Research Article

Al-Iraqia Medical College Journal (AIMCJ)
ISSN (Online): 3104-4565
ISSN (Print): 3104-4557

ARTICLE INFO
Received: 30/ 6/ 2025
Revised: 7/ 8/ 2025
Accepted: 8/ 8/ 2025
Publish online: 15 /08 / 2025

*Corresponding Author Thura Saad Abed Alkareem
Email: thara.s.abedalkareem@aliraqia.edu.iq

CITATION
Alkareem TSA, Khudhair AM. Human herpes virus 6 seroprevalence in pediatric leukemia: a case-control study. *AIMCJ*. 2025;2(2):60-67

Doi: https://doi.org/10.58564/AIMCJ2.2.2025.239

Human Herpes Virus 6 Seroprevalence in pediatric leukemia: A case-control Study

Thura Saad Abed Alkareem[1*], Anfal Mohammed Khudhair [1]

[1] Department of Microbiology, College of Medicine, Al-Iraqia University, Baghdad, Iraq.

Abstract

This study investigates the incidence of primary infections and reactivations of the HHV-6 virus in pediatric leukemia patients, and their possible role in the disease course or in contributing to immunosuppression.

Human Herpesvirus 6 (HHV-6) Primary Infections and Reactivation in Children with Leukemia: A Clinical and Epidemiological Study" illustrates the gaps and problems in the research of HHV-6 viruses in children with leukemia which was the case in the United States and shows the defects in the use of clinics and hospital laboratories and the possible contribution of the systematic research of the pediatric oncology clinic in children suffering from leukemia aiming pediatric population of the country was the first step in Canadian leukemia and HHV-6 viruses in children.

The present case-control study analyzed a total of blood samples. The case group comprised of children aged 1-13 years who were diagnosed with acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) with a sample size of 50. The control group had 50 children who were matched by age and sex with the case group and were healthy without any personal or familial history of hematological malignancies or viral illness. The collected samples were centrifuged and the sera were stored at 4 degrees Celsius till the analysis. hhv-6 specific IgG antibodies were assessed by the EL1SA technique.

The mean serum level of HHV-6-specific IgG antibodies was significantly higher in the leukemia group (77.67 ± 21.79) compared to the control group (14.89 ± 2.93), and this difference was statistically significant ($p < 0.001$).

Our findings indicate a higher prevalence of HHV-6 infection among pediatric leukemia patients. This may reflect viral reactivation due to immunosuppression rather than a direct oncogenic role for the virus.

Keywords: HHV-6 virus, Pediatric leukemia, viral infection.

Introduction

Leukemia is a form of cancer originating in the bone marrow that impacts white blood cells. Leukemia cells proliferate rapidly in the bone marrow, inhibiting the generation of healthy blood cells (1). The myeloid and lymphoid systems are impacted by prevalent forms of leukaemia, including acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML), acute lymphoblastic leukaemia (ALL), and chronic lymphocytic leukaemia (CLL) (2). In 2018, the GLOBOCAN survey identified leukaemia as the 13th most prevalent disease globally, with a concomitant 17% rise in its fatality rate that year. Despite the rising incidence of leukaemia, its exact aetiologies remain unidentified. Leukaemia is profoundly affected by environmental and genetic risk factors, including infections, exposure to ionising radiation, and chemical exposure (3).

Viral infections present a considerable threat to children undergoing therapy for acute lymphoblastic leukaemia (ALL). Children with acute lymphoblastic leukaemia (ALL) are vulnerable to infections due to various factors, including the disease, prolonged neutropenia, and aggressive chemotherapy and steroid treatment (4).

Human herpesvirus 6 (HHV-6), previously referred to as human B-lymphotropic virus, was identified in the blood lymphocytes of individuals with AIDS or lymphoproliferative diseases. Subsequently, the virus was detected in CD4+ cells and classified as a herpesvirus. The organism was designated human herpesvirus six due to its identification as the sixth herpesvirus to be isolated. HHV-6 induces acute, chronic, and persistent infections like other herpesviruses (5). Similar to cytomegalovirus (CMV), human herpesvirus 7 (HHV-7), and human herpesvirus 8 (HHV-8), human herpesvirus 6 (HHV-6) is classified as a beta herpesvirus and comprises two distinct strains (A and B). Seroprevalence studies in Japan and the United States indicate that the predominant strain of the virus is HHV-6 B. Only those with significant immunocompromise are impacted by HHV-6A disease (6). Betaherpesviruses, including HHV-6A, HHV-6B, and HHV-7, establish latency by integrating into the subtelomeric regions of human chromosomes. The failure of genetically engineered herpesviruses lacking telomeric repeat sequences to incorporate into human telomeres efficiently is believed to result from homologous recombination involving telomeric repeat sequences.

Multiple genes associated with HHV-6 latency have been discovered; however, the functional relevance of these gene products remains ambiguous (7).

This study aims to determine the prevalence of HHV-6 infection—specifically primary infections and reactivations—in pediatric patients diagnosed with leukemia, and to explore its potential role in disease progression or immune suppression rather than causation.

Materials and Methods:

The College of Medical Al-Iraqia University has approved the study. I obtained consent from the parents prior to collecting a sample from each child. Blood samples were collected from the Central Teaching Hospital of Paediatrics in Baghdad between November of 2024 to February 2025. The participants ages ranged from 1 to 13 years. IgG antibodies to HHV-6 were determined by ELISA.

Patients and Sampling

This case-control study included 100 participants, 50 pediatric patients recently diagnosed with leukemia and 50 age and sex matched healthy controls. The leukemia diagnoses, either acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) were validated clinically, hematological, and via laboratory assessments. The control group consisted of healthy children who did not have, nor did their families have, a personal or familial history of hematological malignancies or recent viral infections.

Inclusion criteria:

- Individuals were children aged between 1 and 13 years who were recently diagnosed with either ALL or AML.
- Diagnosis made through clinical and laboratory assessments.
- Patients receiving chemotherapy during any phase (induction, consolidation, or maintenance).

Exclusion criteria:

Individuals diagnosed with other hematologic malignancies, and with known disorders of immunodeficiency, or systemic chronic diseases.

ELISA Protocol

Each participant's venous blood sample of 2 mL was collected through a sterile, single-use syringe. The blood samples were placed into gel-separator tubes. Serum was separated by centrifuging the blood samples at 3000 rpm for 10 minutes at room temperature. The serum obtained was then placed into sterile Eppendorf tubes and refrigerated at 4 °C until further analysis was conducted.

The presence of HHV-6 specific IgG antibodies was tested with a commercial sandwich ELISA kit from Sunlong Biotech (Catalog No. SL3292Hu). In this assay, the wells of a 96 well microplate were pre-coated with HHV-6 specific antigens. Serum samples were placed into the wells, where the antibodies were

captured. After washing steps, HRP conjugated antigens were added, followed by an additional incubation period.

After washing steps, TMB was added, and a color change was observed indicating a positive result. Absorbance was measured at the designated wavelength and results were interpreted as per the manufacturer's instruction cut-off values provided in the assay manual.

Statistical Analysis

Data entry, validation, and analysis were performed with SPSS version 26 and STATISTICA version 12. Descriptive analysis included, frequency distributions, and counts and percentages of qualitative variables alongside means, standard deviations, and ranges of quantitative variables.

For inferential analysis:

Quantitative variables of the leukaemia and control groups were compared using unpaired student's t-test. Associations of two or more categorical variables were analyzed with the Chi-square or its alternative, Likelihood ratio test. A p-value of < 0.05 was deemed statistically significant.

Results

Regarding the molecular parameters among study's groups, it has been found that the mean level of viral parameter of Human Herpesvirus-6 (HHV-6) Immunoglobulin-G (HHV-6-IgG) was significantly higher among cases group (leukemic children) than that of the controls group (77.66710 ± 21.788151 vs. 14.89104 ± 2.933393) with significant difference of -62.776060 (t= -20.191, df:98, P= 0.000) respectively (Table 1) (Figure 1).

Participants in the leukaemia group had human herpesvirus-6 (HHV-6) IgG antibodies at a mean serum concentration of 77.67 ± 21.79. Control participants had a substantially lower mean concentration of 14.89 ± 2.93. The total mean difference between groups in HHV-6 IgG antibodies was 62.78 with a t-test value of t = 20.19, df = 98, $p < 0.001$.

Table 1: Mean comparison of Human Herpesvirus-6 Immunoglobulin-G (HHV-6-IgG) among study groups (n=100)

Serological Parameters (Mean ± SD)	**Study groups (leukemia) (n=100)**		**Mean differences**	**Significance [a]**
	Cases (Yes, n=50)	**Control (No, n=50)**		
Human Herpesvirus-6 - Immunoglobulin-G (HHV-6-IgG)	77.66710 ± 21.788151	14.89104 ± 2.933393	-62.776060	*t*= -20.191, df: 98, ***P* 0.000**

a: Unpaired T-Test

Interpretation in the Results Section

The difference in the mean serum concentration of HHV-6 specific IgG antibodies was significantly different between the two groups in the study. The mean concentration in the leukaemia group was significantly elevated (77.67 ± 21.79) compared to the control group's value (14.89 ± 2.93). The mean difference was −62.78 which was significant (t = −20.19, df = 98, p < 0.001). The groups were compared with an independent samples t-test.

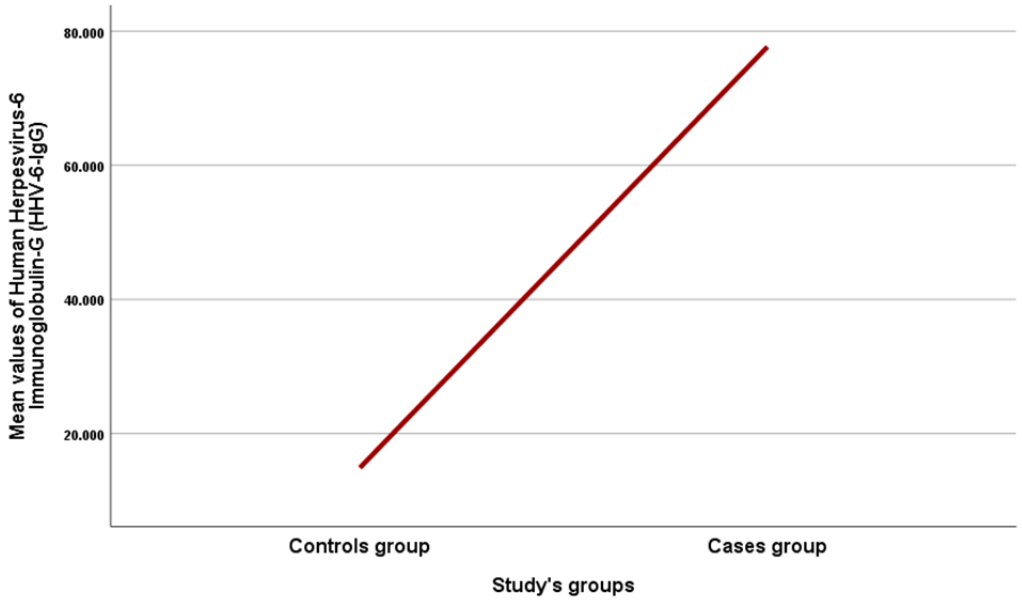

Figure 1. Mean comparison of Human Herpesvirus-6 Immunoglobulin-G (HHV-6-IgG) among study groups (n=100)

Discussion

Viral infections following immunosuppression may result from either the reactivation of latent viruses or the acquisition of new infections. Human herpesviruses (HHVs) are a group of DNA viruses classified into three subfamilies: Alpha herpes virinae, Beta herpes virinae, and Gamma herpes virinae. Herpes simplex virus types 1 and 2 and varicella-zoster virus are categorized as α-herpesviruses; cytomegalovirus (CMV), human herpesvirus 6A and 6B (HHV-6A/6B), and HHV-7 are β-herpesviruses; whereas Epstein-Barr virus (EBV) and HHV-8 fall under γ-herpesviruses (8).

More than 95% of the global population exhibits seropositivity for at least one latent HHV. Following primary infection, HHVs can establish latency in lymphocytes or monocytes without overt clinical manifestations. However, immunosuppressive conditions—such as chemotherapy—may trigger viral reactivation (9).

In the present study, children with leukaemia demonstrated significantly higher mean levels of HHV-6-specific IgG antibodies (77.67 ± 21.79) compared to the control group (14.89 ± 2.93). The mean difference was −62.78, which was statistically significant ($t = -20.19$, $df = 98$, $p < 0.001$), suggesting a strong association between HHV-6 exposure or reactivation and immune suppression during leukaemia treatment.

An important characteristic of HHV-6 is its ability to integrate into human chromosomes—a phenomenon referred to as chromosomally integrated HHV-6 (CI-HHV-6). This integration leads to persistently high levels of HHV-6 DNA in all nucleated cells and may be inherited (10). In leukemia patients, viral loads are often low, with detection primarily in normal lymphocytes or progenitor cells rather than in leukaemic blasts. HHV-6 is more frequently detected during remission or post-chemotherapy periods, supporting the hypothesis of viral reactivation rather than a causal oncogenic role (11).

Previous studies have reported that children with acute leukaemia, particularly older individuals, exhibit HHV-6-specific IgM positivity and high-avidity IgG antibodies, indicating either an inadequately controlled primary infection or reinfection.

Typically, IgM antibodies appear within two weeks of infection and may persist for 2–3 months, whereas IgG antibodies can remain detectable for life (12).

Conversely, other studies have found no significant difference in HHV-6 antibody titers between patients and controls. Some Sero epidemiological evidence suggests that young children naturally exhibit higher HHV-6 antibody levels than adults, implying that the observed association between HHV-6 and acute lymphoblastic leukemia (ALL) in some studies might be confounded by age rather than indicating a direct viral role in disease pathogenesis (13).

Conclusions:

HHV-6 is a pervasive herpesvirus that induces permanent latent infection in most humans. Our data suggest that Human herpesvirus 6 (HHV-6) is more frequently detected in children with pediatric leukemia than healthy controls. Its identification in leukemia patients is prevalent but mainly indicates reactivation resulting from immune suppression (e.g., chemotherapy) rather

than a direct neoplastic influence. The virus can infect early hematopoietic progenitor cells but does not seem to convert them into malignant cells.

Approximately 1% of the population exhibits the integration of HHV-6 DNA into human chromosomes (ciHHV-6), which may exert unidentified effects on cellular physiology; nevertheless, a definitive correlation with leukemia initiation has not been found.

Conflict of Interest:

The authors declare no conflicts of interest.

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References:

1. Ding F, Deng L, Xiong J, Cheng Z, Xu J. Analysis of global trends in acute lymphoblastic leukemia in children aged 0–5 years from 1990 to 2021. *Frontiers in Pediatrics.* 2025 Mar 13;13:1542649.
2. Li J, Wang Y, Dong C, Luo L. Advancements in leukemia management: Bridging diagnosis, prognosis and nanotechnology. *International Journal of Oncology.* 2024 Oct 2;65(6):112.
3. Ibrahim RM, Idrees NH, Younis NM. Epidemiology of leukemia among children in Nineveh Province, Iraq. *Rawal Med J.* 2023 Mar 3;48(1):137.
4. Zawitkowska J, Drabko K, Czyżewski K, Dziedzic M, Jaremek K, Zalas-Więcek P, Szmydki-Baran A, Hutnik Ł, Matysiak M, Czogała W, Balwierz W. Viral infection profile in children treated for acute lymphoblastic leukemia—results of nationwide study. *Pathogens.* 2022 Sep 24;11(10):1091.
5. King O, Syed HA, Al Khalili Y. Human herpesvirus 6. *StatPearls* [Internet]. 2025 May 5. Available from: www.ncbi.nlm.nih.gov/books/NBK540998/#article-23023.s2.
6. Sclerosis AL. Practice Essentials, Background. *Pathophysiology*. 2024.
7. Prusty BK, Gulve N, Chowdhury SR, Schuster M, Strempel S, Descamps V, Rudel T. HHV-6 encoded small non-coding RNAs define an intermediate and early stage in viral reactivation. *NPJ Genomic Medicine*. 2018 Sep 5;3(1):25.
8. Handous I, Achour B, Marzouk M, Rouis S, Hazgui O, Brini I, Khelif A, Hannachi N, Boukadida J. Co-infections of human herpesviruses (CMV, HHV-6, HHV-7 and EBV) in non-transplant acute leukemia

patients undergoing chemotherapy. *Virology journal*. 2020 Mar 17;17(1):37.

9. Xu J, Chen L, Cen T, Zeng Y, Liu Q. HHV-6B-and HHV-7-associated choroiditis secondary to acute myelogenous leukemia: a case report. *Journal of International Medical Research.*2023;51(10):04479.
10. Hubacek P, Muzikova K, Hrdlickova A, Cinek O, Hyncicova K, Hrstkova H, Sedlacek P, Stary J. Prevalence of HHV-6 integrated chromosomally among children treated for acute lymphoblastic or myeloid leukemia in the Czech Republic. *Journal of medical virology*. 2009 Feb;81(2):258-63.
11. Faten N, Agnès GD, Nadia BF, Nabil AB, Monia Z, Abderrahim K, Henri A, Salma F, Mahjoub A. Quantitative analysis of human herpesvirus-6 genome in blood and bone marrow samples from Tunisian patients with acute leukemia: a follow-up study. *Infectious agents and cancer*. 2012 Nov 12;7(1):31.
12. Salonen MJ, Siimes MA, Salonen EM, Vaheri A, Koskiniemi M. Antibody status to HHV-6 in children with leukaemia. *Leukemia*. 2002 Apr;16(4):716-9.
13. Levine PH, Ablashi DV, Saxinger WC, Connelly RR. Antibodies to human herpes virus-6 in patients with acute lymphocytic leukemia. *Leukemia.* 1992 Nov;6(11):1229-31.