RESEARCH ARTICLE

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A Viral–Host Immunogenetic Interaction Model in Hodgkin Lymphoma: Co-Association of HHV-6B Infection and *IL-18 rs1946518* Polymorphism

Aya Raed Abdulwahhab Alheany[1]*, Aws Zamil Abdulkareem[2], Zahraa Ali Abdullah[3], Shakir H. Mohammed Al-Alwany[3]

Abstract

Background: Hodgkin lymphoma (HL) arises from germinal center B cells through complex viral, genetic, and environmental factors. This study examines HHV-6B infection and the *IL-18 rs1946518* polymorphism as potential contributors to inflammation-driven HL susceptibility and risk of disease progression. **Methods:** A case-control study of 180 venous blood samples was conducted, including 90 HL patients and 90 healthy controls. Viral and genomic DNA were isolated using the standard phenol-chloroform protocol, and polymerase chain reaction (PCR) amplification of HHV-6B genomes and the *IL-18 rs1946518* single-nucleotide polymorphism (SNP) was performed. Variants of *IL-18 rs1946518* were confirmed using Sanger sequencing. To compare genotype and allele frequencies between patient and control groups, statistical tests were conducted, including chi-square tests and logistic regression, as appropriate. **Results:** No significant difference in age was observed between HL patients (28.5 ± 9.7 years) and controls (30.6 ± 10.8 years; $P > 0.05$). Males represented 57.8% of HL patients compared to 42.2% females. The presence of HHV-6B DNA was detected in 25.6% (23/90) of HL patients, with 74.4% (67/90) testing negative. Analysis of *IL-18 rs1946518* revealed a significant difference in the frequency of the TT genotype between HL patients and controls ($P = 0.04$, OR = 0.28, 95% CI: 0.09–0.87). The frequency of T and C alleles was observed to be higher in HL patients (T: 70, C: 40) and in controls (T: 60, C: 60), respectively, suggesting a potential increased risk of HL associated with the T allele and a possible protective effect of the C allele. **Conclusions:** Current evidence links HHV-6B infection and *IL-18 rs1946518* variants to the pathogenesis of Hodgkin lymphoma, potentially influencing disease susceptibility and clinical outcomes. Further studies with larger cohorts are needed to clarify the underlying mechanisms.

Keywords: Hodgkin lymphoma- HHV-6B- IL-18 polymorphism- Immunogenetics- Viral–host interaction

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Introduction

Hodgkin lymphoma (HL) is a rare blood cancer that occurs when germinal center B cells are transformed into cancerous Hodgkin Reed-Sternberg (HRS) cells. The factors that contribute to the pathogenesis are complex; they include viral, environmental and genetic factors; however, the precise cause of HL remains unclear, despite tremendous progress in treatment [1].

Roseola infantum's causal agent, Human Herpes Virus-6 (HHV-6), has recently attracted considerable attention in the medical field for its neurotropic and possibly carcinogenic characteristics [1]. Both HHV-6A and HHV-6B are forms of the virus, however they are geographically and disease-associated distinct. Roseola, in rare cases of meningitis and hepatitis, and HHV-6B are the most commonly reported infections [2]. Viral proteins such as pDR7 and pU94 contribute to oncogenic processes, and the virus can persist in a latent state through integration into the host genome [2].

An important pro-inflammatory cytokine, interleukin-18 (IL-18) has a major influence on inflammatory and autoimmune diseases [3]. In sepsis and other inflammatory conditions, IL-18 primarily produced by dendritic cells and macrophages contributes to tissue damage [4]. Its activation depends on caspase-1 through the NLRP3 inflammasome pathway [5]. Dysregulation of IL-18 or IL-1β leading to excessive secretion can result in chronic inflammation and associated oncogenic changes [6].

The rs1946518 polymorphism of the IL-18 gene has not been extensively investigated in relation to

*[1]Department of Microbiology, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq. [2]Department of Science, College of Basic Education, Diyala University, Diyala, Iraq. [3]Department of Biology, College of Science, Babylon University, Babylon, Iraq. *For Correspondence: aya_alheany@ibnsina.edu.iq*

HL susceptibility, although it has been implicated in several cancer types. Alterations in IL-18 expression and inflammatory responses related to this variant may predispose to cancer development and progression. Given the limited data regarding the association between *IL-18 rs1946518* polymorphism and HHV-6B infection in HL, this study aimed to address whether HL risk and disease susceptibility are associated with *IL-18 rs1946518* genetic variation and HHV-6B infection.

Materials and Methods

Study design and participants

The case-control study was carried out in the period between December 2024 and March 2025 in several healthcare facilities in Iraq such as the Iraqi Centre of Blood Diseases, Merjan Teaching Hospital, and Baghdad Medical City. The participants enrolled in the study were 90 Hodgkin Lymphoma patients based on WHO criteria and 90 age- and sex-matched controls who did not have the disease. All samples were taken by using peripheral blood samples in order to have molecular analyses.

HHV-6B detection

The Intron Viral DNA Extraction Kit- Korea was used to extract viral genomic DNA. Specific primers were used to amplify the U95 region of HHV-6B to remove a fragment of 517 bp.

Primers used

U95-HHV6B-F:
5'-CGGGATCAAAACCGCGAATC-3'
U95-HHV6B-R:
5'-TTCATCGCCAGATGCCGTAG-3'

The conditions of PCR amplification were 40 cycles of denaturation at 95°C for at (45s), annealing at 59°C for (45s), and extension at 72°C for (1min) with a final extension at 72°C for (5min).

IL-18 Genotyping

The genomic DNA was extracted from whole blood using the Intron DNA extraction kit. The IL-18 (rs1946518, 607C/A) polymorphism was analyzed using primers designed via the Primer Quest Tool (IDT, USA), validated against reference sequences, and confirmed by restriction site mapping using NEB cutter.

Primers used

IL-18F: 5'-TCAGGACTTCCCCTTCCTCC-3'
IL-18R: 5'-TGCCACCTTGCTAATTCCCT-3'

Having annealing temperature of 53 to 60°C, the expected PCR outcome was 439 bp. PCR reactions were carried out using the following components: 10 µL master mix, 4 µL forward and reverse primers, 2 µL template DNA, and 5 µL nuclease-free water. Amplifications were done with a Biometra thermal cycler (Germany) using the following conditions: an initial denaturation at 95°C, 40 denaturation cycles at 95°C of 1 minute, annealing at 60°C of 1 minute, extension at 72°C of 2 minutes, and a final extension at 72°C of 5 minutes. To do further analysis the amplified products were stored at -20°C. Due to DNA quality and sequencing validation requirements, genotyping analysis was performed on a representative subset of samples (20 patients and 10 controls).

Statistical analysis

The SPSS version 24.0 was used in data analysis. The association between categorical variables was done using the chi-square test. The Spearman rho correlation was used to determine the correlation between the study variables and odds ratios with 95% confidence interval assessed the risk associations. Chi-square goodness-of-fit test was used to assess Hardy-Weinberg equilibrium (HWE) for *IL-18 rs1946518* genotypes in the control group. A p-value < 0.05 was considered statistically significant.

Results

Demographic characteristics

The sample size comprised of HL patients aged between 16 and 68 (mean = 28.5 ± 9.7 years) and controls aged between 30.6 ± 10.8 years. There was no statistically significant difference in terms of age in both groups (P > 0.05). The gender distribution was 57.8 percent males and 42.2 percent females among patients with HL, where the male to female ratio was 1.36: 1. There were no significant differences in the control group where 56.7 percent were male and 43.3 percent female. The age stratification showed that the most common frequency of HL cases was the 36-55-year age group, which included 40% of the patients (20 men and 16 women), followed by the 16-35-year age group (35.5) and the 56-68-year age group (24.5), with an equal representation of men in all age groups.

HHV-6B genome detection

Conventional PCR analysis showed that the 25.6% (23/90) of the patient specimens were infected with HHV-6B genome and 74.4% (67/90) were uninfected (Table 1). This was statistically significant (P = 0.03), which demonstrated that there was a significant presence of HHV-6B in HL patients.

Polymerase chain reaction (PCR) was used to reveal the presence of HHV-6B DNA in peripheral blood samples of Hodgkin lymphoma patients. The presence of HHV-6B was confirmed by the amplification of a 517 bp DNA fragment. This specific DNA product was observed in lanes 1 through 9. Electrophoresis was performed to visualize the PCR products. Each well was loaded with 1.5 µl of a 1.5% agarose gel, which was subjected to an electric current of 75 V and 20 mA for one hour. Following electrophoresis, the gel was stained with a RedSafe solution to enable visualization of the DNA bands under

Table 1. Detection of HHV-6B DNA in Patients with HL (n = 90)

Result	Number	Percentage (%)	P-Value
Positive	23	25.6	0.03
Negative	67	74.4	
Total	90	100.0	

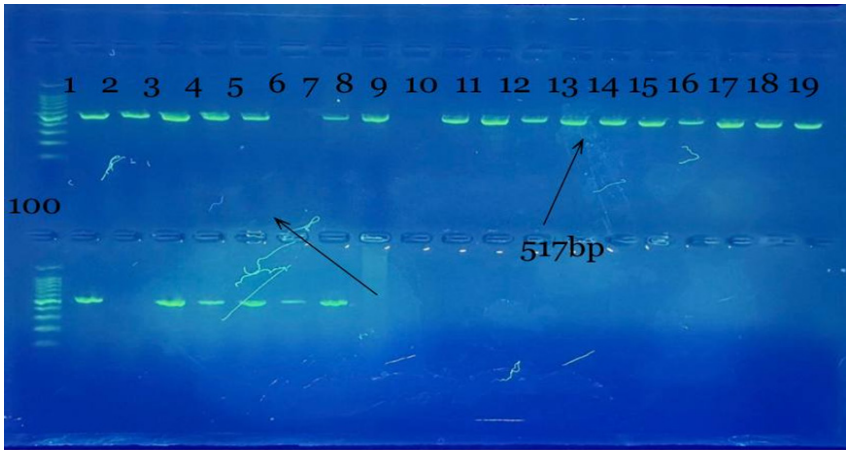

Figure 1. Detection of Human Herpesvirus 6B (HHV-6B) DNA in Peripheral Blood Samples from Patients with Hodgkin Lymphoma by Conventional PCR.

ultraviolet (UV) light (Figure 1).

Age-stratified analysis revealed the highest HHV-6B infection prevalence in the 36–55-year age group (12.2%), followed by the 16–35-year (7.8%) and 56–68-year (5.5%) groups, with significant differences among age strata ($P < 0.05$). Gender-specific analysis showed that 60.9% of male HL patients and 39.1% of female patients tested positive for HHV-6B, with this difference reaching statistical significance ($P = 0.04$).

IL-18 rs1946518 polymorphism analysis

Analysis of the 439 bp fragments of the *IL-18 rs1946518* gene demonstrated differential genotype distributions between HL patients and controls. Among HL patients, genotype distribution was 20% CC, 20% CT, and 60% TT, whereas controls showed 50% CC, 20% CT, and 30% TT distributions (Table 2).

Genotype distribution in the control group was consistent with Hardy–Weinberg equilibrium (χ^2 test, $P = 0.42$). As shown in Table 2, the TT genotype and T allele of *IL-18 rs1946518* were significantly more frequent among Hodgkin lymphoma patients compared with controls, suggesting increased disease susceptibility.

The gel electrophoresis experiment involved the allelic variation study of the *IL-18 rs1946518* gene, after a polymerase chain reaction (PCR) amplification of the target sequence. Separation of amplified DNA fragments occurred on a 1.5% agarose gel under an electric field of 75 V for 55 minutes. In order to visualize the DNA bands, Five microliters (5 µL) of each sample were loaded into separate wells, and the gel was stained with RedSafe solution (Figure 2).

The TT genotype was significantly more frequent in HL patients compared to controls ($P = 0.04$, OR = 0.28), suggesting an association with increased disease risk. Allelic analysis showed the T allele to be more prevalent among HL patients (70% vs. 40% in controls), whereas the C allele was more prevalent among controls (60% vs. 40%) and potentially conferring a protective effect ($P = 0.02$, OR = 0.28).

Sequence submission

The nucleotide sequences of *IL-18 rs1946518* were submitted in the National Center of Biotechnology Information (NCBI) with the accession numbers of LC870667, LC870668, and LC870669.

Correlation analysis

The rho correlation analysis by Spearman showed that there are significant relationships among the study variables (Table 3). There was a significant positive correlation between the HHV-6B presence and the SNP of HL patients *IL-18 rs1946518* ($r = 0.875$, $P = 0.004$). Also, there was a strong correlation between *IL-18 rs1946518* SNP and patient age ($r = 0.795$, $P = 0.002$). However, there was no significant correlation between HHV-6B and *IL-18*

Table 2. Genotype and Allele Frequency Distribution of IL-18 rs1946518 Polymorphism in Hodgkin Lymphoma Patients and Controls (Based on a representative subset of samples)

Genotype/Allele	Patients No. (%)	Control No. (%)	Z-statistics	p-value	OR (95% CI)
Genotype					
CC	4 (20%)	5 (50%)	Reference		
CT	4 (20%)	2 (20%)	0.83	0.4	0.4 (0.04-3.42)
TT	12 (60%)	3 (30%)	1.72	0.04	0.2 (0.03-1.24)
Allele Frequency					
C	12 (30%)	12 (60%)	Reference		
T	28 (70%)	8 (40%)	2.18	0.02	0.28 (0.09-0.87)

Note: Genotype and allele frequency analysis was conducted on a representative subset of samples due to DNA quality and sequencing validation requirements. Percentages are calculated within each group.

Table 3. Spearman's Rho Correlation Analysis of Study Variables

Variable	Age groups (years)		IL-18 rs1946518		Gender		HHV-6B	
	R	P	R	P	R	P	R	P
HHV-6B	0.795**	0.002	0.875**	0.004	0.185	0.07	—	—
IL-18 rs1946518	0.843**	0.005	—	—	—	—	—	—
Gender	0.847**	0.006	—	—	—	—	—	—
Age groups (years)	0.125	0.07	−0.862	0.007	0.123	0.512	0.145	0.034

** Correlation is significant at the 0.01 level (2-tailed)

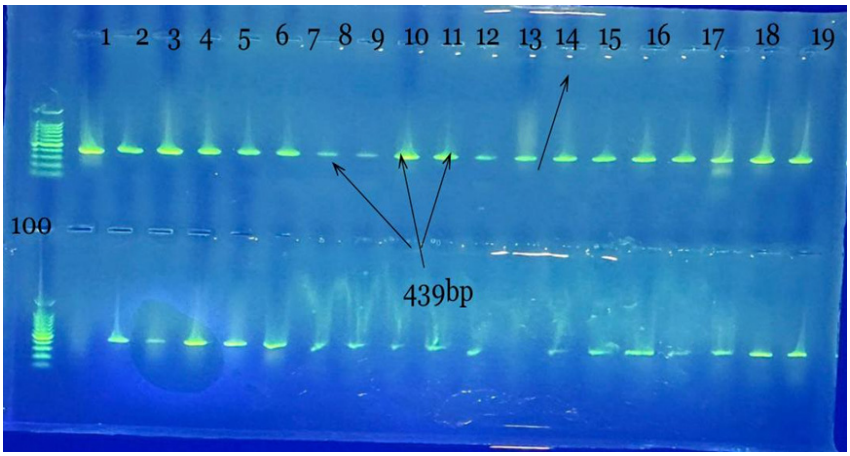

Figure 2. *IL-18 rs1946518* Gene Amplified Product Analysis by Gel Electrophoresis

rs1946518 SNP following a stratification of both genders (r = 0.185, P = 0.07).

Discussion

Hodgkin lymphoma is a complex malignancy characterized by the malignant transformation of germinal center B cells and the presence of Hodgkin–Reed–Sternberg (HRS) cells [7, 8]. Its multifactorial etiology involves genetic susceptibility, environmental influences, and viral infections, among which human herpesvirus-6 (HHV-6) has attracted increasing attention. In the present study, HHV-6B DNA was detected in 25.6% of HL patients, a finding that falls within the wide range of detection rates reported in previous studies. HHV-6 preferentially infects $CD4^+$ T lymphocytes, where it can persist in a latent state or integrate into the host genome [9]. Viral proteins such as pDR7 and pU94 regulate oncogenic pathways, including interference with Ras-mediated transformation, indicating that HHV-6 is contributory to lymphoid and other neoplasms, such as HL, non-Hodgkin lymphoma and acute lymphoblastic leukemia [10]. In this regard, HHV-6 DNA has been detected in lymphoma tissue including lymphocytes, histiocytes and occasionally HRS cells, with reported prevalence ranging from 16.6% to over 70%, based on the subtype of disease and methods of detection [11].

Our findings extend to the existing evidence by showing that HHV-6B infection is significantly associated with the *IL-18 rs1946518* polymorphism, suggesting that viral infection and host immunogenetic susceptibility in HL pathogenesis was interact synergistically to cause the disease, rather than independent effects. A previous study has indicated inconsistent prevalence rates of HHV-6 among HL depending on the detection technique used like PCR or in situ hybridization [11]. Eliassen et al. [11] identified HHV-6 DNA in approximately 54% of HL cases, predominantly HHV-6A with occasional HHV-6B co-infection, and our findings placing within the range of epidemiological expectations.

IL-18 is a pro-inflammatory cytokine involved in tumorigenesis, angiogenesis, metastasis and immune evasion and several of IL-18 gene polymorphisms associated with cancer susceptibility [12]. In this study, HL patients had a higher frequency of TT genotype and T allele of the *IL-18 rs1946518*, while the C allele had a higher frequency among controls, suggesting a potential protective effect. These findings are consistent with previous reports of associating IL-18 polymorphisms with lymphoma risk and outcome including B-cell non-Hodgkin, diffuse large B-cell and follicular lymphoma, where elevated IL-18 levels have been reported at diagnosis [13].

Mechanistically, IL-18 has been implicated in lymphoma progression through its effects on cell proliferation, apoptosis inhibition, and resistance to anti-tumor therapies by regulating genes such as c-myc, BCL-2, TP53, and BAX [14]. Furthermore, IL-18-mediated activation of inflammasome pathways may exacerbate tumor progression and therapeutic resistance. The strong positive correlation observed between HHV-6B presence and *IL-18 rs1946518* polymorphism in this study suggests that individuals carrying specific IL-18 variants may be more susceptible to HHV-6B infection or may experience enhanced inflammatory responses following viral persistence, thereby contributing to HL development.

Limitations and Future Directions

It should be noted that this study has several limitations. The case-control design and the small size of the sample do not allow definitive conclusions regarding the issues of cause and effect. Moreover, it only concentrated on the HHV-6B whereas HHV-6A has also been showed associated with the pathogenesis of HL. Further research is necessary with greater and more heterogeneous groups of patients, and both forms of HHV-6. The HHV-6B infection and IL-18 polymorphisms at rs1946518 should be followed up over a long period to establish whether the polymorphisms contribute to treatment and general survival rates of HL patients. Moreover, the molecular pathogenesis of the identified associations could be studied through functional studies, which would lead to important information about the pathogenesis of the disease.

Clinical Implications

The therapeutic implications of HL management in the conclusions of the study may have therapeutic implications. The possibility exists that the risk classification and tailored treatment plans will become feasible due to the possibility of identifying patients having specific genetic variations and viral infections. Our capacity to understand the role of HHV-6B in pathogenesis of HL may also impact the development of the antiviral treatment options. The use of genetic variables may influence the manifestation of the disease in various age groups as the significant relationship between the age of the patient and the polymorphisms of *IL-18 rs1946518*. This finding highlights the need for further studies to explore the potential benefits of age-specific treatment approaches.

In conclusions, this study revealed that HHV-6B infection and the *IL-18 rs1946518* polymorphism are associated with Hodgkin lymphoma risk. HHV-6B positivity was significantly associated with the T allele/TT genotype, which were more prevalent among patients, while the C allele showed a protective effect, suggesting a synergistic interaction between viral and genetic factors in HL susceptibility. Incorporating IL-18 genetic markers with HHV-6B status may enhance risk stratification, although validation in larger multicenter and longitudinal studies is required to clarify their impact on treatment response and clinical outcomes and to support personalized management strategies.

Author Contribution Statement

A.R.A.A. designed the study and drafted the manuscript; A.Z.A. performed data analysis; Z.A.A. collected samples; S.H.M.A. supervised the research. All authors approved the final manuscript.

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Data Availability

Data supporting the findings are available from the corresponding author upon reasonable request.

Ethical Approval

The study was approved by the Ethical Committee of Ibn Sina University of Medical and Pharmaceutical Sciences (Approval No. M402322) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Conflict of Interest

The authors declare no conflicts of interest.

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