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BMI-1 Gene Expression in Patients with Acute and Chronic Myeloid Leukemia in the Northeast of Iran

Zahra Zafari*, MSc, Hossein Ayatollahi, MD, Tayebeh Sohrabi*, MSc, Pourya Arbab Jafari*, MSc, Habibollah Esmaeili***, PhD, Mohammad Reza Keramati**♦, MD**

*Department of Hematology and Blood Bank, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
**Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
***Social Determination of Health Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

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♦Corresponding Author:
Mohammad Reza Keramati, MD
Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
Tel: +98 51 38521400
Fax: +98 51 38591057
Email: KeramatiMR@mums.ac.ir

Abstract

Background: The B-cell-specific Moloney murine leukemia virus integration site1 (BMI-1) is one of the famous members of the Polycomb ring finger group, which plays a crucial role in the gene transcription regulation through histone changes. Hence, it is believed to be necessary to further clarify the clinical effects of BMI-1.

Method: This cross-sectional study was conducted on 70 acute myeloid leukemia (AML), 70 chronic myeloid leukemia (CML), and 20 healthy individuals, as the control group. We used real-time quantitative polymerase chain reaction in order to assess the BMI-1 level expression and its effect on prognosis in AML patients in the Molecular Pathology Research Center.

Results: The results of the present work indicated that the BMI-1 overexpression was significantly higher in the AML and CML patients compared with that in the healthy controls ($P < 0.001$). Furthermore, a significant relationship was observed between the BMI-1 overexpression and poor prognosis in the AML patients (Hazard ratio=1.749, $P < 0.001$, 95% confidence interval = 1.31-2.32). Additionally, BMI-1high was found in chronic and blastic phase in the CML patients ($P < 0.001$).

Conclusion: We concluded that investigation of BMI-1 gene expression pattern will be conducive to the prognosis and treatment of myeloid leukemia.

Keywords: BMI-1 gene, Acute, Myeloid, Leukemia, Chronic myeloid leukemia, Prognostic effect

Introduction

Acute myeloid leukemia (AML) encompasses a hematological disorder, which has been considered to originate from hematopoietic stem cells. It is characterized by specific clinical and molecular heterogeneous.[1] Disruption in the

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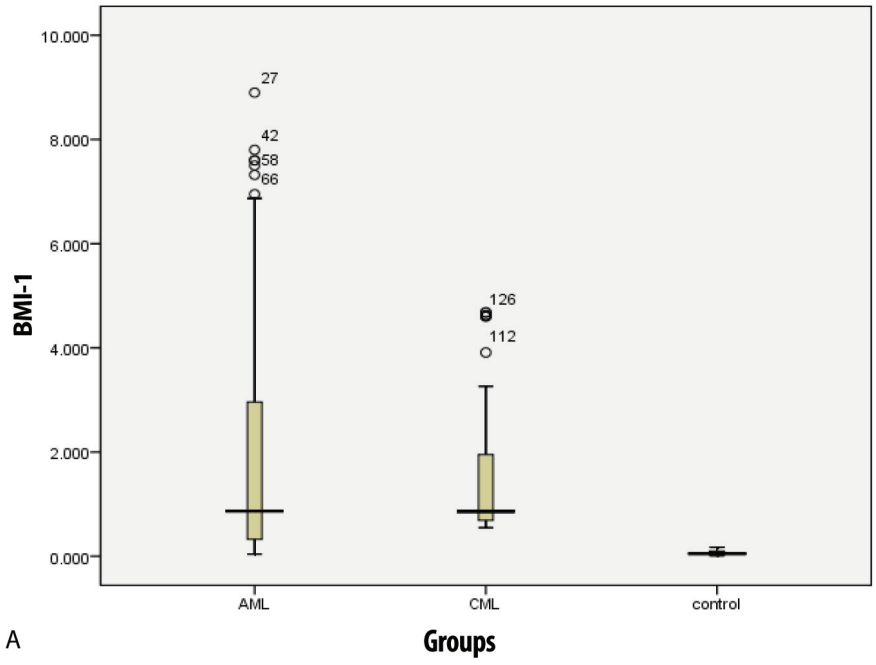
blood progenitor cells differentiation in the early stages of myelopoiesis would lead to increased immature blasts and infiltrate blasts proliferation in bone marrow and peripheral blood, impairing the production of normal blood cells.[2] Chronic myeloid leukemia (CML), as a myeloproliferative neoplasm with the presence of Philadelphia chromosome, accounts for approximately 15% of the newly diagnosed leukemia cases in adults.[3] Several genetic abnormalities, like spot mutations, gene rearrangements, and chromosomal alteration, play an important role in this leukemia development.[4] Philadelphia abnormality leads to the BCR-ABL oncoprotein, which exhibits



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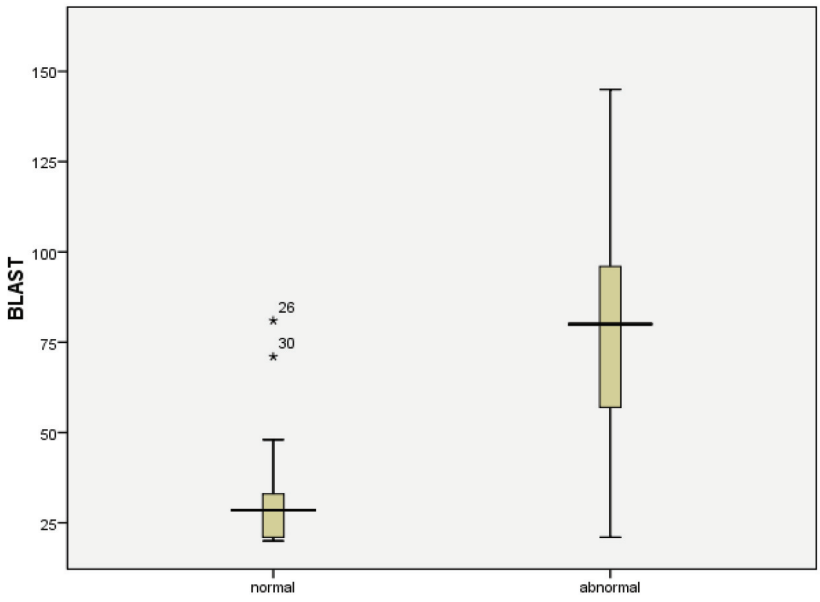

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Figure 1. This figure shows that the expression of BMI-1 in the patients with AML and CML was significantly higher in comparison with that in the healthy donors (A); higher blast counts were found to be more in patients with BMI-1 overexpression (B).
AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; BMI-1: The B-cell-specific Moloney murine leukemia virus integration site1

Table 1. Clinical, cytogenetic, and molecular characteristics of AML and CML patients according to BMI-1 expression status

Variant	No. of the patients (N=140)	BMI-1 expression status		*P*-value
		High expression N=56	Low expression N=84	
Age in groups (year)				
<15	34	19 (34%)	15 (18%)	0.52
16-55	75	29 (52%)	47 (56%)	
56-85	31	8 (14%)	22 (26%)	
Gender				
Female	60	26 (46.4%)	34 (40.47%)	0.74
Male	80	30 (53.57%)	50 (59.52%)	
White Blood Cell ($\times 10^9$/L)				
Mean (±SD)*	95.02 (±74.5)	135.6 (±72.2)	30.47 (±22.38)	<001
Blasts %				
Mean (±SD)	50.4 (±32.07)	76.7 (±31.1)	30.7 (±12.9)	<001
Hemoglobin (g/dl)				
Mean (±SD)	9.39 (±11.51)	6.51 (±1.62)	8.12 (±2.55)	<001
Platelet ($\times 10^9$/L)				
Mean (±SD)	177.7 (±179.8)	55.97 (±17.45)	64.98 (±49.43)	0.138
CML patients	70 (100%)	26 (37%)	44 (63%)	<001
AML (FAB)*				
M0	1 (1.4%)	1 (3.3%)	0 (0%)	0.56
M1	6 (8.6%)	3 (10%)	3 (7.5%)	
M2	21 (30%)	8 (26.7%)	13 (32.5%)	
M3	26 (37.1%)	12 (40%)	14 (35%)	
M4	9 (12.9%)	4 (13.3%)	5 (12.5%)	
M5	5 (7.1%)	1 (3.3%)	4 (10%)	
M6	1 (1.4%)	1 (3.3%)	0 (0%)	
M7	1 (1.4%)	0 (0%)	1 (2.5)	
NPM1 mutation	5 (7%)	3 (10%)	2 (5%)	0.42
FLT3-ITD mutation	12 (17%)	8 (26%)	4 (10%)	0.06
CEBPA mutation	6 (8%)	4 (13%)	2 (5%)	0.21
Karyotype SWOG				
Favorable	19 (27%)	8 (27)	11 (27.5%)	0.65
Intermediate	32 (45%)	15 (50)	17 (42.5%)	
Unfavorable	18 (26%)	6 (20)	12 (30%)	
Missing	1 (2%)	1 (3%)	0 (0%)	

AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; BMI-1: The B-cell-specific Moloney murine leukemia virus integration site1; SD: Standard deviation; FAB:French-American-British classification; NPM1: Nucleophosmin; FLT3: Fms-related tyrosinekinase-3; ITD: Internal tandem duplication; CEBPA: CCAAT/Enhancer Binding Protein α; SWOG: Southwest oncology group

constitutively active tyrosine kinase activity that provokes the myeloid leukemic cells growth.[5] Amongst numerous gene mutations, polycomb group proteins, such as B lymphoma Mo-MLV insertion region 1 (BMI-1), MBLR, and EZH2, have been reported in several papers.[6] BMI-1 is an important protein member of polycomb group, which plays important roles in chromatin modification, stem cell function, DNA damage repair, and mitochondrial bioenergetics.[7, 8] Although BMI-1 gene is expressed in both normal and leukemic stem cells, its overexpression leads to the disease aggressiveness.[9] Earlier research has indicated that the absence of BMI-1 is associated with a profound defect in HSCs' self-renewal.[10] With the development of novel molecular methods, the identification of important prognostic markers has been investigated in patients with AML and CML.[11, 12] These markers participate in epigenetic mechanisms, like polycomb ring finger group, which plays a fundamental role in cell cycle regulation.[13] BMI-1 expression has been reported in several human tumors as a key part in self-renewal and differentiation of HSCs.[14,15,16] BMI-1 is overexpressed in breast and other carcinomas and is associated with poor outcomes.[17] Therefore, this research aimed to investigate the BMI-1 expression in acute and chronic myeloid leukemia and study its effect on prognosis just in AML patients.

Materials and Method

Patients and data collection

We conducted the current cross-sectional study in the Cancer Molecular Pathology Research Center, Ghaem Hospital of Mashhad University of Medical Sciences (MUMS). The study was approved by the Ethics Committee of MUMS and each patient provided informed consent (Ethics code: IR.MUMS.fm.REC.1395.584).

A total number of 70 AML and 70 CML patients, also in addition to 20 healthy individuals with no neoplastic disorders or peripheral blood and bone marrow samples in their background, were included in this study. Unsuitable samples and AML patients who were undergoing chemotherapy, had recurrent leukemia, or uncertain diagnosis were excluded. The subjects comprised 40 males and 30 females in both groups. Morphology and cytogenetics analysis were performed as the routine patients clinical evaluation part.

RNA extraction and cDNA conversion

Peripheral blood samples and bone marrow were obtained in sterile EDTA vacutainers. Through peripheral blood mononuclear cells (PBMCs), the samples were purified by Ficoll gradient centrifugation. Total RNA was attained utilizing the TriPure Isolation Reagent Kit (Roche Diagnostic GmbH, Mannheim, Germany). RNA concentration was determined using the NanoDrop 2000 Spectrophotometer at 260:280 nm (Thermo Fisher Scientific, Wilmington, DE, USA). The samples with low RNA concentration (<20 ng/µL) were excluded from this study. RevertAid ™ H Minus First Strand cDNA synthesis kit (Fermentase, St. Leon-Rot, Germany) was employed for the cDNA synthesis via RT from 1µg of total RNA template.

Quantitative PCR for BMI expression

Primers sequences for BMI gene expressions at a concentration of 0.1µg: forward 5′GTATTCCCTCCA CCTCTTCTTG-3′, reverse 5′TGCTGATGACCCATTTACTGAT3′. qPCRs

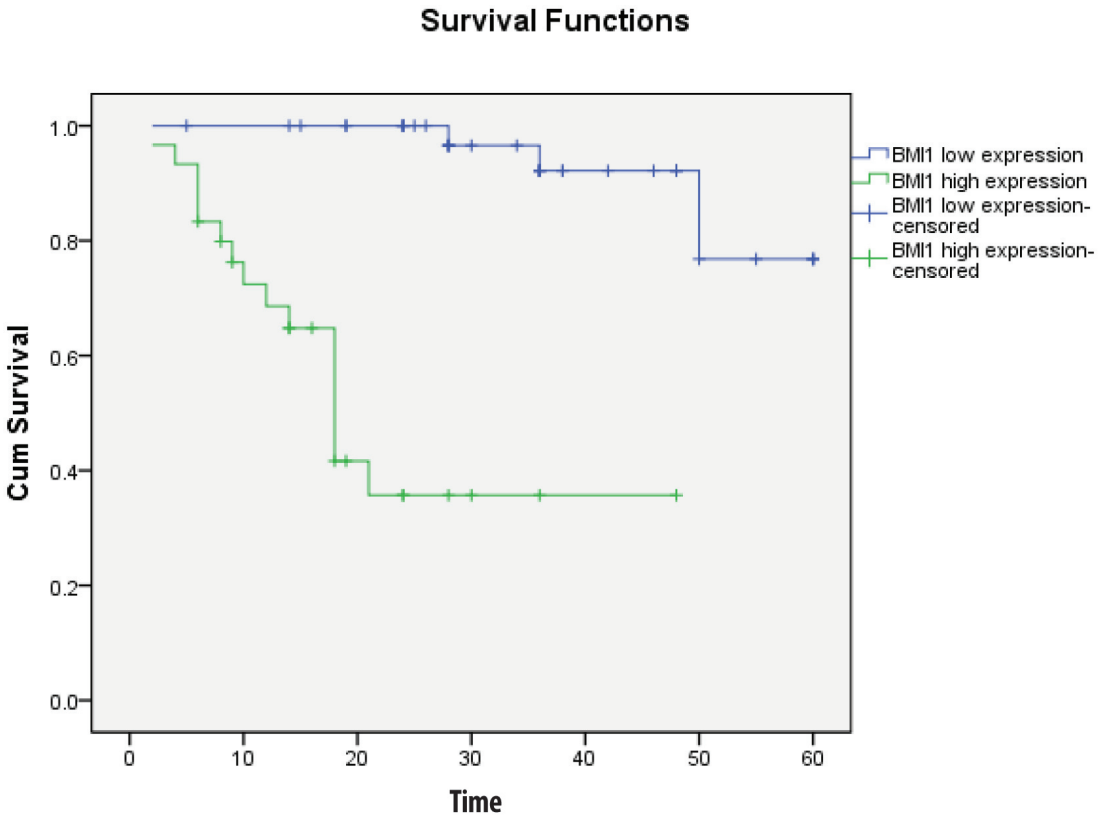

Figure 2. This figure depicts the survival analysis of a number of the AML patients according to BMI-1 expression status. Kaplan-Meier analysis showed a lower overall survival in the patients with BMI-1 overexpression compared with those with low BMI-1 expression.
Cum: Cumulative; AML: Acute myeloid leukemia; BMI-1: The B-cell-specific Moloney murine leukemia virus integration site1

Table 2. Multivariate analysis of OS as the dependent parameter studied with other covariates in the AML cases

Covariates	OS		
	P	HR	CI
Gender	0.21	0.47	0.142-1.554
Age (years)	0.20	1.011	0.990-1.032
Hemoglobin (g/dl)	0.3	1.367	1.033-1.809
BMI-1(High vs. Low)	<001	1.749	1.317-2.32
Blasts	0.3	1.009	0.992-1.027
Karyotype	0.126	0.38	011-1.31
White Blood Cell ($\times 10^9$/L)	0.39	1.003	0.99-1.009

OS: Overall survival; AML: Acute myeloid leukemia; HR: Hazard ratio; CI: Confidence intervals; BMI-1: The B-cell-specific Moloney murine leukemia virus integration site1

were quantified with an ABI VERITI 9902 (Applied Biosystems, Foster City, CA, USA). We identified PCR composition parameters as the following: master mix (20µL for each reaction) contained 0.5 µL ROX, 0.5 µL forward or reverse primers, 7.5 µL H2O, nuclease-free, 10 µL SYBRand1 µL cDNA. At the final stage, the program, which was set up for thermal cycler, was as the following: cycling steps at 50ºC for 2 minutes and 95ºC for 10 minutes, which were followed by the 50 PCR cycles at 95ºC for 15 seconds, and 63ºC for 60 seconds. Meltcurve was set at the temperature of 63ºC for 1 second and at the 95ºC for 1 second. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) gene control primers were evaluated for cDNA qualification confirmation (a housekeeping gene).[11] The relative gene expressions in the controls and patients were determined through the use of the comparative CT ($2^{-\Delta\Delta}$Ct) method. In this research, we used the cutoff value 1, in which the samples ≥1 would be considered as BMI-1-high expression and the samples <1 should be considered as BMI-1^{low} expression.

Statistical analysis

All the statistical analyses were performed using the SPSS v.11.5. In order to determine data distribution (the equality of continuous), we utilized the Kolmogorov–Smirnov test, followed by the Chi-Square and independent sample t-test for comparing the categorical variables and mean of continuous variables, respectively. We compared the subjects via the Mann-Whitney U test for the non-parametric variables, the Pearson *x*2 test for the categorical variables, and the log-rank test for the survival estimation. A *P*-value < 0.05 was considered to be statistically significant. The overall survival (OS) was counted as the time length from either the diagnosis date or the beginning of the treatment for a disease; it was calculated with the Kaplan–Meier curve.

Results

BMI-1 expression levels in healthy individuals and leukemic patients

The cases herein included 40 males and 30 females in both groups with the mean age of 25.6 years old in AML and 42.7 years old in CML. The mean expression ±SD and BMI-1 were 2.11 ± 2.5 (0.04-8.91) in AML and 1.46 ± 1.22 in CML patients (0.54-4.64), which were significantly higher in comparison with the BMI-1 expression in healthy controls (0.06 ± 0.05 (0.01-0.17); $P < 0.001$). 140 patients were detected with BMI-1 expression and subsequently, 56 patients (30 AML and 26 CML) were recognized with BMI-1^{high} expression (Table 1) (Figure 1).

Association with clinical, cytogenetic, and molecular characteristics

There was a significant difference between the BMI-1^{high} expression and white blood count (WBC), and Hb and high BM blast, which were counted in the AML patients ($P < 0.001$). Other parameters, like their age, sex, and Pltand FAB classification, did not imply any significant correlations with the BMI-1transcript. Karyotype and molecular markers, including FLT3, NPM1, and CEBPA expression, did not indicate any remarkable associations. Although FLT3-ITD expression appeared to be higher in those with BMI-1^{high} compared with those with low expression, this condition was not significantly meaningful ($P > 0.05$).

Prognostic impact of BMI-1 expression on AML patients

The 5-year follow-up of 70 AML patients in prognosis analysis revealed that patients with the predicted BMI-1^{high} status had a shorter OS in comparison with those with BMI-1^{low} group (high versus low, 76 vs. 35%; $P < 0.001$) (Figure 2). Moreover, according to the patients with adverse prognosis, out of 19 patients, 6 cases (31.60%) were M2 subclass with BMI-1^{high} expression. Multivariate analysis demonstrated by considering age, sex, WBC, Hb, karyotype, and BMI-1 expression status as covariates for prediction of OS in the subjects with AML. This analysis showed that increased BMI-1 expression is an unfavorable prognostic factor for OS ($P < 0.001$, Hazard ratio = 1.74, 95% CI = 1.31-2.32) (Table 2).

Discussion

In this study, we investigated the level of BMI-1 expression in AML and CML patients and assessed its prognostic effects on patients with AML. Based on the obtained results, BMI-1 expression was higher in the AML and CML patients in comparison with that in the healthy donors and BMI-1 gene overexpression was associated with poor prognosis in the AML patients.

Herein, BMI-1 overexpression prevalence was 42.8% in the AML and 37.1% in the CML patients. Saudy et al. reported 54% and 49% for their AML and CML patients, respectively.[19] Mohammad et al. indicated a high expression level of BMI-1 protein in thier study, which was about 11% of the malignant cells of mantle cell lymphoma.[20, 21]

The correlation between the level of BMI-1 and prognostic effect was controversial

The findings in the current work indicated that patients with BMI-1^{high} expression had short overall survival in the AML cases, which is in agreement with the results reported by Saudy et al., Sawa et al., Chowdhury et al., and M Hiraki.[22, 23, 24] In normal stem cells BMI-1 controls self-renewal and cell cycle by regulating and suppressing $p16^{Ink4a}$ /$p19^{19Arf}$/*MDM2*/ *p53* tumor suppressor pathways. The absence of these proteins would result in the cell cycle progression. BMI-1 over expression promotes the self-renewal and tumorigenic potential of cancer stem-like cells (CSCs). Consequently, BMI-1 overexpression is associated with poor prognosis.[17]

In this research, BMI-1 overexpression was significantly higher in the M2 subclass patients with poor prognosis; this result was in agreement with that reported by M. Chowdhury.[25] Sawa et al. and Saudy et al. reported that AML M0 and M4 patients have higher BMI-1 expression.[19, 23] It seems to be reasonable due to the blast count, genetic, and racial factors in these groups. Interestingly, this finding demonstrated that BMI-1 expression would lead to the myeloid transformation, which could explain the BMI-1^{high} expression in patients with CML. Furthermore, higher blast counts were found to be higher in BMI-1^{high} patients, which explained that overexpression of this gene has a pivotal role in the halt of HSCs differentiation. In the group of patients with higher BMI-1 and normal karyotype, 26% had FLT3-ITD positivity; however, only 4% had FLT3-ITD amongst the patients with lower BMI-1 expression.

As expected, the BMI-1high expression frequency among the subjects aged 16 to 55 years was higher (52%) in comparison with that in the other groups. In this study, there were no significant associations between the BMI-1 expression and chromosomal disorder ($P = 0.28$).

Conclusion

Based on the results of this study, BMI-1 expression increased in the AML and CML groups compared with the healthy subjects and had a prognostic effect on those with AML. Even though molecular markers did not indicate any significant associations with the BMI-1 transcription in this cohort study the expression screening and mutation of other epigenetic molecular could be considered helpful. We believe that adding BMI-1 expression status to the marker prognostic group will be conducive to the patients' treatment and stratification with myeloid leukemia. Nonetheless, a prospective study with a large number of AML and CML cases is required for further evaluation

of BMI-1 role in order to introduce BMI-1 as a novel marker.

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Conflict of Interest

None declared.

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