Evaluation of BAALC gene expression in normal cytogenetic acute myeloid leukemia patients in north-east of Iran

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Abstract
Background: Acute myeloid leukemia (AML) is known as one of the most common leukemia among adults. Environmental and different genetic factors affect disease process, prognosis and treatment. Among different genetic factors NPM1, FLT3, MLL and BAALC genes are the most effective on patient's survival rate. The aim of this study was to assess amount of BAALC gene expression in AML patients, and its relation to survival rate. 

Methods: In this case-control study, from all 94 individuals referred to Ghaem Medical Center during 2012-2015, 47 cases were normal cytogenetic AML and others were healthy individuals that were studied as control group. Real-time PCR method was applied for gene expression evaluation. Other information of patients was extracted from medical documents. SPSS v.21 was used for data processing.

Results: Mean age of studied cases was 31.50 years. The most of BAALC gene expression was seen in M1 and M2 subtypes, and the less was in M5. A significant relation was found between amount of gene expression and patient's survival rate.

Conclusion: BAALC gene expression was increased significantly in AML cases. BAALC expression had reverse relation with patients' survival rate in North-East of Iran.

Keywords: Acute myeloid leukemia, Survival rate, Prognosis

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Introduction
Acute myeloid leukemia (AML) is a malignant clonal disorder of immature myeloid hematopoietic cells that is the most common type of acute leukemia among adults. This malignancy compose 80% of adult leukemia. Reported mean age of AML patients is 60. Some factors such as viruses, radiation, chemotherapy, benzene exposure and smoking increase risk of AML (1-4). Also, agents such as age, molecular and genetic changes such as FLT3, NPM1, MLL disorders and hematologic indices are important prognostic factors in AML process (5-11). In addition to mentioned genes, brain and acute leukemia-cytoplasmic (BAALC) gene is an important factor in treatment and prognosis determination (12-16).

BAALC gene is located in 8q23.3 position. It is 90kb in length which has 8 exons (17, 18). Overexpression of BAALC gene is reported in AML subjects with trisomy 8 abnormalities in gene expression studies (12). It is a new marker in hematopoietic progenitor cells, which its effects are indeterminate on cellular mechanism (12,13,17-19). Different studies emphasis on gene expression level impression on different physiologic and pathologic circumstances.

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prognosis. Generally, BAALC expression is a suitable prognostic marker which can be used to assign survival rate in normal cytogenetic AML (CN-AML) individuals (12,14).

No study was found in AML patients in mentioned field in North East of Iran, thus, the aim of this study was to define amount of gene expression and frequency of up and down regulation of BAALC gene, and to determine the survival rate of affected patients.

Methods
In this case-control study, 94 individuals referred to molecular pathology research center of Ghaem Hospital in Mashhad city during 2012-2015 were recruited in 2016. From all, 47 cases were normal cytogenetic AML and others were healthy individuals that were studied as control group. All patients were categorized based on French-American-British (FAB) criteria. Patients' age and sex and hematological indices were achieved from medical files. Extracted information included peripheral blood and bone marrow blast count, red and white blood cells and platelets, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and clinical signs including splenomegaly, hepatomegaly, and lymphadenopathy and bleeding protests. Three years survival rate were determined by phone call to patients family. It is necessary to mention that this research was approved by Ethics Committee of Mashhad University of Medical Sciences, code 839.

Peripheral blood samples of all studied cases were collected in EDTA container tubes. Peripheral blood mononuclear cell were separated by Ficoll gradient centrifugation method and Sigma Aldrich kit (Sigma Aldrich Company, Saint Louis, MO, USA). In the following RNA extraction was done by Tri-pure solution (Tri-pure kit, Cat No. 11 667 165 001, made by Germany). Mentioned tests were applied according to kit instruction.

RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentase company, thermo scientific 100 reactions) was applied for cDNA synthesis. GPI gene control primers were evaluated for cDNA qualification confirmation, by PCR method; the results were checked on agarose gel. Qualification of synthesized cDNA was evaluated by nano drop instrument (thermo scientific 2000, made in Finland) in 260nm wavelength.

All samples’ concentration was> 450ng/µL. Studied genes sequences were obtained from Baldus .et al study (13). To determine Primers specificity and evaluation of sequence homology BLAST software were applied which is available in NCBI site. Forward primers for GPI gene were 5'-TCTTCGATGCCAACAAGGAC-3' and 5'-CTTTTCAGGCAATTCTATTGCAGCA-3' for BAALC gene. The Reverse primers was 5'-GCAATCAGTCCTCCGTCAC-3' for GPI and 5'-CTTTTGCAAGCATTCTCTTAGCA-3' for BAALC gene. The used probe sequences were 5'-TTCAAGCTTGACCTCAACACCAAC-3' for GPI and 5'-CTTTTTAGCCTCTGTGGTCTGACCAT-3' for BAALC.

Real time PCRs were applied in thermo cycler ABI (made in USA). All samples were tested in duplicate. RT-PCR reaction master mix (20µL for each reaction) contained 10µL taq man master mixes, 0.5µL ROX stain, 1µL of each forward and reverse primers (10pm), 2µL cDNA , 4.5µL double distilled sterile water .Finally the following temperature patterns were planned in termocycler; 50ºC for 2 minutes, 95ºC for 15second, 95ºC for 15 seconds, and 60ºC for 1 minute. The second, third and fourth steps were repeated for 40 times. The amount of gene expression was calculated by ∆CT method. Melting curve analysis was done for products specificity confirmation.

Chi-square, t-test, Kruskal-Wallis and Mann- Whitney tests were applied for analysis based on data distribution (checked using Kolmogorov-Smirnov test), through SPSS v.21. P-value<0.05 was considered
Results
There were 26 (55.3%) male and 21 (44.7%) female in patient group. Gender distribution of controls was same as cases group. Patients were categorized to child and adult; the first one was ≤15 and consisted of 13 (27.5%) and the second was > 15 which consisted of 34 (72.5%) approached subjects. Patients’ mean±SD age was 31.5±23.72 yrs. The youngest one was 2 yrs and the oldest 77 yrs. Table 1 indicates the patients' frequency based on morphologic subtypes. Most of patients were in M1 (29.78%) and M2 (27.65%) subtypes based on FAB categorization. Mean of peripheral blood and bone marrow blasts were 39.0% and 66.2% in AML cases, respectively. Hematologic and clinical findings of studied cases are mentioned in Table 2. Mean of Hb, HCT and RBC indices were significantly different between the two groups (p=0.03), which is justifiable with occurred pancytopenia. In comparison of BAALC gene expression there was no any significant relation between amount of gene expression and clinical and hematological parameters (p=0.02). Among hematologic indices, RBC count had significant invert relation with amount of BAALC gene expression; patients’ age and sex were related to BAALC expression too (p>0.05). Mean of gene expression was 1.20±0.86 in patients and 1.0±0.00 in controls. Gene evaluation was associated with up regulation in 47% of cases and down regulation in 53% of patients. Survival rate was assessed during 36 months in 47 AML patients and it was 26 months in down regulation subjects and 33 months in up regulation individuals. There was significant relation between up regulation of BAALC and survivability reduction (p=0.04).

Discussion
In the recent decades, genomic profile has been used to identify prognostic factors of leukemia. ERG, BAALC, MN1 and WT1 genes are considered as categorization indices based on gene expression patterns; they can be used to distinguish therapeutic intervention and disease process in AML patients (13,20). Although BAALC overexpression mechanism has not been recognized yet, but it is reported in myeloid leukogenesis. This up regulation prevents myeloid blast differentiation and is associated with poor prognosis in AML and acute lymphoblastic leukemia (ALL) individuals. Different research revealed that BAALC up regulation has been observed in glioblastoma, melanoma and gastrointestinal cancers; they suggested an oncogenic role for BAALC (21).

This gene expression is known as a prognostic and survival rate marker in normal cytogenetic AML cases (12). BAALC up

| AML subtypes | Each subtype | Up regulation | Down regulation |
|--------------|--------------|---------------|-----------------|
| M0           | 2.12 (1)     | 2.12 (1)      | 0               |
| M1           | 29.78 (14)   | 57.14 (8)     | 42.85 (6)       |
| M2           | 27.65 (13)   | 53.84 (7)     | 46.15 (6)       |
| M3           | 10.63 (5)    | 0             | 100 (5)         |
| M4           | 12.76 (6)    | 0             | 100 (6)         |
| M5           | 10.63 (5)    | 0             | 100 (5)         |
| M6           | 4.25 (2)     | 50 (1)        | 50.00 (1)       |
| M7           | 2.12 (1)     | 0             | 100 (1)         |

*% (n)

| Variable   | Gene expression | Mean±SD | p    |
|------------|-----------------|---------|------|
| Blast-Blood| Up regulation   | 11.1±51.69 | <0.05|
|            | Down regulation | 10.4±31.12 |      |
| Blast-BM   | Up regulation   | 3.4±65.68  | 0.80 |
|            | Down regulation | 5.2±65.77  |      |

Table 1. Frequency distribution of morphologic subtypes of AML patients

Table 2. Hematological findings of studied cases

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regulation was seen in 47.00% of AML group members of this study; Damiani et al research on 175 AML patients by RT-PCR method revealed up regulation in 50% of studied cases, and recommended it as a suitable marker for minimal residual disease (MRD) assignment (15). High BAALC expression was reported in 48.51% of 125 CN-AML individuals of Yoon's study in Korea too (22).

Baldus study on BAALC gene expression and FLT3 mutation in normal cytogenetic AML patients under 60 years found no significant differences between age, sex and bone marrow blast count and amount of expressed BAALC gene, before treatment; he mentioned poor prognosis of BAALC up regulation too; while cited gene overexpression and peripheral blood blasts increment were seen in M1 and M0 subtypes. In current study, BAALC down regulation was obvious in M5b subtype (13). Parts of our findings were supported by Baldus (13) and weber's (12) studies which expressed that there were no any significant relationships between BAALC expression, gender and hematological parameters (WBC, HB, bone marrow and peripheral blood blasts count).

Yahya Rs et al in their study about prognostic role of BAALC gene in AML patients in Egypt in 2013 represented up regulation in 48.9% of studied cases; while they did not report any relations between BAALC up regulation and clinical parameters. They reported BAALC up regulation in association with patient's survival rate reduction and indicated to its prognostic role (16). We calculated amount of cited gene expression in each morphologic group (based on FAB), the most up regulated cases were M1 and M2; as mentioned before it is reasonable according to blast count in these groups. The most frequent down regulation was seen in M5 subtype. These findings are similar to Baldus et al reported result about normal cytogenetic AML patients under 60; as it mentioned before they observed overexpression of BAALC along with peripheral blood blast increment in M1 and M0 and down regulation in M5b subtypes. Their findings support our results about poor prognosis of BAALC over expression too (13). Liu et al stated that BAALC overexpression is seen in undifferentiated cells; they found the most up regulation in M0, M1 and M2 categories too, same as our findings (23).

**Conclusion**

Current study emphasized that BAALC up regulation is increased in AML patients, and it is more common in M1 and M2 subtypes. It is associated with bad prognosis. BAALC regulation mechanism need more studies in AML patients and in normal hematopoiesis.

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