The Effect of *B-Cell Lymphoma 2* and *BCL2-Associated X* Polymorphisms on the Survival of Acute Lymphoblastic Leukemia Patients: Application of Frailty Survival Models

Navideh Nikmohammadi 1,2, Parvin Sarbaksh 2, Mozghan Moazami Goudarzi 3, Mehdi Talebi 4, Majid Farshdousti-Hagh 3, Jamileh Malakouti 5, *Neda Gilani 2*

1. Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Statistics and Epidemiology, Faculty of Health, Tabriz University of Medical Sciences, Tabriz, Iran
3. Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
4. Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
5. Department of Midwifery, Faculty of Nursing and Midwifery, Tabriz University of Medical Sciences, Tabriz, Iran

*Corresponding Author: Email: gilanin@tbzmed.ac.ir*

(Received 19 Jan 2021; accepted 10 Mar 2021)

**Abstract**

**Background:** *B-cell lymphoma 2* (*BCL2*) and *BCL2-associated X* (*BAX*) polymorphisms are important in the apoptosis process, response to treatment and survival in Acute Lymphoblastic Leukemia (ALL) patients. We aimed to investigate the effect of these genes with other predictors corresponding to the survival of ALL patients with an appropriate frailty survival model.

**Methods:** Our study was performed in 2020 on sixty-two cases of childhood aged 3-16 (year) with ALL disease who were selected by convenience sampling from the two hospitals of Tabriz, Iran. RFLPPCR method was used for genotyping the promoter region of the *BAX* and *BCL2* genes. We used different frailty survival models, to control heterogeneity between individuals due to unmeasured factors affecting their survival. All analyses were implemented using Stata 16.

**Results:** Based on the result of log-logistic model along with frailty gamma, the proportional odds (standard error) of survival for a CC allele of *BCL2* patient compared to a AA allele patient were 6.0 (1.47); *P*<0.001 and for a AC of *BCL2* allele patient were 0.57 (1.23); *P*=0.009. Patients with AG allele of BAX had 2.05 (1.26) times greater odds of surviving than a AA allele patient (*P*=0.003). The odds of survival of patients with abnormal white blood cell (WBC) were 92% less than normal WBC (*P*<0.001).

**Conclusion:** With controlling unmeasured factors affecting, the *BCL2* and *BAX* genes promoter polymorphism are effective in the survival rates for ALL.

**Keywords:** Acute lymphoblastic leukemia; *B-Cell Lymphoma 2*; *BCL2-Associated X*; Frailty models; Survival

**Introduction**

Leukemia is one kind of cancer that originates in the bone marrow and the blood-forming tissues of the body that intercepts typical blood function by abnormal cell partition (1). One form of leukemia...
that is very well-known in children is acute lymphocytic leukemia (ALL) (2).

This malignancy is treated in different manners such as chemotherapy, immunotherapy, and radiation. Clinical therapies firstly perform their anti-tumor activities by stimulating intracellular death planning (3). The apoptosis process is a physiological cell programmed to die. Detection of the key proteins involved in apoptosis exposure is an appealing way to impede the development of many diseases including cancer. Percept how these proteins affect the apoptotic pathways may lead to more efficient cancer treatments and survival of the patients. The discovery of apoptosis pathways and the development of specific molecules that include apoptosis of tumor cells display that cell death can be targeted therapeutically (4). Chemotherapeutic drugs and ionizing radiation (IR) damage DNA cells and they are involved in the apoptosis process. There is an association between increased resistance to chemotherapy and reduction apoptosis activity (5-8). Susceptibility to apoptosis is the main key to response to anti-neoplastic therapy (9).

Proteins P53, B-cell lymphoma 2 (BCL-2), BCL2 associated X (BAX) genes are central to this process of apoptosis. Anti-apoptosis effects of BCL-2 protein are important in multidrug resistance. More expression of BCL-2 initiates the growth factor withdrawal, IR, glucocorticoids, and multiple chemotherapeutic agents, these process prevents cell death (10, 11). High expression of BCL-2 was associated with an appropriate response to therapy (12, 13). BCL-2 is a member of a family of BCL-2 homologs. The role of BCL-2 is important in the apoptosis process. In addition, BAX protein is one of the homogeneous genes versus BCL-2. Its activity is against the anti-apoptosis effect of BCL-2 in the apoptosis process (14). However, the previous studies have evaluated the effect of BCL-2 promoter (SNP -938C>A) genotyping and BAX (SNP G-248A) polymorphism on patient outcomes without considering time to event or frailty term (12, 15).

The notion of frailty offers a suitable way to introduce unobserved heterogeneity and associations into models for survival data. Also, longitudinal repeated measurement data can be including for survival models for accurate predictions (16, 17).

In this study, we utilized different frailty survival models, to control heterogeneity between individuals due to unmeasured factors, to know whether of BCL-2 C-938A (rs2279115) SNPs and BAX G-248A (rs4645878) SNP polymorphism with other covariates can have significant effects on survival of acute lymphoblastic leukemia patients.

Materials and Methods

Study design and participants

Our study was performed in 2020 on sixty-two cases of childhood aged 3-16 (year) with acute lymphoblastic leukemia. They had been diagnosed by bone marrow aspiration, flow cytometry, cell counts. Patients with 3-16 years old were recruited from the two Shahid Ghazi Tabatabai and Children's Hospital of Tabriz, Iran with the convenience sampling method and were seen by a pediatric oncologist. The patient sampling process took 6 months and patients were followed for one year. Among the patients who were diagnosed with acute lymphoblastic leukemia cancer, patients with unstable clinical conditions, people who received blood products within 10 days before sampling and patients with bone marrow transplantation were excluded.

All stages of the work have been carried out by following the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. The written consent was obtained from parents or legal guardians of children. The ethical aspects of this study were approved by the institutional ethics committee of Tabriz University of Medical Sciences, with code IR.TBZMED.REC.1398.1203.

Assessments

Morphological characteristics of the bone marrow and flow-cytometry of all patients were prepared. Expression of CD7, CD10, CD19, HLA-DR, CD20, CD22, CD3, CD34 and CD45 based on
protocols for routine hospital practice was evaluated. Expression of CD3 marker was classified into normal (<20 ng/mL) and non-normal (≥20 ng/mL) groups. The counts of WBC were ranged at two groups normal (abnormal), according to Children’s Reference Ranges for Routine Hematology Tests (18).

**DNA extraction**

DNA from all blood cells was extracted with a salting-out method. DNA concentration and clarity in each sample were measured with a NanoDrop 1000 Thermo scientific Spectrophotometer (Wilmington, DE, USA). DNA extracts with a visual density ratio between 1.6 to 1.9 at 260/280 nm were chosen for the subsequent steps.

**Amplification of the BCL-2 promoter region**

BCL-2 genotype was determined with the Amplification of genomic DNA. Primers were ready by Bioneer (Daejeon, S. Korea). Forward primer: 5’TATCCAGCTTTTCGG-3’ and the reverse primer: 5’GGCCGCAGATGAAATTACAA-3’ were used. SensoQuest Thermocycler (Göttingen, Germany) was used for enzyme chain reactions (PCR), it was a final volume of twenty-five µL, containing 12.5 µL Master Mix Red (Amplicon, Odense, Denmark), 1.25 µL of each primer, six µL dH2O and four µL genomic DNA.

**Amplification of the BAX promoter region**

BAX genotype was such as with the consolidation of genomic DNA. Forward primer: 5’CGGGGTATCTCTGGGC-3’ and the reverse primer: 5’GTGAGAGCCCCGGCTGAAC-3’ were used. PCR was accomplished in a final volume of twenty-five µL containing 12.5 µL Master Combine Red (Amplicon), 1.25 µL of each primer (Bioneer), six µL dH2O and four µL genomic DNA.

**Restriction enzymes analysis of the BCL-2 and BAX genes**

BCL-2: Aliquots of 6 µL of each PCR production were digestible with 1 unit of restriction nuclease at 37°C a nightlong with 1 µL 10× enzyme buffer. Screening of the samples for the BCL-2 C-938A (rs2279115) SNP was performed by restriction enzyme BccI (New England BioLabs, Ipswich, UK). The homozygous CC (wild-type) genotype was unreal as one major 252-bp band. The AC heterozygous genotype showed each the undigested 252-bp band and also the digestible 154- and 98-bp bands, and also the digestible 154- and 98-bp product delineated the AA genotype.

BAX: The PCR productions (6 µL) were incubated a nightlong with one µL restriction nuclease at 37°C. Screening samples for the BAX G-248A (rs4645878) SNP was performed with the restriction enzyme ASCII (New England BioLabs). Three major bands of 352, 256, and 96 bp were been in the homozygous GG (wild-type) genotype. The 256-bp band was a lot of severe. The heterozygous AG genotype resulted in the loss of a limitation site for ASCII in one of the BAX promoters. The genotyping results showed the 352-bp band, 256 bp band, and 96-bp band. The 352-bp band was most intense, versus, the 96-bp band was mostly invisible. The 352-bp band was been in the homozygous AA genotype. For more detailed information and the PCR productions conditions in every step (19).

**Statistical analysis**

Descriptive statistics are reported as mean (±SD) for quantitative data and as frequency and percentage for qualitative data. For inferential section, parametric survival models (with and without frailty) were used to determine effective factors corresponding to the survival of acute lymphoblastic leukemia patients. The other covariates such as WBC, CD3, CD7, gender, and baseline age entered the models to assay the adjusted effect of genes. To compare the different parametric models and choose the best model, the information-theoretic criteria (such as AIC and BIC). The Hazard ratio (HR) with its standard error (SE) have reported for the exponential and Weibull models, and the results of log-logistic models represented by proportional odds (PO) with its SE. Also time
ratio reported for log-normal models (20, 21). Statistical analysis was done by Stata version 16 (College Station, TX: StataCorp LLC; 2019). P-values less than 0.05 were considered statistically significant.

Results

Out of 62 acute lymphoblastic leukemia patients, 41 (66.1%) were male and 21 (33.94%) female. The mean (± SD) of age at diagnosis patients was 7.4 (±3.37) years and the median (±SE) survival time was found 86 (±14.48) months. The mean (±SD) count of WBC was) $4.5 \pm 3.6 \times 10^9$ /L ranging from $1.3 \times 10^9$ /L-$21 \times 10^9$ /L. Genotyping of the promoter region of the BCL-2 gene (C-938A) showed the following allele frequencies (%) in the ALL patients: AA in 33 children (53.23%), AC in 18 (29.03%) and CC in 11 (17.74%). Similarly, for BAX gene (G-248A) the frequencies of AA, AG, GG alleles were 15 (24.2%), 24 (38.7%), 23 (37.1%), respectively. The restricted mean of survival time in patients with different genotypes were AA allele 104 months; AC allele 121 months and CC allele, 303 months; and for AA, AG, GG alleles of BAX gene were 134, 114 and 162 months, respectively. Based on the Fig. 1, for all categories of BAX polymorphism, CC alleles of the BCL-2 polymorphism had a lower rate of mortality.

![Fig. 1: Mortality rate (%) in acute lymphoblastic leukemia Patients alleles of the BCL-2 C-938A polymorphism and BAX G-248A SNP](image)

Tables 1 and 2 represent HR (SE) for univariate and multivariate exponential and Weibull and PO (SE) for log-logistic models and time ratio for log-normal with and without frailty, respectively. Frailty term was significant in most models, including the final model (results not shown). The finding of Table 1 showed that without adjusting for other variables; age is a significant factor under the exponential, log-normal, Weibull and log-logistic without frailty term. This means that older patients had a higher risk of death than others did; (for example Weibull distribution: 1.12(0.06), $P=0.035$). In a multivariate scenario; underlying the best model, WBC was a significant factor in survival rate. The survival of patients with abnormal WBC was less than others were; (0.52 (1.20); $P<0.001$). The survival difference of patients with AC allele and AA allele in BCL-2 polymorphism was significant (0.57 (1.23); $P=0.009$).
Table 1: Results of univariate parametric models with and without frailty

| Variable               | Model       | Without frailty | Gamma frailty | Inv-Gaussian frailty |
|------------------------|-------------|-----------------|---------------|----------------------|
|                        |             | HR\(^\gamma\) | SE\(^\gamma\) | P        | HR\(^\gamma\) | SE\(^\gamma\) | P        | HR\(^\gamma\) | SE\(^\gamma\) | P        |
| Age (yr)               | Exponential| 1.20           | 0.06          | 0.043\(*\) | 1.13       | 0.07          | 0.054    | 1.12       | 0.07          | 0.054    |
|                        | Weibull     | 1.12           | 0.06          | 0.035\(*\) | 1.30       | 0.32          | 0.285    | --         | --            | --       |
|                        | Log-normal  | 0.89           | 0.04          | 0.049\(*\) | 0.90       | 0.05          | 0.138    | 0.92       | 0.05          | 0.182    |
|                        | Log-logistic| 0.89           | 1.05          | 0.030\(*\) | 0.90       | 1.06          | 0.107    | 0.91       | 1.06          | 0.197    |
| Gender (female)        | Exponential| 0.56           | 0.27          | 0.244      | 0.56       | 0.27          | 0.549    | 0.56       | 0.27          | 0.248    |
|                        | Weibull     | 0.54           | 0.26          | 0.216      | 0.62       | 0.89          | 0.724    | --         | --            | --       |
|                        | Log-normal  | 1.49           | 0.61          | 0.327      | 1.09       | 0.43          | 0.823    | 1.10       | 0.34          | 0.745    |
|                        | Log-logistic| 1.62           | 1.53          | 0.262      | 1.01       | 1.55          | 0.980    | 0.99       | 1.46          | 0.988    |
| CD3 (ng/mL)            | Exponential| 0.94           | 0.58          | 0.921      | 0.91       | 0.62          | 0.895    | 0.91       | 0.61          | 0.900    |
|                        | Weibull     | 0.92           | 0.57          | 0.900      | 0.00       | 0.02          | 0.606    | --         | --            | --       |
|                        | Log-normal  | 1.30           | 0.75          | 0.647      | 2.09       | 0.87          | 0.076    | --         | --            | --       |
|                        | Log-logistic| 1.19           | 1.76          | 0.750      | 1.97       | 1.49          | 0.092    | 2.03       | 1.48          | 0.075    |
| CD7 (ng/mL)            | Exponential| 1.00           | 0.01          | 0.979      | 0.99       | 0.009         | 1.00     | 1.00       | 0.009         | 0.996    |
|                        | Weibull     | 0.99           | 0.01          | 0.996      | 0.94       | 0.08          | 0.514    | --         | --            | --       |
|                        | Log-normal  | 1.00           | 0.00          | 0.747      | 1.00       | 0.00          | 0.130    | --         | --            | --       |
|                        | Log-logistic| 1.0           | 0.01          | 0.861      | 1.01       | 1.005         | 0.158    | 1.008      | 1.005         | 0.128    |
| WBC (abnormal)         | Exponential| 1.54           | 0.79          | 0.397      | 1.58       | 0.86          | 0.400    | 1.58       | 0.85          | 0.399    |
|                        | Weibull     | 1.55           | 0.79          | 0.387      | --         | --            | --       | --         | --            | --       |
|                        | Log-normal  | 0.62           | 0.27          | 0.276      | 0.57       | 0.19          | 0.096    | --         | --            | --       |
|                        | Log-logistic| 0.64           | 1.57          | 0.336      | 0.61       | 1.43          | 0.184    | 0.58       | 1.41          | 0.125    |
| BCL2 C-938 A (rs2279115)|           |                |               |           |             |               |          |            |                |          |
| AC                     | Exponential| 0.78           | 0.38          | 0.625      | 0.77       | 0.39          | 0.623    | 0.77       | 0.40          | 0.947    |
|                        | Weibull     | 0.81           | 0.40          | 0.677      | 1.12       | 1.99          | 0.947    | --         | --            | --       |
|                        | Log-normal  | 1.19           | 0.44          | 0.639      | 0.96       | 0.31          | 0.925    | --         | --            | --       |
|                        | Log-logistic| 1.26           | 1.47          | 0.551      | 1.86       | 1.44          | 0.689    | 0.89       | 1.41          | 0.747    |
| CC                     | Exponential| 0.20           | 0.15          | 0.035*     | 0.19       | 0.15          | 0.042*   | 0.19       | 0.15          | 0.041*   |
|                        | Weibull     | 0.17           | 0.17          | 0.022*     | 0.01       | 0.001         | 0.257    | --         | --            | --       |
|                        | Log-normal  | 4.75           | 2.54          | 0.004*     | 5.30       | 2.05          | <0.001*  | --         | --            | --       |
|                        | Log-logistic| 4.53           | 1.71          | 0.006*     | 4.66       | 1.43          | <0.001*  | 4.88       | 1.43          | <0.001*  |
| AA                     | Reference category |        |               |           |             |               |          |            |                |          |
| AG                     | Exponential| 1.60           | 0.85          | 0.374      | 1.65       | 0.94          | 0.373    | 1.64       | 0.92          | 0.374    |
|                        | Weibull     | 1.60           | 0.85          | 0.374      | 1.49       | 2.33          | 0.799    | --         | --            | --       |
|                        | Log-normal  | 0.69           | 0.32          | 0.431      | 0.90       | 0.37          | 0.816    | 0.90       | 0.30          | 0.772    |
|                        | Log-logistic| 0.64           | 1.59          | 0.357      | 0.95       | 1.52          | 0.917    | 0.99       | 1.46          | 0.987    |
| GG                     | Exponential| 1.07           | 0.71          | 0.918      | 1.09       | 0.77          | 0.899    | 1.08       | 0.76          | 0.903    |
|                        | Weibull     | 1.04           | 0.70          | 0.947      | 3.06       | 5.50          | 0.534    | --         | --            | --       |
|                        | Log-normal  | 0.88           | 0.50          | 0.831      | 0.74       | 0.36          | 0.553    | 0.90       | 0.39          | 0.826    |
|                        | Log-logistic| 0.92           | 1.82          | 0.895      | 0.66       | 1.66          | 0.420    | 0.70       | 1.59          | 0.471    |

\(\gamma\) Hazard ratio
\(*\) Standard error
\(\varphi\) Significant at 0.05 level
\(\dagger\) Results of these models are proportional odds with their standard errors.
\(\S\) Results of these models are time ratio with their standard errors.

Available at: [http://ijph.tums.ac.ir](http://ijph.tums.ac.ir)
AG allele in B4X polymorphism was a significant factor in survival rate (2.05 (1.26); \( P=0.003 \)). However, the effect of the GG allele in B4X polymorphism was not statistically significant in survival rate. Also, the survival of patients with CC allele was 6.0 times more than AA allele in \( BCL-2 \) polymorphism (6.0 (1.47); \( P<0.001 \)).

### Table 2: Results of multivariate survival model with and without frailty

| Variable                  | Model        | Without frailty | Gamma frailty | Inv-Gaussian frailty |
|---------------------------|--------------|-----------------|---------------|----------------------|
|                           |              | HR̂             | SẼ            | P                    | HR̂             | SẼ            | P              | HR̂             | SẼ            | P              |
| WBC (abnormal)            | Exponential  | 3.56            | 2.05          | 0.028*               | 3.56            | 2.05          | 0.028*         | 3.56            | 2.05          | 0.028*         |
|                           | Weibull      | 6.31            | 4.31          | 0.007*               | --              | --            | --             | --              | --            | --             |
|                           | Log-normalτ | 0.39            | 0.14          | 0.013*               | --              | --            | --             | --              | --            | --             |
|                           | Log-logistic| 0.36            | 1.49          | 0.013*               | 0.52            | 1.20          | <0.001*        | --              | --            | --             |
| CD3 (ng/mL)               | Exponential  | 0.13            | 0.77          | 0.730                | 0.13            | 0.77          | 0.730          | 0.13            | 0.77          | 0.730          |
|                           | Weibull      | 0.18            | 1.21          | 0.798                | --              | --            | --             | --              | --            | --             |
|                           | Log-normalτ | 1.11            | 4.23          | 0.977                | --              | --            | --             | --              | --            | --             |
|                           | Log-logistic| 1.39            | 3.88          | 0.932                | <0.001          | 2.47          | 0.005*         | --              | --            | --             |
| CD7 (ng/mL)               | Exponential  | 1.03            | 0.08          | 0.639                | 1.03            | 0.08          | 0.639          | 1.03            | 0.08          | 0.639          |
|                           | Weibull      | 1.03            | 0.09          | 0.677                | --              | --            | --             | --              | --            | --             |
|                           | Log-normalτ | 0.99            | 0.05          | 0.920                | --              | --            | --             | --              | --            | --             |
|                           | Log-logistic| 0.98            | 1.05          | 0.846                | 1.10            | 1.03          | 0.003*         | --              | --            | --             |
| Age (years)               | Exponential  | 1.13            | 0.08          | 0.078                | 1.13            | 0.08          | 0.078          | 1.13            | 0.08          | 0.078          |
|                           | Weibull      | 1.18            | 0.09          | 0.034*               | --              | --            | --             | --              | --            | --             |
|                           | Log-normalτ | 0.91            | 0.04          | 0.069                | --              | --            | --             | --              | --            | --             |
|                           | Log-logistic| 0.90            | 1.04          | 0.045*               | 0.99            | 1.02          | 0.681          | --              | --            | --             |
| Gender (female)           | Exponential  | 1.04            | 0.56          | 0.934                | 1.04            | 0.56          | 0.934          | 1.04            | 0.56          | 0.934          |
|                           | Weibull      | 1.08            | 0.59          | 0.880                | --              | --            | --             | --              | --            | --             |
|                           | Log-normalτ | 0.89            | 0.30          | 0.739                | --              | --            | --             | --              | --            | --             |
|                           | Log-logistic| 0.88            | 1.43          | 0.734                | 0.71            | 1.19          | 0.061          | --              | --            | --             |
| AC                        | Exponential  | 0.84            | 0.43          | 0.739                | 0.84            | 0.43          | 0.739          | 0.84            | 0.43          | 0.739          |
|                           | Weibull      | 0.90            | 0.47          | 0.857                | --              | --            | --             | --              | --            | --             |
|                           | Log-normalτ | 1.12            | 0.38          | 0.736                | --              | --            | --             | --              | --            | --             |
|                           | Log-logistic| 1.09            | 1.41          | 0.796                | 0.57            | 1.23          | 0.009*         | --              | --            | --             |
| CC                        | Exponential  | 0.16            | 0.13          | 0.032*               | 0.16            | 0.13          | 0.032*         | 0.16            | 0.13          | 0.032*         |
|                           | Weibull      | 0.08            | 0.08          | 0.006*               | --              | --            | --             | --              | --            | --             |
|                           | Log-normalτ | 5.24            | 2.91          | 0.003*               | --              | --            | --             | --              | --            | --             |
|                           | Log-logistic| 4.94            | 1.71          | 0.003*               | 6.00            | 1.47          | <0.001*        | --              | --            | --             |
| AA                        | Reference category |          |                |                      |               |              |                |               |              |                |
| AG                        | Exponential  | 1.69            | 1.00          | 0.378                | 1.69            | 1.00          | 0.378          | 1.69            | 1.00          | 0.378          |
|                           | Weibull      | 1.88            | 1.47          | 0.505                | --              | --            | --             | --              | --            | --             |
|                           | Log-normalτ | 0.77            | 0.29          | 0.508                | --              | --            | --             | --              | --            | --             |
|                           | Log-logistic| 0.69            | 1.50          | 0.376                | 2.05            | 1.26          | 0.003*         | --              | --            | --             |
| GG                        | Exponential  | 1.50            | 1.11          | 0.583                | 1.50            | 1.11          | 0.583          | 1.50            | 1.11          | 0.583          |
|                           | Weibull      | 1.86            | 1.43          | 0.415                | --              | --            | --             | --              | --            | --             |
|                           | Log-normalτ | 0.89            | 0.43          | 0.816                | --              | --            | --             | --              | --            | --             |
|                           | Log-logistic| 0.750           | 1.64          | 0.562                | 1.90            | 1.45          | 0.083          | --              | --            | --             |
| AA                        | Reference category |          |                |                      |               |              |                |               |              |                |

Y: Hazard ratio  
† Standard error  
τ Results of these models are time ratio with their standard errors.  
*Significant at 0.05 level  
£ Results of these models are proportional odds with their standard errors.

Available at: [http://ijph.tums.ac.ir](http://ijph.tums.ac.ir)
AIC and BIC criterions applied for the multivariate survival models in Table 3. The conclusion of these criterions showed that the Log-logistic with frailty gamma has the best fit among other models. The frailty term in this model had a high level of significance among the other models ($P=0.009$).

**Table 3**: AIC and BIC criterion of the different models of acute lymphoblastic leukemia Patients

| Model               | BIC    | AIC    | RANK |
|---------------------|--------|--------|------|
| Without Heterogeneity |        |        |      |
| Exponential         | 135.542| 114.271| 5    |
| Weibull             | 134.096| 110.698| 4    |
| Log-normal          | 132.337| 108.939| 2    |
| Log-logistic        | 133.267| 109.868| 3    |
| Gamma Heterogeneity |        |        |      |
| Exponential         | 139.670| 116.271| 6    |
| Log-logistic*       | 131.719| 106.193| 1    |
| Inverse Gaussian Heterogeneity | 139.670 | 116.5795 | 6    |

* Best model with high level of significant in frailty term.

**Discussion**

Diagnosis of key proteins in the apoptotic process can be effective in controlling cancer progression. Finding how to affect these proteins in the apoptotic pathways may lead to the best treatments. BAX and BCL2 polymorphism in controlling apoptosis are important factors.

In this paper, we studied the effective association between the survival of acute lymphoblastic leukemia patients and several most common prognosis factors such as alleles of BCL2 C-938A (rs2279115) SNP polymorphism, BAX G-248A (rs4645878) SNP polymorphism, age at diagnosis, WBC and gender. We used frailty models to study heterogeneity among individuals.

Frailty models account for the presence of a latent multiplicative effect on the hazard function. This effect is not directly estimated from the data. When the standard models cannot account for all the variability in the failure times, frailty models can be used instead of standard models. Concept of frailty was discussed in many studies (22-24).

In the study, frailty term was significant. Parametric survival models had good fitting rather than semi-parametric models (25). AIC and BIC criteria indicated that the Log-logistic with frailty gamma model are the best models in multivariate analysis.

We found that age and WBC were effective factors under the most of models. Gender was not a significant factor in the survival rate. Allele CC of the BCL2 polymorphism appears a significant factor in all fitted models, this implies that patients with the CC allele had higher survival time than other patients. The effect of AC allele in BCL-2 polymorphism is a significant factor in the survival rate. However, only the AG allele in BAX polymorphism was an effective factor in survival rate. This showed that BCL-2 polymorphism is an important factor in survival than BAX polymorphism.

Several studies have shown a correlation between high BCL-2 expression and poor response to therapy in specific tumors; against many studies that have shown low BCL-2 expression is related to poor response and shortened survival in lung cancer and childhood acute lymphoblastic leukemia (12, 15, 26-28). A study on acute myeloid leukemia (AML) showed that expression of BAX and BCL-2 does not differ significantly among AML patients in terms of remission, relapse and overall survival (29). BCL-2 effects in remission rates in B-cell chronic lymphocytic leukemia (B-CLL) (30). Other studies confirm the important role of the BCL-2 protein in B-CLL (31, 32). The expression
pattern of BAX, BCL-2, and their ratio differs between various cancers and within the same cancer. Some items such as type of cancer, the source, the sample size, the data variance, the treatment modalities, and the techniques used are effective in the results.

Limitation

Survival analysis was performed for one year after. Long-term follow-up with a larger sample size is required for results that are more accurate. We focused only on BAX and BCL-2 polymorphism. However, different proteins are involved in the apoptosis process. Given the conflicting results, further studies are needed.

Conclusion

With controlling heterogeneity between individuals, the effect of CC allele in BCL-2 polymorphism is more than other alleles. AG allele of BAX polymorphism is a single effective allele at this polymorphism in survival rate. Generally, both of these genes are significant in the survival of patients. WBC and age in prognostic are effective factors. Patients with normal WBC counts and young patients showed better survival.

Journalism Ethics considerations

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

This study is a part of the MSc thesis supported by Tabriz University of Medical Sciences (grant number: 64214). In addition, we would like to thank all participants in this research.

Conflict of interest

No potential conflict of interest was reported by the authors.

References

1. Buffler PA, Kwan ML, Reynolds P, Urayama KY (2005). Environmental and genetic risk factors for childhood leukemia: appraising the evidence. Cancer Invest, 23(1): 60–75.
2. Cook EE, MacMillan A, Gershman ST (2018). Cancer Among Adolescents and Young Adults in Massachusetts from 2004 to 2014. J Adolesc Young Adult Oncol, 7(4):493-498.
3. Troeger A, Siepermann M, Escherich G, et al (2007). Survivin and its prognostic significance in pediatric acute B-cell precursor lymphoblastic leukemia. Haematologica, 92(8):1043-1050.
4. Burz C, Berindan-Neagoe I, Balacescu O, Irimie A (2009). Apoptosis in cancer: key molecular signaling pathways and therapy targets. Acta Oncol, 48(6):811-821.
5. Dive C, Hickman JA (1991). Drug-target interactions: only the first step in the commitment to a programmed cell death?. Br J Cancer, 64(1):192-196.
6. O'Connor PM, Wassermann K, Sarang M, Magrath I, Bohr VA, Kohn KW (1991). Relationship between DNA cross-links, cell cycle, and apoptosis in Burkitt's lymphoma cell lines differing in sensitivity to nitrogen mustard. Cancer Res, 51(24):6550-6557.
7. Lowe SW, Ruley HE, Jacks T, Housman DE (1993). p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell, 74(6):957-967.
8. Haghshenas R, Jamshidi Z, Doaei S, Gholamalizadeh M (2019). The Effect of a High-intensity Interval Training on Plasma Vitamin D Level in Obese Male Adolescents. Indian J Endocrinol Metab, 23(1):72-75.
9. Fisher DE (1994). Apoptosis in cancer therapy: crossing the threshold. Cell, 78(4):539-542.
10. White E, Chiou SK, Rao L, Sabbatini P, Lin HJ (1994). Control of p53-dependent apoptosis by E1B, BCL-2, and Ha-ras proteins. Cold Spring Harbor Symp Quant Biol, 59:395-402.

Available at: http://ijph.tums.ac.ir
11. Fukunaga-Johnson N, Ryan JJ, Wicha M, Nuñez G, Clarke MF (1995). BCL-2 protects murine erythroleukemia cells from p53-dependent and -independent radiation-induced cell death. *Carcinogenesis*, 16(8):1761-1767.

12. Gala JL, Vermilyen C, Cornu G, et al (1994). High expression of BCL-2 is the rule in acute lymphoblastic leukemia, except in Burkitt subtype at presentation, and is not correlated with the prognosis. *Ann Hematol*, 69(1):17-24.

13. Coustau-Smith E, Kitanaka A, Pui CH, et al (1996). Clinical relevance of BCL-2 overexpression in childhood acute lymphoblastic leukemia. *Blood*, 87(3):1140-1146.

14. Oltvai ZN, Millman CL, Korsmeyer SJ (1993). BCL-2 heterodimerizes in vivo with a conserved homolog, BAX, that accelerates programmed cell death. *Cell*, 74(4):609-619.

15. Porwit-MacDonald A, Ruby K, Wilkinson S, Wheatley K, Wong L, Janossy G (1995). BCL-2 protein expression in normal human bone marrow precursors and in acute myelogenous leukemia. *Leukemia*, 9(7):1191-1198.

16. Gilani N, Haghshenas R, Esmaeili M (2019). Application of multivariate longitudinal models in SIRT6, FBS, and BMI analysis of the elderly. *Aging Male*, 22(4):260-265.

17. Gilani N, Kazemnejad A, Zayeri F, Asghari Jm, Izadi Afs (2017). Predicting outcomes in traumatic brain injury using the glasgow coma scale: a joint modeling of longitudinal measurements and time to event. *Iranian Red Crescent Med J*, 19(2) 0-0.

18. Soldin SJ, Brugnara C, Wong EC (2003). Pediatric reference ranges. *Amer Assci for Clinical Chemistry, https://www.healthcare.uiowa.edu/path_handbook/appendix/heme/pediatric_normals.html*

19. Moazami-Goudarzi M, Farshbdousti-Hagh M, Hoseinpour-Feizi A, et al (2016). The acute lymphoblastic leukemia prognostic scoring whether it is possible by BCL-2, BAX gene promoter genotyping. *Caspian J Intern Med*, 7(2):105-113.

20. Hougaard P (2000). *Shared frailty models. Analysis of multivariate survival data*. Springer, p. 215-62.

21. Klein JP, Moeschberger ML (2006). *Survival analysis: techniques for censored and truncated data*. Springer Science & Business Media, 2: 3-5.

22. Duchateau L, Janssen P (2008). *The frailty model*. New York: Springer Verlag.

23. Lancaster TJEotES (1979). Econometric methods for the duration of unemployment. 939-56.

24. Rotolo F, Munda M, Legrand C JrPv (2012). parfm: Parametric Frailty Models. 2(2).

25. Altman DG, De Stavola BL, Love SB, Stepniewska KA (1995). Review of survival analyses published in cancer journals. *Br J Cancer*, 72(2):511-518.

26. Fontanini G, Vignati S, Bigini D, et al (1995). BCL-2 protein: a prognostic factor inversely correlated to p53 in non-small-cell lung cancer. *Br J Cancer*, 71(5):1003-1007.

27. Pezzella F, Turley H, Kuzu I, et al (1993). BCL-2 protein in non-small-cell lung carcinoma. *N Engl J Med*, 329(10):690-694.

28. Haghshenas R (2020). The Effect of Rope Training on the Plasma Level of Angiopoietin 4, Interleukin-6, and Lipid Profile of Overweight Boys. *Iranian J Endoc Met*, 22(2): 162-168.

29. Kulsoom B, Shamsi TS, Afsar NA, Memon Z, Ahmed N, Hasnain SN (2018). BAX, BCL-2, and BAX/BCL-2 as prognostic markers in acute myeloid leukemia: are we ready for BCL-2-directed therapy?. *Cancer Manag Res*, 10:403-416.

30. Nückel H, Frey UH, Bau M, et al (2007). Association of a novel regulatory polymorphism (-938C>A) in the BCL2 gene promoter with disease progression and survival in chronic lymphocytic leukemia. *Blood*, 109(1):290-297.

31. Thomas A, Pepper C, Hoy T, Bentley P (2000). BCL-2 and BAX expression and chlorambucil-induced apoptosis in the T-cells and leukaemic B-cells of untreated B-cell chronic lymphocytic leukaemia patients. *Leuk Res*, 24(10):813-821.

32. O'Brien SM, Cunningham CC, Golenkov AK, Turkina AG, Novick SC, Rai KR (2005). Phase I to II multicenter study of oblimersen sodium, a BCL-2 antisense oligonucleotide, in patients with advanced chronic lymphocytic leukemia. *J Clin Oncol*, 23(30):7697-7702.