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Association of Interleukin-12 and Interleukin-18 Polymorphisms with Acute Lymphoblastic Leukemia Disease in Iraqi Patients

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Abstract

Acute lymphoblastic leukemia (ALL) is a blood cancer where high numbers of abnormal, immature lymphocytes called blasts start over-multiplying in the bone marrow. The lymphocyte cells and interleukins are crucial for controlling the immune response in tumor microenvironment**.** Many of the single nucleotide polymorphisms (SNPs) of some interleukin genes may change protein synthesis or function and regulate the immune response. A lot of these changes have been connected to a high risk of developing cancer. Aim of this study was to investigate the potential association between IL-12 (+1188 A/C) (rs3212227) and IL-18 (–607 A/C) (rs1946518) polymorphism with serum levels of interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) and their genotypes association with the susceptibility to ALL disease in Iraqi patients. The study included 59 ALL patients and 30 healthy controls. The detection of IL-12 and IL-18 SNP genes was determined by ARMS-PCR methods and serum levels of IFN-γ and TNF-α were determined by utilizing enzyme linked immuno-sorbent assay (ELISA) kits. Results observed no significant association of IL-12 and IL-18 polymorphism with serum IFN-γ and TNF-α level. Genotyping frequencies of IL-12 (+1188A/C) CA, AA and CA+AA genotypes showed a strong association with the development of ALL disease, IL-18 genotypes showed no significant association with the disease progression.

Keywords: Acute lymphoblastic leukemia, IL-12 Polymorphism, IL-18 Polymorphism, IFN-γ, TNF-α, Risk Factor.

ارتباط تعدد أشكال الانترليوكين-12 والانترليوكين-18 بمرض سرطان الدم الليمفاوي الحاد في المرضى العراقيين

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الخلاصه

ابيضاض الدم اللمفاوي الحاد(ALL) هو سرطان الدم حيث تبدأ اعداد كبيرة من الخلايا الليمفاوية غير الطبيعية وغيرالناضجة التي تسمى الخلايا الارومية في التكاثر المفرط في نخاع العظم.تعتبر الخلايا الليمفاوية والانترلوكينات ضرورية للتحكم في الاستجابة المناعية في بيئة الورم. وقد تغير العديد من اشكال النيوكليوتيدات المفردة (SNPs) لبعض جينات الانترلوكين في عملية تخليق البروتين او وظيفة تنظيم الاستجابة المناعية وقد ارتبط الكثير منها بخطر الاصابة بالسرطان. الهدف من هذه الدراسة كان التحقق في التاثير المحتمل بين تعدد الاشكال للانترلوكين-12(rs3212227)(+1188 A / C) والانترلوكين -18 (−607 A / C)

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(rs1946518)Cعلى مستويات المصل منالانترفيرون-كاما وعامل نخر الورم-الفا المرتبط بقابلية الاصابة بمرض سرطان الدم الليمفاوي الحاد في المرضى العراقيين. تم التحري عن تعدد الطرز الوراثية في جينات الانترلوكين-12 والانترلوكين-18 بواسطة ARMS-PCR وتم تحديد مستويات IFN-γ و TNF-α في المصل باستخدام طريقة الامتزاز المناعي المرتبط بالإنزيم . اظهرت النتائج عدم وجود ارتباط بين تعدد اشكال الانترلوكين -12 والانترلوكين-18 لانترفيرون-كاما (IFN-γ) وعامل نخر الورم (TNF-α)في مصل الدم. كما اظهرت الترددات الاليلية والجينية للانماط الجينية لانترلوكين-12 CAوAA و / 1188A +)CA+AA(ارتباطا قويا بتطور مرض ابيضاض الدم اللمفاوي الحاد ALL. بينما لم تظهر الانماط الجينية للا نترلوكين-18 اي ارتباط مع تطور المرض.

1. Introduction:

Acute lymphoblastic leukemia (ALL) is a form of cancer which develops when lymphoid progenitors proliferate out of control in the blood, bone marrow and extra medullary sites. While 80% of ALL occurs in children, it also causes a serious disease when it occurs in adults [1]. The real patho-physiological mechanism is unclear. However, inherited genetic susceptibility, particular environmental risk factors, genomic polymorphism in oxidative stress enzyme and alternation in the immune microenvironment of bone marrow, have all shown powerful significant relation with ALL [2]. Cytokines as a crucial component of the leukemia microenvironment, have been linked to the initiation and development of several types of cancers, particularly ALL [3], [4].

Interleukin 12 (IL-12) is a cytokine that was initially recognized as a maturation factor in the cytotoxic lymphocyte and a natural killer cell stimulatory factor (NKSF). According to the reports, IL-12 stimulates NK and T cell proliferation, induces cytokine production, enhances their cytolytic activity and induces cytokine production especially Interferon- γ (IFN-γ) [5]. IFN-γ, plays a crucial role in fighting tumor cells, is released by a component of the innate immune response such as natural killer cells (NK) cells [6]. Production of IFN-γ is partially mediated by IL-12 released from macrophages and dendritic cells (DC) as regulatory immune cells [7]. The stimulation of natural killer cells by IL-18, another cytokine which induces IFN-γ production, increases the immune system's capacity to fight tumor cells [8]. Interleukin-18 (IL-18) is released by numerous cells such as natural killer cells, B and T-lymphocytes, macrophages, monocytes and Langerhans cells [9, 10]. Numerous studies have recently suggested that small variations in the inherited genome, namely single nucleotide polymorphisms (SNPs) in the cytokine, may be crucial in defining a person's susceptibility to ALL [11].

IL-18 in combination with IL-12 induces secretion of IFN-γ in NK and T cells in humans [12]. It is believed that exhibiting the -607A/C, -137G/C haplotype in the IL-18 gene promoter region controls the expression of gene at the transcriptional level and alters the amount of IL-18 level produced [13]

Immune cells such as natural killer cells, neutrophils and T-lymphocytes with activated macrophages secrete TNF-α [14]. TNF- is demonstrated to be produced in the tumor microenvironment, ectopically by malignant/leukemic and immune cells, producing a tumor-supportive environment and being crucial to the onset and development of malignant disease [15].

The aim of this research was to investigate the association of IL-12 and IL-18 polymorphisms with TNF-α and INF-γ production by immune cells in the ALL tumor

microenvironment and the frequencies of IL-12 and IL-18 polymorphisms in ALL. Also to determine the potential association between IL-12 and IL-18 genotypes, and susceptibility to ALL in Iraqi patients

2. Materials and Methods

2.1. Sample collection

The Human Ethics Committee of the College of Science of Salahaddin University, Erbil authorized and approved the current study (Approval No.: 4S/43228/6/2021 Date: 9/6/2021). Informed written agreement was given by each patient for the publication of their details in this research. This study was carried out in Nanakali Hospital for Blood Diseases and Cancers, Erbil, City during August 2021 until June 2022. Peripheral five ml blood samples were taken from 59 ALL patients with median age 17 (4-55) years, including 35 males and 24 females and 30 healthy controls with median age 19.5 (10-30) years that included 15 males and 15 females. All the patients were type-B ALL and in the third cycle of treatment to exclude any differences due to different cycle of treatment. Three ml of the sample was centrifuged for 10 minutes at 3000rpm and relocated to 1.5 ml and then stored at -20°C in Eppendorf tube until the cytokine level was measured. The remaining 2 ml was put in an EDTA tube for polymorphisms of IL-12 and IL-18 genotyping.

2.2. Assessment of Cytokine Serum Levels

Serum IFN-γ and TNF-α levels were measured using ELISA kit (Sunlog Biotech Co., LTD). The assays were carried out in accordance with recommended procedures. The kits sensitivity was 2 pg/ml, inter- and intra-assay evaluations of the reliability of the abovementioned kits were performed.

2.3. Genotyping of IL-12 and IL-18 Polymorphisms

Isolation of genomic DNA from blood samples was done through use of a blood DNA preparation kit (Jena Bioscience, Germany), depending on manufacturer's instructions. An amplification-refractory mutation system PCR (ARMS-PCR) is a simple and rapid method that is utilized for amplification and genotyping of IL-12 and IL-18.

The primer sequences of IL-12 genotyping were those designed by Kazmi *et al.* [16]. The primer used for IL-12 included common primer ATCTTGGAGCGAATGGGC, A allele primer TTGTTTCAATGAGCATTT AGCATCG and C allele primer TTGTTTCAATGAGCATTTAGCATCT. Thermocycling for IL-12 was performed under settings that PCR conditions included an initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30s, annealing temperature at 58°C for 45s, and extension step at 72°C for 45s, followed by a final extension at 72°C for 5 min. The PCR products for IL-12 were 784 bp.

For IL-18 primers (forward outer CCTACAATGTTACAACACTTAAAAT and reverse outer ATAAGCCCTAAATATATGTATCCTTA) and two inner primers (forward inner GATACCATCATTAGAATTTTGTG and reverse inner GCAGAAAGTGTAAAAATTATCAA). The size of products for A allele278 bp and for C allele, were 208 bp, while the product size of the two outer primers (control band) was 440 bp. Sequences of the primers for IL-18 genotyping was designed by Taheri *et al.* [12].

For IL-18 detection polymorphism cycling conditions were included 95°C for 5 min, then denaturation at 95°C for 30s, consisting of 30 cycles, annealing at 53°C for 20s, and then extension step at 72°C for 30s. Final extension step was performed at 72°C for 10 min. Additionally, electrophoresis on a 2% agarose gel consisting 0.5 μg/ml ethidium bromide was used to analyze PCR products and visualized by trans-illumination with UV light.

2.4. Statistical analysis

GraphPad Prism Software (version 8.0.1) was used to perform the statistical analysis. One-way ANOVA was used to study the association between IL-12 and IL-18 with the secretion of TNF-**α** and IFN-**γ** in ALL patients as well as healthy controls. Chi-square test was performed to investigate the relationship between IL-12 and IL-18 genotypes and the occurrence of acute lymphoblastic leukemia and to compare the genotype distribution between patients and the control. A p-value less than 0.05 was considered to be statistically significant.

3. Results

3.1 Role of different IL-12 and IL-18 Genotypes on serum IFN-γ and TNF-α production in ALL patients

Different IL-12 genotypes showed no significant effects on serum levels of IFN-γ (P=0.416) (Table 1) and serum TNF-α levels (P=0.150) (Table 2)

Table 1: Serum IFN-γ level compared in different IL-12 genotypes in ALL patients.

IL-12 SNP	IFN-γ (Mean ± SEM) (pg/ml)	P-value
CC	87.64 ± 9.474	0.416
CA	84.94 ± 3.351	
AA	76.08 ± 1.884	

Table 2: Serum TNF- α level compared in different IL-12 genotypes in ALL patients

IL-12 SNP	TNF-α (Mean ± SEM) (pg/ml)	P-value
CC	265.6 ± 15.85	0.150
CA	267.2 ± 9.118	
AA	233.5 ± 13.67	

Moreover, there were no significant effects of IL-18 genotypes on serum IFN-γ levels (P=0.854) (Table 3) and serum TNF-α levels (P=0.249) (Table 4).

Table 3: Serum IFN level compared in different IL-18 genotypes in ALL patients

IL-18 SNP	IFN-γ (Mean ± SEM) (pg/ml)	P-value
CC	85.36 ± 6.203	0.854
CA	80.06 ± 3.271	
AA	82.35 ± 4.415	

Table 4: Serum TNF- α level compared in different IL-18 genotypes in ALL patients

IL-18 SNP	TNF-α (Mean ± SEM) (pg/ml)	P-value
CC	268.5 ± 10.38	0.249
CA	268.3 ± 15.46	
AA	243.7 ± 9.468	

3.2 Role of IL-12 and IL-18 polymorphism on susceptibility to ALL disease

Allele and genotype frequencies of IL-12 (+ 1188 A/C) CA and AA genotypes showed an association with the development of acute lymphoblastic leukemia with OR (95% CI) = 6.263 (1.615 to 22.04), (2.429 (0.564 to 9.9129) and dominant model OR (95% CI) = 4.636 (1.339 to 15.94) association of risk factor and genotypes (Table 5).

Table 5: Genotypic and allelic frequencies of the IL-12(rs3212227) (C/A) polymorphism in ALL patients and the control subjects.

IL-12 (rs3212227) (C/A) SNP	ALL Patients N=59(No.%)	Control N=30(No.%)	OR	95%CI	P-value
Genotype Frequencies					
Co-dominant					
CC	17(34)	3(10)	1	-	-
CA	19(38)	21(70)	6.263	1.615-22.04	0.005
AA	14(28)	6(20)	2.429	0.564-9.912	0.450
Dominant					
CC	17(34)	3(10)	1	-	-
CA+AA	33(66)	27(90)	4.636	1.339-15.94	0.016
Recessive					
CC+CA	36(72)	24(80)	1	-	-
AA	14(28)	6(20)	0.6429	0.210-1.985	0.424
Alleles					
C	53(53)	27(45)	1.378	0.727-2.664	0.327
A	47(47)	33(55)			

However, there was no statistical association between different IL-18 genotypes and development of ALL as a risk factor (Table 6).

Table 6: Genotypic and allelic frequencies of the IL-18(rs1946518) (C/A) polymorphism in ALL patients and the control subjects.

IL-18 (rs1946518) (C/A) SNP	ALL Patients N=59(No.%)	Control N=30(No.%)	OR	95%CI	P-value
Genotype Frequencies					
Co-dominant					
CC	26(52)	18(60)	1	-	-
CA	9(18)	6(20)	0.963	0.264-2.940	0.951
AA	15(30)	6(20)	0.578	0.178-1.667	0.335
Dominant					
CC	26(52)	18(60)	1	-	-
CA+AA	24(48)	12(40)	0.722	0.288-1.761	0.486
Recessive					
CC+CA	35(72)	24(80)	1	-	-
AA	15(30)	6(20)	0.583	0.192-1.760	0.325
Alleles					
C	61(61)	42(70)			
A	39(39)	18(30)	0.670	0.342-1.350	0.307

4. Discussion:

Acute lymphoblastic leukemia is a malignancy of lymphoid cells considered by the accumulation and the proliferation of hematopoietic progenitor cells in the bone marrow that impedes normal blood production [17]. Great efforts have been directed to study SNP and its relation to immune response and cancer disease susceptibility and its outcome [18]. Immune cells secrete cytokines as a functional protein group which is described to modulate immune response. Some cytokines are linked to the development and spread of tumors [19].

Single nucleotide polymorphism in the regulatory sequences of interleukin genes are thought to be related to the different secretion of cytokines. However, our study showed that IL-12 polymorphism CC, CA, and AA have no significant effects on serum IFN-γ production in ALL patients (Table 1). Yilmaz *et al*. demonstrated that CC homozygous individuals expressed significantly more IL-12 compared to AA homozygotes or AC heterozygotes with IL-10 secretion also proximal promoter −1082 SNP has been reported in Caucasian populations in Turkey [20].

Genetic element and effectors mediator regulate the immune response through interleukin-12 as a pro-inflammatory cytokine produced by antigen-presenting cells, which is a stimulator of IFN-γ production from T and natural killer (NK) cells. Interferon-γ expression reduced in ALL patients leads to the disruption of the immune system in ALL patients enabling tumor cells to evade immune surveillance [4, 21]. In the current investigation, no significant correlations were found between serum levels of TNF-α and IL-12 different genotypes (Table 2). However, Westendrop *et al.* determined the amount of genetics effects on cytokine secretion and its role in the fatal outcomes. They observed the capability to release TNF-α (TNFα) at low levels in patients who had meningococcal disease with a tenfold higher probability of fatal outcomes (OR 8.9, 95% CI 1.8–45) [22].

The secretion of IFN-γ by T cells and NK cells as well as the NK cells cytolytic activity can both be enhanced by interlukin-18 [8]. However, the polymorphism CC, CA, and AA in IL-18 showed no significant effects on serum IFN-γ and TNF-α production by these cells in ALL patients as shown in Tables 3 and 4. In contrast, a study of three single nucleotide

polymorphisms in human IL-18 of 48 multiple sclerosis patients from Swedish population showed that common IL-18 promoter polymorphisms influence potentially the expression of IFN-γ [23].

Furthermore, for the first time in this research we evaluated an association between IL-12 and IL-18 genotypes and susceptibility to ALL in Iraqi patients. This finding showed a significant association between CA and AA genotype of IL-12 (+ 1188 C/A) with the development of acute lymphoblastic leukemia (OR=6.263, 95%CI=1.615-22.04, p=0.005) (OR=2.429, 95%CI=0.564-9.9129, p=0.450) respectively. Also, the dominant CA+AA genotype of IL-12 (+ 1188 C/A) showed a significant association with the development of acute lymphoblastic leukemia (OR=4.636, 95% CI = 1.339-15.94, p=0.016) (Table 5). Numerous researches have investigated the link between IL-12 polymorphism and the risk of cancer, with mixed results. Cancer risk was greater among people with the CC/AC IL-12 genotypes as in breast cancer [24], gastric cancer [25], bladder cancer [26] and cervical cancer [27]. However, no significant association was observed between the IL-12 polymorphism and the risk of hepatocellular carcinoma and/or colorectal cancer development [28].

On the other hand, no significant association between different IL-18 genotypes and the development of ALL as a risk factor was observed in our study (Table 6). Similar to our results, no significant association between different IL-18 (-607C>A) genotypes and development of breast cancer [29] and colorectal cancer [30] have been reported. Few studies have indicated that SNP (-607C>A) in the IL18 gene is associated with increased risk of hepatocellular carcinoma in Egyptian patients [31] and higher risk of prostate cancer in the Iranian population [32].

In conclusion, present study showed that IL-12 and IL-18 polymorphism CC, CA, and AA have no significant effects on serum IFN-γ and TNF-α production in ALL patients and no significant association between different IL-18 genotypes and development of ALL was observed. However, the co-dominant variant CA and AA genotype of IL-12 (+ 1188 C/A) and the dominant CA+AA genotype of IL-12 (+ 1188 C/A) are significantly associated with the development of acute lymphoblastic leukemia and may serve as prognostic predictors for ALL risk among Iraqi patients.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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