# Assessment of BAALC Gene Expression in Acute Myeloid Leukemia Patients Compared to Control Group in North-East of Iran

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**ABSTRACT**

Acute myeloid leukemia (AML) is recognized as one of the most common types of leukemia among adults. This condition is characterized by the uncontrolled proliferation of myeloid progenitors, affecting the normal function of different systems in the human body. Various factors such as genetic abnormalities, exposure to chemicals, and viruses can induce AML. Expression of NPM1, CEBPA, MN1, and BAALC genes is among important genetic factors affecting AML prognosis and diagnosis. The aim of this study was to assess amount of BAALC gene expression in AML patients and its relation to survival rate. This case-control study was performed at Mashhad University of Medical Sciences, Mashhad, Iran. 56 AML patients and 56 healthy individuals were participated in this research. The level of BAALC gene expression was assessed by real-time polymerase chain reaction (RT-PCR) method; GPI gene was used as the control. For statistical analysis, SPSS version 21 was applied. P-value less than 0.05 was considered statistically significant. In total, 55.3% and 44.7% of the participants were male and female, respectively. BAALC gene up- and down-regulation was reported in 28.6% and 71.4% of AML cases, respectively. BAALC gene expression and hematological parameters were significantly different between the two groups ($p< 0.05$). Based on the findings, the survival rate was estimated at 31 and 39 months in patients with BAALC up- and down-regulation, respectively. BAALC gene over expression could be considered as a predictive factor for poor prognosis and reduced survival in AML patients.

**Keywords:** Acute myeloid leukemia, BAALC, RT-PCR, Survival rate, Up-regulation, Down-regulation

**ÖZET**

**Kuzeydoğu İran Bölgesindeki Akut Myeloid Lösemili Hastalarda BAALC Gen Ekspresyonunun Kontrol Grubu ile Karşılaştırmasının Değerlendirilmesi**

Akut miyeloid lösemi (AML), yetişkinler arasında en yaygın lösemi tiplerinden biri olarak bilinir. Bu durum, miyeloid progenitörlerin kontrolsüz çoğalmasıyla karakterizedir ve insan vücudundaki farklı sistemlerin normal fonksiyonlarını etkiler. Genetik anormallikler, kimyasallara maruz kalma ve virüsler gibi çeşitli faktörler AML'yi indükleyebilir. NPM1, CEBPA, MN1 ve BAALC genlerinin ekspresyonu, AML prognozunu ve tanısını etkileyen önemli genetik faktörler arasındadır. Bu çalışmanın amacı AML hastalarında BAALC gen ekspresyonu miktarını ve bunun survival hızı ile ilişkisini değerlendirmektir. Bu vaka-kontrol çalışması, İran'ın Meşhed şehrinde Mashhad Üniversitesi Tıp Bilimlerinde gerçekleştirildi.

Bu araştırmaya 56 AML hastası ve 56 sağlıklı birey katıldı. BAALC gen ekspresyon seviyesi, gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) yöntemi ile değerlendirildi; Kontrol olarak GPI geni kullanıldı. İstatistiksel analizler için SPSS version 21 kullanıldı. P değerinin 0.05'ten küçük olması istatistiksel olarak anlamlı kabul edildi. Katılımcıların %55.3'ü erkek, %44.7'si kadındı. BAALC geni ekspresyonu AML vakalarınının %28.6 oranında düşük ve %71.4 oranında yüksek olduğu rapor edildi. BAALC gen ekspresyonu ve hematolojik parametreler iki grup arasında anlamlı düzeyde farklıydı ($p< 0.05$). Bulgulara dayanarak, survival hızı, BAALC geni ekspresyonu yüksek olanlarda 31 ve düşük eskpresyonu olanlarda 39 ay olarak hesaplandı. BAALC gen aşırı ekspresyonu, AML hastalarında kötü prognoza ve sağkalımı azaltmaya yönelik prediktif bir faktör olarak düşünülebilir.

**Anahtar Kelimeler:** Akut myeloid lösemi, BAALC, RT-PCR, Sağkalım hızı, Ekspresyon artışı, Ekspresyonun azalması

## INTRODUCTION

Leukemia is characterized by the uncontrolled proliferation of immature white blood cells in the bone marrow. This condition is categorized into chronic and acute types. Acute leukemia is an aggressive disorder, induced by malignant changes in hematopoietic stem cells or primary precursors. Acute myeloid leukemia (AML) is a heterogeneous, clonal disorder of hematopoietic precursors with abnormal differentiation or reaction to the regulators of cell proliferation.[1] AML is the most common type of acute leukemia during adolescence and the first months after birth.[2,3]

Several factors such as viruses, radiation, chemotherapy, toxic materials, exposure to Banzen, and smoking can increase the risk of AML.[2,3] Various genetic disorders are also associated with AML, which can affect the diagnosis and prognosis of this condition. Therefore, identification of these genetic disorders can promote the understanding of this disease and help with the selection of treatment strategies.[4]

Molecular study of recurrent cytogenetic abnormalities leads to the identification of genes involved in leukemia.[5] The World Health Organization (WHO) has used the available information to categorize leukemia. So far, many effective genes in AML have been identified, among which NPM1 (on chromosome 5q35) and CEBPA (on chromosome 19q13.1) are associated with a good prognosis in patients. On the other hand, mutations such as FLT3-ITD (on chromosome 13q1), WT1 (on chromosome 11p13), KIT (on chromosome 4q11-q12), ERG (on chromosome 21q22), MN1 (on chromosome 22q12.1), and EVI1 (on chromosome 3q26) are known to result in a poor patient prognosis.[6]

BAALC gene (90 kb in size) is another effective gene in AML, located on chromosome 8q23.3 between ATP6c proximal and fzd6 distal.[7] BAALC gene has been identified in some AML cases.[7-11] However; few studies have introduced BAALC expression as a suitable index for prognosis and survival in patients with cytogenetically normal AML.[11] Considering the scarcity of information on the advantages and disadvantages of BAALC gene expression in the northeast of Iran. The aim of this study was to assess amount of BAALC gene expression in AML patients and its relation to survival rate

## PATIENTS and METHODS

This case-control study was performed at the Molecular Pathology and Cytogenetic Research Center of Mashhad University of Medical Sciences, Mashhad, Iran in 2012-2015. This study was approved by the ethics committee of the university.

### *Sample Selection*

In total, 56 AML patients were allocated to the case group, while 56 non-AML cases were enrolled in the control group, and they were healthy individuals with normal bone marrow samples, which were sent to the laboratory for iron staining.

Patients who met the following criteria (inclusion criteria) were enrolled in the case group: 1) >20% myeloid blasts in the peripheral blood or bone marrow aspiration sample; 2) presence of cell surface markers such as CD13, CD33, CD117, CD64, and CD14, identified by flow cytometery and immunocytochemistry; 3) positive myeloperoxidase and Sudan black staining; 4) positive specific and non-

**Table 1.** Assessment of hematological parameters

| Hematological parameters | Case group Mean ±SD | Control group Mean ±SD | P-value |
|---|---|---|---|
| Red blood cell (×10$^6$/µl) | 2.75±0.78 | 4.44±0.48 | <0.001 |
| White blood cell (×10$^3$/µl) | 38.14±46.25 | 8.90±2.91 | <0.001 |
| Platelet count(×10$^3$/µl) | 65.89±56.20 | 237.82±67.64 | <0.001 |
| Hematocrit (%) | 24.49±6.40 | 39.02±4.34 | <0.001 |
| Hemoglobin (g/dL) | 7.62±2.37 | 13.12±1.59 | <0.001 |
| Mean corpuscular volume (fL) | 89.38±13.46 | 88.40±5.88 | 0.6 |
| Mean corpuscular hemoglobin (pg) | 27.91±4.36 | 29.52±2.55 | 0.01 |
| Mean corpuscular hemoglobin concentration (g/dL) | 30.90±3.06 | 33.30±1.63 | <0.001 |
| Peripheral blood blast | 37.00±14.09 | - | - |
| Bone marrow blast | 64.75±4.64 | - | - |

specific esterase and periodic acid-Schiff staining; 5) detection of AML-related translocations such as t(8; 21), t(15; 17), and inv(16); and 6) AML diagnosis in the primary stage. The exclusion criteria were: the patients already started the treatment, being treated or experienced recurrence, 2) lack of confirmed diagnosis, 3) the patients with clotted, or inappropriate anti-coagulants and those collected without sterile methods, 4) patients without confirmed diagnosis and with real time PCR, elevated GPI curve was not obtained.

The final diagnosis was confirmed by a pathologist (based on FAB and WHO classifications) after consulting with an oncologist, evaluation of stained slides (from the peripheral blood and bone marrow), and performing other related tests including periphery blast, WBC, RBC, platelets counts and indices likewise MCH, MCV, MCHE were done for all studied cases. Besides, demographic information, age, gender, and clinical manifestations including splenomegaly, hepatomegaly, lymphadenopathy and petechia/purpura.were extracted from patients' medical records.

### *RNA Extraction*

Peripheral blood mononuclear cells were separated through Ficoll density gradient centrifugation. Then, RNA was extracted from the separated cells by TriPure solution according to the manufacturer's instructions (TriPure RNA Extraction Kit, Roche Company, No: 11 667 157 001, Germany).

### *cDNA Synthesis and Assessment*

Revert Aid™ H Minus First Strand cDNA Synthesis Kit (No.: K1621; Thermo Company, Finland) was used according to the manufacturer's instructions. Polymerase chain reaction (PCR) method was applied to confirm accurate cDNA synthesis. GPI gene was used as the control, and the results were evaluated on Agarose gel. The quality of synthesized cDNA was assessed by a NanoDrop spectrophotometer (Thermo Scientific NanoDrop2000, Finland) at a wavelength of 260 nm.

### *Probe and Primers*

The nucleotide sequence of BAALC gene was obtained from previous studies.[12] To determine the probe specificity, BLAST online software, which can be found on NCBI website, was used. The following primers were applied for BAALC gene amplification: 5'GCCCTCTGACCCAGAAACAG3' as the forward primer and 5'CTTTTGCAGGCATTCTCTTAGCA3' as the reverse primer; also, the probe sequence was 5'FAM-CTCTTTTAGCCTCTGTGGTCTGAAGGCCAT.

### *Real-Time PCR (RT-PCR)*

Each sample was tested in duplicate by RT-PCR method, using the 10 µl TaqMan Permix (Takara Company, Japan) 1 µl forward primer 10 pmol, 1 µl reverse primer 10 pmol, 1 µl Probe (5 pM), 2 µl cDNA, and 7 µl DW and The thermocycler (ABI, USA) was prepared for the PCR process (at 55$^0$C for 20 min, at 95$^0$C for 10min, at 95$^0$C for 15 sec,

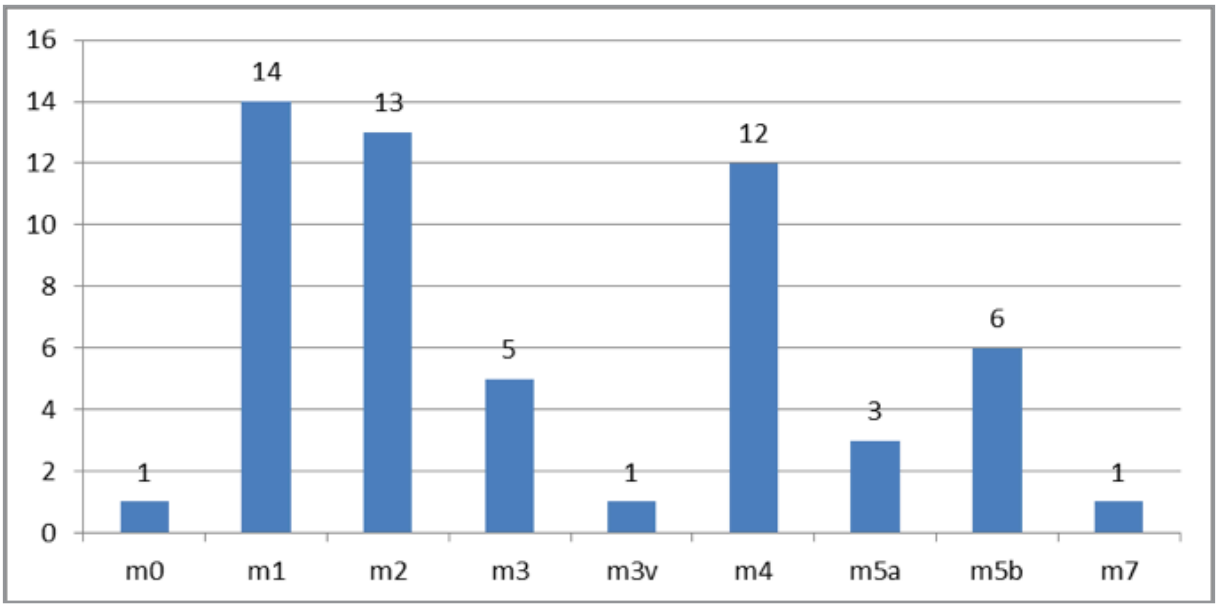


**Figure 1.** Distribution of patients based on WHO and FAB classifications (%)

and at 60$^0$C for 1 min); the last three steps were repeated 40 times. The amount of gene expression was calculated by ΔCT method. Melting curve analysis was done for products specificity confirmation.

### Statistical Analysis

BAALC gene expression and some other parameters such as gender, age, genetic abnormalities, and hematological findings in the studied participants were analyzed by SPSS Version 21.0.

## RESULTS

Among 56 AML patients, 31(55.3%) and 25 (44.7%) cases were male and female, respectively. The sex ratio of the control group was similar to the case group. Therefore, Chi-square test results revealed no significant difference between the two groups in terms of sex (p= 0.48). The mean age of the subjects was 31.12±22.46 years in the case group and 31.78±23.19 years in the control group. Student's t-test showed no significant difference between the two groups in terms of age (p= 0.48). Hematological parameters are presented in Table 1.

Among the evaluated parameters, hemoglobin level, hematocrit level, and red blood cell count were significantly higher in the control group, compared to the case group (p= 0.00). Patient distribution based on the French–American–British (FAB) system classification is described in Figure 1.

Clearly, M1, M2, and M4 were the most frequent subtypes, based on FAB classification, while NOS (no translocation) was the most common subtype, according to WHO categorization. Clinical signs of subjects were evaluated by physicians. Splenomegaly, hepatomegaly, lymphadenopathy, and petechiae/purpurawere reported in 12.5%, 5.4%, 14.3%, and 26.4% of AML patients, respectively.

The mean BAALC gene expression was 0.637±0.961 in the case group and 1.0±0.0 in the control group. Mann-Whitney test results showed a significant difference in gene expression between the two groups (p= 0.04). Based on the assessment of changes in gene expression in the studied population, up-regulation and down-regulation of BAALC gene were reported in16 (28.6%) and 40 (71.4%) subjects in the case group, respectively. No significant association was found between the patients' hematological findings and level of BAALC gene expression (p> 0.05).

BAALC up-regulation was reported in 100% (n= 1), 57.14% (n= 8), and 53.84% (n= 7) of cases with subtypes M0, M1, and M2, respectively; however,

no overexpression was observed in other subtypes. On the other hand, BAALC down-regulation was reported in 100% of cases with subtypes M3, M3v, M4, M5a, M5b, and M7, 23.21% of subjects with subtype M2, and 25% of cases with subtype M1. There was no significant association between gene expression and AML subtype ($p > 0.05$).

BAALC up-regulation was detected in 28.57% (n= 2) of splenomagalic cases, 37.5% (n= 3) of lymphadenopatic cases, and 26.6%[4] of individuals with bleeding manifestations such as petechiae and purpura; however, based on Chi-square test results, none of these findings were significant ($p > 0.05$).

The mean peripheral blast count was 11.14±51.69 and 10.40±31.12 in AML patients with BAALC up- and down-regulation, respectively. In addition, the mean bone marrow blast count was 3.40±65.68and 5.18±65.77 in AML patients with BAALC up- and down-regulation, respectively. A significant difference was found between gene regulation and the percentage of peripheral blood blasts ($p= 0.005$). According to survival rate assessment during four years, it was 39 months in cases with BAALC down-regulation and 31 months among those with BAALC gene up-regulation; overall, the average survival rate was 37 months in all the studied cases.

## DISCUSSION

Leukemia is a malignant condition involving the bone marrow and peripheral blood. As mentioned earlier, some molecular and genetic changes can induce leukemia and affect the prognosis and diagnosis of this condition.[12-14] Despite great developments in the detection of effective genetic markers in AML pathogenesis, some aspects of AML heterogeneity have remained undetermined.

The importance of BAALC gene is attributed to its role as a risk factor in AML pathogenesis, prognosis, and survival.[11] Damiani D, et al. in 2013 reveled that BAALC gene overexpression occurred in more than 50% of AML cases. Our findings showed that 28.6% of subjects in the case group had BAALC gene up-regulation. In fact, BAALC gene expression could be used to assess minimal residual disease (MRD).[15] Moreover, Yahya et al. in 2013 reported BAALC gene up-regulation in 48.9% of AML patients with normal karyotypes. However, they could not find a relationship between clinical parameters and gene expression.[16]

In the present study, gene expression was significantly associated with peripheral blood blast count ($p< 0.05$); in fact, the count was higher in cases with BAALC overexpression. Baldus et al. in 2013 in a study on BAALC expression and FLT3 mutation in 307 patients (younger than 60 years) showed no significant difference between bone marrow blast count and gene expression; however, they reported a significant rise in BAALC expression in M0 and M1 subtypes and a decline in M5b; they also revealed that BAALC overexpression is associated with poor patient prognosis.[12] In addition, in the present study, M0, M1 and M2 subtypes showed the most frequent BAALC overexpression, that this phenomena inhibits the differentiation of myeloid blasts. Due to reduced blast count and differentiation of them, decreased gene expression observed in M3, M4, M5a and M5b.

Besides, the evaluation of the gene expression in the patients showed 28.6% and 71.4% increase and decrease, respectively. Comparison of gene expression with CBC results in the patient showed no significant relationship between increase expression of BAALC and RBC, WBC, platelets counts and other hematologic indices. Comparison of the clinical manifestations between the groups of increased and decreased gene expression as well showed no significant relationship. Thought, peripheral blasts count had a significant relationship with the gene expression amount; there was no relationship with the bone marrow blast count and amount of gene expression.

Similarly, Ferrara et al. in an effort to discover prognostic factors of AML concluded that BAALC up-regulation is associated with disease relapse, poor response to treatment, and poor prognosis.[17] Also, Santamaria et al. revealed that BAALC gene overexpression is a dependent negative prognostic factor, leading to reduced life expectancy among patients.[18]

In addition, Nolte in a study on factors affecting the survival of patients with acute promyelocytic leukemia showed that BAALC up-regulation is associated with a 60% survival rate, where as a sur-

vival rate of 87% was reported in down-regulated cases[19,20], 19 in four year survival assessment, we reported similar results which showed a survival rate of 39 months in up-regulated cases versus a survival rate of 31 months in down-regulated subjects.

Recently new molecular biomarker which is known as miRNA is used for leukemia examination. Actually miRNAs bind to the target mRNA and affect gene and protein expression. These non-invasive markers regulate hematopoesis and subsequently leukemic cells proliferation.[21] It is suggested to perform similar study to investigate the profile of circulating miRNA and to compare the results with gene expression findings in leukemic patients.

## CONCLUSION

The oncogenic role of BAALC in acute leukemia, with inhibiting the differentiation of blasts, especially myeloid trace, reduction of apoptosis, and its correlation with other malignancies is demonstrated. The overexpression of this gene in AML in this study, represent the probable essential role of it in pathogenesis of acute leukemias, specially the AML.

As BAALC gene expresses in the blastic blood cells, it might be usable for predicting the progress of myeloproliferative disorders to malignant leukemias. This gene is also a suitable marker for estimating the disease activity after chemotherapy or bone marrow transplantation. It might enable monitoring the disease recurrence, the blast count in follow-ups. It is recommended to use this gene as a marker for disease recurrence and survival estimation after treatment.

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