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Association of Interleukin-6 Gene Expression and Polymorphism With Incidence of Non-Hodgkin Lymphoma Disease Within the Iraqi Population

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Abstract

Lymphomas are broad tumors originating in the lymphatic system, causing lymph node enlargement. Non-Hodgkin lymphoma (NHL) is a group of blood cancers that includes all types of lymphomas except Hodgkin lymphomas. The Interleukin-*6 (IL-6)* gene is associated with various malignancies and can influence tumor growth and immune responses. This paper aims to study the role of *IL-6* gene expression and polymorphisms in association with increased incidence of non-Hodgkin lymphoma. The study included 100 participants (50 NHL patients and 50 controls). Sex, age (divide into three groups), family history, CBC, and some kidney function tests were studied for both 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 NHL incidence in the Iraqi population. The results showed a significant variation for all studied demographic and clinical data except with sex, family history, creatinine, smoking, and alcohol between NHL patients and healthy controls. Moreover, the median of the fold change of *IL-6* gene expression ($2^{-\Delta\Delta Ct}$) revealed down-regulation in NHL patients (0.04) with respect to control, which was (1). According to studied SNPs, some genotypes and alleles appeared to have significant protective effects while others appeared to be a risk factor that plays a significant role in increasing NHL incidence (large number of deaths for patients). In conclusion, the results of our investigation of an Iraqi Arab population revealed significant associations between the *IL-6* gene and NHL.

Keywords: Non-Hodgkin lymphoma, IL-6 polymorphisms, SNPs, Risk factor.

علاقة التعبير الجيني والتغاير الوراثي لجين *IL-6* مع حدوث اللمفومة اللاهودجكِنية في عينة من المجتمع العراقي

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الخلاصة

الأورام اللمفاوية هي أورام واسعة تنشأ في الجهاز اللمفاوي، مما يسبب تضخم العقد اللمفاوية. سرطان الغدد اللمفاوية اللاهودجكِنية (NHL) هو مجموعة من سرطانات الدم التي تشمل جميع أنواع سرطانات الغدد اللمفاوية باستثناء سرطانات الغدد اللمفاوية الهودجكينيه. جين *الإنترلوكين-6 (IL-6)* مرتبط بأنواع مختلفة من

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الأورام الخبيثة ويمكن أن يؤثر على نمو الأورام والاستجابات المناعية. هدفت البحث الى دراسه علاقه جين *الإنترلوكين- 6* والتغاير الوراثي له وعلاقته بحدوث سرطان الغدد اللمفاوية اللاهودجكينية. شملت الدراسة التالية 100 مشارك .(50 مصاب بNHL و50 اصحاء) .تمت دراسة الجنس، العمر (مقسم إلى ثلاث مجموعات)، التاريخ العائلي، تعداد الدم الكامل، وبعض اختبارات وظائف الكلى لكلا المجموعتين المدروستين. تؤكد هذه الدراسة على دور تعبير جين *IL-6* والبوليمورفزم ، وخاصةSNPs rs1800795 G/C ، rs1800796 G/C، و rs1800797 G/A في حدوث NHL في السكان العراقيين. أظهرت النتائج تباينًا كبيرًا في جميع البيانات الديموغرافية والسريرية المدروسة باستثناء الجنس , تاريخ العائله, مستوى الكرياتينين, التدخين والكحول بين مرضى NHL والأشخاص الأصحاء. علاوة على ذلك، أظهر الوسيط لتغير مضاعف تعبير جين (IL-6 (2−ΔΔCt زيادة في التعبير في مرضى (0.04) NHL مقارنةً بجموعه السيطرة الذي كان (1). وفقًا لل SNPs المدروسة، ظهرت بعض الأنماط الجينية والأليلات كان لها تأثيرات وقائية كبيرة بينما ظهرت أخرى كعوامل خطر تلعب دورًا كبيرًا في زيادة حدوث .NHL في الختام، كشفت نتائج الدراسه في السكان العرب العراقيين عن ارتباطات كبيرة بين جين *IL-6* وسرطان الغدد الليمفاوية غير الهودجكيني

1. Introduction

Cancer is the case of the unregulated proliferation of cells and the spread of abnormal cells to other parts of the body. It is the third leading cause of death worldwide, and WHO anticipates it quadrupling to 10 million by 2040. Cancer is now known to result from DNA abnormalities in cells, causing unchecked growth and spread, classified by its origin [1].

Lymphomas are broad tumors originating in the lymphatic system, causing lymph node enlargement. The lymphatic system is exist in all parts of the human body except the bone marrow, cartilage, and central nervous system [2]. Malignant lymphomas are the third most common group of neoplasms in the head and neck region and have varying degrees of malignancy [3]. The anticipated five-year overall survival rate is 72%, and there is a positive trend of improvement. Dr. Thomas Hodgkin identified lymphoma in 1832, with non-Hodgkin's comprising 85% and Hodgkin's 15% of cases. These are the two main categories [4]. Advances in immunology, genetics, and technology have enhanced the classification and understanding of the varied and complex lymphoma [5].

Depending on the kind of lymphocyte B-cell, T-cell, or NK cell—that is involved, lymphomas are classified [6]. NHL and HL differ in clinical features; NHL, the most common blood cancer, lacks Reed-Sternberg cells, has genetic alterations, and accounts for 3% of diagnostics and fatalities[7]. NHL in children and adolescents is an aggressive malignancy with rapid and brief symptoms, varying by lymphoid tissue location [8]. NHL, some grow quickly and others slowly. The most common types of NHL include B-cell lymphomas like chronic lymphocytic leukemia (CLL), follicular lymphoma, small lymphocytic lymphoma (SLL), Burkitt lymphoma, and diffuse large B-cell lymphoma (DLBCL), T-cell lymphomas like mycosis fungoides, adult T-cell leukemia/lymphoma (ATLL), and anaplastic large cell lymphoma (ALCL), each with distinct features and treatments [4]. They all share these symptoms: Painless adenopathy is a common sign of lymphoma, with fever, unexplained weight loss, and night sweats in advanced stages [9]. Murphy staging classifies illness into four stages: Stage I features nodal or extranodal tumors outside the mediastinum and abdomen. Stage II covers diaphragm-side nodes, external tumors, and ileocecal involvement. Stage III includes mediastinal, pleural, thymic, IBD, paraspinal, and epidural cancers. Stage IV involves CNS or bone marrow abnormalities. These tumors can result from chromosomal translocation, chemical exposure, virus infections, and chronic inflammation [10].

Cytokines are 15–20 kDa proteins essential for immune signaling and regulation, classified by their receptor characteristics [11], and they impact tumor growth by either enhancing or suppressing the immune system, influencing cancer spread [12]. Interleukin-6 (IL-6) is an inflammatory cytokine with several activities, is located at the 7p21-24 chromosomal position, and has five exons and four introns. It has a 303 bp promoter and 185-amino acid cationic glycoprotein [13].

IL-6 has been linked to colorectal, head-and-neck, renal cell, lymphoma, and multiple myeloma inflammation over the years [14]. In many cancers, IL-6 is correlated with inflammation, angiogenesis, and metastasis, which worsens immune responses. It boosts proteins that inhibit tumor cell death and increase blood vessel development to promote tumor growth [15]. Numerous *IL-6* gene SNPs, such as the -174G>C (rs1800795) and −572G (rs1800796) polymorphisms, are linked to cancer risk. The -174G>C polymorphism may block transcriptional activation and glucocorticoid receptor interaction[16].

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

2. Materials and Methods

Study Subject (case control study)

The present investigation was duly permitted and approved by the Human Ethics Committee of the College of Science at Baghdad University (Ref.: CSEC/0224/0020).

A study at the Baghdad Cancer/ Health Directorate/ Baghdad/ Iraq and the Anbar Cancer Center/ Anbar/ Iraq, involved 100 participants (50 NHL and 50 Control) from November 2023 to February 2024. NHL 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. Smoking, and alcohol were assessed for both groups. The age range for both groups was between 30-80) and the male to female number in the patients group was (25/25) while the number in the control group was (27/23).

Blood Collection

Blood samples (7 ml) were collected in three types of tubes: EDTA for CBC and genotyping, TRIzol™ tube for RT-qPCR, and a gel tube for kidney function tests (urea and creatinine).

Clinical markers determination.

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

IL-6 gene expression

Blood samples were collected from all subjects and stored in the TRIzol ™ Reagent tube. The RT-qPCR method was used to evaluate the expression of the *IL-6* gene following the extraction of total RNA. The total RNA was isolated using TRIzol™ Reagent, following the manufacturer's instructions (Thermo Scientific, USA).

Quantus Fluorometer was used to detect the concentration of extracted RNA and the quality of samples for downstream applications (Promega, USA). The separated RNA was

reversely transcribed into complementary DNA (cDNA) using the GoTaq® one-step RT-qPCR System kit (Promega, USA).

Primer design and preparation

The sequence of the *β-Globin* gene was obtained from the National Center for Biotechnology Information (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	Sequence 5'-3'	Annealing Temp. (ºC)
β-Globin-F	ACACAACTGTGTTCACTAGC	65
β-Globin-R	CAACTTCATCCACGTTCACC	
IL-6_exp-F	GGTACATCCTCGACGGCATCT	60
IL-6_exp-R	GTGCCTCTTTGCTGCTTTCAC	

Detection of Gene Expression for IL-6 Genes

The entire process, from cDNA synthesis to PCR amplification, was carried out using the kit of GoTaq® 1-Step RT-qPCR System (Promega, USA), which transpires in a singular tube, where RNA is converted to cDNA using the one-step method. This procedure was performed in a reaction volume of 10 µl, with 1 µl of RNA designated to be transcribed reversely. Primer was newly designed for the *IL-6 gene*, alongside the utilization of *β-Globin* as the housekeeping gene, which serves as an endogenous control to normalize the mRNA levels of the IL-6 gene.

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 of steady-state mRNA levels. Quantitative real-time PCR was performed to verify target gene expression, as shown in Table 2.

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

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

The heat profile as indicated in Table 3 was used to program the cycling protocol for the following optimum cycles.

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

Steps	°C	m: s	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

The real-time cycler programming determined each sample's threshold cycle (CT). After being run in duplicate, the average values of each sample were calculated. The selected gene expression data were normalized against housekeeping genes.

The Livak method was utilized to analyze the data, and the results were presented as fold changes in gene expression as follows:

The CT value difference between each target gene and the housekeeping gene for each sample was calculated. "Ct (control) = CT (gene)-CT" (housekeeping gene)
Ct (patient) = CT (gene) -CT (housekeeping gene). The variance in Ct values for the genes of interest was computed as (Ct) and calculated as follows: Ct = Ct (patient) - Ct (control). To calculate the fold-change in gene expression as follows: Fold change=$2^{-\Delta\Delta Ct}$ [17].

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.

Conventional PCR was used to amplify the (815bp) region of *IL-6* gene SNPs (rs1800795, rs1800796, and rs1800797). 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, as shown in Table 4.

Table 4: Primer designed to amplify 815 bp**.**

Primer Name	Sequence 5`-3`	Annealing Temp. (°C)	Product size (bp)
IL-6-F	TGTAAAACGACGGCCAGTCTGAAGCAGG TGAAGAAAGT	55	815
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 PCR reaction was utterly dependent on the extracted DNA criteria. The reaction was carried out under ideal PCR conditions, with an initial denaturation at 95 ^{0}C for 5 minutes in one cycle, followed by 30 cycles of denaturation, annealing, and extension. Each denaturation cycle lasted 30 seconds at 95 ^{0}C, each annealing cycle lasted 30 seconds at 60 ^{0}C, and the extension cycles lasted 30 seconds at 72 ^{0}C. The ultimate extension procedure was accomplished with a single cycle at 72 ^{0}C for 7 minutes [18].

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), mean, 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 was used to assess 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.

3. Results and Discussion

Demographic characteristics and chemical tests of NHL Analysis

This study compares NHL patients to healthy controls (HC) in terms of demographics and clinical markers (urea, creatinine, and CBC) results. NHL patients are generally older, with 16% aged 15-30 (HC: 32%), 46% aged 30-60 (HC: 54%), and 38% aged 60-80 (HC: 14%); this age difference is significant *(p=0.005*). The sex distribution is similar: NHL patients are 50% male vs. 54% in HC (*p=0.690*). According to previous studies, more men than women are affected. NHL can affect anyone, but most cases occur in people over 65. NHL is most common in 65-74-year-olds and most deadly in 75-84-year-olds [19] . In the current investigation, the family history of NHL is present in 6% of patients (*p=0.080*). Compared to other studies, NHL increased the risk associated with family history, and studies show that those with a family history of NHL have a substantially higher risk of Diffuse Large B-Cell Lymphoma (DLBCL). Family history shows how common exposures (from the shared environment and activities) affect genetic predisposition and strengthen the risk of NHL [20].

Smoking rates are 6% in NHL and 0% in controls (*p=0.080*), and alcohol consumption did not differ significantly (*p=0.317*). However, smoking's negative connection with follicular lymphoma was surprising; smoking increases the risk of follicular lymphoma. However, numerous large cohorts with similar alcohol intake have demonstrated an inverse link between alcohol and NHL risk [21]. Clinical results show NHL patients have high differences, showing significantly lower WBC (4.95 vs. 7.80 × 10^9/L, *p=0.001*), lymphocytes, hemoglobin, and platelets. In contrast, they have higher urea levels (29.50 vs. 25.00 mg/dl, *p=0.042*) but similar creatinine levels (0.70 mg/dl, *p=0.595*), as shown in Table (5).

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

characteristics		Group		*P*.value
		Patients N=50	Control N=50	
Age; year's	15-30	8 (16%)	16 (32%)	**0.005 ****
	30-60	23 (46%)	27 (54%)	
	60-80	19 (38%)	7 (14%)	
Sex	Male	25 (50%)	27 (54%)	0.690 NS
	Female	25 (50%)	23 (46%)	
Family history	Yes	3 (6%)	0 (0.0%)	0.080 NS
	No	47 (94%)	50 (100%)	
Smoking	Yes	3 (6%)	0 (0.0%)	0.080 NS
	No	47 (94%)	50 (100%)	
Alcohol	Yes	1 (2%)	0 (0.0%)	0.317 NS
	No	49 (98%)	50 (100%)	
WBC; $\times 10^9$/L	Median (IQR)	4.95 (2.86-8.30)	7.80 (6.10-9.20)	**0.001****
LYMPH; 10^3/L	Median (IQR)	1.25 (0.43-14.00)	2.45 (1.90-3.00)	**0.022***
Hemoglobin (Hb); g/dl	Median (IQR)	10.02 (8.80-11.30)	13.20 (12.00-14.00)	**0.001****
RBC; $\times 10^9$/L	Median (IQR)	3.63 (2.90-4.04)	4.38 (3.96-4.78)	**0.001****
Platelet; $\times 10^9$/L	Median (IQR)	178 (106-251)	269 (211-325)	**0.001****
Urea; mg/dl	Median (IQR)	29.50 (23.00-41.00)	25.00 (21.40-30.00)	**0.042***
Creatinine; mg/dl	Median (IQR)	0.70 (0.57-0.80)	0.70 (0.59-0.80)	0.595 NS

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

The results in Table 5 clearly showed that the urea and lymphocytes % of NHL patients compared with control revealed significant differences ($p > 0.01$) and highly significant ($P > 0.001$) for Age, WBC, RBC, PLT, and Hb %. In contrast, no significant differences were seen with sex, family history, creatinine, smoking, and alcohol between the studied groups.

In previous studies, anemia and platelets were independently prognostic of worse outcomes in individuals with DLBCL and other forms of NHL. In NHL, hemoglobin levels below 120 g/L are often observed upon diagnosis [22]. In follicular lymphoma, an indolent form of lymphoma, hemoglobin levels below 120 g/L are considered a negative prognostic indicator and are routinely utilized in clinical practice as a component of a scoring system to assess prognosis [23]. Anemia correlated with worse clinical outcomes in intermediate-grade non-Hodgkin lymphomas, particularly DLBCL. Certain variables indicated non-responsiveness to chemotherapy in the pre-rituximab period [24].

Contrary to the results of another research indicating no correlation between anemia and decreased progression-free survival or overall survival. The causative mechanism of anemia commonly associated with chronic inflammation or malignancy is believed to be mediated by IL-6 and the liver-produced peptide hepcidin. Hepcidin inhibits the release of iron from macrophages and enterocytes, so obstructing a sufficient supply of iron for erythropoiesis, ultimately resulting in anemia [25].

Studies in patients with acute leukemia, multiple myeloma, NHL, and Waldenström macroglobulinemia have shown that high serum hepcidin levels are adversely linked with hemoglobin levels. There were no verified associations between platelets and white blood cell counts [26]. All patient groups had anemia and substantial hemoglobin variations from healthy controls. Significant differences in CBC parameters, such as hemoglobin and platelets, were seen between AML patients and controls ($p<0.001$), but WBC levels were inconsistent [27].

Ultimately, renal function parameters, including urea and creatinine levels in patients and controls, were studied, and significant variation was seen with urea only. In a study by Yugi Guo and his colleague, there was no significant correlation between urea and creatinine levels in patients and controls [28].

Gene Expression

After the RNA extraction, reverse transcription was used to synthesize cDNA. The annealing temperature and melting temperature were set, for our target gene *IL-6*. 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 folding ($2^{-\Delta\Delta Ct}$) and ΔCt values, as stated in Figure 1.

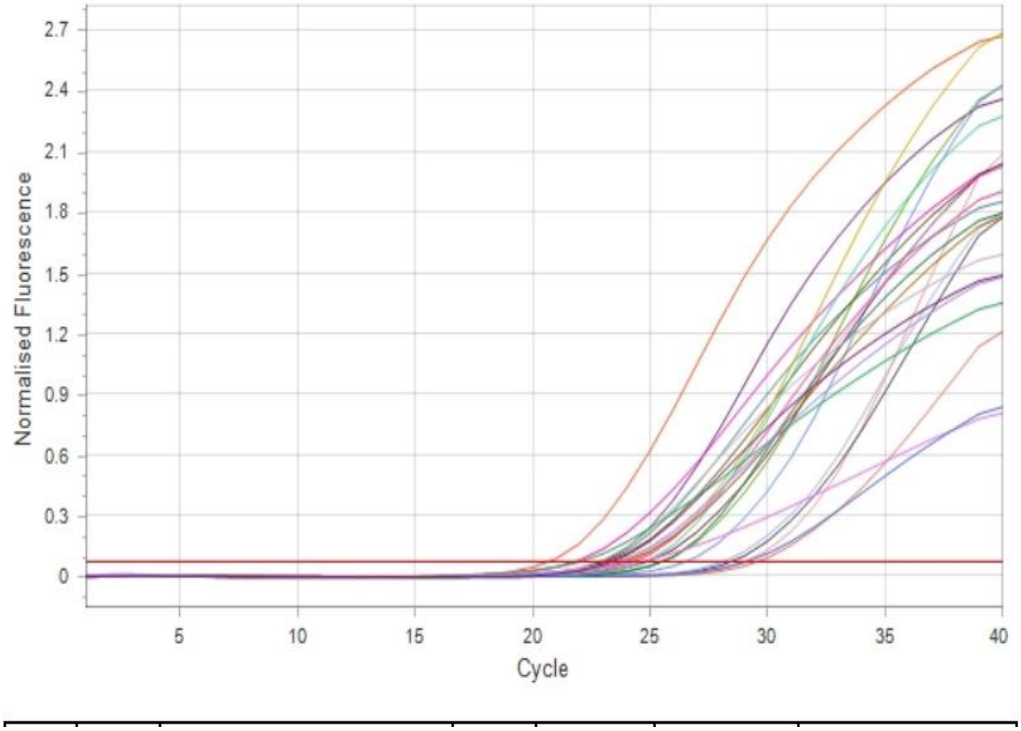

Figure 1 : Amplification plots of *IL-6 gene* by RT-qPCR.

IL-6 expression levels in NHL patients compared to healthy controls were measured. NHL patients have a median ΔCt of 11.68 (IQR: 7.48 to 14.27), while healthy controls have a median ΔCt of 6.65 (IQR: 4.56 to 9.34). Statistically, there were significant differences ($p=0.001$ 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 down-regulation at (0.04).

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

Group	*IL-6* expression group		*P*-value
	Patients N=50	Control N=50	
	Median (IQR)	Median (IQR)	
ΔCt	11.68 (7.48 - 14.27)	6.65 (4.56-9.34)	**0.001 ****
Fold of change ($2^{-\Delta\Delta Ct}$)	0.853 (0.16 - 0.94)	1.00	

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

Studies show that dysregulated cytokine expression, a hallmark of chronic inflammation and autoinflammatory diseases, may cause hematological malignancies. Elevated levels of *IL-6* have been observed in several malignancies, and its high expression is adversely correlated with treatment response [29]. 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). The remaining TCGA cancer codes are as follows: stomach adenocarcinoma, DLBC, AML, Kidney renal papillary cell carcinoma, thyroid carcinoma, kidney chromophobe, head and neck squamous cell carcinoma, pheochromocytoma and paraganglioma, and additional malignancies [30].

Our results are consistent with studies where it was found that IL-6 plays a significant role in hematological malignancies like NHL. Notably, *IL-6* levels significantly decreased in patients post-chemotherapy, but differed from another, which found no significant difference [31]. Another study examined *IL-6* expression levels in patients with ALL and found no significant differences compared to the control group, while in another study with ALL, it was found to be decreased [32]. These variations in *IL-6* gene levels can only be explained by the races and demographic differences. However, there was no prior evidence linking this cytokine to NHL.

SNPs of IL-6 NHL Analysis

Three SNPs with polymorphic frequencies (rs1800795 [G/C], rs1800796 [G/C], and 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 NHL and healthy controls. The rs1800795 SNP was observed with three genotypes in NHL patients (GG, GC, and CC) with frequencies of 60, 30, and 10%, respectively. The corresponding G and C allele frequencies were 75% and 25%, respectively. However, significant variation was observed between the two groups for both GC and CC genotypes with the odds ratio (OR) of 11.95 (95% [CI] = 3.98 – 335.84, p-value = 0.000 (3 x 10^6)) and 12.81 (95% [CI] = 2.06 – 79.44, p-value = 0.01) respectively. The (rs1800795) SNP distributions did not significantly deviate from HWE (*P = 0.157*) in patient groups, as shown in Table 7. Moreover, an additional single nucleotide polymorphism (SNP) 1800796 was observed, revealing three genotypes in NHL patients (GG, GC, and CC) with 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***). While CC genotype is absent in both groups, odds ratio calculations are not possible. The NHL patient genotype frequencies align with Hardy-Weinberg equilibrium (HWE p-value: 0.538NS),

suggesting random mating. In contrast, the control group deviates from HWE (*p-value: 0.018*, significant), indicating possible population structure or selection. This may be due to the small sample size of cases under study. Additionally, rs1800797 SNP was also observed with three genotypes in NHL patients (GG, GA, and AA) with frequencies of 60, 34, and 6%, respectively. The corresponding G and A allele frequencies were 77% and 33%, respectively. However, significant variation was observed in AG group the (OR) of 11.75 (95% [CI] = 3.77 –30.65*, p-value = 0.000(2.7X10-6)*****), no significant variation was observed in AA group the odds ratio (OR) of 0.90 (95% [CI] = 0.13 – 5.04, *p-value = 1.000* NS), and the (rs1800797) SNP distributions did not significantly deviate from HWE (*P = 0.776*) in patient groups, while it was significantly in the control group (*P= 0.001*), as shown in Table (7). Thus, three SNPs were shown to play a significant role in both increasing and decreasing NHL incidences by acting as protective and risk factors.

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

SNPs of *IL-6* Genotype and allele frequencies in NHL	**patients No.= 50**	**Controls No. = 50**	**OR (95 % CI)**	***p*-value**
***rs1800795* G> C**				
GG	30 (60%)	50(100%)	Reference	-
GC	15(30%)	0(0.00%)	11.95(3.98- 35.84)	0.000(3x10^{-6})****
CC	5(10%)	0(0.00%)	12.81(2.06 -79.44)	0.01**
HWE *p*-value	0.157 NS	0.157 NS	-	-
allele G	75 (75%)	100 (100 %)	Reference	-
frequency C	25 (25 %)	0 (0.00%)	9.72(4.21- 22.43)	0.000(1X10^{-8})****
***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	30(60%)	45(90%)	Reference	-
GA	17(34%)	0(0.00%)	10.75(3.77 - 30.65)	0.000(2.7X10^{-6})****
AA	3(6%)	5(10%)	0.90(0.13 - 5.04)	1.000 NS
HWE *p*-value	0.776 NS	< 0.001***	-	-
allele G	77 (77%)	90 (90%)	Reference	-
frequency A	33 (33%)	10 (10%)	3.86(1.71 - 9.31)	0.000(3.1X10^{-4})****

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

In gene polymorphism, *IL-6* has a complex role in carcinogenesis, notably in hematological malignancies, influencing inflammatory settings, host responses, and malignant clones [16]. In comparison to previous research, it is suggested that the *IL-6* gene promoter polymorphism (174G>C) is linked to increased risks of various neoplastic and non-neoplastic disorders, such as Kaposi sarcoma, diabetes, and Crohn's disease. However, findings on its association with lymphoma risk are inconsistent. A case-control study found no link between this polymorphism and non-Hodgkin lymphoma risk [33]. Three further investigations, two involving Caucasians and one concerning an Egyptian Arab community, similarly concluded

that there is no connection between the *IL6* (174 G > C) and *IL6* (597/598G > A) gene polymorphisms and the risk of NHL [34]. While the current investigation of an Iraqi Arab community found that *IL-6* SNPs play a highly significant role in non-Hodgkin lymphoma (NHL), specifically, rs1800795 G>C is linked to an increased NHL risk, while the GC genotype shows a decreased risk. In rs1800796 G>C, NHL patients predominantly have GG genotypes, suggesting the G allele may raise risk, whereas the C allele provides protection. Additionally, rs1800797 G>A is associated with higher NHL risk especially for the GA genotype and A allele. *IL-6* SNPs are identified as major risk factors for NHL, aiding in understanding of its genetic basis and treatment options. The study identifies unique *IL-6* SNPs as substantial risk factors for NHL, with implications for comprehending the genetic underpinnings of the illness and possible treatment applications. Where our result was consistent with Gu *et al.,* [35], it was found that in the Han Chinese population, the risk of NHL is positively associated with IL-6 rs1800795 (GC vs. GG: OR = 3.976, 95% CI: 1.400–11.295, p = 0.006) and *IL-6* rs1800797 (GA vs. GG: OR = 3.976, 95% CI: 1.400–11.295, p = 0.006) variations [35]. However, ethnicity and race may explain the differences.

Haplotype analysis in NHL 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, though there may be many haplotypes for any given stretch of chromosomal DNA at the population level [36]**.** There are significant links between specific *IL-6* gene haplotypes and NHL. NHL patients showed significant variation in only three haplotypes (GCA, GGA, CCA) in comparison with controls (*P<0.05*). Haplotype variations GCG, GGG, CCG, and CGA are less significant in NHL patients, as indicated in Table 8. These findings suggest that *IL-6* haplotypes may influence NHL etiology.

Table 8: Estimated numbers and frequencies of haplotypes (*rs1800795*, *rs1800796* and *rs1800797)* of the *IL-6* gene in NHL 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	0.50	-	-	0.025*
G C G*	0.00	0.00	20.00	0.200	-	-	$3.07x10^{-6}$ NS
G G A*	0.00	0.00	5.00	0.050	-	-	0.025*
G G G*	75.00	0.750	70.00	0.700	1.398	0.742-2.633	0.299 NS
C C A *	6.00	0.060	0.00	0.00	-	-	0.012*
C C G	2.00	0.020	0.00	0.00	-	-	-
C G A*	17.00	0.170	0.00	0.00	-	-	$1.35x10^{-5}$ NS

All frequencies <0.03 were ignored in the analysis. Significantly* (p≤0.05) ; non-significant: NS.

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 [37].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. To assess the linkage disequilibrium

(LD) between various pairs of IL6 and haplotypes, the computation involved the determination of D, accompanied by the Pearson correlation coefficient (r^2). This approach aimed to evaluate the strength of the association[37] .

LD analysis between *IL-6* gene SNPs rs1800795 and rs1800796 shows a weak correlation, indicating independent inheritance. D' and r^2 values are low for these SNPs, meaning one genotype provides little information about the other. In contrast, a substantial linkage disequilibrium exists between rs1800795 and rs1800797, suggesting they may jointly influence *IL-6* genetics and disease risk. The 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 2.

Table 9: Linkage disequilibrium analysis between SNPs of *IL-6* gene in NHL patients**.**

Linkage disequilibrium	***rs1800795***	***rs1800796***	***rs1800797***
rs1800795		D′ 0.142 r^2 0.015	D′ 0.900 r^2 0.586
rs1800796	D′ 0.142 r^2 0.015		D′ 0.238 r^2 0.056
rs1800797	D′ 0.900 r^2 0.586	D′ 0.238 r^2 0.056	

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).

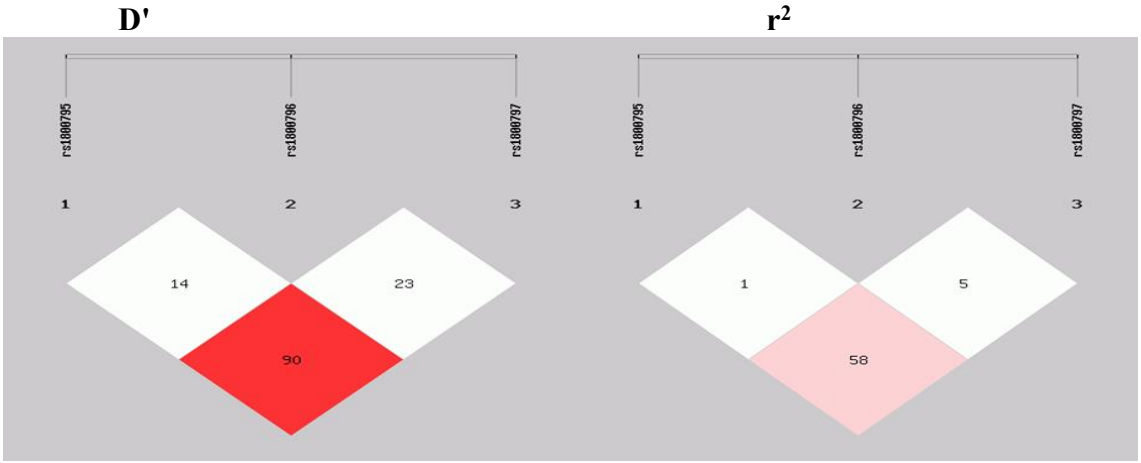

Figure 2 : Pairwise linkage disequilibrium coefficient (D') and correlation coefficient (r^2) between *IL-6* SNPs (*rs1800795*, *rs1800796* and *rs1800797*) in NHL patients and controls.

Conclusion

This research emphasizes the influence of *IL-6* gene expression and polymorphisms on the predisposition to non-Hodgkin lymphoma (NHL). The findings indicate that down-regulation of gene expression and specific SNPs, particularly rs1800795, rs1800796, and rs1800797, are associated with increased NHL risk in the studied Iraqi population. The lower expression fold of *IL-6* in NHL patients relative to healthy controls indicates a complicated relationship between *IL-6* signaling and lymphoma development. These results enhance the comprehension of the genetic determinants affecting NHL 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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Conflict of interest

The authors declare that they have no conflict of interest.

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