institute for the Treatment of Childhood Cancer – ITACI/HCFMUSP (approval no. 5.447.771). Written informed consent was obtained from all legal guardians, and assent was additionally obtained from participants aged six years and older.

Study Design and Sample

A total of 65 individuals aged 3 to17 years were recruited and allocated into two groups: 39 patients who underwent chemotherapy for malignant neoplasms (study group – SG) at ITACI and 26 healthy participants (control group – CG) who were followed at the pediatric dentistry clinic of FOUSP between May 25, 2022, and June 9, 2023.

Inclusion criteria for both groups were age between 3 and 17 years and the ability to provide saliva samples. Participants in the control group had no history of systemic disease or ongoing medication use. Patients receiving concomitant radiotherapy or presenting clinical conditions that prevented sample collection were excluded from the SG.

Two previously calibrated examiners (Kappa>0.81) performed the sample collection. Sociodemographic information, oncological diagnosis, chemotherapy protocols, and complete blood count results (obtained within 30 days of the clinical assessment) were extracted from the hospital's electronic medical records system.

Salivary Sample Collection for Laboratory Analyses

Unstimulated saliva was collected via additive-free collection tubes (Vacuette, 6 mL, Greiner Bio-one Brasil, Americana, São Paulo, Brazil) connected to a portable vacuum pump (Nevoni 5005BRST, NSR Indústria Comércio e Representações LTDA, Barueri, SP, Brazil) via two 19G scalp needles. The needle of each scalp punctured the rubber cap of the collection tube; one adapter was connected directly to the pump hose, while the second adapter was removed, allowing the free end of the extension tubing to be placed into the child's mouth. Approximately 2 mL of saliva was obtained per participant.

The samples were immediately placed on dry ice and transported to a −80°C freezer until analysis.

Laboratory Analyses

Immunological analysis–cytokine quantification

The cytokine profile of all participants was assessed via the Luminex® Cytokine Human 6-Plex Panel (Luminex Corporation, Austin, TX). The assay was performed according to the manufacturer's instructions and involved the use of polystyrene microspheres internally dyed with two fluorophores, generating 100 distinct bead sets. Each bead population carried a unique color-coded signature that was detected by the Luminex® platform, enabling simultaneous quantification of the following cytokines: IL-4, IL-6, IL-8, IL-10, IFN-γ, and TNF-α.

Molecular analysis – Oral shedding

The molecular analysis of the salivary samples was performed at the Virology Laboratory of the Institute of Tropical Medicine, FMUSP. Detection and

quantification of the HHV panel were conducted via real-time polymerase chain reaction (PCR).

Nucleic acid extraction was performed via the Extracta DNA/RNA Viral Kit (Loccus do Brasil Ltda.) on the automated EXTRACTA 32 system. A total of 200 µL of saliva was processed, with a final elution volume of 100 µL. The purified viral DNA was stored at −80°C until analysis.

The detection and quantification of eight human herpesviruses (HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7, and HHV-8) were carried out via quantitative real-time PCR (qPCR), following protocols adapted from the literature (13-15). Reactions were performed on the QuantStudio™ 6 Flex system (Applied Biosystems, USA) in a final volume of 15 µL, which contained GoTaq™ Probe qPCR Master Mix, virus-specific primers and probes, and sample DNA. The thermal cycling profile consisted of initial activation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60 °C for 1 minute.

Target identification was achieved via probes labeled with FAM, VIC, or Cy5 fluorophores and BHQ or MGB-NFQ quenchers, as detailed in Supplementary Table 1. The resulting data were analyzed with QuantStudio Design & Analysis Software v.2.6 (Thermo Fisher Scientific).

Statistical analysis

Statistical analyses were performed using JMP® Pro version 13 (SAS Institute Inc., Cary, NC, USA). The normality of data distribution was assessed prior to analysis. Categorical variables are described as absolute and relative frequencies and were compared via Fisher's exact test. Continuous variables (viral load and cytokine concentrations) are reported as the means and standard deviations and were analyzed via the nonparametric Wilcoxon rank-sum test. Correlations between the viral load and cytokine levels were assessed via Pearson's correlation coefficient and the following classification was applied:

- 0.00–0.19 "very weak,";
- 0.20–0.39 "weak,";
- 0.40–0.59 "moderate,";
- 0.60–0.79 "strong,";
- 0.80–1.00 "very strong".

A significance level of 5% ($p<0.05$) was adopted for all analyses.

RESULTS

The demographic data of all participants are summarized in Table 1. No statistically significant differences were observed between the groups regarding the mean age (SG: 9.54±4.41 years; CG: 9.19±3.18 years; p=0.1832) or sex distribution (p=0.8041) (Table 1).

In the study group, 59.0% (23/39) of the participants had malignant solid tumors, whereas 41.0% (16/39) had hematological malignancies (Table 1).

Table 1. Distribution of the sample according to demographic variables and general clinical characteristics.

Measure	Category	Study Group n=39 (100%)	Control Group n=26 (100%)	p-value
Age	3 to 11 yo	23 (59.0)	20 (76.9)	0.1832
	12 to 17 yo	16 (41.0)	6 (23.1)	
Sex	Female	18 (46.2)	13 (50.0)	0.8041
	Male	21 (53.8)	13 (50.0)	
Type of neoplasia	Hematologic	16 (41.0)	0 (—)	—
	Solid tumors	23 (59.0)	0 (—)	
Chemotherapy regimen	High mucotoxic potential	29 (74.4)	0 (—)	—
	Low mucotoxic potential	10 (25.6)	0 (—)	
Leukocyte count	Leukopenia	25 (64.1)	0 (—)	
	Normal	14 (35.9)	0 (—)	
OM	Grade 0	37 (93.9)	0 (—)	
	Grade 1	2 (5.1)	0 (—)	

*Fisher's exact test.
OM: Oral Mucositis. High-mucotoxic-potential regimens included methotrexate, cyclophosphamide, doxorubicin, and 5-fluorouracil.

Leukopenia was identified in 64.1% (25/39) of the SG participants at the time of sample collection. Additionally, 74.4% (29/39) of the patients in the study group were receiving chemotherapy regimens with high mucotoxic potential, including methotrexate, cyclophosphamide, doxorubicin, and 5-fluorouracil. At the time of the oral examination, 5.1% (2/39) presented grade I oral mucositis, whereas 94.9% (37/39) presented grade 0 (Table 1). No other clinical manifestations were observed.

Leukemia was the most prevalent diagnosis within the study group (15/39; 38.4%), followed by central nervous system tumors (8/39; 20.5%), bone tumors (5/39; 12.8%), hepatic tumors (4/39; 10.2%), and sarcomas (4/39; 10.2%) (Table 2).

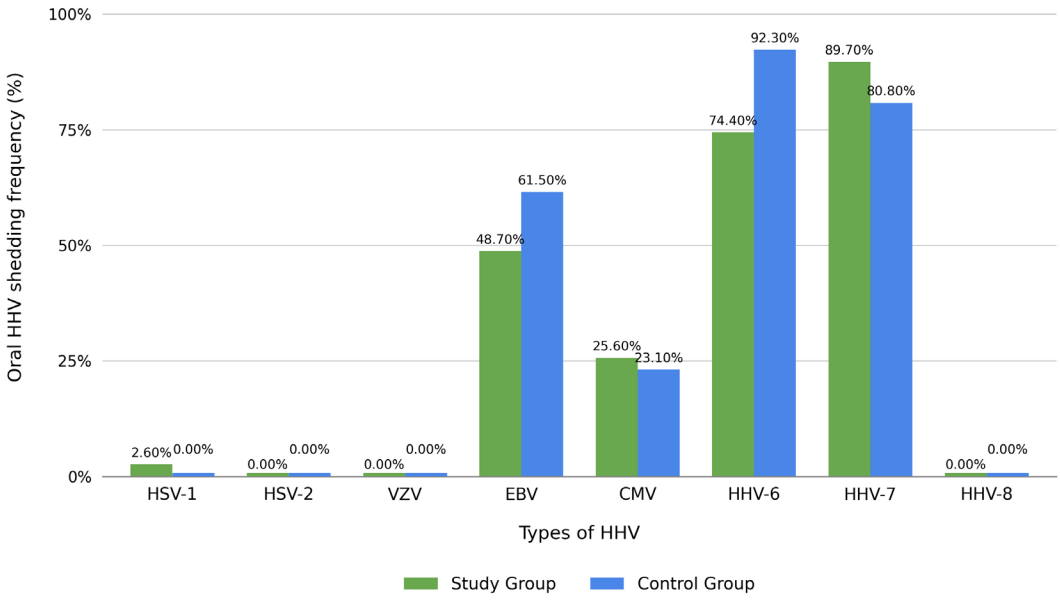
Figure 1 presents the oral shedding patterns of human herpesviruses in the SG and CG. In the SG, the most frequently detected viruses were HHV-7 (89.7%), HHV-6 (74.4%), EBV (48.7%), and CMV (25.6%). Neither HSV-2, VZV, nor HHV-8 was detected in either group. Only one participant (2.6%) in the study group shed HSV-1. Given the extremely low detection frequency, statistical analyses could not be performed for HSV-1, HSV-2, VZV, or HHV-8 because of insufficient variability for robust inference.

Table 3 shows that the mean HHV-7 viral load was significantly greater in the study group (6.5 copies/mL)

Table 2. Distribution of the study group according to oncologic diagnosis.

Diagnosis	(n/39)	% of total
Leukemia	**15/39**	**38.4**
Central Nervous System (CNS) Tumors	**8/39**	
Pineal germinoma	1/39	
Pineal germinoma	2/39	**20.5**
Neuroblastoma	3/39	
Medulloblastoma	2/39	
Bone Tumors	**5/39**	
Osteosarcoma	4/39	**12.8**
Ewing sarcoma	1/39	
Liver Tumors	**4/39**	
Hepatoblastoma	1/39	**10.2**
Hepatocellular carcinoma	2/39	
Primary hepatic sarcoma	1/39	
Sarcomas	**4/39**	
Rhabdomyosarcoma	1/39	
Sarcoma (unspecified subtype)	1/39	**10.2**
Desmoplastic small round cell tumor	1/39	
Atypical lipomatous tumor	1/39	
Renal tumor	**1/39**	**2.5**
Lymphoma	**1/39**	**2.5**
Giant ovarian dysgerminoma	**1/39**	**2.5**

Note: Statistically significant results are in bold.



*Fisher's exact test.
Source: The author.

Figure 1. Frequency and percentage of oral shedding of human herpesviruses (HHVs) in children and adolescents in the chemotherapy group compared with the control group.

than in the control group (5.8 copies/mL) (p=0.0288). In contrast, no statistically significant differences in the shedding frequency or viral load of EBV, CMV, or HHV-6 were detected between the groups.

The mean salivary concentrations of IFN-γ, IL-10, IL-6, IL-8, and TNF-α did not differ significantly between the groups (p>0.05). In contrast, the IL-4 level was significantly greater in the study group (8.4±20.6 pg/mL) than in the control group (7.2±22.9 pg/mL; p=0.0374) (Table 3).

No statistically significant associations were detected between inflammatory cytokines and the shedding of EBV, CMV, HHV-6, or HHV-7.

Overall, the correlations between cytokine levels and viral loads observed were weak or very weak. Statistically significant associations were identified in only two instances: a weak positive correlation between CMV and IL-6 (p=0.0095; r=0.34) and a weak negative correlation between HHV-6 and TNF-α (p=0.0125; r=–0.33). No significant correlations were found between the remaining HHVs and the cytokines analyzed (Table 4).

No statistically significant associations were detected between the presence or salivary viral load of any HHV and participant age, type of neoplasia (solid vs. hematologic), or leukocyte count within the study group (p>0.05).

Table 3. Means and standard deviations of the viral load (cp/mL) and salivary inflammatory cytokines in children and adolescents undergoing chemotherapy compared with those in the control group.

Characteristic	Study group	Control group	p-value
EBV cp/mL	3.4±3.5	4.3±3.5	0.2776
CMV cp/mL	1.9±3.3	1.7±3.2	0.9576
HHV-6 cp/mL	5.3±3.2	6.6±1.9	0.3681
HHV-7 cp/mL	**6.5±2.2**	**5.8±2.9**	**0.0288**
IFN-γ pg/mL	1.1±1.2	1.3±1.5	0.9599
IL-10 pg/mL	6.9±11.5	3.7±3.8	0.1199
IL-4 pg/mL	**8.4±20.6**	**7.2±22.9**	**0.0374**
IL-6 pg/mL	26.7±64.4	12.2±23.0	0.7684
IL-8 pg/mL	978.3±1212.8	660.8±934.1	0.2556
TNF-α pg/mL	13.4±27.2	9.0±17.3	0.9011

Note: Statistically significant results are in bold.
*Nonparametric Wilcoxon rank-sum test.
Source: The author.

DISCUSSION

This study investigated the relationship between oral shedding of human herpesviruses (HHVs) and salivary inflammatory cytokine profiles in children and adolescents undergoing chemotherapy, compared with healthy controls. By simultaneously evaluating eight HHVs and multiple cytokines, this study provides a comprehensive assessment of the oral microenvironment in a pediatric oncology setting.

Saliva has emerged as a valuable biological fluid for monitoring inflammatory responses because of its easy collection, low cost, and noninvasive nature, particularly in vulnerable populations such as pediatric oncology patients[10,12–18]. Furthermore, studies have shown that salivary cytokines reflect both systemic

Table 4. Correlations between salivary inflammatory cytokine concentrations and viral loads.

HHV	Cytokine	Correlation	p-value
EBV cp/mL	IFN-y	0.01	0.9124
	IL-10	0.14	0.2921
	IL-4	-0.20	0.1287
	IL-6	0.22	0.1001
	IL-8	0.14	0.3048
	TNF-a	0.06	0.6739
CMV cp/mL	IFN-y	-0.12	0.3681
	IL-10	0.24	0.0744
	IL-4	-0.06	0.6649
	IL-6	**0.34**	**0.0095**
	IL-8	-0.06	0.6556
	TNF-a	-0.16	0.2425
HHV-6 cp/mL	IFN-y	-0.08	0.5546
	IL-10	0.05	0.7345
	IL-4	0.05	0.7320
	IL-6	0.11	0.4117
	IL-8	-0.09	0.4774
	TNF-a	**-0.33**	**0.0125**
HHV-7 cp/mL	IFN-y	0.10	0.4699
	IL-10	0.13	0.3347
	IL-4	0.09	0.5257
	IL-6	0.08	0.5508
	IL-8	0.09	0.5089
	TNF-a	-0.06	0.6393

Note: Statistically significant results are in bold.
*Pearson's linear correlation coefficient.
Source: The author.

and local inflammatory states and may serve as useful biomarkers for monitoring inflammatory and infectious oral conditions[10,12].

The oral cavity is highly susceptible to manifestations resulting from the toxic effects of oncologic treatment, including oral mucositis (OM), opportunistic infections, candidiasis, and reactivation of latent viruses, particularly those of the Herpesviridae family[2,6,19]. Viral reactivation in immunocompromised patients is well documented, especially in adults undergoing radiotherapy or hematopoietic stem cell transplantation[1,3,4,7]. However, data on viral shedding in pediatric populations receiving conventional chemotherapy remain scarce. On the basis that chemotherapy-induced immunosuppression can promote viral reactivation and trigger local inflammation, we hypothesized that participants in the study group (SG) would exhibit increased viral loads and an exacerbated inflammatory response.

The only statistically significant difference observed was for the HHV-7 viral load, which was greater in the SG (6.5 cp/mL) than in the control group (5.8 cp/mL; p=0.0288). The increased HHV-7 load in the SG may be linked to chemotherapy-associated immunosuppression. This finding is consistent with previous reports showing that patients with cancer or those receiving immunosuppressive therapies often exhibit elevated herpesvirus loads. This observation is particularly relevant, as prior studies have identified HHV-7 as a potential biomarker of OM, dysbiosis, and persistent inflammation in immunocompromised individuals[1,2,8,9]. Unlike qualitative assessments, our quantitative qPCR approach provides a more accurate evaluation of viral shedding intensity.

Despite the higher viral load observed in the study group, no significant differences were found in the frequency of HHV-7 detection between the groups. These findings suggest that reactivation may not necessarily be associated with the qualitative presence of the virus but rather with the intensity of viral replication. This subtle yet important distinction indicates that HHV-7 may remain present in the oral cavity at a basal level in children, regardless of their clinical status, whereas its active replication (as reflected by the viral load) may be linked to the host's immunological condition[1,2,8,9].

The absence of differences in the shedding of other viruses, such as HHV-6, EBV, and CMV, may also be attributed to their latency and shedding patterns, which occur intermittently and do not necessarily increase even under immunosuppressive conditions[4,9]. Additionally, these viruses may sustain low-level replication in extraglandular sites, such as lymph nodes and the peripheral nervous system, which could limit their detectability in saliva[8].

The low detection rate of HSV-1, which was identified in only one patient in the study group (2.6%), contrasts with previous reports that reported prevalence rates ranging from 9 to 60% among children undergoing chemotherapy[20,21].

The absence of HSV-2, VZV, and HHV-8 in both groups is consistent with the findings of previous studies. HSV-2 displays genital tropism and is associated primarily with sexual transmission; VZV, after primary infection, establishes latency in sensory ganglia and is rarely detected in the saliva of children; and HHV-8 is more commonly found in immunosuppressed adults, particularly individuals living with HIV[22–26].

Statistical analysis did not reveal significant associations between the frequency of EBV, CMV, HHV-6, or HHV-7 shedding and the salivary levels of the inflammatory cytokines examined. Moreover, when the viral load was correlated with the inflammatory response, most correlations were not significant, suggesting that the viral loads assessed were not strongly related to cytokine concentrations in saliva.

The only salivary cytokine that was significantly different between the groups was IL-4, with slightly higher levels in the study group (8.4 pg/mL vs. 7.2 pg/mL; p=0.0374). Despite the significant p value, this finding should be interpreted cautiously given the high interindividual variability and wide standard deviation. IL-4 is an anti-inflammatory cytokine associated with Th2-type immune activation and alternative macrophage polarization (M2 phenotype) and plays an important role in immune regulation, tissue repair, and attenuation of excessive inflammatory responses[27].

In the present study, correlations between the viral load and cytokines were mostly weak or absent. However, two associations were noteworthy: a positive correlation between CMV and IL-6 and a negative correlation between HHV-6 and TNF-α. IL-6 is a proinflammatory cytokine broadly involved in innate immune responses, and its elevation in association with CMV may reflect inflammatory activation in response to viral replication (Miranda-Silva et al., 2020)[1]. Conversely, the negative correlation between HHV-6 and TNF-α suggests a potential immunomodulatory effect of HHV-6, which is consistent with studies demonstrating its ability to suppress proinflammatory cytokine production as an immune evasion strategy[28,29].

Importantly, these correlations occurred independently of the clinical group (oncologic or control), indicating that immune modulation by herpesviruses may take place even in healthy individuals. Furthermore, no associations were identified between the viral load and leukopenia, age, or type of neoplasm, suggesting that salivary viral shedding may occur independently of the clinical severity or degree of hematologic immunosuppression. These findings differ from those of Elad et al. (2010)[30], who reported increased viral shedding in patients with neutropenia and hematologic malignancies, differences that may be attributable to methodological and sample-related variations.

Although the findings of this study provide insights into the potential impact of oncologic treatment on the oral microenvironment, the cross-sectional design represents a significant limitation. This approach did not allow causal inferences or longitudinal evaluations throughout treatment. Additionally, the sample size may be considered modest. Nevertheless, the present study provides an initial framework for future longitudinal investigations aimed at elucidating the temporal dynamics of viral shedding and its inflammatory repercussions across different treatment phases. In this context, saliva has emerged as a promising and noninvasive tool for monitoring the oral microenvironment in immunocompromised patients. Moreover, the collection system employed in this study enabled safe, rapid, and patient-friendly sampling, reinforcing the feasibility of saliva-based monitoring strategies in pediatric oncology and their potential contribution to improving patient follow-up and quality of life.

CONCLUSION

This study demonstrated increased salivary HHV-7 viral load and higher IL-4 levels in children and adolescents undergoing chemotherapy, suggesting possible alterations in the oral immune environment. Weak correlations were observed between CMV and IL-6 and between HHV-6 and TNF-α. Overall, salivary HHV viral load showed a limited association with the inflammatory cytokine profile in this population.

AUTHORS' CONTRIBUTIONS

WAC: Data curation, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. PPS: Data Curation, Methodology, Investigation, Resources, Validation. FMMO: Conceptualization, Funding acquisition. LAVSJ: Investigation, Resources. PHBS: Conceptualization, Data Curation, Methodology. MG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

CONFLICT OF INTEREST STATEMENT

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Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: Research Ethics Committee of the University of São Paulo School of Dentistry (CEP-FOUSP) under protocol number 5278459, as well as the Research Ethics Committee of the Institute of Childhood Cancer Treatment (ITACI) at the University of São Paulo Clinical Hospital Medical School (HCFM-USP) under protocol number 5447771.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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