Prognostic impact of inhibitors of DNA binding proteins1 and inhibitors of DNA binding proteins4 genes expression on adult Egyptian patients with acute myeloid leukemia

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Background Acute myeloid leukemia (AML) pathogenesis and treatment are currently being better understood at an accelerated rate. Determining genetic and epigenetic changes that can identify patients who are at risk of poor outcomes is therefore desired to optimize treatment options. Many solid tumors have been reported to overexpress Inhibitors of DNA binding proteins (ID1), but few research has looked at the clinical significance of ID1 expression in AML. Additionally, little research has been focused on the direct role of ID4 in myeloid malignancies, as well as its expression and methylation patterns. The aim of the current study was to assess ID1 and ID4 gene expression in bone marrow (BM) aspiration specimens of 91 AML patients, compared with 14 control donors of bone marrow transplantation (BMT), using real-time polymerase chain reaction (RT-PCR). Data were correlated with patients' clinicopathological features, response to treatment, disease-free survival (DFS), and overall survival (OS) rates.

Results ID1 transcript level was significantly increased in AML bone marrow samples compared with normal controls ($P = 0.002$), while ID4 gene expression showed a nonsignificant difference ($P = 0.717$). In addition, there was a significant increase in ID1 gene expression in fms-like tyrosine kinase 3 (FLT3) mutant group than fms-like tyrosine kinase 3 wild group ($P = 0.010$). The total leukocytic count (TLC) was significantly higher in patients with high ID1 expression ($P = 0.038$) and patients with undetected ID4 expression ($P = 0.025$). No significant associations were detected between ID1 and ID4 expression levels and patients' clinicopathological characteristics and OS rates.

Conclusion In contrast to ID4, overexpressed ID1 can be adopted as a genetic biomarker for diagnosing AML. ID1 and ID4 expressions did not affect the patients' OS or DFS.

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Keywords: Acute myeloid leukemia, genes expression, Inhibitors of DNA binding proteins1, Inhibitors of DNA binding proteins4, leukemia, myeloid

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Introduction

Acute myeloid leukemia (AML) is the most prevalent form of leukemia in adults. It is characterized by the clonal growth of immature 'blast cells' in the bone marrow (BM) and peripheral circulation, which causes BM failure and inefficient erythropoiesis [1]. The cure rates have increased by up to 15% for individuals over 60 and by roughly 40% for patients under 60. However, the prognosis for the older population is still very bad [2].

The inhibitors of differentiation proteins (ID), also known as DNA-binding proteins, were discovered for the first time at 1990. ID proteins are members of the helix-loop-helix (HLH) family. They are divided into four subtypes, namely ID1, ID2, ID3, and ID4 [3]. Cell differentiation and cell linkage commitment are coordinated by ID proteins, which also tightly control the expression of cell cycle regulators. In undifferentiated, highly proliferative, embryonic, or cancer cells, ID gene expression is typically positively regulated, particularly for ID1, ID2, and ID3 [4].

ID1 is considered a tumor promotor and is overexpressed in several tumors. Overexpression of ID1 is seen in acute myeloid leukemia patients. Previous studies demonstrated that the oncogenic tyrosine kinases, such as BCR-ABL, TEL-ABL, TEL-PDGF beta R, and FLT3-ITD, play a major role in the development of hematopoietic malignancy. ID1 was identified as a common downstream target of constitutively activated oncogenic tyrosine kinases [3]. Furthermore, loss of ID1 inhibited t (8;21) leukemia initiation and progression by abrogating AKT1 activation. Additionally, ID1 could immortalize hematopoietic progenitors *in vitro*, and especially common myeloid progenitors, and promote a myeloproliferative disease *in vivo* [5].

On the other hand, ID4 is a tumor suppressor gene since it is found to be epigenetically silenced in several

cancers. However, it is an oncogene in a few cancers [6]. ID4 methylation plays an important role in disease progression in patients with myelodysplastic syndrome which is a myeloid hematopoietic malignant disorder with high susceptibility to transform into AML. High levels of ID4 methylation have been correlated with decreased survival [7]. Different degrees of ID4 gene methylation occur in AML patients with different subtypes and stages, which suggests that ID4 gene methylation may be an early molecular event in the process of AML [8]. However, its expression pattern and role in AML have been scarcely evaluated.

This study was planned to explore the pattern of expression, clinical relevance, and prognostic importance of ID1 and ID4 genes in Egyptian adult patients with newly diagnosed AML.

Patients and methods

This is a prospective clinical study that included 91 adult patients with newly diagnosed AML. The patients were recruited from the Medical Oncology Department of the National Cancer Institute (NCI) from 2015 to 2018 and were compared with 14 healthy age and sex-matched BM donors as controls. This study was approved by the Research Ethics Committee (REC) of Benha Faculty of Medicine for ethical clearance. AML diagnosis was performed based on the WHO criteria [9]. Patients' samples were obtained before receiving any medical treatment. All patients were subjected to history taking, clinical examination, and laboratory investigations including complete blood count (CBC), BM aspiration, immunophenotyping, molecular analysis, and conventional karyotyping.

Blood Samples: For CBC analysis, 2 ml of peripheral venous blood was withdrawn from each patient into a tube containing 1.2 mg/ml k-ethylene diamine tetra-acetic acid (K-EDTA) (1.2 mg/ml). 2 ml of BM aspirate were obtained from each subject. 1 ml was collected on K-EDTA for immunophenotyping and molecular analysis, and 1 ml was collected on sodium heparin for fluorescence *in situ* hybridization (FISH) and conventional karyotyping. Two drops of BM aspirate were obtained for morphology and cytochemistry smear slides. The patients' cytogenetic risk was assessed based on the 2017 recommendations of the European leukemia net (ELN) [10].

RNA extraction and cDNA formation

BM aspirate (1 ml) obtained on K-EDTA was used for RNA extraction using a QIAamp RNA extraction blood mini kit (QIAGEN Austin, TX, USA, catalog no. 52304). The extraction was performed as described in the manufacturer's instructions. The Spectrophotometer NanoDrop (Quawell, Q-500, Scribner, USA) was used to assess the extracted RNA purity and concentration. Samples were stored at -80° C till further assessment. High-capacity cDNA reverse transcription kit (Applied Biosystems, Thermo Fisher Scientific, USA; catalog no. 4368814) was used to prepare complementary DNA (cDNA) based on the manufacturer's instructions. The purity and concentration of cDNA were evaluated, and samples were stored at −20°C until further use.

Real-time PCR (RT-PCR)

Quantification of the ID1 and ID4 mRNA expression was performed using TaqMan Universal PCR Master Mix II (Applied Biosystems, USA, Thermo Fisher Scientific, Cat no. 4440040) and IDI, ID4 Taqman Gene Expression Assay (Applied Biosystems, USA, Thermo Fisher Scientific, Cat no. 4331182, Hs 03676575-S1, Hs 02912975- S1, respectively). ID1 and ID4 expressions were normalized to B-actin as endogenous control. Quantitative RT-PCR was conducted utilizing cDNA with an adjustment of the concentration according to the abundance of mRNA. The thermal reaction conditions were adjusted according to the manufacturer's instructions. Fluorescence was detected using the Step One RT-PCR System (Applied Biosystems, Foster City, CA, USA). Based on Schmittgen and Livak [11], the relative expression of the ID1 and ID4 genes was estimated by the comparative Ct method ($2^{-\Delta Ct}$).

Statistical methods

The data were analyzed using the statistical package SPSS (version 24 for Windows; SPSS Inc, Chicago, IL, USA). The associations between gene expression and the clinicopathological characteristics of the patients were done using Fisher's exact test. The Mann-Whitney test and Kruskal-Wallis test were used to assess the relative expression of the assessed genes between groups. Receiver operating characteristic (ROC) curves were used to assess the gene expression as biomarkers. The patients' cumulative overall and disease-free survival rates were compared by Kaplan-Meier survival analysis and the log-rank test. *P* values less than 0.05 were considered statistically significant. Based on the cutoff values of ID1 and ID4 gene expression obtained by ROC curves, the patients were categorized into low-expression and overexpression groups.

Results

The demographic and clinical characteristics of AML patients are summarized in Table 1.

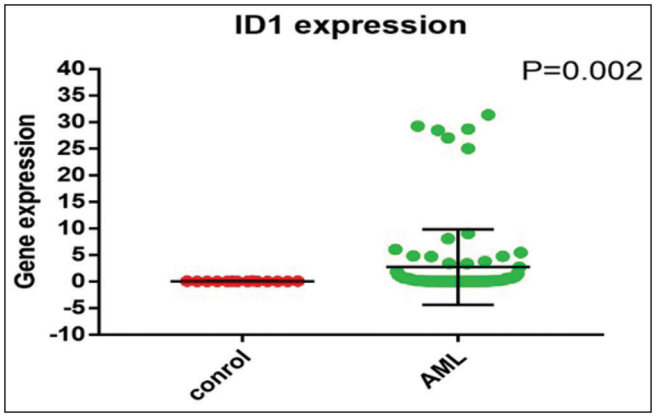
The ID1 expression levels significantly overexpressed in AML patients compared with normal control

Table 1 AML cases general characteristics

Variables	Median	Range	Frequency*	Percent
Sex				
Male			50	54.9%
Female			41	45.1%
Age (years)	33	18-65		
Hepatomegaly			27	29.7%
Splenomegaly			23	25.3%
Lymphadenopathy			30	33.0%
White blood cells count $\times 10^9$/l	38.9	1-440		
Hemoglobin level (gm/dl)	8.0	2.3-13		
Platelets count $\times 10^9$/l	35	5-297		
Peripheral blood blasts (%)	50	0-98		
Bone marrow blasts (%)	70	14-97		
BM cellularity				
Normocellular marrow			14	15.4%
Hypercellular marrow			70	76.9%
Hypocellular marrow			7	7.7%
Cytogenetic risk				
Favorable			18	19.8%
Intermediate			51	56.0%
Adverse			22	24.2%
FLT3				
Mutant			14	15.4%
Wild			77	84.6%
FAB classification				
AML without maturation (M1)			11	12.1%
AML with maturation (M2)			38	41.8%
Acute myelomonocytic leukemia (M4)			24	26.4%
Acute monoblastic leukemia (M5)			17	18.6%
Acute megakaryocytic leukemia (M7)			1	1.1%

*In 91 AML cases.
BM, Bone marrow; FAB, French-American-British classification; FLT3: fms-like tyrosine kinase 3.

Figure 1



Bone marrow ID1 expression levels in adult AML patients and controls.

(P = 0.002). In the control group, it ranged from 0.0 to 0.05 with a median level of 0.03, while in AML patients, it ranged from 0.0 to 31.4 with a median of 0.12 (Fig. 1). Regarding ID4 gene expression in this study, there was no significant difference of its levels in AML patients BM samples compared with normal controls (P = 0.717), it ranged from 0.0 to 0.005 with a median of 0.001 in the control group, while in AML patients, it ranged from 0.0 to 0.04 with a median of 0.0007 (Fig. 2).

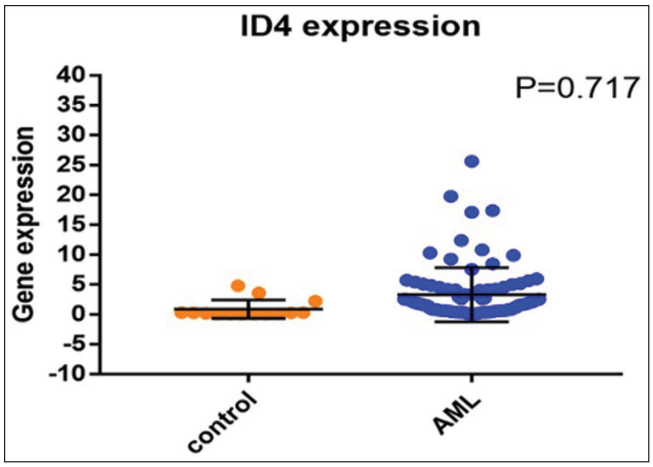
ROC analysis revealed that ID1 distinguished AML patients from controls, with an area under the curve (AUC) of.720 (95% CI: 0.592 -0.847, P = 0.01). In contrast, ID4 showed poor performance with an AUC of.531 (95% CI: 0.375 -0.687, P = 0.71).

Based on ROC analysis, AML patients were categorized according to ID1 expression into low (< 0.038) and high expression (> 0.038) groups and according to ID4 expression into detected and undetected groups. The detected group was divided into low (< 0.001) and high expression (> 0.001).

Regarding ID1 gene expression, the high-expression group demonstrated a significantly higher TLC count than the low-expression group (P = 0.038). On the contrary, no statistically significant difference was found between the low and high expression groups regarding other clinical and laboratory findings, including age, sex, hepatomegaly, splenomegaly, lymphadenopathy,

Hb levels, platelet count, PB blasts, BM blasts, BM cellularity, FAB subtypes, treatment response, and survival status (Tables 2, 3).

Figure 2



Bone marrow ID4 expression levels in adult AML patients and controls.

Regarding ID4 gene expression, there was a significant difference between the low, high, and undetected expression groups regarding TLC (P = 0.025). It was higher in the undetected group than in the other two groups. No significant association was found between ID4 expression and all clinical characteristics (P > 0.05) (Tables 4, 5).

There was a significant increase in ID1 gene expression in fms-like tyrosine kinase 3 (FLT3) mutant group more than FLT3 wild group (P = 0.010) while no significant difference between the same groups regarding ID4 gene expression (P = 0.520). No significant differences were observed between NPM mutant and wild groups, normal and abnormal karyotyping groups, and favorable, intermediate, and adverse cytogenetic risk groups regarding ID1 and ID4 gene expression (Table 6).

Kaplan-Meier revealed no significant difference between different expression levels of ID1 or ID4 regarding OS and DFS (Figs 3–6).

Table 2 Association between ID1 gene expression and clinical findings in AML cases

Variables	ID1 expression frequency** (%)		
	Low (n=25)	High (n=65)	P value
Age	33 (19-62)	36 (19-57)	0.732
Sex			0.638
Male;	15 (60.0%)	34 (52.3%)	
Female	10 (40.0%)	31 (47.7%)	
Hepatomegaly			0.211
No	15 (60.0%)	48 (73.8%)	
Yes	10 (40.0%)	17 (26.2%)	
Splenomegaly			0.282
No	21 (84.0%)	46 (70.8%)	
Yes	4 (16.0%)	19 (29.2%)	
Lymphadenopathy			0.802
No	18 (72.0%)	43(66.2%)	
Yes	7 (28.0%)	22 (33.8%)	
TLC(×109/l)	15.3 (4.9-240)	45 (1-358)	**0.038**
Hb(gm/dl)	7.8 (6-12.1)	8 (4.6-12.1)	0.809
PLT(×109/l)	46 (8-297)	36 (5-208)	0.118
PB Blasts (%)	40 (0-95)	60 (0-94)	0.490
BM Blasts (%)	70 (25-95)	68 (24-93)	0.540
BM cellularity			0.641
Hypercellular	18 (72%)	52 (80.0%)	
Normocellular	5 (20.0%)	8 (12.3%)	
Hypocellular	2 (8.0%)	5 (7.7%)	
Treatment response			
CR	18 (72.0%)	49 (75.4%)	0.79
relapse	7 (28.0%)	16 (24.6%)	
Survival status			
Live	11 (44.0%)	26 (40.0%)	0.813
Death	14 (56.0%)	39 (60.0%)	

P value less than or equal to 0.05 is significant.
*Out of 90AML patients (one case undetected).
BM, bone marrow; CR, complete remission; Hb, hemoglobin level; PB, peripheral blood; PLT, Platelets count; TLC, total leukocytic count.

Table 3 Statistical comparison between low and high ID1 gene expression regarding FAB classification in AML cases

	ID1 expression frequency* (%)		*P* value
	Low (*n*=25)	High (*n*=65)	
FAB grouped			0.469
AML without maturation (M1)	5 (20.0%)	6 (9.2%)	
AML with maturation (M2)	9 (36.0%)	28 (43.1%)	
Acute myelomonocytic leukemia (M4)	8 (32.0%)	16 (24.6%)	
Acute monoblastic leukemia (M5)	3 (12.0%)	14 (21.5%)	
Acute megakaryocytic leukemia (M7)	0 (0.0%)	1 (1.5%)	

P- value less than or equal to 0.05 is significant.
*Out of 90 AML cases (one case undetected).
FAB, French-American-British classification.

Table 4 Association between ID4 gene expression and clinical findings in AML cases

Variables	ID4 expression frequency** (%)			
	Low (*n*=36)	High (*n*=29)	Undetected (*n*=26)	*P* value
Age	36.5 (19-57)	34 (19-62)	31 (18-65)	0.104
Sex				0.743
Male	18 (50.0%)	17 (58.6%)	15 (57.7%)	
Female	18 (50.0%)	12 (41.4%)	11 (42.3%)	
Hepatomegaly				0.222
No	28 (77.8%)	21 (72.4%)	15 (57.7%)	
Yes	8 (22.2%)	8 (27.6%)	11 (42.3%)	
Splenomegaly				0.186
No	29 (80.6%)	23 (79.3%)	16 (61.5%)	
Yes	7 (19.4%)	6 (20.7%)	10 (38.5%)	
Lymphadenopathy				0.358
No	24 (66.7%)	22 (75.9%)	15 (57.7%)	
Yes	12 (33.3%)	7 (24.1%)	11 (42.3%)	
TLC (×109/l)	20.9 (1.7 -358)	33.9 (1-281.7)	79 (2.6-440)	**0.025**
Hb (gm/dl)	7.8 (4.6-12.1)	8.4 (4.9-12.1)	7.9 (2.3-13)	0.140
PLT (×109/l)	35 (5-297)	44 (6-240)	27.5 (8-191)	0.127
PB Blasts (%)	40 (0-95)	61 (9-94)	67.5 (4-98)	0.064
BM Blasts (%)	72 (25-95)	67 (24-93)	75 (14-97)	0.438
BM cellularity				
Hypercellular	25 (69.4%)	24 (82.8%)	22 (84.6%)	0.515
Normocellular	7 (19.4%)	4 (13.8%)	2 (7.7%)	
Hypocellular	4 (11.1%)	1 (3.4%)	2 (7.7%)	
Treatment response				
CR	26 (72.2%)	22 (75.9%)	20 (76.9%)	0.902
Relapse	10 (27.8%)	7 (24.1%)	6 (23.1%)	
Survival status				
Live	14 (38.9%)	13 (44.8%)	10 (38.5%)	0.857
Death	22 (61.1%0	16 (55.2%0	16 (61.5%)	

P value less than or equal to 0.05 is significant.
*Out of 91AML patients.
BM, bone marrow; CR, complete remission; Hb, hemoglobin level; PB, peripheral blood; PLT, platelets count; TLC, total leukocytic count.

Table 5 Statistical comparison between different levels of ID4 gene expression regarding FAB classification in AML cases

	ID4 expression frequency** (%)			*P* value
	Low (*n*=36)	High (*n*=29)	Undetected (*n*=26)	
FAB-Grouped				0.756
AML without maturation (M1)	5 (13.9%)	4 (13.2%)	2 (7.7%)	
AML with maturation (M2)	13 (36.1%)	12 (41.4%)	13 (50.0%)	
Acute myelomonocytic leukemia (M4)	12 (33.3%)	7 (24.1%)	5 (19.2%)	
Acute monoblastic leukemia (M5)	6 (16.7%)	5 (17.2%)	6 (23.1%)	
Acute megakaryocytic leukemia (M7)	0 (0.0%)	1 (3.4%)	0 (0.0%)	

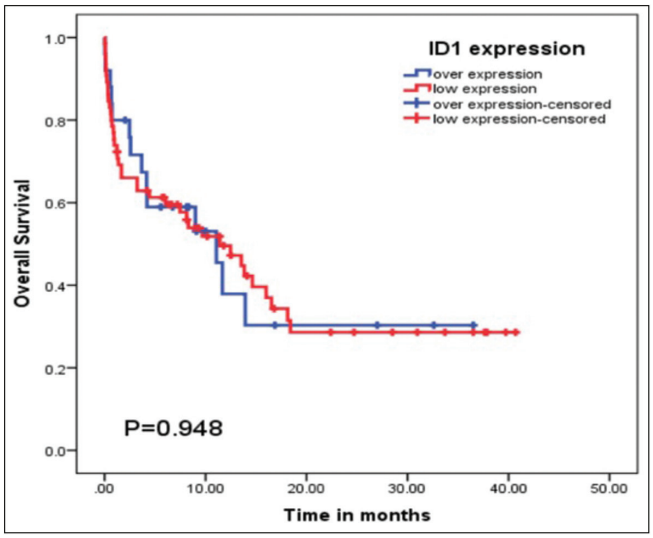
P value less than or equal to 0.05 is significant.
*Out of 91 AML cases.
FAB, French-American-British classification.

Table 6 Statistical Comparison of cytogenetic risk groups and molecular genetics in AML cases according to ID1 and ID4 relative genes expression

	ID1	*P* value	ID4	*P* value
FLT3 mutation				
Wild	0.07 (0-31.4)	0.010	0 (0-0.04)	0.520
Mutant	0.54 (0.01-9.02)		0 (0-0.02)	
NPM mutation				
Wild	0.12 (0-31)	0.829	0 (0-0.02)	0.238
Mutant	0.24 (0-8)		0 (0-0.03)	
Karyotyping				
Abnorma	4 (0-8)	0.365	0.01 (0-0.02)	0.162
Normal	0.12 (0-31)		0 (0-0.03)	
Cytogenetic risk groups				
Favorable	0.02 (0-4.8)	0.152	0 (0-0.02)	0.970
Intermediate	0.14 (0-31.4)		0 (0-0.03)	
Adverse	0.13 (0.01-9.02)		0 (0-0.02)	

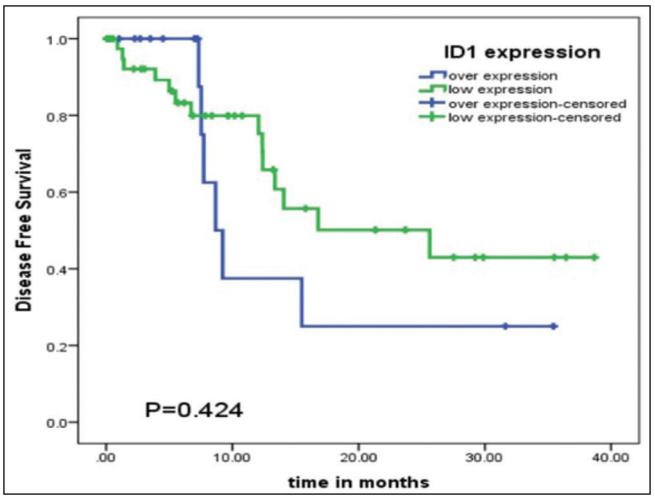
P value less than or equal to 0.05 is significant.
FLT3, fms-like tyrosine kinase 3; NPM, nucleophosmin.

Figure 3



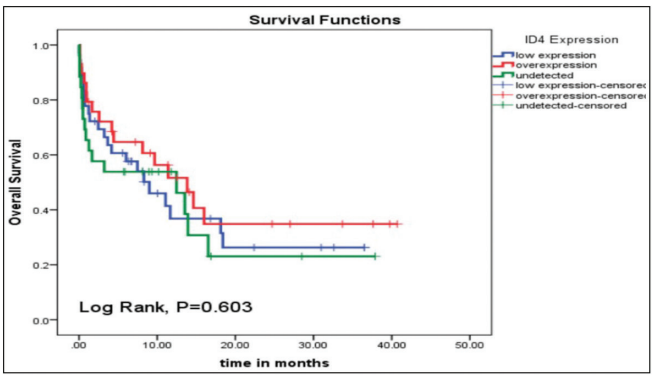
Kaplan-Meier analysis for OS with ID1 expression in AML patients

Figure 4



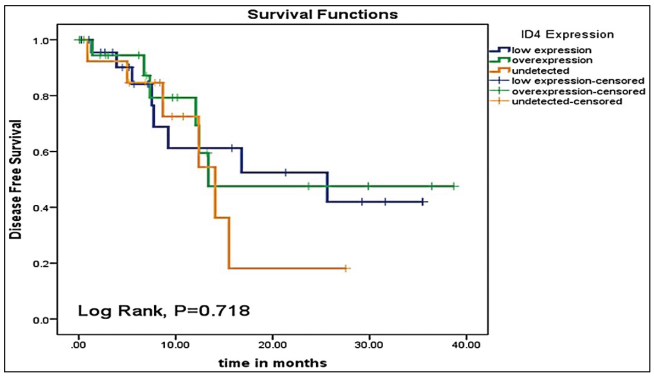
Kaplan-Meier analysis for DFS with ID1 expression in AML patients.

Figure 5



Kaplan-Meier analysis for OS with ID4 expression in adult AML patients.

Figure 6



Kaplan-Meier analysis for DFS with ID4 expression in adult AML patients.

Discussion

In humans, the ID family consists of four members: ID1, ID2, ID3, and ID4. Previous studies have demonstrated that ID proteins play important roles in proliferation, apoptosis, differentiation, invasion,

metastasis, and angiogenesis in various human tumor types Xu and colleagues [12].

ID1 gene has been identified as a potential proto-oncogene that promotes cell proliferation as well as invasion. However, ID4 has emerged as a tumor suppressor based on the evidence that it is epigenetically silenced in many cancers, but in a small number of cancers, ID4 also acts as an oncogene Sharma and colleagues [13].

However, only half of AML patients have cytogenetic abnormalities. Thus, reliable biomarkers are still required in clinical practice Khaled and colleagues [14]. So, it is essential to investigate other molecular aberrations that may affect the pathogenesis of AML or can predict patients' outcomes. The aim of the current study was to assess the expression level of ID1 and ID4 genes in AML patients, regarding their biological role and prognostic and predictive values.

The current work demonstrated a significant overexpression of ID1 gene in AML patients compared with normal control. These findings may lead to the assumption of using ID1 gene as a diagnostic marker for AML patients and it may have a role in the AML pathogenesis. These data are consistent with previously published studies that reported upregulation of ID1 gene in AML patients Zhou and colleagues, Tang and colleagues, Damm and colleagues [15–17]. ID1 gene is overexpressed in over 20 types of cancer, and it is generally considered as a tumor promoter and contributes to tumorigenesis mainly because of its role in regulating proliferation and differentiation Sharma and colleagues [13]. ID1 gene may be important for the pathogenesis of hematologic malignancies caused by oncogenic tyrosine kinases such as FLT3-ITD. FLT3/ITD mutation leads to constitutively activated FLT3 protein which causes proliferation, inhibits apoptosis, and suppresses differentiation of leukemic cells Zhao and colleagues [3].

According to Chen and colleagues [18], ID1 suppresses p21 expression in AML, which modifies the levels of cyclin A and E and promotes cell cycle progression for leukemogenesis. Additionally, ID1 suppresses cell differentiation, which aids in carcinogenesis. Antisense oligonucleotides or chemical inhibitors can be used to block the expression of the ID1 gene, which reduces cancer cell proliferation, encourages cell differentiation, and reduces invasiveness. Therefore, targeting ID1 is a viable anticancer treatment approach.

However, other studies contradict these findings. They reported that ID1 mRNA is not detected in leukemia cells and denied that low-level expression of ID1 gene in AML cells, is due to inactive chromatin structure imposed by histone deacetylation Ishiguro and colleagues, YU and colleagues [19,20].

The present work showed that the high ID1 gene expression group shows a significantly higher TLC count in comparison to the ID1 gene low expression group ($P = 0.038$). Similarly, Zhou and colleagues [15] reported that patients with elevated ID1 expression have a higher total leucocyte count than patients with low ID1 expression ($P = 0.062$). In contrast, Tang and colleagues [16] revealed no significant difference between ID1-positive and negative patients regarding leukocyte count.

Through the activation of some downstream signals, FLT3/ITD mutation results in constitutively activated FLT3 protein, which promotes proliferation, prevents apoptosis, and suppresses leukemic cell differentiation. This could be the cause of high leucocytic count in patients with FLT3/ITD mutations Chauhan and colleagues [21]. The present study revealed that there was a significant increase in ID1 gene expression in the FLT3 mutant group more than in the FLT3 wild group, which may explain the presence of higher TLC in the group with high ID1gene expression than with low gene expression.

In the present study, the patients' clinical features, including age, sex, hepatomegaly, splenomegaly, lymphadenopathy, Hb level, platelet count, the number of blasts, and BM cellularity were not significantly associated with ID1 gene expression. Similarly, Zhou and colleagues [15] reported nonsignificant differences between patients with high and low ID1 gene expression regarding sex, Hb, platelets, and BM blasts. However, patients with elevated ID1 expression were significantly older than those with low expression. Also, Tang and colleagues [16] stated that ID1-positive patients are significantly older than negative patients. Furthermore, Damm and colleagues [17] concluded that high ID1 patients tend to be older.

In this study, there was a significant increase in ID1 gene expression in FLT3 mutant group more than FLT3 wild group ($P = 0.010$), which was in concordance with Tang and colleagues [16], who stated that ID1 expression is significantly associated with FLT3/ITD+ ($P = 0.003$). Additionally, Damm and colleagues [17] demonstrated that highly expressed ID1 in AML patients is significantly associated with FLT3-ITD. In contrast, Zhou and colleagues [15] did not report a correlation between ID1 expression and FLT3-ITD mutations.

One of the fundamental mechanisms in the development of myeloid leukemia is the abnormal activation of signaling pathways that encourage the proliferation and survival of leukemic blasts. Hematopoietic progenitor cells frequently express FLT3, which is involved in the regulation of cellular differentiation and proliferation. FLT3-ITD mutations are mostly responsible for hematopoietic malignancies Walker and colleagues [22]. According to Damm and colleagues [17], ID1 is a target gene shared by several oncogenic tyrosine kinases. ID1 expression and Flt3/ITD had a poor prognosis. This clearly suggests that the ID1 gene may play a role in the development of oncogenic tyrosine kinase-driven hematologic malignancies.

This study did not report an association between ID1 expression and OS or DFS, precluding its use as a survival predictor in AML patients. This finding contradicts Tang and colleagues [16] and Damm and colleagues [17] who confirmed the performance of ID1 overexpression in predicting DFS and OS in patients younger than 60 years.

Regarding ID4 gene expression, the current study revealed no significant difference between AML patients and controls (P = 0.717). In contrast, Zhou and colleagues [23] demonstrated a significant downregulation in AML patients (P = 0.001). Additionally, they reported that the ID4 gene acts as a tumor suppressor in AML. Zhou and colleagues [24] reported significant downregulation of ID4 gene expression in AML patients. This discrepancy may be related to variations in race, assessment methods, or sample size among studies.

We found a significant difference between ID4 low, high, and undetected gene expression groups regarding total leucocyte count (P = 0.025); it was much higher in the undetected group than in the other two groups. Similarly, Zhou and colleagues [24] reported that patients with hypermethylation of ID4 gene have elevated total leucocyte count (P = 0.008). On the contrary, Zhou and colleagues [23], concluded no significant difference in leucocyte count according to ID4 expressions (P = 0.253).

Our study did not show statistically significant differences between different levels of ID4 gene expression (low, high, and undetected) regarding age, sex, hepatomegaly, splenomegaly, lymphadenopathy, platelet count, Hb level, peripheral blasts, BM blasts, and BM cellularity. These results were in line with Zhou and colleagues [23].

According to our study, ID4 gene expression showed no significant association with OS or DFS. This finding contradicts Xu and colleagues [25], who reported that patients with low ID4 expression have a significantly shorter OS and DFS than those with high ID4.

The small sample size may be a limitation of this study. Further research should be conducted with more in-depth insights into the biological behavior of AML and its relation to ID1 and ID4 to confirm and add to our findings.

Conclusion

This study demonstrated a significant overexpression of ID1 gene in AML patients compared with normal control. These results may support the hypothesis that the ID1 gene contributes to the development of AML. Furthermore, ID1 and ID4 cannot predict survival in AML patients. However, further research on a larger sample is needed to investigate the significance of these gene alterations in AML patients and the involved pathogenetic tracks. This will provide new perspectives for novel therapies targeting these genes to improve patients' outcomes.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was approved by the Benha Faculty of Medicine Research Ethics Committee (REC) (Number: MD. 9. 2018).

Consent for publication

All participants agreed to publication.

Conflicts of interest

Competing interests: All authors declare no competing interest.

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