Original Article

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Analysis of BAALC gene Expression as prognosis factor in Pediatric Acute Myeloid Leukemia in Iran

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Abstract

Background: Brain and Acute Leukemia Cytoplasmic (BAALC) is a gene which its expression is confined to progenitor cells; therefore, no expression has been illustrated in mature cells of bone marrow or white blood cells (WBC). In addition, high BAALC expression is associated with poor prognosis in Acute Myeloid Leukemia (AML) individuals and is considered as an important risk factor in Cytogenetic Normal Acute Myeloid Leukemia.

Materials and Methods: This retrospective study was designed to evaluate prognostic importance of BAALC gene expression in pediatric AML patients. Recently, recognized 114 AML Iranian children with age range of 1-15 years were entered in this study during 2012-2015. Real-Time PCR was applied for BAALC gene.

Results: High BAALC gene expression was detected in 47 patients (41.2%) and low expression in 67 patients (58.8%). High BAALC gene expression group (n=47) contained23 males and 24 females. All patients were followed up for 2 years to measure disease prognosis. BAALC expression was a main unfavorable prognostic factor in AML patient's especially normal karyotype. AML cases with high BAALC expression had considerable further cumulative risk after 25 months; it was 39 months in low expressed cases. All of cytogenetic normal acute myeloid leukemias (CN-AML) and high BAALC expressed patients died. High BAALC expression in AML cases was associated with considerable shorter overall survival (OS).

Conclusion: According to our findings, BAALC expression is a significance poor prognostic factor in AML patients with normal karyotype. This study suggests new therapeutic strategies to ameliorate the treat rate of AML patients. Further research with longer follow up and larger sample size is required to definite statistical perusals.

Keywords: BAALC, gene Expression, Pediatrics, prognosis

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease of blood cells which affect differentiation and proliferation of hematopoietic stem cells ; it concludes 15%-20% of pediatric leukemias and also has 70% overall survival (OS) (1-3). Pediatric AML is responsible for more than 50% of hematopoietic malignancies mortality (2). Frequency of AML in Iranian children population is account 30% of childhood malignancy; this statistic is as the same as other region in the world (4).

Treatment protocols and prognosis of this cancer are dependent on genetic markers as well as molecular and cytogenetic abnormalities (1, 2). Cytogenetic information is commonly attainable in 70-80% of children AML patients (5). The most publicized cytogenetic abnormalities are t(8;21)(q22;q22), inv(16)(p13.1q22), t(15;17)(q22;q21), 11q23/MLL, t(1;22)(p13;q13), t(7;12)(q36;p13), and t(11;12)(p15;p13); they are found in nearly half of pediatric AML cases (6,7). Different genes abnormalities such as ERG, MN1, FLT3, CEBPA, NPM1, WT1,

and EVI1 have been discovered in AML patients. Cited genes are known as prognostic markers especially in cytogenetic Normal AMLs (CN-AMLs) (5).

Brain and Acute Leukemia Cytoplasmic (BAALC) is another gene which the expression is confined to progenitor cells; therefore, no expression has been illustrated in mature cells of bone marrow or white blood cells (WBC) (8, 9) In addition, high BAALC expression is associated with poor prognosis in AML individuals and is considered as an important risk factor in cytogenetic normal AML (CN-AML) (5). BAALC gene expression is lower in peripheral blood cells compared to bone marrow. It may operate as a detrimental prognostic factor via exciting proliferation and preventing apoptosis in leukemic cells (8).

Moreover, a high BAALC expression is correlated with higher rate of primitive resistance and relapse in pediatric AML, eventuate in poor overall survival (OS) (8-10).

Accordingly, this study was designed to evaluate prognostic importance of BAALC gene expression in pediatric AML patients.

Materials and Methods

This retrospective study was performed in Cancer Molecular Pathology Research Center of Mashhad University of Medical Sciences (MUMs). It was a pilot study; it means that no similar study has been done in Iran. Recently, recognized 114 AML Iranian children with age range of 1-15 years were entered in this study during 2012-2015. Medical and laboratory histories of the investigated cases were assessed. Two Hemato-pathologists checked the archived slides for probable diagnosis. Information was procured from recorded files; the reports contained hematologic indexes, clinical symptoms such as lymphadenopathy, splenomegaly hepatomegaly, and (CBC). Data were elicited from accessible document files in this center and no isolate sampling was performed from patients, and archived samples were applied for this research.

Inclusion criteria were as follows: 1) present of >20% myeloid blasts in bone marrow aspiration or peripheral blood specimen; 2) attendance of cell surface markers such as CD13, CD33, CD117, CD64, and CD14, recognized by flow cytometery and immunocytochemistry; 3) positive myeloperoxidase and Sudan black staining of prepared slides; 4) finding of AML- pertaining translocations such as t(8; 21),t(15; 17), and inv(16); and 5)AML diagnosis in the early step , without background disease (Denovo AML).

Recurrent translocation

AML recurrent translocations which are mentioned in Table III were assessed in patients under study by real time PCR method. Real time PCR process was done according to Bio Med1 protocol (11).

BAALC Determination

Total RNA was isolated by TriPure RNA Extraction Kit (Roche Company, No: 11667157001, Germany). Complementary DNA (cDNA) was synthesized by applying 1 µg of total RNA by Revert Aid™ H Minus First Strand cDNA Synthesis Kit (No.:K1621; Thermo Company, Finland). Quality of synthesized cDNA was determined by NanoDrop spectrophotometer (Thermo Scientific NanoDrop2000, Finland) at a wavelength of 260 nm. Comparative real-time RT-PCR experiments were carried out as described below; Glucosephosphate isomerase (GPI) and BAALC were amplified in the same tube using 1µL cDNA, 10µ master mix (Takara Company, Japan), 250 nM GPI probe (5'-HEX-TTCAGCTTGACCCTCAACACCAAC-TAMRA-3') , 600 nM GPI forward primer (5'TCTTCGATGCCAACAAGGAC-3') and 600 nM reverse (5'-GCATCACGTCCTCCGTCAC-3') ; 250 nM BAALC probe (5'-FAM-CTCTTTTAGCCTCTGTGGTCTGAAG GCCAT-3'

TAMRA), 600 nM BAALC forward primer (5'-

GCCCTCTGACCCAGAAACAG-3') and 600 nM reverse (5'-CTTTTGCAGGCATTCTCTTAGCA-3') were added to master mix too (10). Real-Time PCR was applied in ABI machine (Applied Biosystems, Foster City, CA). Relative cycle threshold (CT) procedure was performed to characterize the comparative expression level of BAALC. The thermo cycler was set for the PCR procedure , the program was as follows:55°C for 20min, 95°C for 10min, 95°C for 15 sec, and 1 min at 60°C); the ultimate three steps were repeated 40 times. Amount of gene expression was computed by ΔCT method.

Statistical Analysis

The patient's data were collected and statistically analyzed using SPSS (version 12). All Data were reported as mean values ± SD. The variation between two means was analyzed statistically by student (t) test. Chi-square (X2) and Fischer exact tests were applied to determine significance of parameters. The log-rank test was applied for survival evaluation. P value was computed for all parameters (P is significant if\or ≤ 0.05 with confidence interval 95 %). OS was calculated by Kaplan–Meier curve. OS is the time from diagnosis to ultimate follow-up or death due to any reasons.

Results

The patient's specifications are displayed in Table I. High BAALC gene expression was detected in 47 patients (41.2%) and low expression in 67 patients (58.8%). High BAALC gene expression group (n=47) contained 23 males and 24 females. All patients were followed up for 2 years to measure disease prognosis. Moreover, other parameters were compared in Table I. There were no statistically significant disagreements between high and low BAALC expressed groups in terms of sex, WBC, Lymphadenopathy, Hepatomegaly, Splenomegaly, Hemoglobin, and Total Leukocyte Count (TLC) parameters. However, significant differences were found between two groups regarding red blood cell (RBC), Platelet, Blast cell count in peripheral blood and BM, age, hemorrhagia, world health organization (WHO) and French American British (FAB) classifications. More details are mentioned in Table II.

Based on FAB classification, BAALC gene expression was increased in M0, M1, M2, and M4 subtypes. Furthermore, BAALC gene expression was shown the greatest increment in Normal Cytogenetic (CN-AMLs) AML patients. High BAALC expression in AML cases was associated with considerable shorter OS (Figure 1). Low BAALC expressed patients had 42 months OS with 38-41 months confidence interval; But high BAALC expressed cases had 29 months OS, with 27-32.1 months confidence interval. Totally, average survival and confidence interval were 35 and 33-36.8 months, respectively. Table III expresses OS in studied patients.

Mortality rate was higher in patients with high BAALC expression. It has been compared with low expressed cases in Figure1. AML cases with high BAALC expression had considerable further cumulative risk after 25 months; it was 39 months in low expressed cases. All of CN-AML and high BAALC expressed patients died.

Table I: Clinical and molecular characteristics at diagnosis according to BAALC expression status in AML patients.

	N	Mean	Std. Deviation
WBC	114	36.7105	19.95943
RBC	114	2.5226	0.73945
HGB	114	6.9737	1.98004
HCT	114	22.7947	5.63788
mcv	114	92.1137	10.11172
PLT	114	79.63	61.033
sex	114	1.58	0.496
Age	114	7.39	4.517
expression	114	.5877	0.49442
BM.blasts	114	61.9474	5.05342
blood.blasts	114	42.68	12.058
Fold_change	114	1.42320624	1.070827657

Table II: Clinical characteristics at diagnosis in AML patients.

	Std. Deviation	**Overall survival (OS) Log rank P**	**OS (95 % CI)**	**Hazard ratio**	**Hazard ratio (95 % CI)**	**P Value**
High BAALC expression	1.006	29	27-32.1	0.78	0.1-1.1	P< 0.001
Low BAALC expression	0.270	42	38-41	0.1	0-0.2	P< 0.001
Overall	1.182	35	33-36.8	-	-	P< 0.001

Table III: Univariate analysis of molecular and clinical factors.

		High BAALC group (n=47)		Low BAALC group (n=67)		P- value
		N	%	N	%	0.105
Sex	Males	23	20%	43	37.7%	
	Females	24	21%	24	21%	
Age (years)	Mean ± SD	8.38±4.05		6.70±4.72		0.000
hemorrhagia	Present	36	-	12	-	0.001
Lymphadenopathy	Present	18	-	12	-	0.701
Hepatomegaly	Present	18	-	6	-	0.045
Splenomegaly	Present	24	-	12	-	0.157
RBC	Mean ±SD	2.28±.74		2.68±.69		0.001
WBC	Mean ±SD	39.71±54.87		37.52±43.1		0.347
Hb (gm/dl)	Mean ±SD	6.38±2.24		7.38±1.75		0.033
Platelets (x103/mm3)	Mean ±SD	67.32±60.23		88.27±60.542		0.000
TLC (x103/mm3)	Mean ±SD	38.31±22.05		35.58±18.54		0.002
Blast cells (%) in peripheral blood	Mean ±SD	52.64±9.17		35.70±8.38		0.000
BM blast cells (%)	Mean ±SD	63.82±3.42		60.62±5.58		0.005
WHO						0.010
AML with t(8;21)	-	6	5.2%	6	5.2%	
AML with t(15;17)	-	5	4.3%	7	6.1%	
AML with t(6;9)	-	6	5.2%	0	0%	
AML with inv16	-	0	0%	6	5.2%	
AML with normal cytogenetic	-	30	26.3%	48	42.1%	
FAB classification						0.000
M0(6)	-	6	5.2%	0	0%	
M1(12)	-	12	10.4%	0	0%	
M2(36)	-	24	20.8%	12	10.4%	
M3(12)	-	4	3.5%	8	7%	
M4(12)	-	12	10.4%	0	0%	
M5(24)	-	11	9.6%	13	11.4%	
M6(0)	-	0	0%	0	0%	
M7(6)	-	0	0%	6	5.2%	

Discussion.

The prognostic importance of high expression of ERG, MN1, BAALC, FLT3, and WT1 genes have not been completely evaluated in pediatric AML and CN-AML patients (1, 13) Tanner et al., suggested that BAALC gene expression is stage-particular which is mutual amongst progenitor cells in myeloid, lymphoid, and erythroid signaling pathways; it could be considered as a new marker for prognosis determination (14). In addition, Baladus .et al., demonstrated that high BAALC expression is non-random between French–American–British (FAB) subtypes and cytogenetic categories of AML evidently (15).

In the present study, high BAALC gene expression was detected in 47 cases (41.22%).This finding was similar to Yahya et al., results (16) reporting high expression of BAALC gene in 22 out of 45 cases (48.9%) ; Damiani et al., (17) detected high BAALC gene expression in 87 out of 175 (50%) of their studied individuals.

Researchers have shown that M2 and M0/M1 FAB subtypes are correlated with high BAALC expression, while M4 and M5 subtypes are associated with low expression (18); high expression in M2 subtype was only observed in pediatric AML patients. However, High BAALC expression was found in all of FAB subtypes, except M6 and M7; and the most of High BAALC expression was discovered in M2 subtype.

As already noted, there was no significant difference were seen in other parameters such as CBC criteria and FAB classification among cases with BAALC gene expression. This result is in contrast with Yahya .et al., [14] and Adel A .et al., (19) researches; they reported no significant discords between two cited groups regarding clinical items of patients at the time of diagnosis. In this study, there were no significant differences between two groups in terms of WBC count and hemoglobin parameters; but blast cells count in peripheral blood and bone marrow showed meaningful difference between two mentioned groups. Eventually, high BAALC expression was associated with blast cells increment in peripheral blood and bone marrow. This is in disagreement with Elsharnouby .et al., (20) and Adel A .et al., (19)observing no significant differences between high and low BAALC expressed groups regarding WBC as well as platelet and peripheral blood, and bone marrow blast cell count and hemoglobin.

Baldus reported significant association between high BAALC expression and higher WBCs and blast cell countin peripheral blood and bone marrow that is in line withthis study (15). Mizushima Y .et al., (21) did not discover any correlations between BAALC expression level and WBC count in AML cases (n = 114) (P = 0.34).

There were significant disagreements in disease result between two groups; higher rate of death and lower rate of complete remission and OS were observed in high BAALC expressed cases in comparison with low ones. It was as the same as Eid .et al., (22) and Nibourel et al., (21) who explained that BAALC gene expression is an independent bad prognostic factor in CN-AML. Yahya .et al., (16) expressed that high BAALC expression is compatible with lower CR occurrence, higher mortality rate and considerable shorter DFS, and poor OS. According to, Tanner .et al., (14) BAALC expression is associated with worse prognosis in AML patients. It seems that BAALC has an important role in operations that define blasts.

Conclusion

BAALC expression is a significance poor prognostic factor in AML patients with normal karyotype. This study suggests its interpolation into new therapeutic strategies to ameliorate the treat rate of AML patients. Further research with longer follow up and larger sample size is required to definite statistical perusals.

Conflict of interest

The Authors declare no conflicts of interest.

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