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ORIGINAL ARTICLE

Association of IL-27 rs153109 and rs17855750 Polymorphisms with Risk and Response to Therapy in Acute Lymphoblastic Leukemia

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Abstract Interleukin (IL)-27 is a cytokine with important anti-cancer activity. This study has evaluated the effects of IL-27 rs153109 and rs17855750 single nucleotide polymorphisms (SNPs) on risk of acute lymphoblastic leukemia (ALL) development, as well as their impact on prognosis and patient survival. A total of 200 patients and 210 healthy subjects were genotyped by polymerase chain reaction-restriction fragment length polymorphism. We observed a higher frequency of rs153109 AG and rs17855750 TG genotypes and allele G in patients compared to controls ($p < 0.001$). Combined G variant genotypes (AG + GG and TG + GG) also conferred significantly greater risk of ALL. There was a significant correlation between the genotypes of both SNPs with event-free survival (EFS). Patients with GG genotypes of both SNPs and those of rs153109 AG and rs17855750 TG had a shorter EFS than patients with rs153109 AA and rs17855750 TT genotypes ($p \leq 0.035$). Combined G variant genotypes for both SNPs showed poorer response to therapy in all patients ($p < 0.027$) as well as B-ALL (rs153109, $p < 0.001$) and T-ALL (rs153109, $p = 0.048$) patients. In multivariate analysis, rs153109 combined G variant genotype was associated with shorter EFS (relative risk = 9.7, $p = 0.026$). Among those who relapsed, 87.1% had the rs153109 AG genotype and 77.4% had the rs17855750 TG genotype ($p < 0.01$). Patients had higher IL-27 serum levels compared to controls, but this did not differ between genotypes. In conclusion, the association of IL-27 rs153109 and rs17855750 polymorphisms with risk of ALL development and their impact on EFS suggested an important role for this cytokine in biology and response to ALL therapy.

Keywords Interleukin-27 · Acute lymphoblastic leukemia · Polymorphism

Introduction

Acute lymphoblastic leukemia (ALL) is a malignancy marked by overproduction of lymphoblasts. This disease is the most common malignancy in childhood; 2–4 cases per 100,000 children live with ALL. The rate of ALL in Fars Province, Iran has been reported as 30 cases per million with a peak incidence in children under the age of 4 years [1, 2]. This disease can be a malignant disorder of precursor T cell (T-ALL) in 15%–20% or B cell (B-ALL) origin in 80%–85% of cases. The main mechanism that causes ALL is not well known, but chromosomal translocations, abnormalities in the signaling pathways, in addition to genetic and environmental factors are suggested as contributing factors [3].

Cytokines are small proteins that play important roles in immune responses. The interleukin (IL)-12 family of cytokines, comprised of IL-12, 1 L-23, IL-27 and 1 L-35, acts as a bridge between innate and adaptive immunity [4]. B cells, dendritic cells, monocytes, and macrophages mostly secrete members of the IL-12 family. IL-12, the major cytokine of this family is mainly produced by dendritic cells and has the

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capacity to differentiate T cells toward T helper (h)1 cells [5, 6]. This cytokine is composed of two p40 and p35 subunits which bind to IL-12-receptor (R)B1 and IL-12-RB2, respectively. The subunit p40 is shared with other members of this family. Among the members of IL-12 cytokine family, IL-27 is a heterodimeric cytokine with two subunits of the IL-12 p35-related protein (p28) and the IL-12 p40-related protein [(Epstein-Barr induced molecule 3 (EBI3)] [7]. This cytokine is mainly secreted from antigen presenting and processing cells, such as macrophages and dendritic cells [7]. The IL-27 receptor, which is a complex that consists of WSX-1 and glycoprotein 130 (gp130), expresses on endothelial cells, monocytes, mast cells, fibroblasts, and T and B cells [8–10]. The role of IL-27 in regulating the activity of T cells is controversial; both proinflammatory and anti-inflammatory activity have been reported [11]. This cytokine has initially been shown to induce naive T cell proliferation and promotion toward the Th1 immune response by inducing phosphorylation of signal transducers and activators of transcription (STAT)1, STAT3, and T-bet expression [12]. Recent studies report its direct inhibitory effects on Th2 and Th17 cell activities by inhibition of their related transcription factors GATA3 and retinoic acid receptor-related orphan receptor (ROR)γt [13]. In addition, IL-27 is a major stimulus for T cell production of IL-10 [14, 15].

Various studies have investigated the effect of IL-27 on tumor immune response [16–18]. IL-27 can augment antitumor cytotoxic T cells (CTL) responses through direct stimulation of $CD8^+$ T cells and programming CTLs into memory effector cells. This cytokine can increase CTL survival without reduction of cytolytic activity. In addition it stimulates human natural killer (NK) cell effector functions [15, 16]. IL-27 has been shown to act directly against multiple myeloma cells and inhibit angiogensis by activating production of chemokines such as CXCL10 and CXCL9 [18]. Based on these studies IL-27 has been suggestedas a novel, promising therapeutic agent for patients affected by different oncologic diseases.

The IL-27 gene is located on chromosome 16p11 and consists of five exons [19]. This gene has several single nucleotide polymorphisms (SNPs). Some have associations with various malignancies [20–23]. Among various polymorphisms, two common rs153109 and rs17855750 SNPs have been the subject of most studies. The associations of these SNPs with breast [20], esophageal [21], cervical [22], and ovarian cancer [23] have been shown. However, to the best of our knowledge, their relationship with hematological malignancies such as ALL has not been investigated. Considering the important regulatory role of IL-27 cytokine in tumor immunity, we sought to assess the contribution of IL-27 rs153109 and rs17855750 SNPs to ALL susceptibility. In addition, we investigated the relationship between these variants to prognostic factors, and response to therapy.

Materials and Methods

Subjects

A total of 200 ALL patients admitted between 2010 to 2016 to Shiraz University of Medical Sciences affiliated hospitals, Shiraz, Iran enrolled in this study. Histopathological, immunological, and clinical findings confirmed the disease diagnosis. The patients' ages ranged from 1 to 18 years (6.5 ± 5.0 years). Of patients, there were 40 new cases that had received no chemotherapy at enrollment. We used the samples from these patients for IL-27 serum measurements. The laboratory and clinical characteristics recorded at presentation consisted of gender, age, white blood cell (WBC) counts and platelet numbers, immunophenotype, hemoglobin (Hb) levels, percentages of blast in the bone marrow and peripheral blood, and extra-medullary involvement (EMI) that included splenomegaly, hepatomegaly, lymphadenopathy, and CNS involvement. Due to incomplete documentation, we could not record clinical and laboratory information of all the patients, which included cytogenetics data. Patients received treatment according to the Children's Oncology Group (COG) protocol [24]. During the follow-up period, we evaluated response to therapy and outcome by measuring relapse rate, and event-free survival (EFS), and overall survival (OS). We divided the patients according to the protocol and on the basis of prognostic factors into two groups: standard (WBC: $<50 \times 10^3/\mu l$ and age 1 to <10 years) and high-risk (WBC: $\geq 50 \times 10^3/\mu l$ or age < 1 and ≥10 years) [25].

The control group included 210 healthy age and sex-matched subjects who referred to Shahid Motahari Outpatient Clinic and Namazi Hospital for regular examinations and had no signs of familial malignancy or other serious diseases. Of these, we used serum samples of 40 age- and sex-matched subjects as the control for serum IL-27 measurement. All patients and controls or their parents gave informed consent. The Shiraz University of Medical Sciences Ethics Committed approved this study.

Patient Samples and Genomic DNA Extraction

We obtained 2 ml whole blood in EDTA from all patients and controls in order to extract genomic DNA. Extraction was performed using a Genetbio DNA Blood Kit (South Korea) according to the manufacturer's instructions. Final preparation was quantified by measuring absorbance at 260 and 280 nm, after which we stored the extracts at −20 °C until use. In addition, 2 ml of blood was also collected from 40 new cases and matched controls to measure serum IL-27 levels.

Genotyping

The IL-27 rs153109 and rs17855750 polymorphisms were analyzed using polymerase chain reaction-restriction fragment

length polymorphism (PCR-RFLP). Sequences of the primers selected from the previous studies [21] were as follows: SNP rs153109 [forward]: 5-TCAGTCAGTGACCAGGATCG-3′, [reverse]: 5-ACCAAGAAACCCCATCCTCT-3′; SNP rs17855750, [forward]:5-CCTGGTTCAAGCTGGTGTCT-3′, [reverse]:5-GGGGCAAGGTCTGTTAGTGG-3′. Each PCR amplification was prepared in a total reaction volume of 20 μl containing: 10 μl 2XTEMPase Hot Start Master Mix Blue [Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 3.0 mM $MgCl_2$, 0.2% Tween 20, 0.4 mM of each dNTP, 0.2 unit/μlTEMPase Hot Start DNA Polymerase, blue dye and stabilizer] (Ampliqon, Denmark), 0.5 μM of each primer (Bioneer, South-Korea), and 5–40 ng genomic DNA. A PCR reaction was applied as negative control to detect contamination of PCR products. The PCR reactions were as follows: Initial denaturation at 95 °C for 15 min and then 35 cycles [denaturation at 95 °C for 30 s, annealing at different temperatures (63.5 °C for rs153109 and 68.5 °C for rs17855750) for 30 s, extension at 72 °C for 30s], and a final extension at 72 °C for 5 min. The PCR products size (rs153109, 224 bp; and rs17855750, 225 bp) were visualized on a 2% agarose gel stained by safe stain dye (CinnaGen, Iran) to detect the quality of the amplification. The PCR products were digested with restriction endonuclease AVAI (Eco881) (Fermentase, Lituania) for rs153109 and Hpy188I (Biolabs, UK)] for rs17855750. After incubation at 37 °C for overnight, digested products were separated by electrophoresis on 3.5% agarose gel (Invitrogen, UK) and the bands were visualized by an ultraviolet transilluminator (Upland, CA) (Fig. 1). The sizes of fragments produced by restriction enzymes were as follows: rs153109, a 224 bp fragment (AA), two fragments of 183 bp and 41 bp (GG) and three fragments of 183 bp, 41 bp and 224 bp (AG); rs17855750, a 225 bp fragment (GG), two fragments of 118 bp and107 bp (TT) and three fragment of 118 bp, 107 bp and 225 bp (TG).

Enzyme-Linked Immunosorbent Assay (ELISA)

Sera from 40 untreated patients at the time of diagnosis and 40 age and sex-matched controls were obtained for measuring IL-27 using commercial ELISA kit (Bioscience, Boston, MA) according to the manufacturer's protocol. The sensitivity of IL-27 kit was 64 pg/ml.

Statistical Analysis

Hardy–Weinberg equilibrium for all genotypes was evaluated by Arlequin (ver 3.1) software. Genotypes and the distribution of alleles between patients and controls were tested with Pearson's chi-square ($\chi 2$) and Fisher's exact tests. Haplotype analysis was performed by Arlequin software and analyzed with Epi-Info 2002 software (CDC, Atlanta, GA). Other statistical analyses were accomplished using SPSS version 23 for Windows (SPSS Inc., Chicago, IL). We calculated the percentages of patients who obtained complete remission (CR) after initial therapy and those who relapsed. The EFS was defined as the time from CR achievement to relapse or death. OS was determined from the date of diagnosis to the recorded date or date of death. We estimated OS and EFS by the Kaplan–Meier method. Univariate analysis was carried out using the log rank test and multivariate analysis by Cox's regression model. The Mann-Whitney U test was used to compare serum IL-27 levels between patients and controls. *P*-values less than 0.05 were considered statistically significant. All data were presented as mean ± standard error (SE) unless otherwise specified.

Results

Patient Characteristics

Table 1 summaries the ALL patients' clinical and paraclinical characteristics. Of 200 ALL patients, there were 133(66.5%) males and 67(33.5%) females. Most patients (75.4%) had B-ALL and 24.6% had T-ALL. The initial WBC counts for patients was 29.3 ± 4.4 × 10^3/μl and the platelet number was 132 ± 11.8 × 10^3/μl. Patients had 84.5 ± 0.9% bone marrow blasts and 56.9 ± 3.2% peripheral blood blasts. EMI was present in 55.6% of patients as follows: splenomegaly (32.6%),

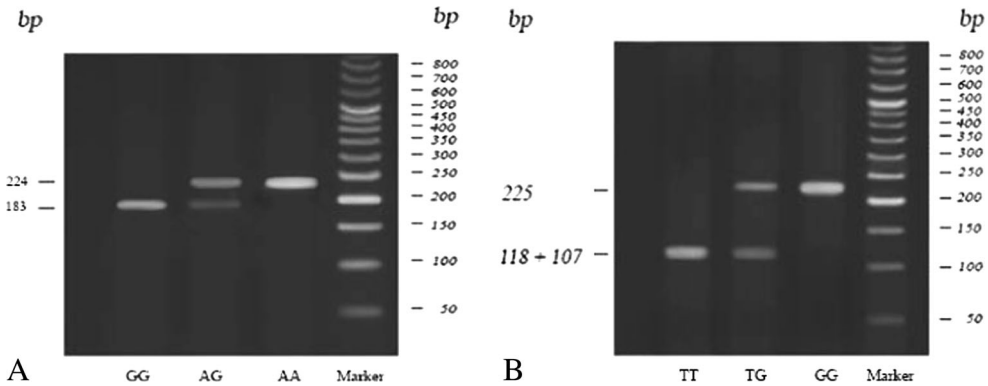

Fig. 1 Electrophoresis of the PCR products showing IL-27 rs153109 (**a**) and rs17855750 (**b**) polymorphisms, in a DNA sample, on agarose gel. bp, base pair

Table 1 Laboratory and clinical characteristics of patients with acute lymphoblastic leukemia (ALL)

Variables	Total	B-ALL	T-ALL
Patients (n)	200	107(75.4)	35(24.6)
Age (years)	6.5 ± 5.0	5.9 ± 4.5	7.0 ± 5.1
Sex: Male	133 (66.5)	78 (72.3)	28(80)
Female	67 (33.5)	29 (27.6)	7 (20)
WBCs ($\times 10^3/\mu l$)	29.3 ± 4.4	18.5 ± 3.5	69.7 ± 14.0
Platelets ($\times 10^3/\mu l$)	132 ± 11.8	138 ± 14.1	118 ± 20.4
Hb (g/dl)	8.5 ± 0.1	8.0 ± 0.2	9.7 ± 0.4
BM blasts (%)	84.5 ± 0.9	84.5 ± 1.1	85.0 ± 1.9
PB blasts (%)	56.9 ± 3.2	55.5 ± 3.9	61 ± 5.3
Patients with EMI	75(55.6)	46(52.6)	19(55.8)

Data are represented as number, (%) or mean ± SE, with the exception of age, which is reported as SD

WBC White blood cells; *Hb* Hemoglobin; *BM* Bone marrow; *PB* Peripheral blood; *EMI* Extra-medullary involvement

CNS involvement (12.6%), hepatomegaly (31.1%), and lymphadenopathy (8.1%). After diagnosis, 78.6% of patients entered remission during 27.6 ± 41 days of chemotherapy. During the follow-up, 19.5% of patients relapsed. Patients had an EFS of 1019 ± 64 days (median: 892 days) with an OS of 1166 ± 63 days (median: 1230 days). A total of 93.4% (death rate: 6.6%) of patients survived until the end of the study. There were 60% of patients assigned to the standard group and 40%in the high-risk group. The cytogenetics information of 86 patients were available of whom 39 patients (45.3%) had abnormal karyotypes, including 11 hyperdiploid covering chromosomes +x, +y, +4, +6, +9, +10, +11, +14, +17, +18, +21, +22, 4 hypodiploid, monosomy 7, 12, 13 and 24 pseudodiploid including deletions in chromosomes 2, 3, 8, 9, 11, 12, PAX5 and translocations t(9;22)Ph^+, t(12;21), t(1;19), t(4;11), t(8;12), t(1;14), t(11;19), t(9;11).

Genotypic and Allelic Distribution of the Il-27 Polymorphism

Table 2 shows the genotype and allelic frequencies of IL-27 rs153109 and rs17855750 SNPs. The Hardy–Weinberg *p*-values in both ALL cases and controls in each SNP exceeded 0.05. Evaluation of the genotype distribution for rs153109 showed that 30% of patients had the AA genotype, 68% had the AG genotype, and 2% had the GG genotype. The frequencies of these genotypes in the controls were as follows: AA (67.15%), AG (27.15%), and GG (5.7%). The allele frequencies were 64% for the A allele and 36% for the G allele in patients compared to 80.7% for the A allele and 19.3% for the G allele in controls. A significant difference existed in genotype [$p < 0.001$; odds ratio (OR):5.7; 95% confidence interval (CI): 3.72–8.72)] and allele frequencies ($p < 0.001$; OR: 0.42; 95% CI:0.31–0.58) for this SNP between patients and controls. We observed higher numbers of patients with the AG genotype and G allele compared to the controls. In patients, the genotype frequencies for the rs17855750 SNP were as follows: TT (17%), TG (78.5%), and GG (4.5%). In controls these values were 33.8% (TT), 59% (TG), and 7.2% (GG). A total of 56.25% of patients had the T allele and 43.75% had the G allele. In controls, there were 63.3% who had the T allele and 36.7% with the G allele. Statistical analysis revealed significant differences in genotypic distribution ($p < 0.001$; OR: 2.53; 95% CI: 1.63–3.91) and allelic frequencies ($p = 0.04$; OR: 0.74; 95% CI: 0.56–0.98) for this SNP between patients and controls. The TG genotype and allele G was higher in patients compared to controls. The result of genotype combination analysis showed a significantly higher frequency of AG versus AA + GG ($p < 0.001$) and TG versus TT + GG ($p < 0.001$) in patients compared to controls. The combined G variant genotypes (AG + GG and TG + GG) also conferred significantly greater risk of ALL ($p < 0.001$). A comparison of the IL-27 haplotype frequencies of rs153109/rs17855750 in patients and controls showed significant differences ($p < 0.001$). We observed an association with the AG, GT, and GG haplotypes to increased ALL susceptibility (data not shown).

Genotype Distribution and Allele Frequencies in Relation to Patient Characteristics and Prognostic Factors

We analyzed the impact of each genotype on established prognostic factors. As shown in Table 3, the genotype, rs17855750 TG was associated with higher numbers of WBCs ($33.1 \pm 5.2 \times 10^3/\mu l$) and lower numbers of platelets ($120 \pm 12.9 \times 10^3/\mu l$) in peripheral blood. A total of 88.5% of patients with platelets $\leq 100 \times 10^3/\mu l$ and 88.5% of patients with WBCs $>10 \times 10^3/\mu l$ had the rs17855750 TG genotype. Of patients with bone marrow blasts ≥50%, 71.6% had the rs153109 AG genotype and 83.5% had the rs17855750 TG genotype. Analysis of these SNPs for possible associations with age, sex, Hb, and EMI showed no significant results. The relationship between genotypes and prognostic factors in B-ALL and T-ALL groups of patients was also examined. Most B-ALL patients with ≥50% bone marrow blasts had the AG genotype (72.5%; $p = 0.04$). In the T-ALL group, patients with the rs17855750 TG genotype had the lowest number of platelets ($92.8 \pm 17.9 \times 10^3/\mu l$) compared to the other genotypes ($>270 \times 10^3/\mu l$;$p = 0.003$). All16 of these patients had platelet numbers less than $100 \times 10^3/\mu l$ ($p = 0.024$). A significant association between patients' cytogenetics and rs153109 genotypes but not rs17855750 genotypes was found. Cytogenetic abnormalities was observed in all four patients with GG genotype and in 35 patients (42.7%) with AA + AG genotypes ($p = 0.03$) (Supplementary Table 1). No significant association existed between these polymorphisms and the risk groups were found.

Table 2 Genotype and allele frequencies of the IL-27 gene polymorphisms in acute lymphoblastic leukemia (ALL) patients

IL-27 SNPs		Patients (n = 200)	Controls (n = 210)	OR (95% CI)	p-value
rs153109	Genotype				
	AA	60(30)	141(67.15)	1.00 (reference)	
	AG	136(68)	57(27.15)	5.7(3.72–8.72)	<0.001
	GG	4(2)	12(5.7)	0.33(0.10–1.06)	0.09
	Alleles				
	A	256(64)	339(80.7)	1.00 (reference)	
	G	144(36)	81(19.3)	0.42 (0.31–0.58)	<0.001
	Genotype				
	AA	60(30)	141(67.1)	1.00 (reference)	
	AG + GG	140(70)	69(32.9)	0.21 (0.14–0.32)	<0.001
	Genotype				
	AG	136(68)	57(27.1)	5.7 (3.73–8.73)	<0.001
	AA + GG	64(32)	153(72.9)	1.00 (reference)	
	Genotype				
	GG	4(2)	12(5.7)	1.00 (reference)	
	AG + AA	196(98)	198(94.3)	0.33(0.11–1.06)	0.091
rs17855750	Genotype				
	TT	34(17)	71(33.8)	1.00 (reference)	
	TG	157(78.5)	124(59)	2.53(1.63–3.91)	<0.001
	GG	9(4.5)	15(7.2)	0.61(0.26–1.43)	0.35
	Alleles				
	T	225(56.25)	266(63.3)	1.00 (reference)	
	G	175(43.75)	154(36.7)	0.74 (0.56–0.98)	0.04
	Genotype				
	TT	34(17)	71(33.8)	1.00 (reference)	
	TG + GG	166(83)	139(66.2)	0.40 (0.25–0.64)	<0.001
	Genotype				
	TG	157(78.5)	124(59)	2.53 (1.63–3.91)	<0.001
	TT + GG	43(21.5)	86(41)	1.00(reference)	
	Genotype				
	GG	9(4.5)	15(7.1)	1.00 (reference)	
	TG + TT	191(95.5)	195(92.9)	0.61 (0.26–1.43)	0.35

SNP Single nucleotide polymorphism; *OR* Odds ratio; *CI* Confidence interval

Table 3 IL-27 gene polymorphism association with white blood cellsand platelet numbers and bone marrow blast percentage in patients with acute lymphoblastic leukemia (ALL)

Variables	rs153109				rs17855750			
	AA	AG	GG	p-value	TT	TG	GG	p-value
Number (%)	60(30)	136(68)	4(2)		34(17)	157(78.5)	9(4.5)	
WBCs ($\times 10^3 \mu l$)	27.5 ± 7.1	30.5 ± 5.8	83.0 ± 4.1	0.69	11.3 ± 5.7	33.1 ± 5.2	14.1 ± 4.7	0.04
≤10	22(27.5)	55(68.75)	3(3.75)	0.4	14(17.5)	62(77.5)	4(5)	0.02
>10	12(23.1)	39(75.0)	1(1.9)		4(7.7)	46(88.5)	4(9.1)	
Platelets ($\times 10^3 \mu l$)	124 ± 22.4	140 ± 14.5	65 ± 32.2	0.5	196 ± 37.1	120 ± 12.9	184 ± 68.9	0.06
≤100	22(28.2)	53(67.9)	3(3.9)	0.5	7(9.0)	69(88.5)	2(2.5)	0.04
>100	12(22.2)	41(75.9)	1(1.9)		11(20.4)	39(72.2)	4(7.4)	

Data are represented as number, (%), or mean ± SE

WBC White blood cells, *BM* Bone marrow

Genotype Distribution and Allele Frequencies in Relation to Response to Therapy and Survival

We examined the influence of IL-27 gene polymorphisms on response to therapy in patients with ALL. Among those who relapsed, 87.1% had the rs153109 AG genotype ($p = 0.001$) and 77.4% had the rs17855750 TG genotype ($p = 0.01$). As shown in Table 4, we observed a significant difference in EFS between the different genotypes of both SNPs. There was shorter EFS in patients with the GG genotypes of both SNPs and those with rs153109 AG (814 ± 74 days; $p < 0.001$, log rank test) and the rs17855750 TG (843 ± 70; $p = 0.035$) compared to those with the rs153109 AA (1291 ± 111 days) and rs17855750 TT (1577 ± 143 days) genotypes. Although patients with the rs153109 GG and AG genotypes had shorter OS than the AA genotype, this was not a significant finding (Table 4). We observed the same for the rs17855750 SNP in which the genotypes showed no significant correlation with OS. In B-ALL patients, we found a significantly shorter survival in GG (853 ± 391 days) and AG (826 ± 93 days) patients compared to those with AA (1364 ± 149 days; $p = 0.001$). In T-ALL, although the pattern was the same, we did not find any correlation of the three genotypes with EFS and OS. Evaluation of survival analysis in patients with the combined G variant genotypes showed that no significant association existed in either of the genotypes with OS. However, we observed significantly shorter EFS (815 ± 71 days; $p < 0.001$) for the rs153109 AG + GG compared to the AA genotype. Similarly, patients with the TG + GG genotype had shorter EFS (830 ± 67 days) than those with the TT genotype ($p = 0.027$). Combined G variant genotypes of rs153109 SNP in B-ALL patients paralleled the results obtained in total patients (Table 4). In T-ALL patients, the rs153109 combined G variant genotypes showed significant correlation with shorter EFS ($p = 0.048$). Survival curves for patients with various genotypes were plotted according to the Kaplan–Meier methods. Figure 2 shows those combine G variant genotypes which had significant findings. As shown, both rs153109 and rs178555750 genotypes in total patients correlated with EFS. In multivariate analysis, the influence of rs153109 combine G variant genotype remained significant ($p = 0.026$; RR = 9.7).

IL-27 Serum Levels

IL-27 serum levels in patients before receiving any chemotherapy were measured and compared with an age- and sex-matched control group by ELISA. As seen in Fig. 3, a significant difference existed in IL-27 serum levels between patients and healthy controls; patients had higher IL-27 levels (440 ± 38.5 pg ml) than the controls (298 ± 21.8 pg/ml; $p = 0.002$). However, we observed no significant correlations between IL-27 levels and different genotypes of patients and controls. Analysis of combined G variants showed a higher, nonsignificant level of IL-27 serum level in patients with the rs17855750 TG + GG genotype (459 ± 42.8 pg/ml) compared to the TT genotype (312 ± 43.5 pg/ml).

Discussion

ALL is a type of blood cancer that progresses rapidly and creates immature blood cells [2]. A number of studies have investigated the importance of different molecules in the development and prognosis of ALL [26–28]. IL-27 is a member of the IL-12 family with two distinct inflammatory and anti-inflammatory functions. This cytokine has an important role in anti-cancer activity [19]. The present study has examined

Table 4 IL-27 gene polymorphism association with response to therapy in acute lymphoblastic leukemia (ALL) patients and patients with B cell (B-ALL) and T cell (T-ALL)

	rs153109						rs17855750					
Variable	AA	AG	GG	*p*-value	AG + GG	*p*-value	TT	TG	GG	*p*-value	TG + GG	*p*-value
Total ALL patients												
EFS	1292 ± 111	814 ± 74	837 ± 303	<0.001*	815 ± 71	<0.001*	1577 ± 143	843 ± 70	681 ± 215	0.035*	830 ± 67	0.027*
OS	1358 ± 110	1039 ± 75	837 ± 303	0.83	1028 ± 73	0.85	1655 ± 138	1014 ± 71	847 ± 231	0.39	1001 ± 67	0.23
B-ALL												
EFS	1364 ± 149	826 ± 93	853 ± 391	0.001*	827 ± 90	<0.001*	1658 ± 167	842 ± 90	551 ± 248	0.1	829 ± 86	0.34
OS	1425 ± 150	1002 ± 94	853 ± 391	0.61	993 ± 91	0.44	1727 ± 163	983 ± 92	735 ± 283	0.49	961 ± 87	0.13
T-ALL												
EFS	1145 ± 154	761 ± 155	774 ± 0	0.11	762 ± 148	0.048*	1429 ± 297	819 ± 125	809 ± 307	0.16	819 ± 121	0.18
OS	1234 ± 145	1043 ± 162	774 ± 0	0.86	1032 ± 156	0.46	1561 ± 276	1013 ± 124	1008 ± 0	0.41	1007 ± 119	0.7

Data are represented as number, (%) or mean ± SE. Survival duration and CRD are in days. *p*-value, log rank test. Combined G variant genotypes (AG + GG and TG + GG) compared to AA or TT genotype

EFS Event-free survival; *OS* Overall survival

*Significant results

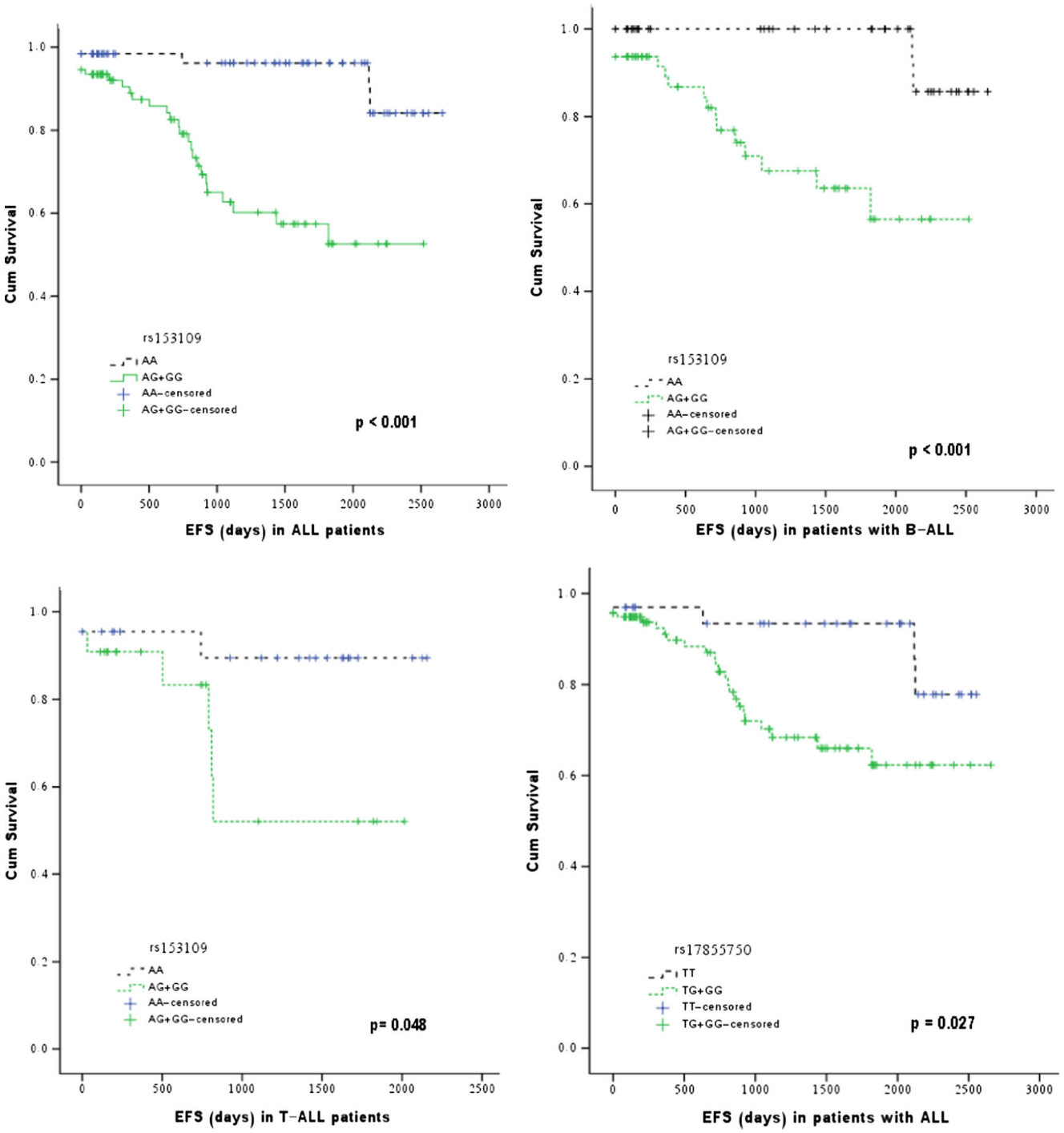

Fig. 2 Kaplan–Meier curves for event-free survival (EFS) of patients in relation to rs153109 and rs17855750 genotypes. We observed significant differences between the combined G variant genotypes (AG + GG) versus AA and (TG + GG) versus TT calculated by the logrank test

the association between IL-27 gene polymorphisms (rs153109 and rs17855750) with risk of ALL and the relation to prognosis and response to therapy.

We observed a significant difference in allele and genotype distributions of rs153109 and rs17855750 between patients and controls. Patients had significantly higher rs153109 AG genotype and G allele compared to controls. There was a higher frequency of the rs17855750 TG genotype and G allele observed in patients compared to the healthy subjects. In genotype combination analysis, we observed a significantly higher frequency of the rs153109 AG + GG genotypes versus AA and the rs17855750 TG + GG genotypes versus TT which indicated a higher risk of developing ALL for those with rs153109 G and rs17855750 G alleles. In previous studies,

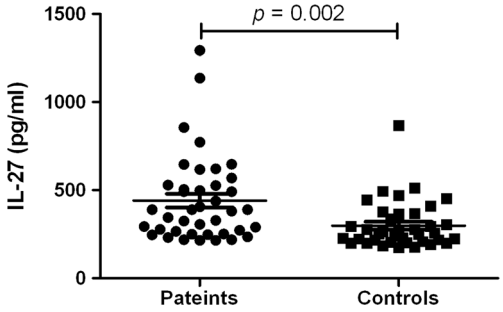

Fig. 3 Serum levels of IL-27 in patients and healthy subjects

Pu et al. have reported a similar result in patients with renal cancer [29]. They observed a significant association of renal cancer risk with the G allele of both rs153109 and rs17855750 SNPs. These researchers also found an increased risk of cancer with the AG genotype compared to the AA/GG genotypes. Lyu et al. reported significantly higherrs153109 GG genotype and G allele in patients with colorectal cancer compared to healthy subjects in the Chinese Han population [30]. Another study conducted on endometrial cancer indicated that the G allele had an association with susceptibility to disease [31]. These data implied the importance of the G allele in relation to cancer development.

The rs153109 (964A > G) polymorphism assessed in the current research is located in the promoter region of the IL-27gene [32]. Studies have shown that the promoter might play an important role in regulating the process of transcription and protein expression. Considering the important role of IL-27 in deviation of T cells toward Th1 cells and according to the various reports on association of this SNP with different cancers, it has been suggested that this polymorphism is functional [33]. Another SNP, rs17855750, is located in exon 2. A change of the T to G allele (2905 T > G) leads to the conversion of the amino acid serine to alanine [34].This SNP, by changes in the IL-27p28 gene, has an association with renal, endometrial, and bladder cancers [29, 31, 35]. However, there is scant information regarding the association of these two SNPs with IL-27 serum protein expression. Zhao et al. [36] and Tao et al. [21] have not detected any associations between serum IL-27 levels to IL-27 rs153109 and rs17855750 polymorphisms in glioma and esophageal cancer. In the present study, we measured IL-27 serum levels in a group of 40 new ALL cases and found no significant correlation with patient genotypes, which suggested that IL-27 rs153109 and rs17855750 polymorphisms might not influence IL-27 release. The effect of other genes could be assumed as a reason for this lack of association between the polymorphisms and IL-27 serum levels. As an example, IL-12 gene 16,974 A/C polymorphism has shown to influence IL-27 production [35]. Moreover, IL-27 production comprises a complex pathway that involves not only alterations at DNA level but also changes at transcription or post-transcriptional levels [37]. Of note, patients with the combined G variant rs17855750 genotype (TG + GG) had higher mean IL-27 serum levels compared to the TT genotype. Although not significant, we recommend that this relationship be examined in further studies with higher numbers of patients. A comparison of IL-27 serum levels in patients and control groups showed a significant difference due to higher levels of IL-27 in patients compared to controls. A few studies have reported the relationship between IL-27 serum levels and cancer. A study by Tao et al. on esophageal cancer reported less IL-27 serum concentrations in patients compared to healthy controls [21]. Zhao et al. reported a lower concentration of IL-27 in patients with glioma than controls [36]. In contrast, two studies on lung and breast cancers have reported greater concentrations of IL-27 in patients compared to healthy controls [38, 39]. The reason for this inconsistency is unknown. Apart from the genetic background of the patients, the biology of tumors and the unique pro- and anti-inflammatory functions of IL-27 in immunity might be effective. IL-27 can act as an antitumor factor by its link with the development of Th1 responses and stimulatory effects on CD8+ T cell function [11]; on the other hand, it has also been suggested to have a tumor-promoting activity by inducing IL-10 and programmed death ligand (PD-L) [40].

There are limited studies on the role of IL-27 in leukemia. Zorzoli et al. have shown that IL-27 can inhibit growth of leukemic cells from acute myeloid leukemia patients. These cells were injected intravenously in non-obese diabetic/severe combined immunodeficient (NOD/SCID)/Il2rγ-deficient mice [41]. Leukemia cells harvested from these mice showed significant reductions in their spreading and angiogenic activity. Cannel et al. used a mouse model of acute leukemia to determine the antitumor activity of IL-27 against B-ALL cells. This group showed that IL-27 inhibited the spread of pediatric B-ALL cells in a preclinical mice model. In vitro treatment of B-ALL cells with IL-27 has been shown to decrease angiogenesis and cell proliferation [42]. With these data, it is reasonable to assume that the increased IL-27 in patients with ALL might be a protective mechanism to inhibit the proliferation of cancer cells and induction of cytotoxic T cells, NK cells, and Th1 cells.

We investigated the possible relation of the genotypes to prognostic factors and response to therapy. We found a significant association between the rs17855750 TG genotype with a higher number of WBCs (in total patients) and lower numbers of platelets (in total and T-ALL patients). Both rs153109 AG (in total and B-ALL patients) and rs17855750 TG genotypes (in total) were more frequent in patients with bone marrow blasts greater than 50%. These parameters are considered poor prognostic factors, hence it might be suggested that patients with the rs153109 AG genotype and rs17855750 TG genotype possibly have a poorer prognosis. Another finding of this study on the association of rs153109 AG and rs17855750 TG genotypes with a greater rate of relapse in patients supported this result.

On evaluation of the possible relationship between genotypes and patients' cytogenetics, we observed that all patients with rs153109 GG genotype had abnormal cytogenetics suggesting a possible impact of this genotype on disease prognosis.

We compared the differences in OS and EFS in various genotypes and found that patients with GG genotypes of both SNPs and the 153,109 AG and rs17855750 TG genotypes had shorter EFS compared to those with the 153,109 AA and rs17855750 TT genotypes. A similar pattern of association existed with the rs153109 genotypes and EFS in B-ALL patients. With respect to T-ALL patients, this group consisted of a lower number of patients and we did not find any correlation between the genotypes and OS or EFS. Possibly, an increased number of T-ALL patients would give a significant result. The EFS of all patients with various genotypes presented in Table 4 shows the following order: AA > AG > GG for rs153109 and TT > TG > GG for rs17855750. This order leads us to presume that the greater G allele in a genotype would be associated with a poorer response to therapy. We have observed this finding with our results on shorter EFS in patients with combined G variant AG + GG and TG + GG genotypes. In multivariate analysis the impact of rs153109 polymorphism on EFS remained significant, which suggested the rs153109 combined G variant genotype as an independent factor for shorter EFS in ALL.

This study had some limitations which included the relatively low number of patients for analyzing IL-27 serum levels and T-ALL patients for their relationship with response to treatment and disease outcome.

In conclusion, the result of this study showed a significant association of IL-27 gene polymorphisms with risk of ALL development. Despite the higher serum levels of IL-27 in patients compared to controls, we observed no significant association with the different genotypes. Both the rs153109 AG and rs17855750 TG genotypes showed an association with greater relapse rate and other unfavorable prognostic factors such as higher number of WBCs and bone marrow blasts, and lower numbers of platelets. Although the combined G variant genotypes of both SNPs showed no significant effects on disease outcome (OS), they were associated with shorter EFS. In this regard the impact of rs153109 polymorphism was stronger than rs17855750 polymorphism on response to therapy in ALL patients. These results provided evidence for the importance of studied IL-27 polymorphisms on risk, prognosis and response to therapy in ALL patients.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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