Oncology and Radiotherapy ©
Vol.17 Iss.12: 001-007 • RESEARCH ARTICLE

IL-27 rs153109 Polymorphisms and serum level as prognostic risk factor to relapse and resistance in adult patients with Acute Lymphoblastic Leukemia (ALL)

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Abstract:

Background: Acute Lymphoblastic Leukemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor B cell (BCP-ALL) or T cell (T-ALL) origin that invade the bone marrow, blood and extramedullary sites. While many studies addressed the outcome of adult ALL in developed Western countries, there is paucity of such prospective studies from developing Mediterranean ones. This study conducted at Baghdad Teaching Hospital/ Hematology center in Iraq to detect Interleukin (IL)-27 polymorphism and serum level because this cytokine has important anti-cancer activity.

Aim: our aim of the present study is to investigate the possible role of IL27 gene polymorphism as risk factors for the development and whether they effect on the clinical outcome of ALL in Iraqi adult patients , along with possibilities for using IL27 amount in blood for monitoring relapse and resistant patients

Materials and Methods: A case control study have been conducted and based on : 70 adult Iraqi patients diagnosed clinically by physician as having ALL and 40 healthy control to genotyping of IL27 (rs153109) single nucleotide polymorphisms (SNPs) which was accomplished using a polymerase chain reaction–restriction fragment length polymorphism (RFLP-PCR) assay and ELISA for measure serum level IL27 .

Results: Comparison of frequencies of IL-27-AG genotypes and alleles between control group and group of response show Genotypes AA and A/G were not significantly associated with response group , but alleles showed significant variation in such a way that allele A was against response and allele G was with response , Comparison of IL-27 AG genotypes and allele frequencies between group of response and group of no response there was no significant difference in genotypes or allele frequencies thus, no genotypes or allele can be regarded as a risk or a protective factor. Regarding IL-27 level in serum, there was significant difference among groups ($p<0.001$) in such a way the levels in relapse and resistance groups higher than both control group and response group ($p<0.05$).

Conclusion: IL27 is good biomarker for prognosis relapse in patient that complete treatment and therapy resist and must continuously doing checkup , also open agate for using them as new therapeutic target .

Key words: Lymphoblastic Leukemia, bone marrow

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Word count:4582 **Tables:** 09 **Figures:** 01 **References:** 33

Received:-25 July, 2023, Manuscript No. OAR-23-108931
Editor assigned:-28 July, 2023, Pre-QC No. OAR-23-108931 (PQ)
Reviewed:-15 August, 2023, QC No. OAR-23-108931 (Q)
Revised:-23 August, 2023, Manuscript No. OAR-23-108931 (R)
Published:-05 September, 2023, Invoice No. J-108931

INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is uncontrolled proliferation of lymphoblast, decreased number of mature lymphocyte cells in the Bone Marrow (BM) [1]. Relapsed and/or Refractory ALL still remains with a very low survival and high morbidity associated with its treatment and the disease still remains a challenge in developing countries [2-4]. Unfortunately, overall survival in adults with ALL is worst, even though most adult patients can reach initial complete remission using recently developed treatments [5].

The potential mechanisms that cause relapse involve clonal evolution, the ability of ALL cells to escape the immune-suppressive tumor response and innate and acquired chemoresistance [6].

IL-27 is one of the limited cytokines that play a role in HSC regulation. IL-27 act as a potent stimulus for lymphocyte expansion and survival and there are reports that address the impact of IL-27 on haematopoesis [7]. And induced proliferation of human naive CD4 T cells and B cells [8]. Implies that IL-27 may be an important molecule in controlling immune checkpoint mechanisms that operate in cancer [9].

The genes responsible for cytokine generation are highly influenced by the presence of Single Nucleotide Polymorphisms (SNP) in main regions such as regulatory sequences or in promoter regions, contributing to disease susceptibility and evolution [10]. (rs153109) is a functional polymorphism, located 964 bp upstream to the transcription site of the IL-27 gene, consisting of the transition of A to G, this transition leads to the formation of a new binding site in the IL-27 gene promoter, consequently changing the IL-27 gene expression pattern [11].

The intronic SNP (rs 153109) in IL-27 gene has three genotypes (AG, GG and AA) the association of IL-27 rs153109 polymorphisms with risk of ALL development suggested an important role for this

cytokine in biology and response to ALL therapy [12-14].

MATERIALS AND METHODS

A case-control study was done during the period from January 2021 to October 2022 , number of patients in the study was 70 Iraqi patients diagnosed clinically by physician as having ALL their ages between (14- 72) years groups as follows: First group 40 patients were diagnosed with ALL (reach complete remission and still on treatment) , 30 patients as second group they were (10 resist and 20 relapsed) . Also, the 40 samples of healthy individuals were enrolled in this study, their ages between (14 - 72) years. The study protocol was approved by the Ethics Committee of the Iraqi Ministry of Health and written informed consent was obtained from all participants before entering the study.

Whereas, RFLP PCR Technique was used for SNP primers (IL 27 SNP rs153109), F 5-TCAGTCAGTGACCAGGATCG-3′, R 5-ACCAAGAAACCCCATCCTCT-3′ with prodect size 224bp was design in this study by using NCBI-Database]15]. Primers was provided by Macrogen company, and using Restriction Enzyme (AVAI) (AA: 224bp; GG: 183bp, 41bp; AG: 183bp, 41bp, 224bp)

Collection of Blood Samples

Blood Samples has been collected by vein puncture under aseptic condition using sterile syringe, 3ml of blood has been separated into two tubes 1st tube with EDTA for SNPS, 2nd without EDTA for serum separation in order to measure seum concentration by ELISA test, then stored in a deep freeze at -70℃ for molecular analysis.

Then Determination of gDNA concentration was measured the optical density at 260 nm with A spectrophotometer Uv-1900i, (dsDNA= 50 µg/ml) [16]. DNA purity was measured by calculating the ratio of at OD260/OD280, the ratio should be 1.8 ±0.2 [17].

RFLP-PCR technique was performed for detecting (IL 27 SNP rs153109) in ALL patients and in healthy control blood samples as the following steps:

PCR master mix was prepared by using (Promega kit) and this master mix done according to company instructions as following (Table 1).

Tab 1. PCR Master Mix

PCR Master mix	Volume
DNA template	6 µl
Forward primer (10pmol)	2 µl
Reveres primer (10pmol)	2 µl
Free nuclease water	2.5 µl
Total volume	12.5µl

After that, these PCR master mix component that mentioned in table above placed in standard Promega Kit that contains all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and loading dye). Then, all the PCR tubes transferred into vortex centrifuge at 3000 rpm for 3 minutes. Then placed in PCR Thermocycler. PCR thermocycler conditions were done for each gene (IL-27) as following (Table 2).

Tab 2. PCR Thermocycler program

PCR step	Temp.	Time	Repeat
Initial denaturation	95 °C	3 min	
Denaturation	95°C	0.30 sec	36 cycle
Annealing	55°C	0.30 sec	
Extension	72°C	0.30 sec	
Final extension	72°C	5 min	
Hold	4°C		

The PCR products were analysed by Agarose gel electrophoresis. RFLP-PCR mix preparation. RFLP-PCR mix was for (IL 27 SNP rs153109) were prepared by using (AVAI restraction enzyme) this master mix done independent according to company instructions as following (Table 3).

Tab 3. RFLP-PCR master mix preparation for each reaction

RFLP-PCR Master mix	Volume
PCR product	10µl
Restriction enzyme buffer 10X	2 µl
Restriction enzyme (10 unit)	1 µl
Nuclease free water	7 µl
Total volume	20 µl

After that, this master mix placed in vortex centrifuge at 3000 rpm for 2 minutes, then transferred into incubation at 37°C for 15 min. After that, RFLP-PCR product was analysis by 3% agarose gel electrophoresis methods that mention in PCR product analysis.

RESULTS

Comparison of frequencies of IL-27-AG genotypes and alleles between control group and group of response is shown in (Table 4). Genotypes AA and A/G were not significantly associated with response group (p = 0.945), but alleles showed significant variation (p = 0.003) in

such a way that allele A was against response (odds ratio 0.98) and allele G was with response (odds ratio = 1.02).

Tab. 4. Comparison of frequencies of IL-27-AG genotypes and alleles between control group and group of response

IL-27-AG	Control	Response	p	OR	95 % CI
	n = 30	n = 40			
Genotypes					
AA, n (%)	14 (46.7 %)	19 (47.5 %)	0.945 C	0.97	0.37 - 2.50
A/G, n (%)	16 (53.3 %)	21 (52.5 %)	NS	1.03	0.40 - 2.67
Alleles	Control	Response	p	OR	95 % CI
	n = 60	n = 80			
A, n (%)	44 (73.3 %)	59 (73.8 %)	0.003 C **	0.98	0.46 - 2.09
G, n (%)	16 (26.7 %)	21 (26.3 %)		1.02	0.48 - 2.18

n: number of cases; C: chi-square test; NS: not significant; **: significant at p ≤ 0.01; OR: odds ratio; CI: confidence interval

Comparison of frequencies of IL-27-AG genotypes and alleles between control group and group of relapse is shown in (Table 5). Genotypes AA and A/G and alleles A and G were not significantly associated with relapse group (p>0.05).

Comparison of frequencies of IL-27-AG genotypes and alleles between control group and group of resistance is shown in (Table 6). Genotypes AA and A/G and alleles A and G were not significantly associated with resist group (p>0.05)

Tab. 5. Comparison of frequencies of IL-27-AG genotypes and alleles between control group and group of relapse

IL-27-AG	Control	Relapse	p	OR	95 % CI
	n = 30	n = 20			
Genotypes					
AA, n (%)	14 (46.7 %)	4 (20.0 %)	0.054 C	3.5	0.94 - 12.97
A/G, n (%)	16 (53.3 %)	16 (80.0 %)	NS	0.29	0.08 - 1.06
Alleles	Control	relapse	p	OR	95 % CI
	n = 60	n = 40			
A, n (%)	44 (73.3 %)	24 (60.0 %)	0.161 C	1.83	0.78 - 4.30
G, n (%)	16 (26.7 %)	16 (40.0 %)	NS	0.55	0.23 - 1.28

n: number of cases; C: chi-square test; NS: not significant; OR: odds ratio; CI: confidence interval

Tab. 6. Gender distribution for the weight of lumber vertebral marrow metastasis patients

IL-27-AG	Control	Resistant	p	OR	95 % CI
	n = 30	n = 10			
Genotypes					
AA, n (%)	14 (46.7 %)	6 (60.0 %)	1.000 F	0.58	0.14 - 2.50
A/G, n (%)	16 (53.3 %)	4 (40.0 %)	NS	1.71	0.40 - 7.34
Alleles	Control	resist	p	OR	95 % CI
	n = 60	n = 20			
A, n (%)	44 (73.3 %)	16 (80.0 %)	0.551 C	0.69	0.20 - 2.37
G, n (%)	16 (26.7 %)	4 (20.0 %)	NS	1.45	0.42 - 5.01

n: number of cases; C: chi-square test; F: Fischer exact test; NS: not significant; OR: odds ratio; CI: confidence interval

Comparison of Genotypes and Allele Frequencies between Group of Response and Group of No Response

Comparison of IL-27 AG genotypes and allele frequencies between group of response and group of no response is shown in Table 7 and there was no significant difference in genotypes or allele frequencies (p >0.05). Thus, no genotypes or allele can be regarded as a risk or a protective factor.

Tab. 7. Comparison of IL-27 AG genotypes and allele frequencies between group of response and group of no response.

IL-27-AG	Response	No response	p	OR	95 % CI
	n = 40	n = 30			
Genotypes					
AA	19	10	0.234 C	1.81	0.68 - 4.82
A/G	21	20	NS	0.55	0.21- 1.47
Alleles	Response	No response	p	OR	95 % CI
	n = 80	n = 60			
A	59	40	0.362 C	1.4	0.68 - 2.92
G	21	20	NS	0.71	0.34 - 1.48

n: number of cases; C: chi-square test; NS: not significant; OR: odds ratio; CI: confidence interval

Tab. 8. Comparison of serum levels of, IL-27 according to response to treatment after merging relapse and resistant group into one common group (no response group)

Characteristic	**Control**	**Response**	**No response**	**p**
	n = 30	**n = 40**	**n = 30**	
IL-27				
Median (IQR)	86.81 (29.86)	101.46 (45.61)	211.88 (96.56)	<0.001 K ***
	B	B	A	
Range	52.53 - 133.46	26.48 - 157.32	25.89 - 380.02	

n: number of cases; IQR: inter-quartile range; K: Kruskall Wallis test; *: significant at p ≤ 0.05; **: significant at p ≤ 0.01; ***: significant at p ≤ 0.001; Capital letters (A, B and C) were used to show the level of significance following conduction of post hoc Dunn's multiple comparison test so that similar letters indicated no significant difference; whereas, different letters indicated significant difference.

Comparison of serum levels of IL-27 according to response to treatment is shown in (Table 8). With respect to IL-27, there was significant difference among groups (p =0.006) in such a way that the level was not significantly different between control group and response group (p>0.05) and the levels in relapse and resistance groups were not significantly different from each other, but were significantly higher than both control group and response group (p<0.05) (Figure 1).

Tab 9. Comparison of serum levels of IL-27 according to response to treatment

Characteristic	Control	Response	Relapse	Resistant	p
	n = 30	n = 40	n = 20	n = 10	
IL27					
Median (IQR)	86.81 (29.86)	101.46 (45.61)	222.57 (65.95)	192.36 (189.08)	
	B	B	A	A	0.006 K**
Range	52.53 - 133.46	26.48 - 157.32	25.89 - 380.02	37.68 - 303.4	

n: number of cases; IQR: inter-quartile range; K: Kruskall Wallis test; *: significant at p ≤ 0.05; **: significant at p ≤ 0.01; ***: significant at p ≤ 0.001; Capital letters (A, B and C) were used to show the level of significance following conduction of post hoc Dunn's multiple comparison test so that similar letters indicated no significant difference; whereas, different letters indicated significant difference.

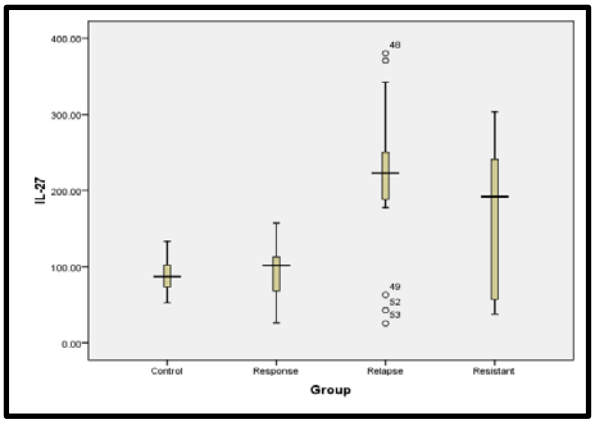

Fig. 1. Box plot showing comparison of serum levels of IL-27 according to response to treatment

Regarding IL-27 level in serum, there was significant difference among groups (p<0.001) in such a way the levels in relapse and resistance groups higher than both control group and response group (p<0.05) (Table 9).

DISCUSSION

Interleukin-27 (IL-27) has been recognized as a pleiotropic cytokine with both pro- and anti-inflammatory properties, few studies have investigated polymorphisms and serum/plasma levels of IL-27 in diseases including cancers [12].

The effect of IL-27 on hematopoietic stem cells (HSCs) remains unknown, but results suggest that IL-27 is one of the limited cytokines that play a role in HSC regulation [7].

Risk of relapse represents the major drawback of ALL due to IL-27 ability to act on leukemia initiating cells that are unresponsive to chemotherapeutic drugs, administration of IL-27 may be used in those patients which tend to relapse or as preventive therapy in combination, IL-27 appears to be a good candidate for ALL therapy due to its multifaceted activity, thus, IL-27 targets both B-ALL blasts and TICs, inhibiting leukemia growth and dissemination , the first demonstration of this issue comes from IL-12 family cytokines whose immune-regulatory functions are well known and whose direct anti-leukemic properties have been recently characterized , receptors for these cytokines can be expressed on leukemia cells and represent potential therapeutic targets for pediatric ALL [18].

Xu et al suggested that IL-27 polymorphism enhanced cancer susceptibility in Chinese population, in addition, results also demonstrated that IL-27 polymorphism increased colorectal cancer risk, however, large and well-designed studies based on homogeneous cancer patients are needed to confirm these findings [18,19].

We didn't identified significant associations between IL-27 SNP and susceptibility to ALL or relapse and resist , but alleles showed significant variation (p = 0.003) in such a way that allele A was against response (odds ratio 0.98) and allele G was with response that's disagree with (Ghavami et al) who observed a significant difference in allele and genotype distributions of rs153109 between patients and controls , also they suggested that patients with the rs153109 AG genotype possibly have a poorer prognosis also finding of his study on the association of rs153109 AG with a greater rate of relapse in patients supported this result (13).

Allele in previous studies have a far from our results result in patients with renal cancer , they observed a significant association of renal cancer risk with the G allele of rs153109 SNPs , these researchers also found an increased risk of cancer with the AG genotype compared to the AA/GG genotypes [20].

Similar results with about G allele in patients with colorectal cancer compared to healthy subjects in the Chinese Han population but disagree reported significantly higherrs153109 GG genotype [21].

Results of IL27 serum level was exactly agree with they measured quantitative serum levels in pediatric with ALL (before and after induction of the chemotherapy

required for treatments) and has been found that the levels of this cytokine was higher in all ALL patients as compared with their level in the control group and were also higher in relapse group than those in remission cases and conclude that this group of cytokine might be playing a role as a biomarker for pediatric ALL and an indicator of disease prognosis as well, it might keep our attention to be considered as a guide for treatment response [22].

Also we show similar results with there was higher serum levels of IL-27 in patients compared to controls [23] .

The heterogeneity of present study with previous studies might attribute to difference in age, gender, living environment and life styles of each groups. Therefore, the precise relationship of IL-27 polymorphism and ALL risk should be investigated in larger sample size case–control studies.

IL-27 is produced early after activation antigen-presenting cells, and is thought of as an immunostimulatory cytokine due to its capacity to induce Th1 differentiation, however, many studies have also identified various immunosuppressive effects of IL-27 signaling, including suppression of Th17 differentiation and induction of co-inhibitory receptors on T-cells [23, 24].

Interleukin 27 (IL-27) is mediates its biologic functions via a heterodimeric cytokine and plays an important role in immune homeostasis, binding of IL-27 to cell surface receptors, IL-27Rα (WSX1) and gp130, results in activation of receptor-associated Janus Kinases and nuclear translocation of Signal Transducer and Activator of Transcription 1 (STAT1) and STAT3 transcription factors and mitogen-activated protein kinase (MAPK) signaling [25-27]. The activation of STAT1 is linked to inhibition of GATA-3 and RoRγt but upregulation of PD-L1, T-bet and IL-10, the ability to engage STAT3 is linked to increased proliferation as well as IL-10 while the MAPK pathway intersects with AHR to promote IL-10 and IL-21 [28].

IL-27 was initially reported as an immune-enhancing cytokine that supports $CD4^+$ T cell proliferation, T-helper (Th)1 cell differentiation, and IFN-γ production, acting in concert with IL-12 (IL-27 synergizes with IL-12 to potentiate IFN-γ production by activated naïve T-cell and natural killer-cell populations) , however, subsequent studies demonstrated that IL-27 displays complex immune-regulatory functions, which may result in either proinflammatory or anti-inflammatory effects in relationship to the biological context and experimental models considered, several pieces of evidence, obtained in preclinical tumor models, indicated that IL-27 has a potent antitumor activity, related not only to the induction of tumor-specific Th1 and Cytotoxic T Lymphocyte (CTL) responses , Therefore, IL-27 is thought to promote Th1 polarization but also to direct inhibitory effects on tumor cell proliferation, survival, invasiveness, and angiogenic potential l , nonetheless, given its immune-regulatory functions, the effects of IL-27 on cancer may be dual and protumor effects may also occur [29,30].

In addition, transgenic over-expression of IL-27 leads to anticancer activity in colon cancer and lymphoma, which is mainly mediated through $CD8^+$ T cells with enhanced Cytotoxic T Lymphocyte (CTL) activity [31, 18].

Another anti-cancer mechanism of IL-27 may involve IL-10 and programmed death ligand (PD-L), IL-27 can stimulate the production of IL-10 in CD8+ T cells which contributes to tumor rejection [32]. IL-27 is an anti-inflammatory cytokine that triggers enhanced antitumor immunity, particularly cytotoxic T lymphocyte responses thus, IL-27 is a feasible approach for enhancing $CD8^+$ T cells' antitumor immunity and can be used as a therapeutic adjutant for T-cell adoptive transfer to treat cancer [33].

SNPs of IL-27 may influence the anti-cancer activity of IL-27, which might account for etiology of cancer may contribute to various cancer susceptibility [14].

To the best of our knowledge, this is the first study in Iraq investigated the association between IL-27 polymorphism, IL27 serum level and ALL risk in adult patients. However, several limitations of our study should be noted. First, the analysis of results should be interpreted with caution because of the small sample size. Second, few studies found for compared results. Third, our results was based on unadjusted estimates, while a more precise analysis should be performed on individual data such as, smoking status, obesity, environmental factors , and other lifestyle also, treatment protocol , history of family

CONCLUSION

IL27 is good biomarker for prognosis relapse in patient that complete treatment and therapy resist and must continuously doing checkup , also open agate for using them as new therapeutic target.

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