Evaluating the expression of ERG gene in acute myeloblastic leukemia in north east of Iran

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Introduction: Erythroblast transformation specific Related Gene (ERG) is a member of ETS transcription factors family, which act as a regulator of primary hematopoietic cell differentiation. Previous researches suggested proto-oncogene for response to mitogenic signal of different cell with MAP kinase and help to tumorgenesis, apoptotic and prognostic role for ERG gene in acute myeloid leukemia (AML) patients. The aim of his study was to evaluate ERG gene expression level in AML cases compare to control group.

Materials and methods: For analysis of gene expression, total RNA was isolated from bone marrow (BM) and peripheral blood of AML `s patient**.** This study was conducted on 54 AML patients and 54 healthy individuals as control group. ERG gene expression and reference gene glucose-6-phosphate isomerase (GPI) was assessed by using real-time polymerase chain reaction (RT-PCR) method. Clinical and laboratory findings studied cases were extracted from medical documents.

Results: This study results indicated that the level of ERG gene expression in AML patients had no significant differences compare to control group (1.81±2.41) ($P < 0.05$), Also ERG gene expression was correlated to patients hematocrit ($P < 0.05$).

Conclusion: This research recommends that there were no significant relation between ERG expression and control groups. It seems that ERG gene assessment is not a good index for AML patient's evaluation.

Keywords: ERG gene, Acute myeloid leukemia, RT-PCR method

Introduction

Acute myeloid leukemia (AML) is a molecular and cytogenetically heterogeneous disease which is characterized by clonal proliferation of myeloid precursors and actually it happens due to maturation arrest. AML has an overall survival (OS) of 70% in adults and 15-20% in paediatrics patients (1, 2). AML patients aged 60 years or more have poor outcome and only 7-15% of them can achieve long-term survival; one of the reasons is the presence of specific genetic alterations, such as gene mutations and alteration gene expression (3). Approximately 20% of childhood AML and 45% of adult AML are characterized with normal cytogenetic, which are named cytogenetically normal AML (CN-AML),

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they constitute the largest AML subset and classified as a homogenous intermediate-risk group (1,3). Among this cytogenetic subgroups mutations in the CEBPA gene, NPM1 and FLT3 mutation can occur, and the lower expression level of the ERG and BAALC also can see. Combinations of two or more of these molecular alterations have been used to predict prognosis in CN-AML patients (2, 3). ETS-related gene (ERG), that is located in chromosome band 21q22.3 are downstream effectors of signaling transduction pathways which involved in the regulation of cell proliferation, apoptosis and differentiation. ERG plays a significant role in first hematopoiesis and hematopoietic stem cell (HSC) maintenance, ETS gene was shown to be involved in leukemogenesis as a fusion partner in AML patients with t (16; 21)(p11;q22) (4, 5).ERG overexpression is frequently seen in AML patients with complex karyotypes and cryptic amplification of chromosome 21, it was found in CN- AML cases and is associated with poor prognosis and clinical outcome (6). It can be applicable as a prognostic factor in cited cases and T-ALL (7, 8). In this study we aimed to evaluate ERG expression and it correlation with overall survival and potential use in newly diagnosed AML cases.

Materials and methods

This study was conducted in the Qaem medical center, Mashhad medical University of medical science (MUMS) during the time period of February 2014 to March 2015. A total number of 54 newly diagnosed acute myeloid leukemia cases were involved in this study. This clinical study was approved by the ethics committee of participating institutions and each patient provided informed consent.

Morphological analysis: Morphologic analysis of bone marrow (BM) and smear preparations was done at the Hematology Unit of the Clinical Pathology Department of Qaem Medical Center, MUMS, Iran. BM aspirate and peripheral blood (PB) smear preparation is part of the routine diagnostic work-up of AML suspected patient.

May-Gru¨nwald Giemsa or a Wright-Giemsa stain slides of PB and BM of studied individuals were examined, it is recommended to count 100 leukocytes of PB smear and 500 nucleated cells of BM, according to WHO proposal (9), presence of >20% blasts is necessary in BM, except for AML with t (15; 17), t (8; 21), inv (16) or t (16; 16).

RNA isolation and complementary DNA synthesis: Pretreated BM or PB samples were available from 54 AML patients and 54 healthy controls. Bone marrow or PB mononuclear cells were extracted from 1-2 ml EDTA samples, (they were obtained by using Ficoll-Hypaque 1077 (Sigma method). Cell pellets were kept at -80°C until RNA extraction was performed. Extraction of total RNA from mononuclear cells was carried out using the RNeasy mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. Concentration and purity of RNA was measured at 260 & 280 & 230 nm using Nano Drop 2000 Spectrophotometer (Thermo Scientific, USA). Ratio of A260/A280 = 1.8 - 2.1 and A260/A230 = 1.8 - 2.1 indicated highly pure RNA.

Extracted RNA was reverse transcribed into Complementary DNA (cDNA), using RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentase, USA) according to the instruction protocol. We used random hexamer primer for cDAN were inside the kit. The PCR reactions were done in a total volume of 20ul, in each reaction, 5 μL of RNA was amplified,

Quantitative real-time polymerase chain reaction (RT-PCR): Complementary DNA and amplification of nucleic acid by (One Step RT-PCR sequence detection system supplied by Applied Bio-systems AB Co) were done. All relative real-time quantitative reactions were performed on Real- Time PCR System. Each reaction

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contained 10 µL of master mix (Taqman), 2 µL of CDNA ,1 µL of each primer and probe, Add sterilized water to reach a final volume of 20 µL,40 cycles of 95°C for 10 min and denaturation at 95°C for 15 s followed by extension at 60°C for 1 min were applied. Primer forward and reverse and probe presented below using the following primers as follows: *ERG Forward primer* 5′-CACGAACGAGCGCAGAGTTA- 3′, *ERG Reverse primer* 5′-CTGCCGCACATGGTCTGTAC-3′, *ERG probe* FAM-CGTGCCAGCAGATCCTACGCTATGG -QFB, glucose-6-phosphate isomerase (GPI) *Forward primer* 5′-TCTTCGATGCCAACAAGGAC-3′, *GPI Reverse primer* 5′-GCATCACGTCCTCCGTCAC-3 and *GPI probe* FAM-TTCAGCTTGACCCTCAACACCAAC-QFB. Commercially available primers and probes for GPI mRNA were used for normalization. PCR reactions were performed on The Applied Bio-system Step One plus Real- Time PCR Systems (Applied Bio-systems). Comparative CT method was used to determine relative expression of both gene (Δ CT = gene CT – GPI CT). If the gene expression failed to reach the software threshold sample is considered negative and if GPI amplification fails the sample was omitted. The CT is reversely related to the amount of target molecules in the reaction.

Data analysis: The threshold cycle data (Ct) and baselines were determined using auto settings. The relative quantification of ERG expression was calculated by comparative CT method ($2^{-\Delta\Delta CT}$). ΔΔCT is the difference of ΔCT value between leukemia and control (ΔΔCT = ΔCT leukemia gene – ΔCT control gene), and ΔCT is the difference of CT value between the target (gene) and endogenous reference (GPI) gene (ΔCT = CT Target gene – GPI gene).

Statistical analysis

The statistical analysis of data was done by using excel program and SPSS version 16, Qualitative data were described in the form of numbers and percentages. Quantitative data were described in the form of mean ± standard deviation (SD). Evaluation of prevalence of the studied marker among the studied groups was done by using Fisher Exact test. All P values were two sided. Significant level was $P < 0.05$.

Results

54 de novo adult acute myeloid leukemia patients and 54 healthy age and sex matched controls were included in this study who presented to the Qaem Medical Center, in the period between February 2014 and March 2015. They were 26 females (26.50) (52%) and 24 males (24.50) (48%), their ages rang was from 16 to 72 with median of 38 years. The clinico-hematological characteristics of the 54 adult AML patients included in this study are shown in Table1. ERG m-RNA level was studied in the 54 AML patients, we classified the patients based on ERG expression in to up and down regulated groups (Table 2).

50% of patients had ERG up regulation and the rest had ERG down regulation. Hematological parameters of two cited groups are compared in Table 2. There were no statistically significant differences between ERG up and down regulated groups except for HCT parameter (P=0.04). Finally, we analyzed the overall survival according to ERG gene expression levels.There was not statistical significant differences between expression level and median survival (P=0.98).

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Table 1. Different parameters in the patients under study.

Parameter	Value
Age (year)	26(range 1-76)
Gender	70M /56 F
WBCs(x 109 /L)	36(range 0.7-202)
HB (gm/dl)	7(3-15)
PLT(x 109 /L)	65(6-205)
Lymphadenopathy	9(16%)
Splenomegaly	7(13%)
Hepatomegaly	4(7%)
Purpora	14(25%)
T(8;21)	7(12.9%)
T(15;17)	4(7.4%)
Inv16	2(3.7%)
Without translocation	41(75.9%)

T, translocation; Inv, inversion.

Table 2. Different hematological parameters in relation to ERG gene expression.

CBC parameters	ERG upregulated patients	ERG downregulated patients	P value
RBC ×106/µl	2.99±0.82	2.63±0.79	0.07
WBC ×103/µl	31.50± 27.93	41.52±49.02	0.72
PLT ×103/µl	74.30±64.49	63.15±55.86	0.91
HCT (%)	25.54±6.95	23.38±6.04	0.04
Hb (gr/dl)	8.23±2.59	7.34±2.29	0.11
MCV (fl)	90±9.33	90.38±12.71	0.52
MCH (pg)	27.54±3.98	28.32±4.83	0.62
MCHC (gr/dl)	30.70±3.64	31.20±2.53	0.78

ERG, Erythroblast transformation specific related gene.

Survival was 38 months for those who had lower gene expression and it was 37 months for those who had upper gene expression. So, there was not significant difference between upregulated and down regulated patients.

Discussion

Cytogenetic disorders play a basic role in leukemia clinical outcomes prediction, but 45% of AML patients have normal karyotype, these cases should follow by other Prognostic factor like ERG gene expression .According to other studies (10), ERG gene expression can predicts clinical outcomes of AML disease (11). In this study 54 patients evaluating in comparison with the healthy donors presented that Patients were 30 males (56.55%) and 24 females (44.44%) in AML and control groups with the mean age of 31 years old. In this study the ERG showed a fifty percent increase. In this study the overexpression of ERG was 50%. A study in 2009 by Klause H Metzeler and his colleagues studied 210 AML patients, 25% of them had increased expression of ERG gene as important prognostic marker, this gene is known as a prognostic marker in normal cytogenetics AML patients, they revealed that ERG overexpression in AML patients who had normal cytogenetic is associated with lower response to chemotherapy (12). In the study of Guido Marccuci and colleagues on 72 AML patients in 2007, approximately 76% of patients had significantly increased expression of ERG (13). Mean ERG expression in patients with acute myeloid leukemia were 2.41 ± 1.81, compared to healthy group significant differences of ERG expression was observed in all patients and controls (P= 0.33).

The relative levels of gene expression (Fold change RQ) were compared. Those who had more than 1 Fold change in expression (up regulation) and those who had less than 1 down regulation,50% AML studied patients had increased ERG

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expression and 50 % of them were down-regulated.

In this study, all patients with AML were classified according to WHO criteria and FAB and frequency of common translocations such as: t (15; 17), inv 16, t (8; 21) and t (6; 9). Patients who had none of these common translocations were 75.93% and the rest had following translocations: t (8, 21) 13%, t (15, 17) 7.41 %, inv16 3.7 %. Comparison of mean ERG gene expression in patients with mentioned chromosomal abnormalities and some without chromosomal abnormalities in AML patients was conducted, there were no significant differences between these two groups. Relationship between ERG gene expression and morphological FAB subgroups examined. The most frequent morphological subgroups of FAB were M1 with 24.1% and M2, M4 with 22.2%. No significant differences were observed between the averages of gene expressions in FAB subgroups. All hematological findings and indices had significant differences between AML and control groups due to leukemia leukocytosis, followed by anemia and thrombocytopenia. Mean WBC was in normal subjects 3.022 ± 8.95 thousand per microliter and the average WBC in AML 39.83 ± 36.45 thousand per microliter, respectively. In acute myeloid leukemia this figure could reach about 50 thousand per microliter. Anemia occurs due to lower concentration of hemoglobin (Hb), or Hematocrit (HCT) according to age, sex and other factors such as geographical reach. In this study ,in AML cases, the correlation between ERG gene expression in relation with red blood cell count, white blood cells and other hematological parameters were measured, only in HCT , significantly different were seen, but in other hematological parameters (WBC, RBC, Hg, MCV, MCHC, PLT) there was no significant difference. In the Leukemic patients because of leukocytosis , arrest maturation and differentiation, complications such as organomegaly, lymphadenopathy, purpura and bleeding from the gums and tissues, including the liver and spleen were seen, after reviewing hematologic indices, 13% of patients have splenomegaly, hepatomegaly 7.40%, lymphadenopathy 16.66%, 25.93% petechiae and purpura . In the relationship between ERG gene expression with clinical signs only in lymphadenopathy significant correlation between clinical symptoms and gene expression levels were observed (P = 0.001). To be considered ERG as a proto-oncogene, it should have significant expression differences between control and case groups; but we don't find any important different between two groups.

Conclusion

There were no significant relation ERG expressions between patient and control groups. It seems that ERG gene assessment would not be a good index for AML patient's evaluation.

References

1. Soliman A, Aal AA, Afify R, Ibrahim N. BAALC and ERG expression in Egyptian patients with acute myeloid leukemia, relation to survival and response to treatment. Open Access Maced J Med Sci. 2016;4(2):264-70. doi: 10.3889/oamjms.2016.058.
2. Farag SS, Archer KJ, Mrózek K, Ruppert AS, Carroll AJ, Vardiman JW, et al. Pretreatment cytogenetics add to other prognostic factors predicting complete remission and long-term outcome in patients 60 years of age or older with acute myeloid leukemia: results from Cancer and Leukemia Group B 8461. Blood. 2006;108(1):63-73. doi: 10.1182/blood-2005-11-4354.
3. Fröhling S, Schlenk RF, Kayser S, Morhardt M, Benner A, Döhner K, et al. Cytogenetics and age are major

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determinants of outcome in intensively treated acute myeloid leukemia patients older than 60 years: results from AMLSG trial AML HD98-B. Blood. 2006;108(10):3280-8.
4. Estey E. Acute myeloid leukemia and myelodysplastic syndromes in older patients. J Clin Oncol. 2007;25(14):1908-15. doi: 10.1182/blood-2006-04-014324.
5. Mrózek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood. 2007 Jan 15;109(2):431-48. doi: 10.1182/blood-2006-06-001149.
6. Taoudi S, Bee T, Hilton A, Knezevic K, Scott J, Willson TA, et al. ERG dependence distinguishes developmental control of hematopoietic stem cell maintenance from hematopoietic specification. Genes Dev. 2011;25(3):251-62. doi: 10.1101/gad.2009211.
7. Eid M, Attia M, Abdou S, EL-SHAZLY S, Elahwal L, Farrag W, et al. BAALC and ERG expression in acute myeloid leukemia with normal karyotype: impact on prognosis. Int J Lab Hematol. 2010;32(2):197-205. doi: 10.1111/j.1751-553X.2009.01168.x.
8. Baldus CD, Burmeister T, Martus P, Schwartz S, Gökbuget N, Bloomfield CD, et al. High expression of the ETS transcription factor ERG predicts adverse outcome in acute T-lymphoblastic leukemia in adults. J Clin Oncol. 2006;24(29):4714-20. doi: 10.1200/JCO.2006.06.1580.
9. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937-51. doi: 10.1182/blood-2009-03-209262.
10. Döhner K, Schlenk RF, Habdank M, Scholl C, Rücker FG, Corbacioglu A, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood. 2005;106(12):3740-6. doi: 10.1182/blood-2005-05-2164.
11. Fröhling S, Schlenk RF, Stolze I, Bihlmayr J, Benner A, Kreitmeier S, et al. CEBPA mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. J Clin Oncol. 2004;22(4):624-33. doi: 10.1200/JCO.2004.06.060.
12. Metzeler KH, Dufour A, Benthaus T, Hummel M, Sauerland M-C, Heinecke A, et al. ERG expression is an independent prognostic factor and allows refined risk stratification in cytogenetically normal acute myeloid leukemia: a comprehensive analysis of ERG, MN1, and BAALC transcript levels using oligonucleotide microarrays. J Clin Oncol. 2009;27(30):5031-8. doi: 10.1200/JCO.2008.20.5328.
13. Marcucci G, Maharry K, Whitman SP, Vukosavljevic T, Paschka P, Langer C, et al. High expression levels of the ETS-related gene, ERG, predict adverse outcome and improve molecular risk-based classification of cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B Study. J Clin Oncol.2007;25(22):3337-43. doi:10.1200/JCO.2007.10.8720.

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