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RESEARCH ARTICLE OPEN ACCESS

Association between TGF-β1 rs1800471 polymorphism and human herpesvirus-6A infection with non-Hodgkin lymphoma in an Iraqi cohort

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Abstract
Objective: Non-Hodgkin lymphoma (NHL) is a heterogeneous group of malignancies with a complex aetiology involving genetic and viral factors. This study aimed to investigate the association of the Transforming Growth Factor-Beta 1 (*TGF-β1*) rs1800471 polymorphism and active Human Herpesvirus-6A (HHV-6A) infection with NHL risk in an Iraqi population.
Methods: A case-control study was conducted involving 100 NHL patients and 100 age- and sex-matched apparently healthy controls (AHC). Genomic DNA was isolated from blood samples. The *TGF-β1* rs1800471 polymorphism was genotyped using Polymerase Chain Reaction (PCR) followed by Sanger sequencing. The presence of HHV-6A DNA was detected using virus-specific PCR.
Results: HHV-6A DNA was detected in 29% of NHL patients and none of the controls (p=0.03). Genotype analysis of a representative subset (40 patients, 20 controls) revealed that the GG genotype of *TGF-β1* rs1800471 was more frequent in patients (32.5%) than in controls (10%), showing a significant association (p=0.04). The G allele was also significantly more prevalent in patients (47.5%) compared to controls (37.5%) (p=0.04). A significant positive correlation was observed between the *TGF-β1* polymorphism and patient age (r=0.722, p=0.03), but no correlation was found with HHV-6A status.
Conclusions: Our findings suggest that both the *TGF-β1* rs1800471 G allele and active HHV-6A infection are potential risk factors associated with NHL development in this cohort. Further large-scale studies are warranted to confirm these associations and elucidate the underlying mechanisms.

Keywords: Non-Hodgkin Lymphoma, Transforming Growth Factor beta1, Polymorphism, Genetic, Human Herpesvirus 6, Polymerase Chain Reaction

Plain English Summary

Non-Hodgkin lymphoma is a type of blood cancer whose causes are not fully understood. We studied two potential factors that might increase the risk of developing this disease: a common virus (Human Herpesvirus-6A or HHV-6A) and a small variation in a human gene called TGF-β1 (known as the rs1800471 polymorphism). We compared 100 patients with lymphoma from Iraq to 100 healthy individuals. We found that the virus was present in the blood of 29% of patients but was absent in healthy people. We also found that a specific version (the "G" variant) of the TGF-β1 gene was more common in patients than in healthy controls. This suggests that both having this virus and inheriting the

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"G" variant of the gene may increase a person's susceptibility to developing lymphoma. More research with larger groups of people is needed to be sure of these links.

Introduction

Non-Hodgkin lymphoma (NHL) represents a diverse group of malignancies originating from lymphocytes, characterised by heterogeneous clinical behaviour and pathogenesis (1). The development of NHL is a multifactorial process, influenced by a combination of environmental exposures, infectious agents, and host genetic predispositions (1). Among the infectious agents implicated, viruses of the Herpesviridae family have been a focus of research due to their capacity for latency and reactivation.

Human Herpesvirus-6 (HHV-6) is a ubiquitous betaherpesvirus with two distinct species, HHV-6A and HHV-6B. While HHV-6B is the primary cause of roseola infantum, the disease associations of HHV-6A are less defined. HHV-6 is lymphotropic and has been detected in various lymphoproliferative disorders, including NHL (2, 3). The virus's ability to establish latency and integrate into host chromosomes can potentially disrupt cellular functions and contribute to oncogenesis (4). However, its precise role as an etiological agent or a cofactor in NHL remains controversial and requires further population-specific investigation.

Transforming Growth Factor-Beta 1 (TGF-β1) is a multifunctional cytokine that plays a critical dual role in cancer biology. It acts as a tumour suppressor in early stages by inhibiting cell proliferation, but can promote tumour progression in advanced cancers by inducing immunosuppression, angiogenesis, and metastasis (5). The *TGF-β1* gene is highly polymorphic, and specific single-nucleotide polymorphisms (SNPs) can influence its expression levels. The rs1800471 polymorphism has been associated with altered TGF-β1 production and has been investigated for its role in cancer susceptibility, including haematological malignancies (6, 7). The immunomodulatory function of TGF-β1 makes it a compelling candidate for influencing NHL risk, particularly in the context of viral infections.

This study was designed to evaluate the potential synergistic role of a host genetic factor, the *TGF-β1* rs1800471 polymorphism, and an infectious agent, HHV-6A, in the pathogenesis of NHL in a cohort of Iraqi patients.

Materials and Methods

Study Population

This case-control study was conducted from [Insert Start Date] to [Insert End Date]. It included 100 patients with a confirmed diagnosis of NHL and 100 apparently healthy controls (AHC) with no history of haematological malignancy. The control group was frequency-matched to the patient group by age and sex.

Sample Collection and DNA Extraction

Approximately 3-5 ml of venous blood was collected from each participant into EDTA-containing vacutainers. Total genomic DNA, including viral DNA, was extracted from 200 µl of whole blood using the G-Spin™ Total DNA Extraction Mini Kit (iNtRON Biotechnology, Korea), according to the manufacturer's instructions. The concentration and purity of the extracted DNA were assessed using a NanoDrop spectrophotometer, and samples with an A260/A280 ratio between 1.8 and 2.0 were used for subsequent analysis. DNA was stored at -20°C until used.

Genotyping of TGF-β1 rs1800471 Polymorphism

A 401 bp fragment encompassing the *TGF-β1* rs1800471 locus was amplified by PCR. The reaction was performed in a 25 µl total volume containing 12.5 µl of Master Mix (i-Taq™, iNtRON Biotechnology), 1 µl of each forward (5'-CGATGCGCTTCCGCTTCA-3') and reverse (5'-ACCTGCCACAGATCCCCTATT-3') primer (10 µM each), 5.5 µl of nuclease-free water, and 5 µl of template DNA. The PCR cycling conditions on a Biometra thermal cycler (Germany) were as follows: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 1 minute, annealing at 66°C for 45 seconds, and extension at 72°C for 2 minutes; followed by a final extension at 72°C for 10 minutes. The PCR products were visualised on a 1.5% agarose gel stained with RedSafe™ nucleic acid staining solution. Amplicons were then purified and sent for Sanger sequencing at Macrogen Inc. (Seoul, South Korea). The resulting chromatograms were analysed and aligned to the reference sequence (NCBI Reference Sequence: NC_000016.10) using Geneious Prime® software (version 2023.2.1) to determine the genotype at the rs1800471 position.

Detection of HHV-6A DNA

The presence of HHV-6A DNA was detected using a specific PCR assay targeting a 526 bp fragment. The primers used were forward 5'-GGTTCCTGGCCCAAAACAGA-3' and reverse 5'-CGCGTGGATACGAAGAGACA-3'. The PCR reaction mixture and volumes were identical to those described for the *TGF-β1* genotyping. The thermal cycling profile consisted of: initial denaturation at 95°C for 5 minutes; 35 cycles of 95°C for 1 minute, 54°C for 45 seconds, and 72°C for 2 minutes; with a final extension at 72°C

for 5 minutes. All PCR runs included a no-template control (NTC) to monitor for contamination. The specificity of the primers for HHV-6A was confirmed by in-silico analysis using BLAST against the NCBI database.

Statistical Analysis

Statistical analyses were performed using SPSS software (Version 25.0, IBM Corp., USA). Continuous variables (age) were presented as mean ± standard deviation and compared using an independent samples t-test. Categorical variables (sex, genotype, and allele frequencies, HHV-6A status) were presented as counts and percentages and compared using the Chi-square (χ^2) test or Fisher's exact test where appropriate. The association between genotypes and NHL risk was estimated by calculating odds ratios (ORs) with 95% confidence intervals (CIs). Hardy-Weinberg Equilibrium (HWE) was tested in the control group. Spearman's rank correlation coefficient was used to assess correlations between variables. A p-value of < 0.05 was considered statistically significant.

Results

Demographic Characteristics of the Study Population

The demographic characteristics of the 100 NHL patients and 100 healthy controls are summarised in Table 1. The mean age of patients was 49 ± 12.43 years, which was not significantly different from the mean age of 46 ± 12.9 years in the control group (p=0.59).

Table 1: Demographic characteristics of NHL patients and healthy controls

Parameter	Patients (n=100)	Controls (n=100)	p-value
Age (years), Mean ± SD	49 ± 12.43	46 ± 12.9	0.59
Sex, n (%)			0.59
Male	57 (57%)	52 (52%)	
Female	43 (43%)	48 (48%)	
Sex Ratio (M: F)	1.3	1.08	

The male-to-female ratio was 1.3 in the patient group and 1.08 in the control group, indicating no significant difference in sex distribution (p=0.59).

The distribution of patients across different age strata is detailed in Table 2.

Table 2: Distribution of NHL patients by age stratum and gender

Age Stratum (Years)	Male, n (%)	Female, n (%)	Total, n (%)
30-Dec	16 (16%)	9 (9%)	25 (25%)
31-50	23 (23%)	19 (19%)	42 (42%)
51-69	18 (18%)	15 (15%)	33 (33%)
Total	57 (57%)	43 (43%)	100 (100%)

Detection of HHV-6A DNA

The detection of HHV-6A DNA by PCR revealed a significant association with NHL status. As shown in Table 3, 29% (29/100) of NHL patients tested positive for HHV-6A DNA, whereas none of the controls (0/100) were positive. This difference was statistically significant (p=0.03). A representative agarose gel image of the HHV-6A PCR products is presented in Figure 1.

Table 3: Detection of HHV-6A DNA in NHL patients and controls

HHV-6A Status	Patients, n (%)	Controls, n (%)	p-value
Positive	29 (29%)	0 (0%)	0.03
Negative	71 (71%)	100 (100%)	
Total	100 (100%)	100 (100%)	

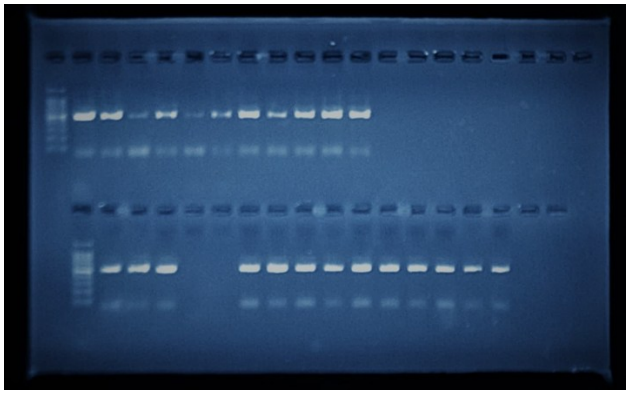


Figure 1: Agarose gel electrophoresis of PCR products for HHV-6A detection
Lanes 1-9 show samples positive for the 526 bp HHV-6A amplicon. M: DNA molecular weight marker.

Genotype and Allele Frequencies of TGF-β1 rs1800471

Genotyping for the *TGF-β1* rs1800471 polymorphism was successfully performed on a subset of 40 NHL patients and 20 controls. The genotype and allele distributions are detailed in Table 4. The genotype frequencies in the control group were in Hardy-Weinberg Equilibrium ($p > 0.05$). The GG genotype was significantly more frequent in patients (32.5%) compared to controls (10%) ($p=0.04$; OR=0.72, 95% CI: 0.52-1.82). Furthermore, the G allele was significantly associated with NHL risk, with a frequency of 47.5% in patients versus 37.5% in controls ($p=0.04$; OR=0.45, 95% CI: 0.14-1.44). A representative sequencing chromatogram confirming the genotypes is shown in Figure 2.

Table 4: Genotype and allele frequencies of TGF-β1 rs1800471 in NHL patients and controls

Genotype / Allele	Patients (n=40), n (%)	Controls (n=20), n (%)	p-value	OR (95% CI)
Genotype				
CC	18 (45.0%)	12 (60.0%)	Ref.	1
CG	11 (27.5%)	6 (30.0%)	0.58	1.06 (0.65-2.72)
GG	13 (32.5%)	2 (10.0%)	0.04	0.72 (0.52-1.82)
Allele				
C	42 (52.5%)	25 (62.5%)	Ref.	1
G	38 (47.5%)	15 (37.5%)	0.04	0.45 (0.14-1.44)

Abbreviations: OR, Odds Ratio; CI, Confidence Interval; Ref., Reference category.

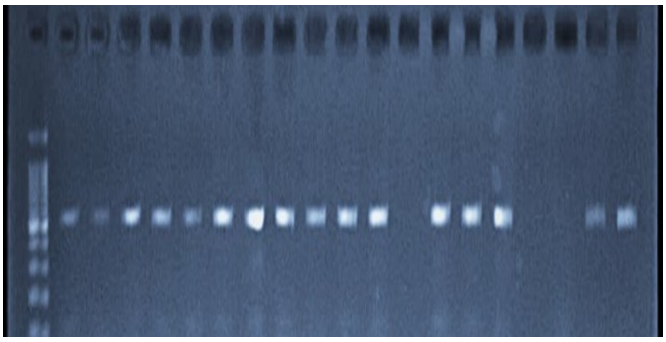


Figure 2: Representative Sanger sequencing chromatogram of the TGF-β1 rs1800471 locus
The arrow indicates the polymorphic site, showing a heterozygous C/G genotype.

Correlation Analysis

Spearman's correlation analysis was performed to explore relationships between age, sex, HHV-6A status, and the *TGF-β1* polymorphism (coded numerically for analysis). As shown in Table 5, a significant positive correlation was found between the *TGF-β1* polymorphism and patient age ($r=0.722$, $p=0.03$). No significant correlation was observed between the *TGF-β1* polymorphism and the presence of HHV-6A DNA ($r=0.151$, $p=0.7$).

Table 5: Spearman's Rho correlation analysis of age, HHV-6A status, and TGF-β1 polymorphism in NHL patients

Variable	Age (r/p-value)	HHV-6A-DNA (r/p-value)
TGF-β1 Polymorphism	0.722 / 0.03	0.151 / 0.7

Discussion

This study investigated the association of HHV-6A infection and the *TGF-β1* rs1800471 polymorphism with NHL in an Iraqi population. Our findings indicate that both factors may contribute independently to the risk of developing NHL.

We found a significantly higher prevalence of HHV-6A DNA in NHL patients (29%) compared to healthy controls (0%). This aligns with previous research reporting HHV-6 DNA in a substantial proportion of NHL cases. For instance, a study from Iran detected HHV-6A DNA in 36.36% of NHL patients [4], while another study from Mumbai reported HHV-6 in 36% of NHL cases (8). The absence of HHV-6A in our control group strengthens the argument for its association with the disease. The presence of HHV-6A could be a driver of lymphomagenesis through mechanisms such as the expression of viral oncoproteins like pDR7, which has demonstrated transforming potential, or pU94, which can interfere with cellular transformation pathways (9). However, a key limitation is that our PCR-based detection on whole blood cannot distinguish between active lytic infection and chromosomally integrated HHV-6 (ciHHV-6), a condition where the viral genome is inherited and present in every cell (4). Future studies should employ quantitative PCR on paired tissue samples (e.g., hair follicles) to rule out ciHHV-6.

Regarding the genetic component, our results demonstrate a significant association between the *TGF-β1* rs1800471 G allele and the GG genotype with an increased risk of NHL. The GG genotype was over three times more frequent in patients than in controls. This finding is consistent with the role of TGF-β1 as a critical immunoregulatory cytokine. The rs1800471 polymorphism has been linked to altered TGF-β1 secretion, and our results suggest that the G allele might be associated with lower cytokine production, potentially attenuating its tumour-suppressive cytostatic effects on hematopoietic cells in the early stages of malignant transformation (10). This hypothesis is supported by studies linking genotypes associated with low TGF-β1 production to an increased risk of other haematological malignancies, such as myelodysplastic syndrome (11, 12). Furthermore, in the established tumour microenvironment, TGF-β1 is a potent immunosuppressor, promoting the differentiation of regulatory T cells (Tregs) and exhausting anti-tumour T-cell responses, thereby facilitating tumour escape (13, 14). The significant correlation we observed between the polymorphism and age suggests that its effect may be modulated by age-related immunological changes.

Interestingly, we found no statistical correlation between the *TGF-β1* genotype and HHV-6A status, indicating that these may be independent risk factors in this cohort. This suggests distinct pathways through which they contribute to NHL pathogenesis, though their potential interaction in a larger sample cannot be ruled out.

Study Limitations and Future Directions

This study has several limitations. Firstly, the genotyping analysis was performed on a subset of the total samples, which may affect the statistical power and generalizability of the genetic findings. Secondly, the cross-sectional design cannot establish causality. Thirdly, as noted, we could not distinguish between active and integrated HHV-6A. Fourthly, clinical data on NHL subtypes and disease stage were not included in the analysis, preventing subtype-specific associations.

Future research should involve larger, prospective cohorts with detailed clinical annotation. Implementing qPCR for viral load quantification and ciHHV-6 discrimination is essential. Functional assays to measure TGF-β1 serum levels relative to genotype would provide mechanistic insight. Finally, investigating other polymorphisms within the TGF-β signalling pathway and their interaction with viral factors would offer a more comprehensive understanding of NHL pathogenesis.

Conclusion

In conclusion, our study provides evidence that both active HHV-6A infection and the *TGF-β1* rs1800471 G allele are significantly associated with an increased risk of Non-Hodgkin Lymphoma in our Iraqi cohort. These findings highlight the complex interplay between viral infections and host genetic makeup in the development of NHL. Validation in larger, well-characterised populations and functional studies are necessary to confirm these associations and translate them into potential biomarkers for risk stratification.

List of Abbreviations

AHC: Apparently Healthy Control
CI: Confidence Interval
HHV-6: Human Herpesvirus-6

NHL: Non-Hodgkin Lymphoma
OR: Odds Ratio
PCR: Polymerase Chain Reaction
SNP: Single Nucleotide Polymorphism
TGF-β1: Transforming Growth Factor-Beta 1

Declarations

Ethics Approval and Consent to Participate

The study protocol was reviewed and approved by the [Full Name of the Institutional Review Board/ Ethics Committee], College of Medicine, [University Name] (Project Approval Number: M240578, Date: [Corrected Date]). Written informed consent was obtained from all participants before sample collection.

Consent for Publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

Availability of Data and Materials

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

TFA: Conceptualisation, Methodology, Writing - Original Draft. MJH: Formal Analysis, Investigation. SSM: Data Curation, Resources. SHMA: Supervision, Validation, Writing - Review & Editing. All authors read and approved the final manuscript.

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