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Research Article

Association between IL1R1 rs2234650 Polymorphism in Patients with Acute Lymphoblastic Leukemia Infected with HHV-6A

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Abstract

Background: Everyone contracts the common infection known as human herpesvirus 6 (HHV-6). Particularly associated with encephalitis, human herpesvirus 6B (HHV-6B) poses a risk of morbidity and death to recipients of allogeneic hematopoietic stem cell transplants. IL-1β and interleukin-1α (IL-1α) are critical for enhancing infection resistance. ***Objective***: To ascertain whether HHV-6A and the *IL1R1* rs2234650 gene polymorphism are related and could be risk factors for Iraqi infants developing acute lymphoblastic leukemia (ALL). ***Methods***: This case-control study included 150 blood samples from 100 patients diagnosed with ALL and 50 from healthy subjects. The *IL1R1* rs2234650 gene polymorphism was identified using the Sanger sequencing method, and *HHV-6A* using PCR. ***Results***: Age group and sex were found to differ significantly. Of 100 samples, 32 percent had HHV6A. The polymorphism of the *IL-1R1* rs2234650 gene revealed that the distribution of DNA polymorphisms according to C\C, C\G, G\G, C\T, T\T, and G\T was 30%, 28%, 16%, 8%, 6%, and 12%, respectively, in patients with ALL and 33.33%, 26.67%, and 16.67%, respectively. 3.33%, 13.33%, and 6.67% in the group that was deemed to be in apparently healthy control (AHC). The frequency of the polymorphism's genotype distribution varied significantly between the patient and control groups. ***Conclusions***: HHV6A and *IL1R1* rs2234650 polymorphisms of ALL variations may be risk factors in the pathophysiology of ALL in Iraqi children.

Keywords: Acute lymphoblastic leukemia, Human herpesvirus 6-A**,** Interleukin-1 receptor type 1. rs2234650**,** PCR, Sequencing.

الارتباط بين IL1R1 rs2234650 تعدد الأشكال في المرضى الذين يعانون من ابيضاض الدم الليمفاوي الحاد المصابين ب HHV-6A

الخلاصة

الخلفية: يصاب الجميع بالعدوى الشائعة المعروفة باسم فيروس الهربس البشري 6 (HHV-6). يرتبط فيروس الهربس البشري 6B بشكل خاص بالتهاب الدماغ، ويشكل خطر الإصابة بالمراضة والوفاة لمتلقي عمليات زرع الخلايا الجذعية المكونة للدم الخبيثي. IL-1β و IL-1α ضروريان لتعزيز مقاومة العدوى. **الهدف**: للتأكد مما إذا كان تعدد الأشكال الجيني HHV-6A و rs2234650 *IL1R1* مرتبطين ويمكن أن يكونا عوامل خطر لدى الأطفاللعراقيين الذين يصابون بابيضاض الدم الليمفاوي الحاد (ALL). **الطرائق**: تضمنت دراسة الحالة والشواهد هذه 150 عينة دم من 100 مريض تم تشخيص إصابتهم بابيضاض الدم اللمفاوي الحاد و 50 من أشخاص أصحاء. تم تحديد تعدد الأشكال الجيني rs2234650 *IL1R1* باستخدام طريقة تسلسل Sanger ، و *HHV-6A* باستخدام تفاعل البوليميراز المتسلسل (PCR). **النتائج**: وجد أن الفئة العمرية والجنس يختلفان اختلافا كبيرا. من بين 100 عينة، كان 32 في المائة مصابين بفيروس HHV6A. كشف تعدد أشكال الجين rs2234650 *IL-1R1* أن توزيع تعدد أشكال الحمض النووي وفقا ل T\T ، C\T ، G\G ، C\G ، C\C ، و G\T كان 30٪ و 28٪ و 16 و 8 و 6 ٪ و 12٪ على التوالي، في المرضى الذين يعانون من ابيضاض الدم اللمفاوي الحاد و 33.33 و 26.67٪ و 16.67٪ على التوالي. 3.33٪ و 13.33٪ و 6.67٪ في المجموعة التي اعتبرت في حالة صحية على ما يبدو. تباين تواتر توزيع النمط الجيني لتعدد الأشكال بشكل كبير بين مجموعة المريض والمجموعة الضابطة. **الاستنتاجات**: قد تكون تعدد الأشكال HHV6A و rs2234650 *IL1R1* في ابيضاض الدم اللمفاوي الحاد من عوامل خطر في الفيزيولوجيا المرضية في ابيضاض الدم اللمفاوي الحاد لدى الأطفال العراقيين.

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INTRODUCTION

A common pathogen belonging to the subfamily β-herpesvirinae, which also contains HHV-7 and HCMV, is the human herpesvirus. The two HHV-6 species, HHV-6-A and HHV-6-B, differ from one another in terms of their genetic, immunological, and biological characteristics [1]. Childhood HHV-6B infection affects almost 90% of the general population [2]. Although it can infect bone marrow and peripheral mononuclear cells, HHV-6 preferentially replicates in CD4þT lymphocytes [3]. CD46 is a ubiquitous membrane cofactor protein that HHV-6A employs as a receptor [4]. It was recently discovered that CD134 is an entrance receptor unique to HHV-6B [5]. All herpesviruses reside as latent episomes in tolerant host cells. Depending on a few variables, the viral lifecycle may develop into a

productive infection that causes the host cells to lyse. New research suggests that about 1% of people may be affected by HHV-6. This virus can join the human genome and be passed down in a Mendelian way when it does this in germ cells [6]. Immunocompromised individuals, such as those undergoing chemotherapy or transplantation, may have HHV-6 reactivation [7]. To manage the primary infection of HHV-6 and its reactivation, cellular immune responses are crucial. However, research into the immunological response to HHV-6 infection has been relatively limited [6]. Studies indicate that there is a T-cell proliferative response to HHV-6 infection, highlighting T-cell role in the immune response against this virus [8]. CD4 and CD8 T lymphocytes specific to HHV-6 have been described in several studies [9]. Recent research suggests that HHV-6 may make regulatory T cells to avoid antiviral immune responses and keep infected hosts' immune systems weak and dormant [10]. The peripheral blood of healthy people has been used to characterize most T-cell responses to HHV-6. Nonetheless, immunological responses are weakened in B-ALL patients. Additionally, leukemia treatment may result in severe immunosuppression for a few weeks, which raises the possibility of HHV-6 reactivation in blood and saliva [8-11]. Interleukin-1 (IL-1) was discovered in the late 1970s and is referred to as "the master cytokine of inflammation." This cytokine has since been extensively studied in connection with several physiological functions and diseases [12]. Notable members of the IL-1 family include IL-1α and IL-1β, two cytokines that bind to the same receptor. The primary role of IL-1 is to mediate systemic and local inflammatory responses, which help to protect against infections. When IL-1 is dysregulated, however, tissue damage and inflammation increase. IL-1 is also a key part of both "homeostatic" and "emergency" hematopoiesis, which lets responses change depending on the need, like when there is an infection [11]. There are approximately three situations in which the IL-1 pathway has been demonstrated to be implicated in cancer patients. First, the pathophysiology of both IL-1β β and IL-1α α is associated with certain solid tumors and hematological malignancies. In leukemic stem cells (LSCs), innate cytokine signaling is increased and not working properly, and antitumor immunity is weakened, among other problems. Furthermore, following anticancer treatment, IL-1 might promote potentially advantageous immune responses; this process is known as immunogenic cell death. This emphasizes how intricate immune response is in relation to cancer treatment. 1.5 seconds after receiving severe chemotherapy and radiation therapy, IL-1 plays a part in the development of cancer cachexia and treatment-related adverse effects, such as intestinal mucositis and cardiotoxicity. Finally, IL-1 and other dysregulated proinflammatory responses are a big part of the inflammatory side effects that can happen after a stem cell transplant, such as sepsis, acute lung injury, and GVHD [13]. These advancements are not only contributing to improved outcomes in the treatment of intestinal mucositis and also enhancing overall patient care, anthracycline-induced cardiotoxicity, and cancer cachexia, but there has also been a notable decrease in the initiation and spread of hematological malignancies, particularly multiple myeloma [14]. By competitively binding to *IL-1R1*, *IL-1Ra* inhibits the activities of IL-1α and IL-1β. Monocytes and macrophages produce IL-1Ra, which the liver then synthesizes during the acute phase response. In inflammatory processes, it acts as a regulator of negative feedback [15]. This study aimed to determine the association between HHV-6A and the IL1R1 rs2234650 gene polymorphism as potential risk factors for the onset of acute lymphoblastic leukemia (ALL) in Iraqi children.

METHODS

Study design and setting

This multi-center case-control study includes 100 patients with ALL with ages between 2 and 12 years who are clinically diagnosed by specialist physicians between January 2024 and December 2024 in the Oncology Department at Imam Al-Sadiq Teaching Hospital, the Iraqi Hematology Center, Baghdad Teaching Hospital, Medical City Center, and other hospitals. It also includes 50 apparently healthy people (AHC) with ages between 2 and 12 years without any history of ALL as a control group. Information for both patients and control groups regarding age, gender, and history was obtained from medical records. About 5 ml of blood were collected and placed in suitable, clean, and sterile EDTA as well as gel tubes and immediately refrigerated until viral genome and human DNA extraction.

Human DNA extraction

The extraction of genomic DNA was conducted for each study subject. First, blood samples were lysed and mixed with buffer containing RNases and proteases K; then, the lysates were transferred to spin columns and treated with specific buffers to enable efficient genomic DNA binding. Finally, the DNA was eluted after washing by using the G-Spin Total DNA Extraction Kit (iNtRON Biotechnology Co., Korea) and following the instructions of the manufacturer. This extraction process was performed in the laboratory of the Faculty of Science, Babylon University.

Primer selection

The detection of *IL1R1* rs2234650 gene polymorphism by using the primer sequence

F- “5′ TGTTCCTGCTAAGGTGGAGG 3′ ” . R- “5′ AGGTTCCCTATCAAGTTTCACCA 3′ .” With PCR product (480 bp).

PCR analysis

The conventional thermal cycler (Germany) was used for PCR amplification; the total volume was

about 25 µL, which included 12.5 µL of master mix, 5 µL of DNA template, 1.0 µL of each forward and reverse primers, and 5.5 µL of nuclease-free water, as described in Table 1.

Table 1: concentrations and volumes for PCR reaction

No.	Contents of PCR Reaction Mixture	Volume (µl)
1	Master mix	12.5
2	Template DNA	5
3	Forward primers	1.0
4	Reverse primers	1.0
5	Nuclease free water	5.5
Total		25

Detection of HHV-6A genome

The condition summarized in Table 2 was used to conduct the PCR amplification procedure. After completing the amplification process, an agarose gel (1.5%) was used for the gel electrophoresis, and a transilluminator was used for analysis.

Table 2: The PCR conditions used for genome amplification

Initial denaturation	Denaturation	Annealing	Extension	Final extension
95 ºC/5 min	95 ºC/ 1.0 min	59 ºC/45 sec 40 cycles	72 ºC/2 min	72 ºC/5min

Extraction and amplification of HHV-6A

Using a specific viral DNA/RNA extraction kit (Intron/Korea), the viral genome was extracted, purified, and migrated using agarose gel from the blood specimens as a first step to amplify the target Human Herpes virus-6A-DNA with the primers F-"5′ TGTTCCTGCTAAGGTGGAGG 3′" and R-"5′ AGGTTCCCTATCAAGTTTCACCA 3′." To perform the PCR, each 25 µl of reaction mixture contains 12.5 µl master mix, 6.5 µl distilled water, 5 µl of DNA, and 2 µl of each forward and reverse primer. The following conditions were used: 95 ºC for 5 min, 95 ºC for 2 min, 54 ºC for 45 seconds, 72 ºC for 1 min for 40 cycles, and 72 ºC for 5 min.

Ethical considerations

The present study followed the principles of the Declaration of Helsinki. Before sample collection, verbal and written consent was obtained from the patients. A local ethics commission reviewed and approved the study protocol, consent form, and subject information on April 10, 2024, under project number M240401.

Statistical analysis

SPSS version 24 software was used to analyze the genotypes and allele frequencies of the *IL1R1* rs2234650 SNP polymorphism between patients with AML and control groups. To figure out how likely it is that a polymorphism will cause ALL or HHV6A infection, we used the ANOVA and LSD tests to find the significant differences between the IL1R1 rs2234650 SNP polymorphism. A p-value of less than 0.05 was considered statistically significant. The odds ratios were also calculated.

RESULTS

The characteristics of the 100 patients with ALL and 50 healthy control groups are summarized in Table 3.

Sequencing

The PCR products of 50 patients with ALL and 30 AHC were sequenced via automated sequencing utilizing the PCR forward (F) primer as the sequencing primer. Sequencing was conducted at Macrogen Company in Geumcheon, Seoul, South Korea, and the sequences were analyzed using Geneious Bioinformatics software version 2 for sequence data analysis and alignment, relying on the DNA sequences available in the NCBI reference database.

Table 3: Clinicopathological Characteristics of ALL Patients with healthy control groups

Variables		patients	Control	*p-value*
Age (year)	Median Range	7.3 (2-12)	6.2 (2-12)	0.06
Sex	Male Female	44 56	20 30	0.04

The mean ages of ALL patients and the control group were 7.3±12.9 years and 6.2±12.6 years, respectively. A non-significant difference was found (p= 0.06) between patients and control according to ages. Among the 100 ALL patients (44 males and 56 females), there is a significant difference between patients according to the ALL gender (p= 0.04). A single band (480 bp) of the target sequence of the *IL-1R* rs2234650 gene was found in the agarose gel (**Figure 1**) after the genomic DNA was amplified using particular primers for *IL-1R* rs2234650 genotyping.

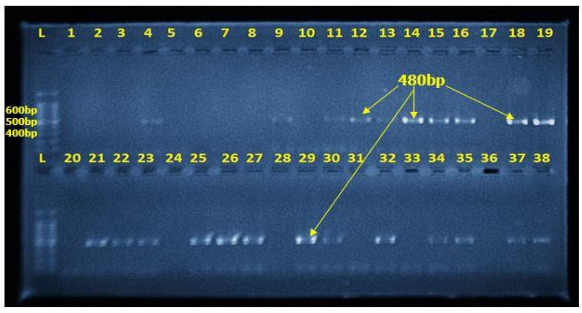

Figure 1: Agarose gel electrophoresis of an amplified product patterns of *IL-1R1 rs*2234650 *located on Chromosome 2 in* exon4 region. Electrophoresis conditions, 1.5% agarose, 85 V, 20 mA for 1h (5 µl in each well), stained with red safe solution.

To sum up the results from the sequenced 480 bp fragments, Table 4 shows the exact locations of the differences that were seen in the NCBI reference sequences. For the IL-1R1 rs2234650 gene polymorphism, the DNA polymorphism distributions for C\C, C\G, G\G, C\T, T\T, and G\T were 30%, 28%, 16%, 8%, and 12%, respectively, in patients with ALL and 33.33%, 26.67%, 16.67%, 3.33%,

13.33%, and 6.67%, respectively, in the group that seemed to be healthy.

Table 4: Genotype and allele frequency of rs2234650 in ALL patients and controls.

Conformational Polymorphism of *IL-1 R1rs1419620062*	Type of Mutation	Study group		OR	*P value*
		ALL n=50	AHC n=30		
C\C	Transition	15(30)	10(33.33)	0.74 (0.40-1.94)	0.06
C\G	Transversion	14(28)	8(26.67)	1.04 (0.75-2.87)	0.04
G\G	Transition	8(16)	5(26.67)	0.64 (0.50-1.64)	0.04
C\T	Transition	4(8)	1(3.33)	0.85 (0.90-2.76)	0.04
T\T	Transition	3(6)	4(13.33)	0.43 (0.33-1.22)	0.06
G\T	Transversion	6(12)	2(6.67)	0.95 (1.40-2.99)	0.03
TOTAL		50(100)	30(100)		
ALLELE					
C		35	17		0.4
G		65	23		

Values were expressed as frequency and percentage.

The difference in frequency of genotype distribution of the polymorphism between patients and control groups was statistically significant (Table 4). As shown in Table 5 and **Figure 2**, the positive result for amplification detection of HHV-6A by PCR technique in samples from patients with ALL was 27% (27 out of 100 cases), while the negative result was 73% (73 out of 100 cases).

Table 5: The positive results of PCR for HHV-6A- infection in patients with AL

HHV6A	n(%)	*p*- value
Positive	27(27)	0.03
Negative	73(73)	
Total	100(100)	

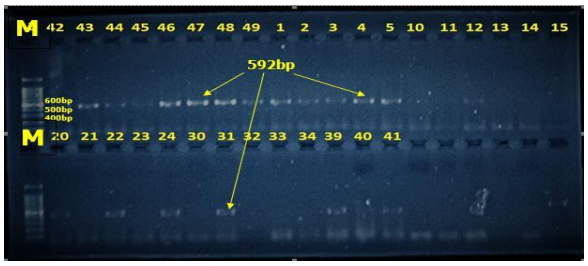

Figure 2: PCR detection of the HHV-6A gene; band (592pb) molecular size in ALL patients. M: A 100–1100 bp DNA ladder. After migrating into 2% agarose at 75V and 20 mA for 120 minutes, the PCR-amplified products were stained with ethidium bromide and placed in 15 µl per well.

Table 6 displays the HHV-6A-DNA detection results by sex for patients with ALL. Males made up 38.6% (17 out of 44) of the ALL cases, while females made up 17.8% (10 out of 56). According to statistics, there were notable differences between the sexes of patients with ALL who had positive PCR results for HHV-6A-DNA detection (*p*= 0.04).

Table 6: Percentage of HHV-6A infection in patients with ALL according to their sex

Patients with ALL	n(%)	HHV-6A Infection	
		Positive n(%)	Negative n(%)
Male	44(44)	17(38.6)	27(61.4)
Female	56(56)	10(17.8)	46(82.2)
p-value		0.04	

The association between HHV-6A and SNP *IL-1R1* (rs2234650) in ALL was found to be very significant and to be strongly positive (r= 0.797, *p*= 0.06). Also, a strong positive correlation was found between IL-1R1 (rs2234650) SNPs and the age of ALL patients (r= 0.927, *p*= 0.01). However, as shown in Table 7, there were no significant associations between HHV-6A and *IL-1R1* SNPs (rs2234650) based on the sex of ALL patients in the current investigation.

Table 7: Spearman's Rho Statistical Testing for Age, Sex, HHV-6A, and *IL-1R1* (rs2234650) SNPs in the Population Groups under Study

Spearman's rho		Age group (year)	*IL-1R1* rs*2234650*	Sex	HHV-6A
HHV-6A	r	0.927	0.797	0.175	
	p	0.01	0.06	0.8	
IL-1R1rs2234650	r	0.856			
	p	0.03			
Sex	r	0.825			
	p	0.04			
Age groups (year)	r	0.255	-0.449	0.345	0.237
	p	0.09	0.08	0.07	0.06

DISCUSSION

The weakened immune systems of leukemia patients allow HHV-6 to reactivate [16]. Using the amplified detection of HHV-6A by PCR technology, the current study showed that 27% of the samples from patients with ALL had positive results, whereas 73% had negative results. HHV-6 has been detected and quantified in the blood of B-ALL patients in the past, and the results showed that HHV-6 is more prevalent during remission than at the time of the leukemia's initial diagnosis. These findings are in line with HHV-6 reactivation in immunocompromised patients after treatment. Previous studies indicated that HHV-6 was present in 20% of B-ALL patients, compared to 8% and 12% of healthy people [17-19]. It has previously been noted that a small percentage of ALL patients had low HHV-6 virus levels [20,21]. One (P451) of these eleven samples exhibited an HHV-6 copy number that was in line with ciHHV-6 [18]. Geographical location, sample size, and illness state all affect the prevalence of ciHHV-6, which ranges from 0.2 to 3% [22]. However, when only studies with a sample size larger than 500 people are

considered, the incidence of ciHHV-6 in healthy adults from the United States or the United Kingdom is approximately 1%. HHV-6B is the integrated virus in two-thirds of ciHHV-6 infections [23]. Based on the evidence we have, there is no link between HHV-6 levels in the blood and levels of pro- and anti-inflammatory cytokines. This means that HHV-6 is not significantly linked to the development of B-ALL or involved in its pathogenesis. [23]. A study of the immune response to HHV-6 showed that people with B-ALL had a low number of T cells that were specific for the virus. On the other hand, all patients who are seropositive have CD4½ T cells that are specific to the virus. The frequencies in healthy people and long-term stable transplant recipients are about the same at replication (1.70%, range 0.14%–40.96%) as they are in healthy people [24]. Similarly, it was found that healthy donors had 0.04%–0.12% of CD4þ T cells that selectively responded to HHV-6 viral antigens (mean 0.06–0.07%) [25]. The low frequency of HHV-6-specific T cells in our study could be related to the way they were measured. It's also possible that viral antigens didn't properly activate T lymphocytes that are specific to HHV-6 because they weren't stimulated or presented properly. Conversely, chronic viral replication in the blood may change certain T cells' functional characteristics and decrease their reactivity [25]. Due to an increased production of anti-inflammatory cytokines, patients with ILRN2 have been linked to a bad result. Infections are the primary cause of morbidity and mortality in more than 70% of children with juvenile acute lymphoblastic leukemia. DNA polymorphism distributions according to C\C, C\G, G\G, C\T, T\T, and G\T were 30%, 28%, 16%, 8%, 6%, and 12%, respectively, in patients with ALL and 33.33%, 26.67%, 16.67%, 3.33%, 13.33%, and 6.67%, respectively, in the AHC group, according to the results of the *IL-1R1* rs2234650 gene polymorphism. There was a statistically significant difference in the frequency of the polymorphism's genotype distribution between the patient and control groups. [26]. Steven *et al*. reported the genotype distribution to be 10.5% for homozygote IL-1RN2, 50.9% for homozygote IL-1RN1, and 38.6% for heterozygote ILRN1/ILRN2 [27]. Even when age, gender, and leukemia risk were taken into account, patients who were homozygote IL1RN2/IL1RN2 and heterozygote ILRN1/ILRN2 had a much higher chance of showing signs of septic shock (p= 0.001). Similar to this, the study shows a link between ITP vulnerability and the rs16944 IL-1B polymorphism's mutant homozygous (GG) and heterozygous (AG) genotypes. Additionally, children with the mutated G allele were 4.6 times more sensitive to ITP. Yadav *et al*. discovered that severe ITP was substantially linked to the homozygous mutant genotype of IL-1B31 when compared to healthy controls in primary ITP [28]. Additionally, the IL-B-31 mutant allele was associated with a significant risk of severe ITP, but the IL-1B-511 genotype was not associated with either severe or non-severe ITP. The current study found that the IL-1RA polymorphism was much more common in cases compared to the control group. This was true for heterozygous, mutant homozygous, and mutant allele-containing genotype variants. Additionally, patients had a significantly higher prevalence of the mutant allele of the IL-1Ra polymorphism than did controls. By analogy, patients had significantly higher rates of mutant genotype and allele than controls [29,30]. Additionally, our results are in line with previous studies [31,32] that linked ITP to a polymorphism of an IL-1R antagonist. El Amawy and Shahin [31] also conducted a case-control study on IL1B polymorphisms (IL-1B-31, IL-1B-511) and IL1RA SNPs. The genotype distributions or alleles of IL-1B-511, IL-1B-31, and IL-1Ra exhibit a strong association with ITP. Elsaadany *et al.* [32] looked at how IL-1B genetic variants affected Egyptian newborns' development of primary ITP. Another possible confounder is the redundancy in the immune system, where previous studies have shown that the *IL-1R1* gene not only affects the production of *IL-1R1* but also the *IL-1α* levels and that the gene coding for *IL-1β* also may affect the *IL-1Ra* production. The clinical utility of assessing gene variants in innate immune molecules is also hampered by many different outcome measurements concerning children with ALL [33].

Study limitations

The study design and the methods of data analysis may include a few limitations.

Conclusion

HHV6A and *IL1R1* rs2234650 polymorphisms of ALL variations may be risk factors in the pathophysiology of ALL in Iraqi children.

Conflict of interests

The authors declared no conflict of interest.

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The authors did not receive any source of funds.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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