Original Article

# Comparison of FAIM3 gene expression between new cases of ALL and relapsed ALL

**Mohsen Valikhani[1], Mohammad Faranoush[2, 4], Soodeh Namjoo[1], Mohammad Reza Rezvany[1, 2, 3*]**

*[1] Department of Hematology, School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran.*
*[2] Pediatric growth and development research center, Institute of Endocrinology, Iran University of medical sciences, Tehran, Iran*
*[3] Department of Oncology-Pathology, Immune and Gene Therapy Lab, Cancer Center Karolinska (CCK), Karolinska University Hospital Solna and Karolinska Institute, Stockholm 17176, Sweden.*
*[4] Iran university of Medical Sciences, Rasool Akram complex medical center and MAHAK Hospital*

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## Abstract

**Background:** Acute lymphoblastic leukemia (ALL) is one of the major lymphoid malignancies and the most common hematologic malignancy in children. ALL is characterized by the presence of malignant lymphoblasts in the blood so that immature lymphocytes cannot become mature and thus do not have an adult cell function. Although it is not unusual in adults, it usually affects children. Most children with this disease are recovered from therapeutic protocols. But the relapse is common after recovery or during the treatment. Various factors are supposed to contribute to the relapse of the disease. One of these factors that is likely to be effective in the recurrence of ALL is the FAIM3 protein (an FCuR), or the Fas inhibitory molecule-3 (FAIM3). The aim of this study was to investigate FAIM3 (TOSO) as a new prognostic factor in ALL. **Materials and methods:** In this study, 19 patients with newly diagnosed and 17 patients with relapsed ALL were included. FAIM3 gene expression was measured with the qRT-PCR method. **Results:** The expression level of FAIM3 in relapsed patients was 5.44 folds higher than newly diagnosed ALL patients. **Conclusion:** Prognosis of ALL is usually well-proven in children and can be cured. However, recurrence of the disease is common. At the molecular level, there are several factors that are referred to as the "factor involved in the relapse" of the disease. These factors increase the survival of the leukemic cells. According to the results of the present study, gene expression level of FAIM3 as an anti-apoptotic factor has increased in relapsed ALL lymphoblasts, compared with new diagnosed patients. Therefore, FAIM3 can be considered as a contributing factor in the relapse of the disease.

**Keywords:** ALL, FAIM3, relapse, FCuR, Toso.

***Corresponding Author:** Mohammad Reza Rezvany; Email: : mohrezrez@yahoo.com, rezvani.mr@iums.ac.ir
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## Introduction

Acute lymphoblastic leukemia is one of the most common types of leukemia. This type of leukemia affects the immature lymphatic cells that make up lymphoid tissues and has an acute process[1].

In abnormal bone marrow leukemia, it produces a large number of blood cells. These cells differ in normal and do not function properly[2]. On the other hand, leukemia cells also affect the production of other types of blood cells made by the bone marrow, including red blood cells that transfer oxygen to the tissues of the body, and platelets that prevent blood clots. Therefore, in leukemia, the immune deficiency, Anemia and coagulation disorders are common[3, 4].

Symptoms include tiredness, weakness, fading, bleeding or bruising, enlargement of the lymph nodes, spleen testis etc. or, in some cases, bone

pain[5]. Signs and symptoms of leukemia are often associated with infiltration of the leukemic cells in the normal tissue and ultimately cause bone marrow deficiency (anemia, neutropenia, and thrombocytopenia), or infiltration into specific tissues (lymph nodes, liver, spleen, brain, bone, skin, gums and testicles)[6]. Clinical examination often indicates lymphadenopathy and hepatosplenomegaly, and the symptoms of central nervous system involvement. The latter is rare at the time of diagnosis of acute lymphoblastic leukemia[4]. The testicles are the most common site of extra-bone involvement for ALL; the painless enlargement of one or both testicles may be seen [4, 7, 8].

ALL treatment usually starts with chemotherapy, which in most cases causes a remission of the disease. After the initial remission, according to existing protocols, chemotherapy usually lasts more than a year to achieve the results of the flow-cytometry of the minimal residual disease (MRD) to the desired level[9, 10].

The disease has an acute process and it has a desire prognosis in children. So that most children with this disease will improve through their treatment protocols[11, 12]. But the relapse is common after recovery or during treatment. Various factors have contributed to the relapse of the disease, and various studies have examined various factors in this regard. These factors usually play a role in the pathways for DNA repair, cell cycle regulation and apoptosis. These factors increase the survival of leukemia cells during recurrence[13, 14]. Among the studied genes in patients with leukemia FAIM3 or FcμR gene or the Fas inhibitor molecule 3 (Fas inhibitory molecule3 or FAIM3), is more commonly studied in patients with chronic lymphocytic leukemia (CLL) as an anti-apoptotic factor[15, 16].

This protein belongs to the membrane type I protein which has an immunoglobin-like domain and contains 390 amino acids (17 amino acids of the signal peptide and 234 amino acids in the extracellular region and 21 amino acids in the membrane and 118 amino acids in the cytoplasmic tail). Studies have shown that one-third of its molecular weight consists of carbohydrates and sialic acid [17-19]. FAIM3 is a single copied gene located on the chromosome 1q32.2 and in the vicinity of two other genes coding for IgM receptors. This gene contains 17.6 kb nucleotide and 8 exons. It has been shown that FAIM3 is a Fas-dependent inhibitor of apoptosis[20]. However, recent studies have shown that this index does not directly inhibit FAS-dependent apoptosis. But only when an anti-FAS Ab of IgM isotype is used, it exhibits an inhibitory effect, and when anti-FAS Ab is from IgG it doesn't[18].

FAIM3 gene expression in acute lymphoblastic leukemia appears to be involved in the relapse of the disease. One of the studies in this field that attempts to compare the expression of FAIM3 expression between newly affected ALL and relapsed ALL is done by STAAL.

STAAL et al., In a comparative study between newly affected ALL and relapsed ALL, has demonstrated that six genes, including the FAIM3 gene, is up regulated in the relapse of the disease[21]. Also considering the role of this molecule in apoptosis, it is likely that it becomes the target molecules in the treatment process. Considering the fact that in Iran, the relationship between this gene and recurrence of disease in ALL patients has not been investigated, this research attempts to reveal this issue.

# Methods

***Patients.*** 19 patients with ALL that are newly affected and 17 patients with relapsed ALL was included in this study (Table1). After informed consent was given peripheral blood samples was obtained from 36 patients fulfilling diagnostic criteria for ALL. Total RNA was extracted from lymphocytes using Tripure isolation reagent (Roche, Basel, Switzerland), based on the manufacture's instruction. One microgram RNA was used to invitro transcribed to cDNA by Revert Aid First strand cDNA synthesis kit (pars tous, cat. no.A101161). CDNA from patients with new ALL and relapsed ALL patients were evaluated using quantitative real-time PCR (qRT-PCR) method to determine the presence of FAIM3 mRNA. The results of the qRT-PCR test were checked on electrophoresis gel to ensure the presence of a specific FAIM3 bond. cDNA was made from RNAs through the recommended protocol of underlying cDNA synthesis kit. To confirm the synthesized cDNA, the housekeeping gene was used during the

real-time PCR. The gene used in this study was GAPDH. It is recommended to use conventional PCR and PCR product electrophoresis to confirm the cDNA, but in this study, to reduce the cost and timing, cDNA confirmation was done by GAPDH graph analyzing of real-time PCR.

**Table 1.** Patient Demographic Information, M= Male, F=Female, N=New case, R=Relapse

| No. | Gender | Clinical Stage |
|---|---|---|
| 1 | M | N |
| 2 | M | N |
| 3 | M | N |
| 4 | F | N |
| 5 | F | N |
| 6 | M | N |
| 7 | F | N |
| 8 | M | N |
| 9 | F | N |
| 10 | F | N |
| 11 | M | N |
| 12 | M | N |
| 13 | F | N |
| 14 | M | N |
| 15 | M | N |
| 16 | M | N |
| 17 | F | N |
| 18 | F | N |
| 19 | F | N |
| 20 | M | R |
| 21 | M | R |
| 22 | M | R |
| 23 | M | R |
| 24 | F | R |
| 25 | M | R |
| 26 | F | R |
| 27 | F | R |
| 28 | F | R |
| 29 | F | R |
| 30 | M | R |
| 31 | M | R |
| 32 | M | R |
| 33 | M | R |
| 34 | F | R |
| 35 | F | R |
| 36 | M | R |

***Quantitative real-time PCR.*** All samples were subjected to qRT-PCR in a duplicate manner. Primers were designed by using the Clone Manager software and were checked on the site http: // www. ncbi nlm.nih. gov / blast. The sequence is shown in the table below (Table2). The Forward and Reverse primers of the FAIM3 gene and GAPDH were each mixed separately before work by an equal amount.

**Table 2.** Primers

| Gene | Forward | Reverse |
|---|---|---|
| **GAPDH** | 5'GAAGGTGAAGGTCG GAGTC3' | 5'GAAGATGGTGAT GGGATTTC3' |
| **Length** | 19 | 20 |
| **Tm(˚C)** | 57.18 | 53.72 |
| **GC%** | 57.89 | 45 |
| **FAIM3** | 5'CCACTTTACTTCCTG CCAGTATC3' | 5'GGCCCTTGTATTC TGCCTTG3' |
| **Length** | 23 | 20 |

The materials were mixed into the nuclease free microtubes on ice. First, DEPC treated water, then Master Mixer, Primer and finally cDNA was added. The PCR protocol was run according to following circumstances (Table3).

**Table 3.** PCR circumstances

| Phase | | Temperature (oC) | Time (S) | Number of cycles |
|---|---|---|---|---|
| **Denaturation** | | 95 | 290 | **1** |
| **PCR** | **Denaturation** | 95 | 15 | **38** |
| | **Annealing** | 64 | 60 | |
| | **Extension** | 72 | 30 | |
| **Melting** | | 97 | 1 | **1** |

***PCR product electrophoresis.*** The PCR products were subjected to electrophoresis. A 1% TAE buffer was loaded on the gel of electrophoresis cast. Then samples were loaded on the gel and 80 voltage flow of electricity was applied for 1.5 to 2 hours. In one of the special well of gel the ladder was loaded. Electrophoresis results are presented.

# Results

Melting curve (Figure1) and Amplification curve (Figure2) of the samples are given.

The result of the real-time PCR reaction was taken on an agarose gel, as shown in Figure 3 and Figure 4. The FAIM3 gene expression rate was calculated using the following formula through using REST 2009 software. This software also calculates P value. Ex. Fold =2-ΔΔCt.

Ex. Fold = expression Fold; ΔΔCt = ΔCt Relapsed –ΔCt New case.

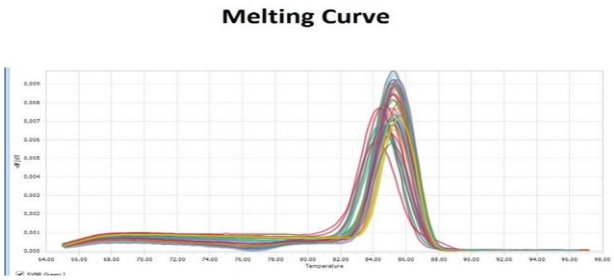


**Figure 1.** Melting curve of both GAPDH and FAIM3 genes

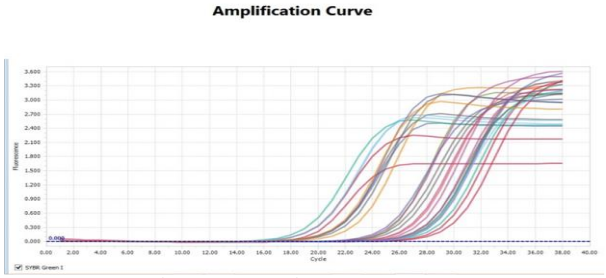


**Figure 2.** Amplification curve of both GAPDH and FAIM3 genes, ΔCt=CtGAPDH – Ct FAIM3.


**Figure 3.** Electrophoresis of real-time PCR products of FAIM3. Numbers of 1, 2 and 3 correspond to new cases samples. Numbers of 4, 5 and 6 correspond to relapsed samples.

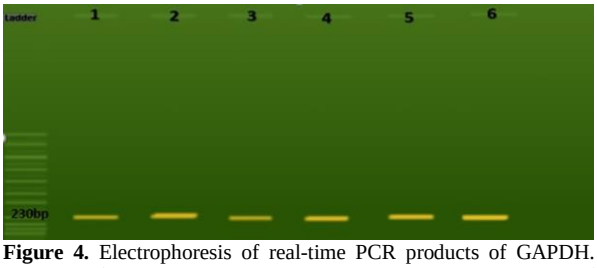


**Figure 4.** Electrophoresis of real-time PCR products of GAPDH. Numbers of 1, 2 and 3 correspond to new cases samples. Numbers of 4, 5 and 6 correspond to relapsed samples.

The analysis of real-time PCR data by this software showed that the expression of FAIM3 expression in relapsed ALL patients was 5.44 fold higher of that of newly diagnosed ALL, (Figure5), (Pvalue=0.001).

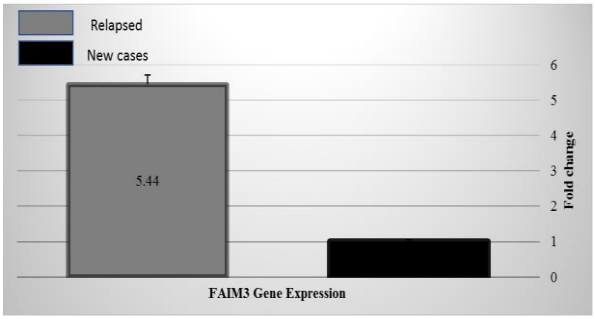


**Figure 5.** Comparison of FAIM3 gene expression between new cases of ALL and relapsed ALL ( fold change ±SD).

## Discussion

Studies that try to determine the role of FAIM3 have different implications for this protein. Sang and Jacob studies have shown that FAIM3-expressing cells (Jurkat) are resistant to Fas-dependent apoptosis [22]. On the other hand, FAIM3 play a role in the internalization and transfer of IgM to lysosomal cells in CLL [23]. FAIM3 also plays an important role in resistance to bacterial infections since in a study it has been demonstrated that FAIM3 negative mice are susceptible to Listeria monocytogenes infection and death [24]. FAIM3 is also involved in the differentiation and maturation of inflammatory dendritic cells (iDC) and viral infection control [25].

Regarding the presence of FAIM3 in the other white blood cells, in 2012, Lang et al studied the role of FAIM3 in granulocyte, monocyte and macrophage. Obtained results showed that FAIM3 is expressed in the granulocytes and monocytes and state that FAIM3 plays a role in granulocytic phagocytosis and the production of inflammatory cytokines such as IL-6 and TNF [26]. FAIM3 negative mice were not able to produce cytokine and died due to Listeria monocytogenes infection, highlighting antibacterial role of FAIM3 [24]. Unlike Lang et al study, Honio and colleagues, found that FAIM3 cell distribution is exclusively restricted to B, and NK cells, and its role in IgM hemostasis is further should be considered for the survival of B lymphocytes and humoral immune responses [27]. In a recent study, Lang et al have described that temperature and stress influence granulocytes by alteration of the FAIM3 expression. Also, the antibody affinity which is used in the flow

cytometry technique, the granulocyte enrichment method and the staining protocols, are the effective factors in the diagnosis and evaluation of FAIM3 and lead to variation in the results [26].

Another category of studies has examined how FAIM3 up regulation or down regulation in various cell types or diseases. Expression of mRNA of FAIM3 gene in recent studies has been studied in CLL patients. In a study by Pallasch et al. patients with CLL showed that the expression of FAIM3 gene was 6.8 fold higher healthy individuals [16]. Marginal zone lymphoma, Hodgkin's lymphoma, and T cell lymphoma have also been investigated in terms of FAIM3 expression, and in these cases, no increase in expression has been recorded [16]. In the case of splenic marginal zone lymphoma (SMZL), Elena Ruiz-Ballesteros and colleagues showed that FAIM3 gene expression increases by 2.72 fold [28]. On the other hand, Richter et al have exhibited that siRNA-mediated FAIM3 gene repression can increase the sensitivity of IL-2 activated T-lymphocytes to FAS-dependent apoptosis [29]. Another study have demonstrated that activation of TLRs is a factor in reducing the expression of FAIM3 at both protein and mRNA levels [23]. The expression of FAIM3 in ALL has not been widely evaluated so that STAAL et al have investigated the role of five other genes on relapse. This study suggests that FAIM3 is up regulated in relapsed ALL patients compared with new cases of ALL. It has been shown by others FAIM3 have an anti-apoptotic role that contributes to the survival of leukemic cells [21].

## Conclusion

Many factors contribute to the pathways of DNA repair, cell cycle regulation, and apoptosis. These factors increase the survival of the leukemic cell. According to the results of this study, FAIM3 as an anti-apoptotic factor may have a role in relapse of ALL. Therefore, FAIM3 can be considered as a contributing factor in ALL.

## Conflicts of Interest

None.

## Acknowledgment

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