THE EXPRESSION PATTERN OF BMI-1 GENE IN ACUTE MYELOID LEUKEMIA

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ABSTRACT

Objectives: B-cell specific moloney murine leukemia virus integration site-1 (BMI-1) gene is a stem cell gene that modulates stem cell pluripotency and is also implicated in the regulation and accumulation of leukemic stem cells (LSCs). The current study aimed at characterizing BMI-1 gene expression in de novo AML patients before the start of chemotherapy and in those who achieved complete remission (CR).

Methods: Real-time polymerase chain reaction was used to assess the gene expression in 54 de novo AML patients: 43 AML and 11 in CR as well as in 21 non malignant bone marrow samples.

Results: AML patients showed a higher BMI-1 median fold change in expression (median=0.157) as compared to AML-CR patients (median=0.000) but this difference was not statistically significant. A higher median fold change was observed in patients with intermediate/unfavourable risk groups (2.381) than with favourable risk (0.000). BMI-1 expression levels were not seen to be influenced by clinicopatholoical factors of the disease or to affect response to first induction, overall and disease-free survival.

Conclusion: The role of BMI-1 in myeloid leukemogenesis needs further delineation to determine its significance in acute leukemia pathogenesis. Our results point to its possible role in AML risk stratification but further studies are needed.

*Keywords***:** BMI-1, Haematopoietic stem cells, Acute Myeloid leukemia.

INTRODUCTION

Acute myeloid leukemia (AML) is a cell autonomous or intrinsic disorder in which the genetic events leading to malignant transformation of a hematopoietic cell are found within that cell and are both necessary and sufficient for leukemo-genesis. Most adult patients with AML die from their disease despite achieving initial complete remission (CR), including those treated with aggressive multi-agent chemotherapy and allogeneic stem cell transplantation. Thus it is obvious that certain leukemic cells are resistant to currently available treatment modalities **(Roboz and Guzman, 2009)**.

Research suggests that a group of AML cells, which are mainly stem/progenitor cells called leukemic stem cells (LSCs), are the origin of the leukemic blasts. They show subtle molecular differences when compared with the normal haematopoietic stem cell and are not targeted under normal chemotherapy protocols **(Guzman and Allan, 2014)**.

The B-cell-specific moloney murine Leukemia virus integration site-1 (BMI-1) gene is an important member in the family of polycomb group genes (PcG), and it directly plays a role in regulation of cell proliferation and cell growth. The polycomb group gene BMI-1 was found to control both normal and leukemic stem cells and several studies have implicated it in cancer cell proliferation, invasion/ metastasis, chemosensitivity, and patient survival. Furthermore, BMI-1 is involved in the maintenance of self-renewal, tumor initiation and prevention of inappropriate differentiation of cancer stem cells (CSCs) by participating in multiple signaling pathways, suggesting that BMI-1 is important in maintaining the CSC properties **(Wang et al., 2015)**.

The aim of the current study was to characterize the expression pattern of BMI-1 gene in newly diagnosed patients with acute myeloid leukemia and in patients who have complete remission.

SUBJECTS AND METHODS

This study comprised 54 AML patients. Cases were selected from the Medical Oncology Department, Kasr Al-Ainy School of Medicine, Cairo University. The research protocol was approved by the Research Ethics committee of the Clinical Pathology Department, Cairo University, and informed consent was obtained from all participants.

AML patients were either newly diagnosed (n=43), before receiving induction therapy, or in CR (n=11). CR was defined as: bone marrow blasts <5%, absence of blasts with Auer rods, absence of extramedullary disease, absolute neutrophilic count $>1.0 \times 10^9$/L, platelet count $>100 \times 10^9$/L and independence of red cell transfusions. Some patients were in CR with incomplete recovery (CRi) defined as: all CR criteria except for residual neutropenia ($<1.0 \times 10^9$/L), or thrombocytopenia (100×10^9/L) **(D?hner et al., 2010)**.

Twenty-one age and sex matched control subjects were also selected in this study. They were patients undergoing bone marrow aspiration for reasons other than malignancy.

Patient medical records were reviewed for history and clinical data. In AML, complete blood count, bone marrow examination, immunophenotyping, and cytogenetic and molecular studies were done to establish diagnosis, classification and prognosis. Demographic and clinical data of patient and control groups are presented in **(Table 1)**.

Table (1): Demographic & Clinical Data of AML patients.

		AML- de novo n=43	AML-CR n=11
Age(MEAN±2SD)		40.4±14.8	33.2±11.5
Sex (M:F)		24:19	7: 4
Organomegaly n (%)		17 (39.5%)	4 (36.4%)
FAB subtype n (%)	*M0*	1 (2.3)	1 (9.1)
	M1	2 (4.7)	1 (9.1)
	M2	20 (46.5)	4 (36.4)
	M3	3 (7)	2 (18.2)
	M4	8 (18.6)	2 (18.2)
	M5	7 (16.3)	1 (9.1)
	M7	2 (4.7)	0 (0)
CD34 expression n (%)		21/43 (48.8)	4/11 (36.4)
Risk group(%)	*Favourable*	11/32 (34.4)	3/11 (27.2)
	Intermediate	17/32 (53.1)	6/11 (54.5)
	Unfavourable	4/32 (12.5)	2/11 (18.2)
Response after first induction n (%)	*Favourable (CR)*	24 (55.8)	8/11 (72.7)
	Unfavourable (PR or Death)	12 (28)	3/11 (27.3)
	Lost to follow-up	7 (16.2)	0 (0)

Treatment:

Treatment regimen of AML:

Patients with newly diagnosed AML (except M3) received induction chemotherapy with combination of 7 days of cytosine arabinoside as continuous infusion of 100mg/m^2 and 3 days of daunorubicin (45-60mg/m^2) OR mitoxantrone (12 mg/m^2 IV). Patients who achieved CR, and had favourable cytogenetics (inv16; t 8;21) received consolidation chemotherapy with high-dose Ara-C (3gm/m2 IV infusion over 3 hours/12 hours for 3 days).

Patients with high risk cytogenetics (monosomy 7 or 5, deletion of 5q and abnormalities of 3q and those with a complex karyotype) or intermediate risk cytogenetics (those with normal cytogenetics and other changes not associated with high risk or favorable groups) were transferred for allogenic bone marrow transplantation if they had matched sibling donor after achieving CR.

Patients with acute promyelocytic leukemia (APL) were treated according to the AIDA protocol (**Lo-Coco et al., 2010**).

Methods:

Detection of expression levels of BMI-1 gene by SYBR Green Real-Time PCR:

Mononuclear cells (MNCs) were separated from EDTA anticoagulated venous blood sample or bone marrow by density gradient using 1.077g/ml Ficoll Hypaque *(Invitrogen, USA)* and stored at -20 ^{0}C for later use in RNA extraction. RNA was isolated using QIAamp RNA Blood Mini Kit, *(Qiagen, Germany).* Total RNA was reversely transcribed in a total volume of 20?l reaction using high capacity cDNA reverse transcription kit *(Qiagen, Germany).*This was followed by amplification of cDNA by RT-PCR using *Quantitect SYBR Green Master Mix (Qiagen, Germany).* The real time cycler *(Applied Biosystems 7500, USA)* was programmed as follows: initial denaturation for 95^{0}C for 2 minutes, followed by 40 cycles of 60 ^{0}C for 40s and finally extension at 95^{0}C for 15s. The signal from the RT-PCR product is normalized to the signal from the internal control (β2-microglobulin) which is amplified by another set of primers. The primer sequences used were: *for BMI-1 gene:* **F-**5'- *TTCATTGATGCCACAACCAT-3'.* R-*5'CAGCATCAGCAGAAGGATGA-3'* (**Reinisch et al., 2006**); for β2-microglobulin: *F 5'- TACACTGAATTCAC CCCCAC-3';R 5'- CATCCCAATCCAAA TGCGGCA -3'* (**Shen et al., 2012**).

The relative expression of BMI-1 was determined using the delta C_T method. A comparative threshold cycle (C_T) was used to determine the gene expression relative to a normal control (calibrator) and used for comparison between patients at different stages of the disease. Briefly, each sample of either patient or control was normalized for the expression of β2 microglobulin (housekeeping gene) using the formula $\Delta C_T = C_T$ of the gene – C_T of β2 microglobulin. The mean expression of the control samples was then chosen as a normal calibrator, and relative BMI-1 expression for every patient was calculated using $2^{-\Delta\Delta CT}$ formula, where $\Delta\Delta C_T = \Delta C_T$ sample – ΔC_T calibrator. BMI-1 expression levels were expressed as an *n-fold difference relative to the calibrator.* Therefore; a $2^{-\Delta\Delta CT}$ value of > 1 is considered as a high expression of the gene as compared to the control and a value of <1 is considered low expression of the gene (**Schmittgen and Livak, 2008**).

Statistical methods:

Data was analyzed using IBM SPSS advanced statistics version 22 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA) then post-Hoc "Schefe test" was used for pair-wise comparison based on Kruskal-Wallis distribution. Spearman-rho method was used to test correlation between numerical variables. Survival analysis was done

using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. All tests were two-tailed. A p-value < 0.05 was considered significant.

RESULTS

BMI-1 mRNA transcripts were detected in 52.4% (11/21) of healthy controls. It was detected in 50% (21/42) of AML patients, and in 30% (3/10) of AML-CR patients **(Table 2).** No statistically significant difference was seen between the controls and patient groups (p=0.502). When comparing AML de novo and AML- CR patients; AML de novo showed a higher median fold change

(median=0.157) as compared to AML-CR patients (median=0.000) (**Figure 1**). However, this difference did not reach a statistical significance (p=0.963) and in both groups there was no fold increase of the BMI-1 expression was found among AML patients in comparison to the control.

No relationship was found between BMI-1 gene expression in AML de novo patients and the patients' age, sex, presence of organomegaly, FAB subtype, CD34, expression, risk group, response after first induction as well as OS and DFS of these patients.

Table (2): Comparison of BMI-1 gene expression detection between patient and normal controls**.**

Disease group	BMI-1		p-value
	Detected n (%)	Not detected n (%)	
Controls (n=21)	11 (52.4)	10 (47.6%)	0.502
AML de novo (n=42)	21 (50)	21(50)	
AML-CR (n=10)	3 (30)	7 (70)	
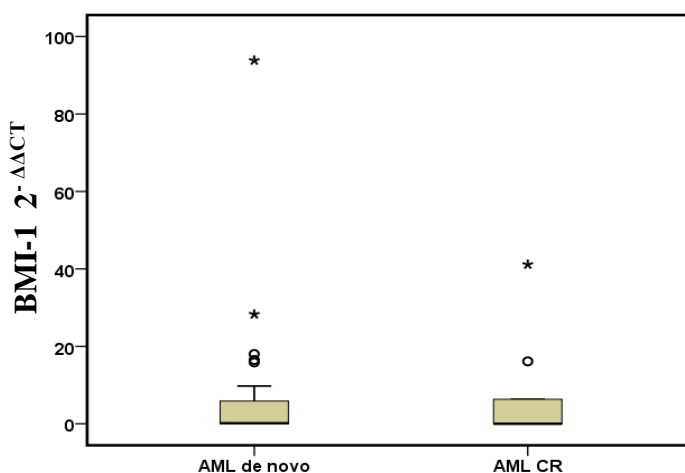
Figure (1): BMI-1 gene expression ($2^{-\Delta\Delta CT}$) in AML patient groups.

DISCUSSION

In our study, AML patients did not show a statistically significant difference from AML-CR patients as regards BMI-1 gene expression. Both patient groups did not show any fold change from controls. **Sawa et al. (2005)** studied the expression of BMI-1in different AML subtypes and found that expression of AML samples was higher than controls. **Shen et al. (2012)** reported a significantly higher expression of BMI-1 in de novo AML patients (M2, M3 & M5), than in controls. However, their AML-CR patients did not differ significantly from controls. Further studies on a larger sample of patients in each FAB subtype are required to validate the role of BMI-1 in acute leukemia pathogenesis.

In our study, although higher levels of BMI-1 gene expression were found in AML patients stratified as intermediate/ unfavourable risk group (median= 2.381) as compared to those in the favourable risk group (median=0.000), and this difference showed a borderline significance (p=0.059). This was also in accordance with **Chowdury et al. (2007)** where high BMI-1 gene levels were weakly correlated with unfavourable karyotypes. However, significantly higher levels of BMI-1 were found in patients with unfavourable karyotype in a study by **Nishida et al. (2014)**.

BMI-1 levels had no influence on OS and DFS. This was in accordance with other studies **Sawa et al. (2005) and Saudy et al. (2014)** but not with **Chowdury et al. (2007) and Nishida et al. (2014)** where BMI-1 levels were correlated with shorter OS times. However, BMI-1 protein was measured rather than mRNA. The difference could be due to the fact that BMI-1 is modified at the post-translational stage **(Sparmann and Van Lohuizen, 2006)**.

In conclusion, the role of BMI-1 in myeloid leukemogenesis needs further delineation to determine its significance and its correlation with cytogenetic and molecular risk groups. However, our results indicate that it may help to refine acute myeloid leukemia risk stratification.

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نمط التعبير عن جين البى ام آى-١ فى سرطان الدم النخاعي الحاد

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يوجد جين البي ام آى-١   فى الخلايا الجذعية ويعتبر جين أساسي في تعدد قدرات الخلايا الجذعية على التمييز و هو أيضاً مسئول عن ضبط و تراكم الخلايا الجذعية السرطانية. وبناءا على ما سبق فإن الهدف من هذا البحث هو تحديد نمط التعبير عن جين بي ام آي-١ في حالات سرطان الدم النخاعى الحاد قبل بدء العلاج بالإضافة إلى حالات التعافي التام من المرض بعد العلاج الكيميائي.

تم دراسة التعبير عن جين بي ام آي-١ بواسطة تفاعل البلمرة المتسلسل المعكوس الكمي ثلاثة و أربعون مريض بسرطان الدم النخاعى الحاد قبل بداية العلاج و احد عشر مريض بسرطان الدم النخاعى الحاد في مرحلة التعافي التام من المرض.و قد تم الاستعانة بواحد وعشرون من المتطوعين الذين لا يعانون من مرض سرطان الدم كمجموعة ضابطة للبحث.

أظهرت الدراسة وجود متوسط أعلى للتعبير عن جين بي ام آى-١ في مرض سرطان الدم النخاعى الحاد قبل تلقي العلاج (٠،١٥٧) بالمقارنة مع المرض في مرحلة التعافي المتكامل (٠،٠٠٠) ولكن هذا الفارق لم يكن ذو دلالة إحصائية. كان متوسط التعبير عن جين بي ام آى-١ أعلى في المرضى المقسمين إلى مجموعة متوسطة\عالية الخطر مقارنة بالمرضى المقسمين إلى مجموعة المنخفضة الخطورة ، وهذا الفارق كان يميل إلى الدلالة الإحصائية. لم تظهر دلالة إحصائية بين التعبير عن جين بي ام آى-١ وعوامل مرض سرطان الدم الميلودي الحاد في مرحلة ما قبل العلاج مثل أعمار المرضى أو جنسهم أو الخصائص الإكلينيكية والمعملية أو الاستجابة للعلاج.

لذلك نوصي بـ جراء هذه الدراسة على مجموعة أكبر من المرضى للتأكد من هذه النتائج وتحديد أهمية هذا الجين في تحديد خطورة المرض.