Iraqi Journal of Biotechnology, 2026, Vol. 25, No. 2, 171-187

The Expression and Genetic Polymorphisms of *IL-6* Gene Association with Hodgkin Lymphoma in A Sample of Iraqi Population

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Abstract

Background Lymphomas are broad tumors originating in the lymphatic system, causing lymph node enlargement. Hodgkin lymphoma (HL) is a type of blood cancers distinct from non-Hodgkin lymphomas. The *interleukin-6* (*IL-6*) gene is associated with various malignancies and can influence tumor growth and immune responses. **Aim** this paper aims to study the role of *IL-6* gene expression and polymorphisms in association with the increased incidence of Hodgkin lymphoma and the possibility of using this interleukin as a tool in diagnosis and treatment follow-up in patients with HL. **Methods** the study included 100 participants (50 HL patients and 50 controls), which collected from December 2023 to March 2024. Sex, age, family history, Complete Blood Count, and some kidney function tests were assessed for both studied groups. This study emphasizes the role of *IL-6* gene expression and polymorphisms, particularly the SNPs rs1800795 G/C, rs1800796 G/C, and rs1800797 G/A with HL incidence in the Iraqi population. **Results** showed varying differences between significant for family history smoking, hemoglobin, platelet, urea and creatinine and non-significant for other studied demographic and clinical data between HL patients and healthy controls. Moreover, the median fold change of *IL-6* gene expression ($2^{-\Delta\Delta Ct}$) revealed up-regulation in HL patients (1.23) concerning to the control, which was (1). According to studied SNPs, some genotypes and alleles appeared to have significant protective effects. In contrast, others appeared to be risk factors that play a significant role in increasing HL incidence. **Conclusion**, our investigation of an Iraqi Arab population revealed significant associations between the *IL-6* gene and HL.

Keywords: Hodgkin lymphoma (HL), Expression, Polymorphisms, *IL-6* gene, SNPs.

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Introduction

Cancer is a pathological condition characterized by uncontrolled cell division and the spread of abnormal cells to other organs (1). Cancer is now known to arise from DNA abnormalities, causing uncontrolled cell growth, and is classified by origin (2). Lymphomas are diverse neoplasms arising from the lymphatic system, resulting in lymphadenopathy (3). Malignant lymphomas are considered the third most common group of head and neck tumors, and they vary in malignancy (4). Additionally, the anticipated five-year overall the survival rate is almost 72%, and there is a positive tendency for improvement (5).

Dr. Thomas Hodgkin identified lymphoma in 1832 (6). The WHO classified lymphoma into two main categories: non-Hodgkin's accounts for 85% of instances, while Hodgkin's constitutes 15%(3). They are classified based on the type of lymphocyte—B-cell, T-cell, or NK cell—present (7). Non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) differ in clinical features (8). The rare cancer HL attacks the lymphatic system and lymph

nodes. It is diagnosed in individuals aged 15-30, with a higher rate in those 55 or older (9).

Classic Hodgkin Lymphoma (CHL) is classified into four different types: nodular sclerosing, mixed cellularity, lymphocyte rich, and lymphocyte deficient, and Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) (5)NLPHL is a type of lymphoma in which lymphocyte-predominant cells are present and Reed-Sternberg cells (RS) are absent. (9) HL subtypes differ in lymphoma cell morphology, immunophenotype, microenvironment, and clinical behavior (10). Hodgkin lymphoma can spread via blood vessels(11). mononuclear Hodgkin (H) and multinucleated (RS) cells are seen within HL in an inflammatory environment. This distinguishes HL from NHL lymphomas (6),(5). Moreover, RS cells have a unique immunophenotype, and their origin is unclear. RS cells exhibit a rare co-expression of various hematopoietic cell markers (10). Current HL therapy has improved, with an 80% cure rate (9).

The source and risk of HL remain understood. HL attaches to immune cells, viruses like HIV and EBV (typically including EBV genomes in HL tumors), and genetics show a ten times greater risk in siblings and monozygotic twins, suggesting a hereditary pattern(11). The stages of Hodgkin's lymphoma are:*Stage I: Cancer in a single lymph structure, node, organ, or extranodal site (IE).*Stage II: Cancer in two lymph nodes or spreading from one organ to others, still limited above or below the diaphragm.*Stage III: Cancer in lymph nodes above and below the diaphragm, possibly affecting nearby tissues.*Stage IV: Advanced stage affecting multiple organs and several parts of it, including the liver, lungs, bones, and lymph nodes (3).

Cytokines are polypeptides between 15 to 20 kDa in molecular weight and functions in immune signaling and regulation either with their receptor types or characteristics (12),(13), they impact tumor growth by enhancing or suppressing the immune system, influencing cancer spread (14). IL-6 is an inflammatory cytokine that has several activities (15), it's located at 7p21-24 chromosomal position (16),(17), along with a 185-amino acid cationic glycoprotein (18). Macrophages, lymphocytes, endothelial cells, fibroblasts, and dendritic cells can secrete IL-6 (19). Known as hybridoma/plasmacytoma growth factor, hepatocyte stimulating factor, and IFN-β2 and other names (20). It is produced by tumor cells, T, B, macrophages, and endothelial cells (14). IL-6 has been connected to inflammation in multiple neoplasia (21). Overproduction of IL-6 synthesis by transcription factors is linked to cancers and severe inflammation(22). Numerous *IL-6* gene SNPs, such as -174G>C (rs1800795) and −572G (rs1800796), are linked to tumor risk (23).

This paper aims to study the role of *IL-6* gene expression and polymorphisms in association with the increased incidence of Hodgkin lymphoma and the possibility of using this interleukin as a tool in diagnosis and treatment follow-up in patients with HL.

Materials and Methods

A study at the Anbar Cancer Center in Al-Anbar, Iraq, involved 100 participants (50 NHL and 50 Control). Hodgkin lymphoma patients were approved with lymphoma after several diagnostic and clinical tests and approval by a physician. The control group was free of lymphoma, hormone issues, or chronic conditions and had no history of alcohol or smoking.

Blood Collection

Blood samples (7 ml) were collected in three types of tubes: EDTA for CBC and genotyping, TRIzol™ tube for RT-qPCR(24), and a gel tube for kidney function tests (urea and creatinine). We distributed the blood samples for each patient and control involved in this investigation into tubes following the manufacturer's instructions for the kit used in the technique to be performed.

Clinical markers determination.

Urea and creatinine were calculated according to the manufacturer's protocol by using (the Boditech kit, Korea).

Gene Expression of IL-6 gene

Blood samples were collected from the individuals and preserved in the TRIzol™ Reagent tube(24).The RT-qPCR technique was employed to assess the expression of the *IL-6* gene after total RNA extraction. According to the manufacturer's guidelines, total RNA was extracted utilizing TRIzol™ Reagent (Thermo Scientific, USA). The Quantus Fluorometer (Promega, USA) was utilized to ascertain the extracted RNA concentration and evaluate the samples' quality for further applications(25). The isolated RNA was reverse transcribed into complementary DNA (cDNA) with the GoTaq® two-step RT-qPCR System kit (Promega, USA)(26, 27), which operates in a single tube, converting RNA to cDNA in a one-step procedure. The method was performed in a reaction volume of 10 µl, with one µl of RNA designated for reverse transcription. A primer was developed for the *IL-6* gene with the *β-Globin* housekeeping gene, serving as an endogenous control for normalizing mRNA levels of the *IL-6* gene.

Primer design and preparation

The cDNA sequences of the *IL-6* gene are newly designed by the National Center for Biotechnology Information (NCBI) for this study, and the *β-Globin* gene was designed by the (NCBI) Gene Bank database. *β-Globin* was utilized as a housekeeping gene. The Premier 3 software was utilized to design RT-qPCR primers, which had an annealing temperature ranging from 60 to 65 degrees Celsius and primer lengths between 20 and 22 nucleotides (Macrogen Company, Korea), as stated in Table (1).

Table (1): Primers used for relative quantifying *IL-6* gene and *β-Globin* gene expression by RT-qPCR.

Primer Name (Target gene)	Sequence 5`-3`	Annealing Temp. (ºC)	Product size	Primer reference
β-Globin-F	ACACAACTGTGTTCACTAGC	65	110	Designed for this study
β-Globin-R	CAACTTCATCCACGTTCACC			
IL-6_exp-F	GGTACATCCTCGACGGCATCT	60	815	Newly designed for this study.
IL-6_exp-R	GTGCCTCTTTGCTGCTTTCAC			

Quantitative Real-Time PCR (qRT– PCR):

The expression levels of the *IL-6* gene were assessed using reverse transcription-quantitative polymerase chain reaction (qRT-PCR), a sensitive technique for determining steady-state mRNA levels(27). Quantitative real-time PCR was performed to verify target gene expression, as shown in Table (2). The heat profile, as indicated in Table (3), was used to program the cycling protocol for the following optimum cycles.

Table (2): Component of the reaction of quantitative real-time PCR.

Master mix components	Stock (Unit)	Final (Unit)	Volume (Unit)
Master Mix	2 (X)	1 (X)	5 µl
Forward primer	10 (µM)	0.3 (µM)	0.3 (µM)
Reverse primer	10 (µM)	0.3 (µM)	0.3 (µM)
Nuclease Free Water			3.4 µl
cDNA	(ng/µl)	(ng/µl)	1(ng/µl)
Total volume			10µl

Table (3): PCR conditions for amplification of *IL-6* gene expression by RTq-PCR.

Steps	Temperature (°C)	Time (m: s)	No. of cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:20	40
Annealing	60 or 65	00:20 Acquiring on Green	
Extension	72	00:20	

Calculation of expression

Each sample's threshold cycle (CT) was determined using the real-time cycler program. The mean values of each sample were computed after being run in duplicate. - Selected gene expression data were standardized against housekeeping.

The ΔΔCt method as advised, to analyze the data, and the outcomes were expressed as folding changes in gene expression as follows: The difference between each target gene's CT value and the housekeeping gene's CT value for each sample was computed. "Ct (control) = CT (gene)-CT" (HKG) Ct (patient) = CT (gene) -CT (HKG). For the genes of interest, the variation in Ct values expressed as (Ct) was calculated as follows: Ct = (patient) Ct - Ct (control) to compute the fold-change in gene expression as follows: Fold change=$2^{-\Delta\Delta Ct}$ (28),(29).

➢ DNA Polymorphism

Genomic DNA Isolation and Quantification

Using the ReliaPrep™ Blood gDNA Miniprep System from Promega, USA, genomic DNA was isolated from a blood sample. A Quantus Fluorometer (Promega, USA) measured extracted DNA concentration and sample quality for subsequent usage. To amplify the (815bp) region of *IL-6* gene SNPs (rs1800795, rs1800796, and rs1800797), conventional PCR was used. Primers were optimized using the identical primer pair (Forward) (Reverse) at 55, 58, 60, 63, and 65°C to identify the optimal primer annealing temperature. The PCR Express (Thermal Cycler, Veriti, USA) cycled PCR, as shown in Tables (4) (30, 31).

Table (4): Primer designed to amplify 815 bp.

Primer Name	Sequence 5`-3`	Annealing Temp. (°C)	Product size (bp)	Primer reference
IL-6-F	TGTAAAACGACGGCCAGTCTGAAGCAGG TGAAGAAAGT	55	815	Newly designed to this study
IL-6-R	CAGGAAACAGCTATGACCTTGTGGAGAA GGAGTTCATAG			

Polymerase chain reaction:

The PCR reaction was performed in a final volume of 25 µl, which included 12.5 µl GoTaq green Master mix, 7.5 µl nuclease-free distilled water, 1µl of each primer Forward and Reverse (10 µM), and 3 µl DNA sample (20-29 ng). After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. The results of the amplification of *IL-6* are shown in Figure (1). The PCR reaction was utterly dependent on the extracted DNA criteria. The the reaction was carried out under ideal PCR conditions, with an initial denaturation at 95 0C for 5 minutes in one cycle, followed by 30 cycles of denaturation, annealing, and extension. Each denaturation cycle lasted 30 seconds at 95 0C, each annealing cycle lasted 30 seconds at 60 0C, and the extension cycles lasted 30 seconds at 72 0C. The ultimate extension procedure was accomplished with a single cycle at 72 0C for 7 minutes.

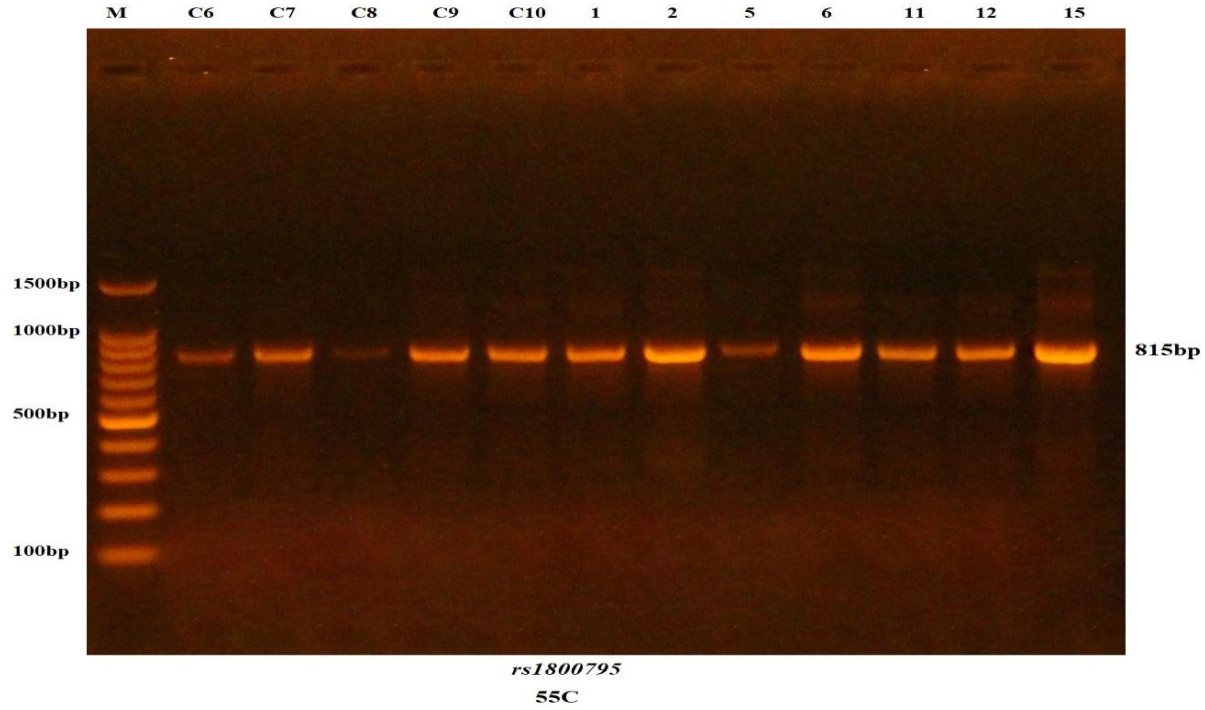

Figure (1): Results of the amplification of *IL-6* specific region of sample species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes C6-15 resemble 815 bp PCR products.

PCR products sequencing:

PCR products were sent for Sanger sequencing using ABI3730XL, an automated DNA sequencer by Macrogen Corporation Korea, to reveal *IL-6* SNPs after alignments with reference DNA sequences available in the National Center for Biotechnology Information (NCBI). The results were received and then analyzed using Geneious software.

Statistical Analysis

Data analysis was conducted using the available statistical package SPSS-26 and WinPepi software. The Data were presented using basic statistical metrics such as frequency, percentage, median (minimum-maximum values), and standard deviation. For qualitative variables (Folding of gene expression), data for normality was tested (Shapiro-Wilk and Kolmogorov-Smirnov Tests). A probability of 0.05 or less was considered significant. The p-value assessed deviations from the Hardy-Weinberg Equilibrium for all genetic variations. WinPepi software estimated the odds ratio and 95% confidence interval for the genotypes and allele frequencies(32, 33).

Results

Demographic characteristics and chemical tests of HL Analysis

This study compares HL patients to healthy controls (HC) in terms of demographics and clinical markers, including Sex, age (divided into three groups), family history, CBC, and some kidney function tests (urea and creatinine) results, as shown in Table (5). The results showed significant differences in the family history of smoking, hemoglobin, platelet, urea, and creatinine. Moreover, non-significant for other studied demographic and clinical data between HL patients and healthy controls. HL patients are generally older, with 42% aged 15-30 (HC: 32%), 46% aged 30-60 (HC: 54%), and 12% aged 60-80 (HC: 14%).Sex, higher proportion of males in the HL group 74% male vs. 54% in HC (p=0.038). In European communities, there is a little greater prevalence in females compared to males, affecting 60–70% of cases(34). HL is most common in 15–30-year-olds, although it also rises in 55-year-olds (9). Two smaller HL peaks occur in youngsters under 15 and elderly persons over 50, being more common in youth. (34). Smoking rates are 32% in HL, and 0% in controls (p=0.001), and alcohol consumption did not differ significantly (p=0.042). Studies suggest that smoking, obesity, and hypertension increase HL burden while drinking alcohol does not. Consuming alcohol was not associated with HL in an English study of 16–69-year-olds with HL(35). The relationship between smoking and cHL risk varies by study(36). As is known, tobacco smoking damages the immune system and may only affect particular cHL subtypes (37). Hodgkin lymphoma risk increased with smoking, especially for nodular sclerosis and mixed cellularity subtypes(36). This supports earlier individual-level studies on Hodgkin lymphoma risk factors and common risk factors. Across all ages, smoking increases the risk of mixed cellularity and EBV-positive Hodgkin lymphoma (cHL), but not nodular sclerosis or EBV-negative forms. (36). In our investigation, the family history of HL is present in 32% of patients (p=0.001). Also, large population-based case-control studies using validated family cancer diagnoses revealed the increased risk of NHL and HL in first-degree relatives of hematological malignancies. Family history was associated with a statistically significant increase in the risk of other NHL and HL subtypes, Leukemia, and multiple myeloma(38),(39).

Table (5): Demographic characteristics and chemical tests of HL patients and healthy controls (HC).

characteristics		Groups		*P*.value
		Patients N=50	Control N=50	
Age; year's	15-30	21 (42%)	16 (32%)	0.344 NS
	30-60	23 (46%)	27 (54%)	
	60-80	6 (12%)	7 (14%)	
Sex	Male	37 (74%)	27 (54%)	0.038 NS
	Female	13 (26%)	23 (46%)	
Family history	Yes	16 (32%)	0 (0.0%)	0.001**
	No	34 (68%)	50 (100%)	
Smoking	Yes	16 (32%)	0 (0.0%)	0.001**
	No	34 (68%)	50 (100%)	
Alcohol	Yes	4 (8%)	0 (0.0%)	0.042 NS
	No	46 (92%)	50 (100%)	
WBC;× 10^9/L	Median (IQR)	7.10 (5.00-10.83)	7.80 (6.10-9.20)	0.804 NS
Hemoglobin(Hb); g/dl	Median (IQR)	11.25 (10.60-13.30)	13.20 (12.00-14.00)	0.001**
RBC;× 10^9/L	Median (IQR)	4.26 (3.78-4.81)	4.38 (3.96-4.78)	0.394 NS
Platelet;× 10^9/L	Median (IQR)	338 (228-462)	269 (211-325)	0.013*
Urea; mg/dl	Median (IQR)	29.50 (24.00-40.00)	25.00 (21.40-30.00)	0.025*
Creatinine; mg/dl	Median (IQR)	0.79 (0.60-1.05)	0.70 (0.59-0.80)	0.025*

***p-value*= Probability value NS: non-significant, * significant, ** highly significant**

According to clinical results, individuals with HL had lower hemoglobin levels(p=0.001**), higher platelet counts (p = 0.013), elevated urea (p = 0.025), and creatinine (p = 0.025). These findings suggest that anemia evaluation and renal monitoring are necessary. In contrast, HL patients show no significant changes, as seen by Table (5), with WBC (p=0.804), and RBC counts remaining constant (p=0.394). Five of 17 renal lymphoma patients showed increased serum creatinine. Renal function must be monitored since renal insufficiency affects survival (40). Most of the kidney lymphoma in HL cases are from The B-cell lineage(41). Hodgkin's disease affects less than 1% of patients' kidneys. The radiological presentation often shows perirenal invasion. HL usually affects lymph nodes, and HL extranodal involvement is more uncommon than NHL(42),(43). Another study detected no renal infiltration(44). Up to 90% of

leukemia autopsies show renal involvement(45). Postmortem multiple myeloma investigations often show kidney damage(46). Previous study data indicates that the prevalence and severity of anemia in HL patients, as well as IL-6 and serum iron levels, were comparable to those in the DLBCL group. Compared to DLBCL and HL patients, non-anemic patients had higher serum hepcidin levels than controls. Hepcidin and hemoglobin levels in the HL cohort were exclusively adversely correlated in anemic individuals. Hepcidin may help explain anemias associated with chronic inflammation, which can resemble anemias associated with cancer. Increased production of cytokines, particularly IL-6, is linked to a variety of tumor forms. Hemoglobin levels in MM, HL, AL, and WM patients are adversely

correlated with higher blood hepcidin levels(47, 48)and(49).

Gene Expression

After the RNA extraction, reverse transcription was used to synthesize cDNA. The annealing temperature and melting temperature were set for our target *IL-6* gene. RT-qPCR measured the gene expression of *IL-6*; the level of gene expression was adjusted to the level of a housekeeping gene and quantified using the ΔCt values and folding ($2^{-\Delta\Delta Ct}$), as stated in Figure (2).

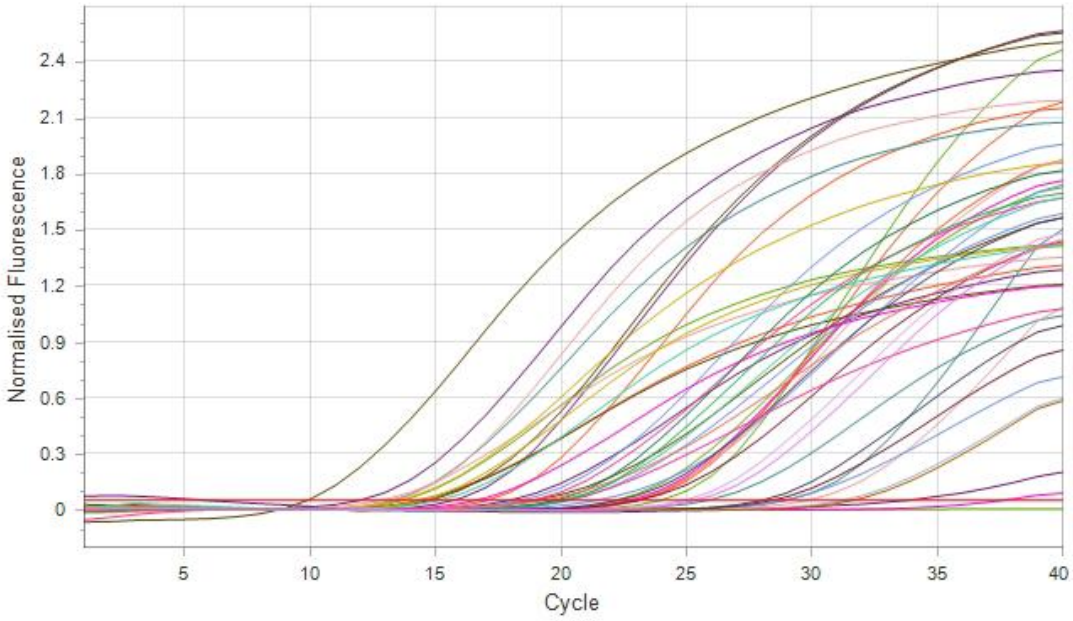

Figure (2). Amplification plots of *IL-6 gene* by RT-qPCR

The *IL-6* expression levels in HL patients compared to healthy controls were measured. HL patients have a median ΔCt of 6.54 (IQR: 5.53 to 8.35), while healthy controls have a median ΔCt of 6.65 (IQR: 4.56 to 9.34). Statistically, there was a significant difference (p =0.001 in ΔCt for the *IL-6* gene between the two groups, as shown in Table (6). Moreover, the median of fold change in gene expression ($2^{-\Delta\Delta Ct}$) revealed up-regulation at (1.23).

Table (6): *IL6* expression is given as a fold change in HL patients compared to healthy controls (HC).

Group	*IL-6* expression				***P*.value**
	HL; n = 50		HC; n = 50		
	Median	IQR	Median	IQR	
ΔCt	6.54	5.53- 8.35	6.65	4.56-9.34	
Fold of change ($2^{-\Delta\Delta Ct}$)	1.23	0.35-2.47	1.00		**0.001****

***P-value*= Probability value, ** highly significant.**

Studies show that dysregulated cytokine expression, a hallmark of chronic inflammation and autoinflammatory diseases, may cause hematological malignancies(50). Elevated levels of *IL-6* have been observed in several malignancies, and its high expression is adversely correlated with treatment response. In comparison to original tumors, recurring tumors and metastatic lesions exhibit

elevated levels of *IL-6* expression (51). However, the molecular mechanism that induces *IL-6* production in cancer is not entirely understood. *IL-6* was detected in pancreatic cancer, testicular germ cell tumors, esophageal carcinoma, and glioblastoma(GBM) (52).

High *IL-6* levels are found in most cancer types and are linked to various aspects of cancer progression. Higher *IL-6* concentrations can reduce the effectiveness of treatment and are associated with different tumor phases and survival rates. It serves as a prognostic marker in pancreatic carcinoma, DLBCL, advanced HL, colorectal cancer, and metastatic renal carcinoma. Some studies detected *IL-6* overexpression in AML patients(53). This is consistent with our investigation, where we found an increase in gene expression for *IL-6*. The newly diagnosed AML patients had higher *IL-6* levels, which promote inflammatory diseases and regulate hematopoiesis. In 2015, Van Loo et al. found higher *IL-6* levels in all AML patients' blood. In 2020, Hou et al. discovered that AML cells may induce HS-5 cells to release increased levels of *IL-6*. These results are identical to those of the study (54). Lymphocytes, monocytes, and macrophages synthesize *IL-6*. This multifunctional cytokine regulates many cellular processes and contributes to the pathogenesis of several diseases, including myeloma, Ki-positive large-cell anaplastic lymphoma, immunoblastic lymphoma, small lymphocytic lymphoma, and acute leukemia. *IL-6* activity was similarly significantly enhanced in ALL and AML sera by Luo et al. They found that *IL-6* promotes leukemia cell growth (55). Numerous other studies demonstrate that acute leukemia's genesis is linked to high IL-6 levels. These studies link plasma IL-6 levels with patient survival and event-free survival, whereas low levels indicate a positive prognosis (56).

However, these variations in *IL-6* gene levels can only be explained by the races and demographic differences. However, few previous data correlated this cytokine as a gene with HL.

SNPs of IL-6 HL Analysis.

Three SNPs with polymorphic frequencies (rs1800795 [G/C], rs1800796 [G/C], rs1800797 [G/A]) were assigned in the DNA sequence of the PCR-amplified region (815 bp), Table (7).

The sequencing result of rs1800795 SNP, which had G/C genotypes. The study examined the alleles and genotype frequencies of the *IL-6* SNP (rs1800795) in Iraqi patients with HL and healthy controls. The rs1800795 SNP was observed with three genotypes in HL patients (GG, GC, and CC) that had frequencies of 86, 14, and 0.00%, respectively. The corresponding G and C allele frequencies were 93 and 7%, respectively. However, significant variation was observed between the two groups, with the odds ratio (OR) of 8.41 (95% [CI] = 1.82 – 38.77, *p-value* = 0.012*), and thus, this genotype GC and C allele was shown to act as a significant risk factor for increasing HL incidence; while CC genotype is absent in both groups, odds ratio calculations are not possible. The (rs1800795) SNP distributions did not significantly deviate from HWE (*P* = 0.594) in patient groups, while it was in the control group (*P*= 0.157). This may be due to the small sample size of cases under study, as shown in Table (7). Moreover, an additional single nucleotide polymorphism (SNP) 1800796 was observed in Table (7), which revealed three genotypes in HL patients (GG, GC, and CC) that had frequencies of 84, 16, and 0.00%, respectively. The corresponding G and C allele frequencies were 92 and 8%, respectively. The odds ratio (OR) of GC is 0.19 (95% [CI] = 0.07 – 0.53, *p-value* =

0.001**), and thus, this genotype GC and C allele were shown to act as significant protective factors for decreasing HL incidence; while CC genotype is absent in both groups, odds ratio calculations are not possible. Additionally, rs1800797 SNP was also observed with three genotypes in HL patients (GG, GA, and AA) that had frequencies of 86, 14, and 0.00%, respectively. The corresponding G and A allele frequencies were 93 and 7%, respectively. However, significant variation was observed in the GA group the (OR) of 7.61 (95% [CI] = 1.64 –35.27, *p-value* = 0.013*), thus, this genotype GA was shown to act as a significant risk factor for increasing HL incidence, no significant variation was observed in AA group, the odds ratio (OR) of 0.00 (95% [CI] = 0.00 – 1.22, *p-value* = 0.059 NS), and the (rs1800797) SNP distributions did not significantly deviate from HWE (*P*= 0.594) in patient groups, while it was significantly in the control group (*P*= <0.001***), indicating potential population structure or selection or could be due to the small sample size. This may be due to the small sample size of cases under study, as shown in Table (7). Thus, three SNPs were shown to play a significant role in both increasing and decreasing HL incidences by acting as protective or risk factors.

Table (7): Numbers and percentage frequencies of *IL-6* genotypes, alleles, and their Hardy-Weinberg equilibrium (HWE) in HL patients compared with control groups.

SNPs of *IL-6* Genotype and allele frequencies in HL	patients No.= 50	Controls No. = 50	OR (95 % CI)	*p*-value
rs1800795 G> C				
GG	43(86%)	50(100%)	Reference	-
GC	7(14%)	0(0.00%)	8.41(1.82 - 38.77)	0.012 *
CC	0(0.00%)	0(0.00%)	-	-
HWE *p*-value	0.594 NS	0.157 NS	-	-
allele G	93(93%)	100 (100 %)	Reference	-
frequency C	7(7%)	0 (0.00%)	7.86 1.75 - 35.40)	0.014*
rs1800796 G>C				
GG	42(84%)	25(50%)	Reference	-
GC	8(16%)	25(50%)	0.19(0.07 - 0.53)	0.001***
CC	0(0.00%)	0(0.00%)	-	-
HWE *p*-value	0.538 NS	0.018*	-	-
allele G	92(92%)	75 (75%)	Reference	-
frequency C	8(8%)	25(25%)	0.26(0.10 - 0.64)	0.002 **
rs1800797 G>A				
GG	43(86%)	45(90%)	Reference	-
GA	7(14%)	0(0.00%)	7.61(1.64 - 35.27)	0.013*
AA	0(0.00%)	5(10%)	0.00(0.00 - 1.22)	0.059 NS
HWE *p*-value	0.594 NS	< 0.001***	-	-
allele G	93 (93%)	90 (90%)	Reference	-
frequency A	7(7%)	10 (10%)	0.68(0.21- 2.07)	0.631 NS

Significantly* ($p \leq 0.05$); significantly ($p \leq 0.01$); non- significant: NS; OR: Odds ratio; 95% CI: 95% confidence interval; *p*: Two-tailed Fisher exact probability; Hardy-Weinberg equilibrium (HWE).**

Recent studies have linked *IL-6* promoter polymorphisms to cancer risk. IL-6 is a multiple pro-inflammatory cytokine linked to cardiovascular disease. Researchers have linked increased *IL-6* and its primary effector to the start, promotion, malignant conversion, invasion, and metastasis of cancer (57). *IL-6* has a pivotal role in the development of Kaposi sarcoma and multiple myeloma. Recent studies show that circulating *IL-6* levels increase Hodgkin lymphoma risk. Promor polymorphisms imply that *IL-6* affects breast cancer prognosis genetically(58).

Most Reed-Sternberg cells, the neoplastic cells for HL, express *IL-6*, which is associated with Hodgkin lymphoma. Higher *IL-6* levels correspond with "B" symptoms (fever, night sweats) in untreated patients(59). IL-6, a strong cytokine that activates B cells, may help Hodgkin-Reed-Sternberg cells form and/or proliferate in numerous ways. Lymph node germinal centers generate HRS cells. Thus, Elevated *IL-6* may autocrinely enhance HRS cell proliferation and survival(60). Hodgkin lymphoma (HL) in young people is negatively associated with the *IL-6*-lowering C allele of the -174G>C promoter polymorphism. More C alleles at this site reduce HL risk. In a study, Cozen et al. observed that more C alleles at location -174 decreased HL incidence(59),(61).The *IL-6* polymorphism locus at position -174 had prognostic significance. Later, researchers found no survival difference by *IL-6* genotype in the Scotland and Newcastle Lymphoma Group Registry (61). Due to a G/C polymorphism in the *IL-6* gene promoter, *IL-6* production increases, and prognosis worsens. *IL-6* may create T- and B-cells, stimulate cytotoxic cells, and help produce lymphokine-activated killer cells (58).Research indicates that CC homozygotes have lower *IL-6* levels than GG and GC homozygotes, which may protect genetically predisposed youth from Hodgkin lymphoma. Although EBV enhances *IL-6* release, most young patients' tumors are EBV DNA negative, suggesting it does not affect this subtype. Nodular sclerosis is the most common histological type in this group, with some epidemiologists considering it a distinct disease(59). It has been suggested that EBV-positive HL is associated with delayed EBV exposure in young people and disturbance of the balance between latent EBV infection and immunity in older people(61). G alleles were prevalent in young NLPHL patients, while C alleles showed a non-significant decrease in classical HL risk. Young adult NLPHL patients had fewer C alleles than controls, with no differences based on EBV status. The *IL-6* genotype did not impact overall or disease-specific survival after adjusting for age, sex, and stage, contradicting prior research “Cozen et al., 2004”. The study involved twin pairs, enabling *IL-6* testing in the unaffected twin, who was rarely affected(62).

A substantial association was found between the *IL-6* promoter and cancer risk and prognosis. Subgroup analysis showed that rs1800795 polymorphism was significantly associated with an increased risk of cervical, colorectal, breast, prostate, lung, glioma, non-Hodgkin's, and Hodgkin's lymphoma, but not gastric or multiple myeloma. Furthermore, rs1800796 was markedly correlated with an elevated risk of lung cancer, prostate cancer, and colorectal cancer but not gastric cancer(60). gestational diabetes This study found the *IL-6* rs1800796 G allele(63). Additionally, rs1800797 was significantly associated with breast cancer, NHL, B-cell lymphoma, and diffuse large B-cell lymphoma but not gastric cancer(60). The cancer risk of rs1800795 and rs1800796 was significantly higher in both Asian and Caucasian groups. However, rs1800797 was only higher in Caucasians. Furthermore, *IL-6* promoter polymorphisms significantly

affected cancer prognosis and thus, the *IL-6* promoter variations rs1800795, rs1800796, and rs1800797 may be tumor indicators for cancer therapy(60). In conclusion, Ethnicity and race may explain the differences between results.

Haplotype analysis in HL patients

A haplotype is defined as the mixture of alleles for diverse polymorphisms that take place on the same chromosome, and an individual will have two haplotypes for any given stretch of chromosomal DNA; However, there may be many haplotypes for any given stretch of chromosomal DNA at the population level. There are significant links between specific *IL-6* gene haplotypes and HL. HL patients showed significant variation in only three haplotypes (GCA, GGA, CCA) in comparison with controls (P<0.05). Haplotype variations GCG and GGG are less significant in HL patients, as indicated in Table (8). These findings suggest that *IL-6* haplotypes may influence HL etiology.

Table (8): Estimated numbers and frequencies of haplotypes (rs1800795, rs1800796, and rs1800797) of the *IL-6* gene in HL patients and controls.

Haplotype (rs1800795, rs1800796 and rs1800797)	Patients (No.=50)		Controls (No.=50)		Odd Ratio	95% CI	*p*-value
	No.%	frequency	No.%	frequency			
G C A*	0.00	0.00	5.00	0.050	-	-	0.023*
G C G*	1.00	0.10	20.00	0.200	0.040	0.005-0.388	$1.20X10^{-5}$
G G A*	0.00	0.00	5.00	0.050	-	-	0.023*
G G G *	92.00	0.920	70.00	0.700	4.929	2.129-11.412	$7.43X10^{-5}$
C C A *	7.00	0.070	0.00	0.00	-	-	0.007**

All frequencies <0.03 were ignored in the analysis

Linkage disequilibrium (LD), the nonrandom association of alleles from different loci, can provide valuable information on the structure of haplotypes in the human genome and is often the basis for evaluating the association of genomic variation with human traits among unrelated subjects (64). The delta (D), relative delta (D′), and p-value were employed as metrics to assess the magnitude of the link. The D′ is a numerical value ranging from 0 to 1, where a value of 1 indicates the highest level of linkage. A positive D value, in conjunction with a D' value and a large p-value, signifies a substantial degree of linkage disequilibrium (LD). The evaluation of linkage disequilibrium (LD) between different pairings of *IL6* and haplotypes involves calculating D and the Pearson correlation coefficient (r^2). This method sought to assess the robustness of the correlation(65).

LD analysis between *IL-6* gene SNPs rs1800795 and rs1800796 shows a strong correlation, indicating dependent inheritance. D' and r^2 values are high for these SNPs, meaning one genotype provides high information about the other. However, a high linkage disequilibrium also exists between rs1800795 and rs1800797, suggesting they may jointly influence *IL-6* genetics and

disease risk. The medium D' and r^2 values for rs1800796 and rs1800797 indicate these SNPs are likely inherited separately, affecting *IL-6* gene characteristics independently, as shown in Table (9) and Figure (3).

Table (9): Linkage disequilibrium analysis between SNPs of *IL-6* gene in HL patients

Linkage disequilibrium	rs1800795	rs1800796	rs1800797
rs1800795		D′ 1.000 r^2 0.184	D′ 1.000 r^2 0.390
rs1800796	D′ 1.000 r^2 0.148		D′ 0.619 r^2 0.180
rs1800797	D′ 1.000 r^2 0.390	D′ 0.619 r^2 0.180	

D′: Scaled D value (D value represents linkage disequilibrium for each pair of SNP) with an interval between (-1, 1). **r^2:** Correlation coefficient between each pair of SNP (0-1).

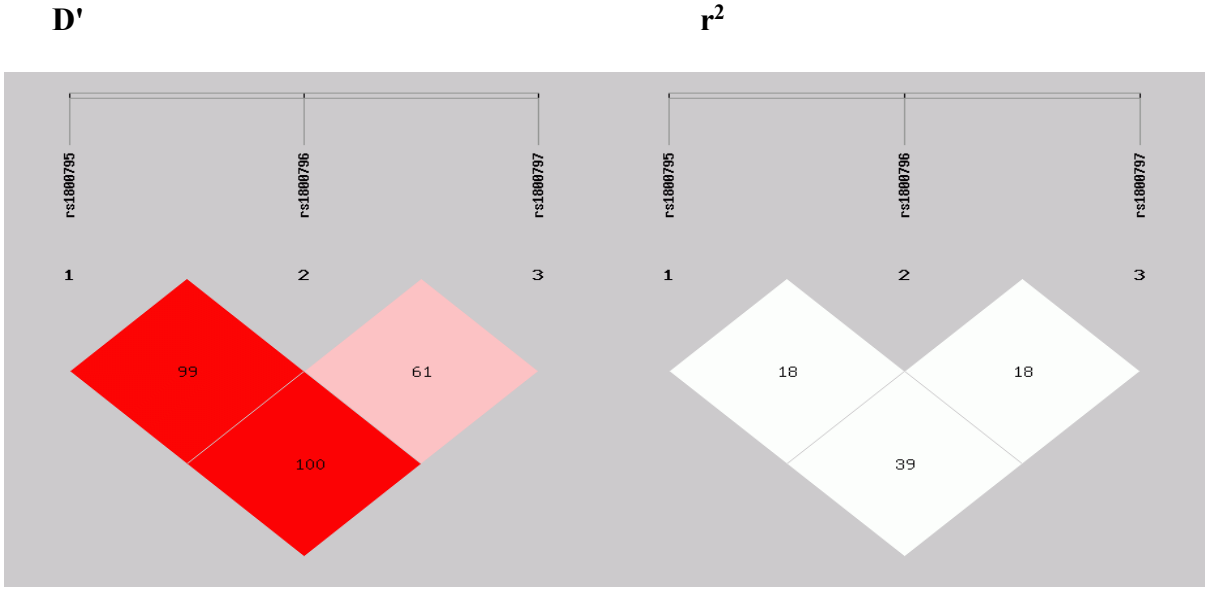

Figure (3): Pairwise linkage disequilibrium coefficient (D') and correlation coefficient (r^2) between *IL-6* SNPs (rs1800795, rs1800796 and rs1800797) in HL patients and controls.

Discussion

Clinical findings in Hodgkin Lymphoma (HL) patients show significantly reduced hemoglobin alongside elevated platelets, urea, and creatinine, highlighting a critical need for regular anemia evaluation and renal monitoring. The pathogenesis of HL is heavily driven by the overexpression of the inflammatory cytokine IL-6, which is secreted by neoplastic Reed-Sternberg cells to autocrinally promote tumor proliferation and survival.

Elevated IL-6 expression strongly correlates with worse treatment response, tumor progression, and aggressive "B" symptoms, serving as a reliable prognostic biomarker for disease survival.

Genetic susceptibility to HL is linked to specific IL-6 promoter single nucleotide polymorphisms (SNPs), including rs1800795, rs1800796, and rs1800797, which vary considerably based on racial demographics. Notably, the C allele of the -174G>C polymorphism acts as a protective genetic factor in young populations by reducing IL-6 production and lowering the overall risk of developing HL. Chronic inflammation and tumor-driven IL-6 production stimulate elevated serum hepcidin levels, offering a molecular explanation for the severity of cancer-associated anemia observed in these patients. Finally, haplotype analysis reveals distinct genetic variations (GCA, GGA, CCA) in HL cases, supported by strong linkage disequilibrium (LD) between specific SNPs that indicates dependent inheritance and shared disease risk.

Conclusion

This research emphasizes the influence of *IL-6* gene expression and polymorphisms on the predisposition to Hodgkin lymphoma (HL). The findings indicate up-regulation of gene expression and specific SNPs, particularly rs1800795, rs1800796, and rs1800797, are associated with increased HL risk in the studied Iraqi population. The higher expression levels of *IL-6* in HL patients relative to healthy controls indicate a complicated relationship between *IL-6* signaling and lymphoma development. These results enhance the comprehension of the genetic determinants affecting HL and may guide future diagnostic and treatment approaches. This research highlights *IL-6* as a possible biomarker, emphasizing the significance of genetic differences in cancer risk assessment and therapy personalization.

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