Roles of *DNMT3B* and *PARP1* Genes Expression in Cytogenetically Normal Acute Myeloid Leukemia

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ABSTRACT

BACKGROUND: Acute myeloid leukemia (AML) has a heterogeneous molecular profile, clinical presentations, and response to treatments and outcomes. DNA methylation is conducted by DNA methyltransferases including DNMT3B. Poly ADP-ribose polymerase 1 belongs to a family of enzymes that mediate important cellular processes including DNA repair, transcription, and cell death/cell proliferation, and it is involved in the development, spread, treatment, and prognosis of some cancers. The objective of this study is to assess the impact of *PARP1* and *DNMT3B* genes expression on laboratory characteristics, response to treatment and survival in Egyptian cytogenetically normal AML patients.

METHODS: This study included 67 Egyptian CN-AML patients in addition to 8 healthy bone marrow donors. Measurement of *DNMT3B* and *PARP1* gene expression was done on bone marrow samples via real-time semiquantitative polymerase chain reaction.

RESULT: Expression of both *DNMT3B* and *PARP1* genes was significantly upregulated in AML ($P = .001$, $P = .036$, respectively). Upregulated *DNMT3B* was associated with higher total leukocyte count (TLC), PB, and BM blast cell%. Also, upregulated *PARP1* correlated with higher TLC, PB, and BM blast cell%. High expression of both *DNMT3B* and *PARP1* correlated with greater frequencies of *FLT3-ITD*. High *DNMT3B* expression, and combined upregulation of both *PARP1* and *DNMT3B* genes associated significantly with ELN stratification. But no correlation was found with response (CR), overall survival (OS), disease-free survival (DFS), or event-free survival (EFS).

CONCLUSION: Our findings highlight the importance of considering *DNMT3B* and *PARP1* expression levels as potential prognostic biomarkers for progression and aggressiveness of CN-AML patients in AML. Assessing their expression levels could be an indicator to guide treatment decisions and potentially improve patient outcomes.

KEYWORDS: Acute myeloid leukemia, *DNMT3B*, *PARP1*, gene expression, real-time PCR

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Background

Acute myeloid leukemia (AML) is an abnormal clonal proliferation of myeloid progenitors in the bone marrow and/or peripheral blood and other tissue.[1] Acute myeloid leukemia derives from a dominant mutation, and then develops cooperative transformative mutations and other mechanisms such as epigenetics modifications leading to neoplastic transformation and clinical/biological heterogeneity.[2] The unfavorable prognosis of AML is primarily due to the heterogeneity of leukemic cells and their ability to develop resistance to chemotherapy. Therefore, it is crucial to explore novel therapeutic approaches that target specific genes involved in apoptosis and tumor progression.[3]

Epigenetic modifications are inheritable changes in gene expression that occur without altering the DNA sequence. These modifications include DNA methylation, histone deacetylation, and miRNA regulation.[4] DNA methylation is carried out by DNMT enzymes, which include DNMT1, DNMT3A, and DNMT3B. These enzymes regulate epigenetic changes by methylating cytosine, predominantly at CpG dinucleotides, resulting in the formation of 5-methylcytosine. Abnormalities in DNA methylation are closely linked to tumor progression and patient prognosis, particularly in hematological malignancies such as AML.[5]

Poly (ADP-ribose) polymerases (PARPs) are essential enzymes that regulate various cellular processes, including the DNA damage response, chromatin remodeling, and transcriptional regulation, thereby preserving genetic stability. Approximately 90% of poly-ADP-ribosylation (PARylation) within a cell is catalyzed by PARP1. PARP1 facilitates PARylation by covalently attaching ADP-ribose units to specific amino acid residues on target proteins. While PARP1 was originally recognized for its role in detecting and repairing single-strand DNA breaks, its PARylation activity also promotes the recruitment of other DNA repair proteins to sites of damage.[6]

DNMT3B and *PARP1* have been implicated in cancer progression and their expression levels have been observed to be altered in various types of cancer.[4,7,8] In AML, few studies

explored the expression of *DNMT3B* and its prognostic significance, whereas, limited research investigated the role of *PARP1* and postulated that it may function as a standalone prognostic determinant.[9]

Notably, results from published clinical trials revealed that PARP inhibitors (PARPi) in hematopoietic cancers are promising therapies, however, patient-specific factors should be considered, and further studies on the regulation and expression of PARPs are necessary to provide more accurate data on their ideal therapeutic use.[4] Therefore, we conducted a prospective analytic study with the aim of this study sought to measure *DNMT3B* and *PARP1* genes expression in de novo adult AML patients, and to assess their utility as prognostic biomarkers by correlating the results with established prognostic parameters and with patients' clinical outcome.

Patients and Methods

The study population comprised 67 newly diagnosed (de novo) adult CN-AML patient who presented to the outpatient clinic of the National Cancer Institute (NCI), Cairo University, from April 2019 to May 2020. The range of patients' ages was (19-73) years. In addition, 8 age and sex matched healthy subjects were chosen from the BM donors at the bone marrow transplantation (BMT) unit and included as a control.

Exclusion criteria included Pediatric AML, previous treatment, AML M3 subtype and association with other neoplasms. All cases were diagnosed based on standard diagnostic methods of examination and cytochemical staining of the BM, immunophenotyping and cytogenetic analyses according to WHO classification, 2017. Patients were classified according to ELN risk stratification of AML[10] into favorable risk (FR), and intermediate risk (IR).

The standard care of treatment in our institute for AML fit patients younger than 56 years (apart from APL) remains intensive chemotherapy with 7 + 3 protocol (Ara-C/Idarubicin or Doxorubicin) aiming for remission induction, while unfit or elderly patients who are ineligible for intensive chemotherapy protocol, receive less aggressive treatment on palliative intent including HMAs or low dose Ara-C. Indications of HSCT in AML patients in our center, patients with unfavorable cytogenetics (eg, FLT3) in the first CR & Refractory/Relapsed Patients after achieving complete remission.

Response to induction therapy was assessed both clinically and by BM examination at day 28. Complete remission was defined according to the standard criteria,[10] then the patients were divided into responsive (CR), and nonresponsive (No CR). Patients were followed up for at least 1 year after treatment.

RNA extraction, and cDNA synthesis and qPCR reaction

A sample of 1 to 2 mL of BM was collected on EDTA vacutainers under complete aseptic precautions before the start of treatment. Total cellular RNA from Human BM was extracted by using QIAamp RNA blood Mini Kit for total RNA purification (QIAGEN®Austin, Texas, USA) following the manufacturer's instructions. RNA concentration and samples' purity were detected by using the Nanodrop spectrophotometer. One microgram of RNA was used to perform Reverse transcription via Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA), then cDNA was stored at −20°C until required.

For amplification of the cDNA to detect *DNMT3B*, *PARP1* genes, the TaqMan® Universal PCR Master Mix at 2× concentration (Catalog no.: 4440040, Thermo Fisher Scientific, Applied Biosystems, USA) was used in addition to readymade assay, TaqMan primer probes for test genes *DNMT3B* (Hs00171876_m1), *PARP1*(Hs00242302_m1) *and* β-*Actin* gene as a reference gene. Real time PCR amplification was performed with programming the computerized thermocyclers (ABI step one-Applied Biosystems) as follows: Initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, and (Combined Annealing/Extension) at 65°C for 1 minute.

Data from the amplification plot was obtained and analyzed. Using the $2^{-\Delta\Delta Ct}$ method, the data are presented as the fold change in *DNMT3B*, *PARP1* genes. Acute myeloid leukemia patients were categorized into high and low expressers according to the median mRNA expression of *PARP1*[9] and DNMT3B.[5]

Statistical analysis

Data management and analysis were conducted using SPSS version 24. Numerical data were summarized as means with standard deviations or medians with ranges, depending on the distribution. Categorical data were presented as frequencies and percentages. Normality of numerical data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Group comparisons for categorical variables were made using the Chi-square or Fisher's exact tests, as appropriate. The Kaplan-Meier method was employed to estimate EFS, DFS, and OS, while predictor and prognostic variables were analyzed in relation to survival using the log-rank test. All tests were 2-sided, with P-values < 0.05 considered statistically significant.

Results

Patients' characteristics

The current study comprised 67 patients, out of them 43(64%) were male patients, and 24(36%) were females. The range of patients' ages was (19-73) years with mean ± SD of 44 ± 14.15 years. Patients' characteristic and initial laboratory findings were shown in Supplemental Table 1.

Expression levels of DNMT3B *and* PARP1 *in BM of AML patients*

PARP1 and *DNMT3B* mRNA transcript expressions were tested in all cases and control groups. *DNMT3B* expression was successfully detected in 64 cases while *PARP1* detection

Table 1. Expression levels of *DNMT3B* and *PARP1* in BM of AML patients.

MARKER	CONTROL GROUP MEDIAN (RANGE)	AML GROUP MEDIAN (RANGE)	*P* VALUE
DNMT3B	1.32 (0.09-9.36)	11.78 (0.05-97.93)	**.001**
PARP1	0.65 (0.25-2.56)	2.26 (0.05-7.33)	**.036**

was successful in 65 cases. *DNMT3B* expressions showed significant upregulation in AML cases [median: 11.78, range: 0.05-97.93] compared with the control group [median: 1.32, range: 0.09-9.36, $P = .001$]. Similarly, *PARP1* expressions were significantly upregulated in the patients [median: 2.26, range: 0.05-7.33] compared with the control group [median: 0.65, range: 0.25-2.56, ***P* = .036**] (Table 1).

Association between the expression DNMT3B *and* PARP1 *and patients' characteristics*

As regards *DNMT3B*, 32 (50%) of cases were above 11.77 (high expression group) and 32 (50%) were below or equal to 11.77 (low expression group), while for *PARP1*, 30 cases (46.2%) were above 2.26 (high expression group) and 35 (53.8%) were below or equal to 2.26 (low expression group). As regards the combined expression of *PARP1* and *DNMT3B*, both were downregulated in 20 (32.3%) cases, both were upregulated in 18 (29%) cases, upregulated *PARP1* and downregulated *DNMT3B* in 10 (16.1%) cases and 14 (22.6%) cases was upregulated *DNMT3B* and down regulated *PARP1* in relation to control group.

Correlation between the expression DNMT3B *and patients' characteristics.* Patients with high *DNMT3B* expression had higher TLC levels ($P = .031$), more blasts in the PB and BM ($P = .004$, $P = .005$, respectively) and more frequent *FLT3-ITD* (41.4% vs 11.5%, $P = .013$) than those with low expression. Also, high *DNMT3B* expression associated significantly with intermediate risk (IR) stratification according to ELN classification (93.1% vs 69.2% in the low expressers; $P = .035$), but *DNMT3B* expression did not significantly correlate with any other clinicolaboratory variable (Table 2).

Correlation between PARP1 *gene expression and patients' characteristics.* Patients overexpressing *PARP1* displayed increased TLC levels ($P = .024$), more PB and BM blasts ($P = .012$, $P = .007$, respectively) and more frequent *FLT3-ITD* (50% vs 10.3%, $P = .001$) compared with those with under-expression. However, *PARP1* levels showed no significant association with any of the remaining parameters (Table 3).

Correlation of combined expression of both gene and patients' characteristics. We found that cases with downregulation of both *PARP1* and *DNMT3B* genes associated with lower TLC ($P < .001$), decreased PB blast count (0.01) and lower BM blast cell count ($P = .037$), compared with cases with upregulation of both genes and cases with upregulation of one gene and downregulation of the other. On the other hand, upregulation with of both *PARP1, DNMT3B* genes correlated with more prevalent *FLT3-ITD* (0.006) and higher frequency of ELN intermediate risk ($P = .023$) compared with downregulation of both genes and to upregulation of one gene and downregulation of the other, but no statistically significant association was found with the other clinicolaboratory variables (Table 4).

Impact of PARP1 *and* DNMT3B *expression on CR.* No statistically significant difference was found between high and low expression levels of either *PARP1*, *DNMT3B* genes, or the combined expression of both genes and CR ($P = .27$, $P = .81$, $P = .76$, respectively). Also, no significant association was found between the levels of expression of *PARP1*, *DNMT3B*, or the combined expression of both genes and relapse ($P = 1$, $P = .96$, $P = 1$, respectively).

Impact of DNMT3B *and* PARP1 *expression on patients' survival.* The patients' median OS was 1.18 months (range, 0.03-38.8 months). No significant difference could be detected between low or high expressers of either *DNMT3B*, or *PARP1* in overall survival (OS), disease-free survival (DFS) or event-free survival (EFS). As regards *DNMT3B*, [median OS of the low expressers: 0.16 months vs 1.1 months for the high expressers ($P = .281$)], [the median DFS of the low expressers: 6.1 months vs 3.3 months for the high expressers ($P = .53$)], and [median EFS of the low expressers: 3 months vs 1 month for the high expressers ($P = .21$)] (Supplemental Figure 1).

Regarding *PARP1*, [median OS of the low expressers: 0.86 months vs 1.35 months for high PARP1 expressers ($P = .42$)], [median DFS of the low expressers: 6.8 months vs 6.1 months for the high expressers ($P = .51$)], and [the median DFS of the low expressers: 1.3 months vs 3 months for the high expressers ($P = .36$)] (Supplemental Figure 2).

In univariate analysis, better DFS associated significantly with lower blast percentage in the BM [median DFS: 13.1 months vs 4.4 months for the patients with higher blast percentage ($P = .009$)] (Figure 1), and better EFS significantly associated cases with negative expression of CD64 [median EFS: 1.6 months vs 1 months for the patients with

Table 2. Correlation between *DNMT3B* gene expression and patients' characteristics.

		DNMT3B NUMBER (%)		*P* VALUE
		LOW GENE EXPRESSION (N = 32)	HIGH GENE EXPRESSION (N = 32)	
Gender	Female	7 (22%)	14 (43.8%)	.062
	Male	25 (78%)	18 (56.2%)	
Age (years)	Mean ± SD Median (range)	44.13 ± 14.15 45 (19-73)	43.18 ± 15.02 44 (20-66)	.83
	⩽60	27 (84.4%)	29 (90.6%)	.45
	>60	5 (15.6%)	3 (9.4%)	
TLC (10^9/L)	Mean ± SD Median (range)	14.11 ± 11.15 13.9 (2.2-97.8)	36.11 ± 31.65 29.7 (6.5-151.4)	**.031**
HB (g/dL)	Mean ± SD Median (range)	8.7 ± 2.14 8.8 (5.5-13)	8.9 ± 2.34 8.2 (5.9-15.5)	.95
PLTs (10^9/L)	Mean ± SD Median (range)	84.13 ± 74.15 52.5 (3-321)	88.16 ± 78.19 69 (14-317)	.84
PB blasts %	Mean ± SD Median (range)	36.83 ± 22.15 24 (1-78)	57.13 ± 21.32 42 (3-80)	**.004**
BM cellularity at diagnosis	Hypercellular for age	26 (81.3%)	29 (96.7%)	.199
	Hypocellular for age	3 (9.4%)	0 (0.0%)	
	Normocellular for age	3 (9.4%)	1 (3.3%)	
Bone marrow blast (%)	Median (range)	50 (21-91)	68 (23-94)	**.005**
***FLT3-ITD* (molecular)**	Mutant	3 (11.5%)	12 (41.4%)	**.013**
	Wild	23 (88.5%)	17 (58.6%)	
***NPM1* (molecular)**	Mutant	8 (32.0%)	5 (18.5%)	.262
	Wild	17 (68.0%)	22 (81.5%)	
***IDH1* (molecular)**	Mutant	6 (27.3%)	6 (20.7%)	.583
	Wild	16 (72.7%)	23 (79.3%)	
Genetic risk according to ELN	FR	8 (30.8%)	2 (6.9%)	**.035**
	IR	18 (69.2%)	27 (93.1%)	
Response to induction therapy	CR	12 (70.6%)	12 (75%)	.81
	No CR	5 (29.4%)	4 (25%)	
Relapse	Yes	5 (15.6%)	6 (18.7%)	.96
	No	27 (84.3%)	26 (81.3%)	

Abbreviations: FR, favorable risk; IR, intermediate risk.

positive expression of CD64 (*P* = **.032**)], (Figure 2). However, in multivariate analysis these associations could not be detected.

Discussion

Despite recent advancements in refinement of predictive and prognostic markers, the mortality rate of AML has remained largely unchanged over the past 2 decades with an approximate median overall survival of 8.5 months, representing the fifth worst cancer type in terms of 5-year OS. This underscores the urgent need to identify novel biomarkers and molecular mechanisms driving leukemogenesis and AML progression to develop innovative therapeutic strategies that can improve patient outcomes.[11]

Table 3. Correlation between *PARP1* gene expression and patients' characteristics.

		PARP1 NUMBER (%)		*P* VALUE
		LOW GENE EXPRESSION (N = 35)	HIGH GENE EXPRESSION (N = 30)	
Gender	Female	14 (40.0%)	10 (33.3%)	.579
	Male	21 (60.0%)	20 (66.7%)	
Age (years)	Mean ± SD Median (range)	44.72 ± 15.81 48 (19-66)	39.18 ± 17.22 40.5 (19-73)	.433
	⩽60	30 (85.7%)	27 (90.0%)	.6
	>60	5 (14.3%)	3 (10.0%)	
TLC (10^9/L)	Mean ± SD Median (range)	13.61 ± 11.16 13.2 (2.2-120.8)	38.11 ± 32.44 29.7 (3-151.4)	**.024**
HB (g/dL)	Mean ± SD Median (range)	8.9 ± 2.64 9 (5.5-13)	8.8 ± 2.98 8.3 (5.9- 15.5)	.87
PLTs (10^9/L)	Mean ± SD Median (range)	83.33 ± 75.25 67.5 (12-321)	86.16 ± 79.19 45.5 (3-317)	.92
PB blasts %	Mean ± SD Median (range)	35.13 ± 29.15 23 (0-80)	60.18 ± 27.32 42 (5-77)	**.012**
BM cellularity at diagnosis	Hypercellular for age	28 (82.4%)	27 (93.1%)	.517
	Hypocellular for age	2 (5.9%)	1 (3.4%)	
	Normocellular for age	4 (11.8%)	1 (3.4%)	
Bone marrow blast (%)	Median (range)	47 (21-91)	67 (31-94)	**.007**
***FLT3-ITD* (Molecular)**	Mutant	3 (10.3%)	14 (50.0%)	**.001**
	Wild	26 (89.7%)	14 (50.0%)	
***NPM1* (molecular)**	Mutant	6 (22.2%)	6 (22.2%)	1
	Wild	21 (77.8%)	21 (77.8%)	
***IDH1* (molecular)**	Mutant	4 (14.8%)	8 (30.8%)	.165
	Wild	23 (85.2%)	18 (69.2%)	
Genetic risk according to ELN	FR	5 (17.2%)	4 (14.3%)	.85
	IR	24 (82.8%)	24 (85.7%)	
Response to induction therapy	CR	10 (58.8%)	13 (76.5%)	.27
	No CR	7 (41.2%)	4 (23.5%)	
Relapse	Yes	6 (17%)	5 (16.7%)	1
	No	29 (83%)	25 (83.3%)	

Abbreviations: FR, favorable risk; IR, intermediate risk.

It has been discovered that *PARP1* is overexpressed in several human malignancies, including nasopharyngeal carcinoma, gastric cancer, hepatocellular carcinoma, breast cancer, and prostate cancer. In addition, the overall prognosis is inversely linked with its overexpression. It is believed that *PARP1* uses a variety of molecular routes to carry out its carcinogenic function.[12-15] While DNMT3A mutations are common in AML occurring in approximately 1/5 of the de novo patients and causing changes in DNA methylation, the incidence of DNMT3B mutation in AML is rare and the influence of *DNMT3B* expression on AML treatment and outcome is poorly defined and controversial.[5]

The current study showed significant upregulation of *DNMT3B* expression in AML cases compared with the control

Table 4. Correlation of combined expression of both *PARP1* and *DNMT3B* and patients' characteristics.

		COMBINED *PARP1* AND *DNMT3B* NUMBER (%)				*P* VALUE
		BOTH ARE DOWN REGULATED	BOTH ARE UPREGULATED	UPREGULATED *PARP1 AND* DOWN REGULATED *DNMT3B*	UPREGULATED *DNMT3B* AND DOWN REGULATED *PARP1*	
		(N = 20)	(N = 18)	(N = 10)	(N = 14)	
Gender	Female	5 (25.0%)	7 (38.9%)	2 (20.0%)	9 (64.3%)	.081
	Male	15 (75.0%)	11 (61.1%)	8 (80.0%)	5 (35.7%)	
Age (years)	⩽60	17 (85.0%)	17 (94.4%)	8 (80.0%)	12 (85.7%)	.689
	>60	3 (15.0%)	1 (5.6%)	2 (20.0%)	2(14.3%)	
TLC (10^9/L)	Lower value*	18_a (90.0%)	4_b (22.2%)	3_b (30.0%)	$7_{a,b}$ (50.0%)	**<.001**
	Higher value*	2_a (10.0%)	14_b (77.8%)	7_b (70.0%)	$7_{a,b}$ (50.0%)	
HB (g/dL)	Lower value*	8 (40.0%)	12 (70.6%)	5 (50.0%)	6 (42.9%)	.267
	Higher value*	12 (60.0%)	5 (29.4%)	5 (50.0%)	8 (57.1%)	
PLT (10^9/L)	Lower value*	9 (45.0%)	8 (47.1%)	7 (70.0%)	5 (35.7%)	.414
	Higher value*	11(55.0%)	9 (52.9%)	3(30.0%)	9 (64.3%)	
PB blasts %	Lower value*	15_a (75.0%)	4_b (22.2%)	$4_{a,b}$ (40.0%)	$8_{a,b}$ (57.1%)	**.01**
	Higher value*	5_a (25.0%)	14_b (77.8%)	$6_{a,b}$ (60.0%)	$6_{a,b}$ (42.9%)	
BM cellularity at diagnosis	Hypercellular for age	15 (75.0%)	17 (100.0%)	9 (90.0%)	12 (92.3%)	.251
	Hypocellular for age	2 (10.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	
	Normocellular for age	3 (15%)	0 (0.0%)	0 (0.0%)	1 (7.7%)	
Bone marrow blast (%)	Lower value*	15_a (75.0%)	5_b (27.8%)	$5_{a,b}$ (50.0%)	$7_{a,b}$ (50.0%)	**.037**
	Higher value*	5_a (25.0%)	13_b (72.2%)	$5_{a,b}$ (50.0%)	$7_{a,b}$ (50.0%)	
FLT3-ITD (molecular)	Mutant	1_a (6.3%)	10_b (58.8%)	$2_{a,b}$ (22.2%)	$2_{a,b}$ (16.7%)	**.006**
	Wild	15_a (93.8%)	7_b (41.2%)	$7_{a,b}$ (77.8%)	$10_{a,b}$ (83.3%)	
NPM1 (molecular)	Mutant	3 (20.0%)	2 (12.5%)	4 (44.4%)	3 (27.3%)	.351
	Wild	12 (80.0%)	14 (87.5%)	5 (55.6%)	8 (72.7%)	
IDH1 (molecular)	Mutant	3 (21.4%)	5 (29.4%)	3 (42.9%)	1 (8.3%)	.346
	Wild	11 (78.6%)	12 (70.6%)	4 (57.1%)	11 (91.7%)	
Genetic risk according to ELN	FR	3_a (20%)	0_b (0%)	3_a (44.4%)	$2_{a,b}$ (15.4%)	**.023**
	IR	12_a (80%)	17_b (100%)	5_a (55.6%)	$11_{a,b}$ (84.6%)	
Response to induction therapy	CR	6 (60%)	8 (80%)	5 (83.3%)	4 (66.7%)	.76
	No CR	4 (40%)	2 (20%)	1 (16.7%)	2 (33.3%)	
Relapse	**Yes**	4 (20%)	3 (16.7%)	2 (20%)	2 (14.3%)	1
	No	16 (80%)	15 (83.3%)	8 (80%)	12 (85.7%)	
Protocol of treatment	Not 3 + 7	1 (7.1%)	0 (0%)	1 (10%)	1 (8.3%)	.690
	3 + 7	13 (92.9%)	16 (100%)	9 (90%)	11 (91.7%)	
BMT	**Yes**	1 (5%)	4 (22.2%)	1 (10%)	0 (0%)	.169
	No	19 (95%)	14 (77.8%)	9 (90%)	14 (100%)	

P value <.05 is considered statistically significant.
Cells that are sharing 2 letters are not statistically significant.
*The relevant median value of the variable is used as a cutoff.

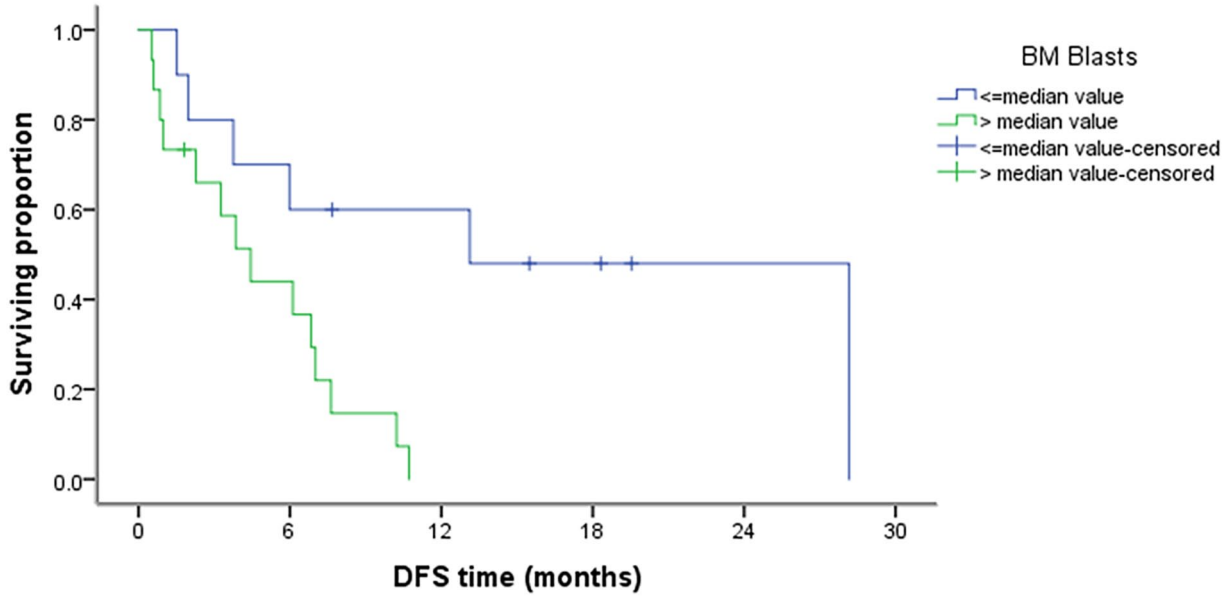

Figure 1. DFS in relation to blast % in the BM ($P = .009$).

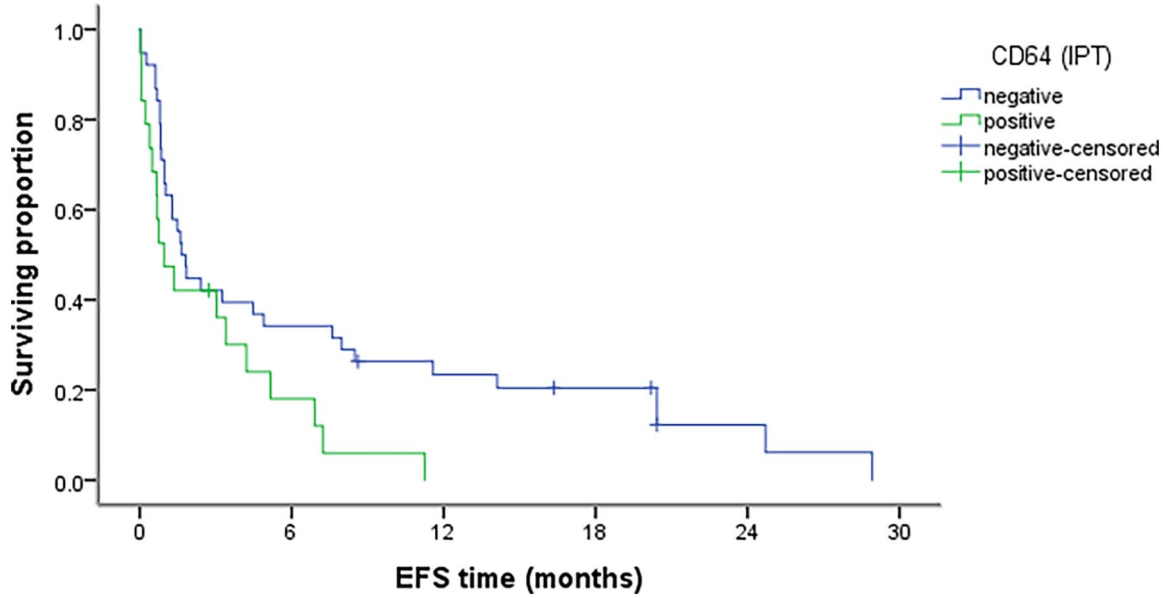

Figure 2. EFS in relation to CD64 ($P = .032$).

group ($P = .001$), a significant correlation between *DNMT3B* overexpression and higher total leukocyte count (TLC) ($P = .031$), as well as higher percentages of bone marrow and PB blasts ($P = .004$, $P = .005$, respectively). Our observation aligns with previous research suggesting a potential involvement of *DNMT3B* in AML pathogenesis and a potential correlation between elevated *DNMT3B* expression and higher proliferation rate or disease progression and aggressiveness.[16,17]

Overexpression of *DNMT3B* in AML was also reported by Mizuno et al, who, in addition, showed that AML cells with methylated $p15^{INAK4B}$ had a tendency to express greater levels of *DNMT3B*. They concluded that, increased expression of

DNMT3B might assist leukemogenesis via the induction of abnormal regional hyper methylation.[18] Likewise, a study on pediatric AML by Lamba et al,[19] reported that increased *DNMT3B* expression was linked to worse prognosis and higher genome-wide methylation burden (GWMB) that might be implicated in the development and hostility of the disease.

In contrast to our results, Zhang et al,[5] found that the expression of *DNMT3B* was reduced in AML patients and *DNMT3B* down regulation associated with higher TLC and lesser PB blasts. Others suggested that *DNMT3B* had a basic role in the pathogenesis of AML yet, may not behave as an oncogene based on observations from earlier studies that showed a tumor suppressive function for *DNMT3B in* inv(16)(p13;q22) AML and MLL-AF9 AML.[20,21] On the contrary, *DNMT3B* was oncogenic in AML cell lines and mouse models; therefore, Wong et al,[8] proposed that the role played by *DNMT3B* in AML appeared to be subtype specific.

Also in this study, higher *DNMT3B* expression was significantly linked to the presence of *FLT3-ITD* ($P = .013$). However, no significant association between *DNMT3B* expression and *NPM1 or IDH1* could be identified. It should be noted that cases harboring *FLT3-ITD* are stratified as intermediate risk (IR) patients in the recent ELN risk classification regardless of the allelic ratio or the presence of concurrent *NPM1* mutation.[10] On stratifying our population according to the recent ELN classification, we noticed that *DNMT3B* overexpression significantly correlated with (IR) ($P = .035$), hence, we confirm *DNMT3B* expression levels may be a useful prognostic biomarker in AML with *FLT3-ITD*, and the incorporation of its quantification might be helpful in the genetic profile used for the categorization of patients into specific therapeutic strategies.

Our findings agree with Hayette et al[16] and Niederwieser et al,[17] who reported *DNMT3B* expression associated with *FLT3ITD* mutations through the association with *HOXA9*. The association of *DNMT3B* expression with the *HOXA* family of genes was also reported by Lamba et al,[19] in pediatric AML suggesting that epigenetic modifications of the *DNMT3B* locus could take a part in transcriptional deregulation that possibly helps the evolution of the disease. In contrast Zhang et al,[5] reported that AML with *DNMT3B* underexpression displayed decreased frequencies of *IDH1* but *DNMT3B* levels did not significantly correlate with *NPM1 or FLT-ITD*.

As regards the prognostic value, we did not observe any correlation between *DNMT3B* expression and disease-free survival, event-free survival, or overall survival in our studied AML patients which is in agreement with Zhang et al,[5] who stated that *DNMT3B* gene expression may not be a significant prognostic biomarker and its potential role in AML remained poorly defined. On the contrary, overexpression of *DNMT3B* was reported as a significant independent poor prognostic indicator.[16,17] The divergent outcomes observed between the results could plausibly be attributed to different population' demographic features (young or older AML patients), dissimilarities in AML characteristics, different sample sizes, and methodologies employed. Furthermore, discrepant outcomes may have originated from the specific cutoff values used to define high or low genes expression. We suggest larger multicenter studies should be conducted for better evaluation and standardization.

The present study demonstrated significant upregulation in *PARP1* expression levels in AML patients compared with the controls ($P = .036$), significant associations between increased *PARP1* expression and higher TLC ($P = .024$), higher counts of blasts in the peripheral blood and bone marrow ($P = .012$, $P = .007$, respectively). Our results are concordant with previous studies [9,15,22,23] that showed overexpression of *PARP1* in AML, particularly in the poor prognosis genetic sub-group and inhibition of *PARP1* suppressed the proliferation of AML cells, brought about apoptosis in vitro, and enhanced the prognosis in mice. These results suggest a potential involvement of *PARP1* in AML development.

Furthermore, our results revealed that elevated *PARP1* expression significantly correlated with *FLT3-ITD* ($P = .001$), but, did not significantly associate with either *NPM1* or *IDH1*. These findings are congruent with Li et al[9] and suggest that *PARP1* expressions may hold potential prognostic value in AML. Interestingly, some research studies have provided valuable insights into the molecular mechanisms and possible associations of *PARP1* in AML. Where, some studies have shown an increase in genomic instability in *FLT3-ITD* mutated AML through *PARP1* associated with *MYC* expression.[4]

In addition, a favorable correlation between *PARP1* expression and myeloproliferative leukemia virus oncogene (MPL) was previously demonstrated and overexpression of both *PARP1* and *MPL* associated with poor outcome suggesting that activation of *MPL* by *PARP1* is another mechanism by which *PARP1* affects AML prognosis.[15] Furthermore, Pashaiefar et al[23] found overexpression of *PARP1* in AML patients with chromosomal translocations when compared with those without translocations, and in patients with 2 or more translocations when compared with those with an isolated translocation, suggesting that *PARP1* is most likely associated with severity of genetic risk.

Moreover, analysis of concomitant *DNMT3B* and *PARP1* expression revealed that cases with the downregulation of both genes displayed significantly reduced TLC, and blast count in peripheral blood and in BM, in comparison to those with upregulated *DNMT3B* and *PARP1* genes. Conversely, the elevated levels of *DNMT3B* and *PARP1* genes were correlated with a higher TLC ($P < .001$), and elevated blast count in the bone marrow ($P = .037$), and peripheral blood ($P = .01$).

To the best of our knowledge, our results are the first one to demonstrate a significant association between the combined upregulation of *DNMT3B* and *PARP1* and the presence of *FLT3-ITD* mutations in AML patients. Notably, a higher proportion of cases exhibiting upregulated expression of both

genes were found to harbor *FLT3-ITD* mutations (58.8%), as opposed to those with downregulated expression (6.3%) ($P = .006$). These observations imply a plausible association between the combined expression of *DNMT3B* and *PARP1* and the occurrence of *FLT3-ITD* mutations in AML patients. In addition, upregulation of both *PARP1* and *DNMT3B* genes associated with higher frequency of ELN intermediate risk (IR) ($P = .023$). However, a significant correlation between the combined expression of *DNMT3B* and *PARP1* and the presence of *NPM1* or *IDH1* mutations could not be revealed.

On the same line, a previous study found the use of combination of drugs that traps PARP1 and inhibits DNMT promoted apoptosis of the lymphomatous and leukemic cells via various mechanisms including inhibition of cell productivity and expansion, and increase of reactive O_2 molecules.[24]

Limitations of this study

The main limitation of this study was small sample size (67) and the unavailability of involving patients from centers other than NCI, and we focused on gene expression levels without delving into the mutational status of *DNMT3B* and *PARP1* in AML due to limited budget. Multi-center study including large number of patients is necessary to approve our results.

Conclusions

Our results confirm results from previous studies and provide valuable insights into the significance of *DNMT3B* and *PARP1* gene expression in AML, suggesting a potential role in disease progression and aggressiveness and supports their interplay in AML. Our study's findings confirm the potential of *DNMT3B* and *PARP1* as predictive markers for identifying AML patients with *FLT3-ITD* and highlight their involvement in AML pathogenesis.

Our findings might have significant implications in identifying high-risk AML patients who may benefit from targeted treatments or more intensive therapies. By assessing the expression levels of *DNMT3B* and *PARP1*, clinicians can obtain additional prognostic indicators to guide treatment decisions and potentially improve patient outcomes.

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Author contributions

HAM and RSAA designed the study and wrote the original drafts and coordinated specimen collection and transport. SKAB and RSAA managed kits purchasing tasks and supervised laboratory analysis workflow. HAM, MMK, and RSAA performed the laboratory analysis. AMF ensured appropriate patient selection and supervised data collection. All authors have read and approved the final manuscript.

Availability of data and materials

All data and materials are available and can be submitted when needed. Corresponding author is the responsible person who should be contacted if someone wants to request the data from this study.

Ethical approval

Approval (no.2306-403-026) of the current study was obtained from review board (IRB) of NCI, Cairo University. All the measures were performed in accordance with Helsinki guidelines.

Informed consent

The study objectives were explained then and written informed consents were got from all contributors (patient and control) before bone marrow sampling. Confidentiality of patient data was guaranteed.

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Supplemental material

Supplemental material for this article is available online.

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