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Reductive regulation of BECN1 gene in adult Egyptian patients with do novo AML

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Abstract

Background: Acute myeloid leukaemia (AML) is a clonal haematopoietic disease characterized by the proliferation of immature blast cells in the bone marrow and peripheral blood. Autophagy is an inherent cellular route by which waste macromolecules are engulfed within autophagosomes prior to their fusion with cytoplasmic lysosomes for degradation. The *BECN1* gene encodes the Beclin-1 protein, which regulates autophagy. Few reports have investigated *BECN1* gene expression and its value in AML patients.

Results: This randomized case-control study included 50 newly diagnosed AML patients, in addition to 20 subjects as a control group. *BECN1* gene expression was assessed using real-time quantitative polymerase chain reaction (qRT-PCR).
The median level of *BECN1* gene expression in AML patients was 0.41 (IQR 0.29–1.03) in comparison to 1.12 (IQR 0.93–1.26) in the control group ($P = 0.000$). Seventy-two percent of AML patients showed reduced *BECN1* gene expression, which was highly significantly associated with intermediate and adverse cytogenetic risk. Reduced *BECN1* gene expression was associated with older age, higher total leukocyte counts, the presence of peripheral blood blast cells, a higher percentage of bone marrow blast cells, and higher expression of CD34 and CD117. FLT3-ITD mutation was detected in 14 patients (38.9%), all of whom showed reduced *BECN1* gene expression ($P = 0.006$). *BECN1* gene expression was also reduced in non-responder AML patients, with a highly statistically significant difference ($P = 0.002$).

Conclusion: A reduction in *BECN1* gene expression might indicate a poor prognosis in adult Egyptian patients with de novo AML. Decreased *BECN1* gene expression is associated with a higher risk of resistance to treatment. Targeting autophagy pathways may help in the treatment of AML patients.

Keywords: Autophagy, *BECN1*, AML, Prognosis

Background

Autophagy is a conserved protein-degradation pathway that plays an important role in haematopoietic stem cell survival, proliferation, and differentiation. Autophagy is an adaptive and protective cellular programme activated during metabolic stress to maintain cellular homeostasis and recycle damaged organelles [1].

The *BECN1* gene encodes the Beclin-1 protein. Beclin-1 regulates autophagy and is a component of the phosphatidylinositol-3-kinase (PI3K) complex, which mediates vesicle-trafficking processes. Indeed, Beclin-1 plays a role in multiple cellular processes, including tumorigenesis, neurodegeneration, and apoptosis. Additionally, implicated in autophagic programmed cell death, it appears that reduction in *BECN1* expression in different types of human cancers is associated with accelerated tumour growth, metastasis, and weak prognosis [2]. However, the exact role of autophagy in leukaemogenesis is still not fully understood.

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This work aimed to investigate the expression status of the *BECN1* gene as a key autophagy regulator gene in newly diagnosed adult Egyptian AML patients in comparison to a healthy control group. We also sought to assess correlations of *BECN1* expression with various haematological parameters and clinical outcomes, especially the response to induction chemotherapy.

Methods

Patients

This study included 50 newly diagnosed adult patients with de novo AML. All patients were admitted to and followed up in the authors' affiliated institution during the period from May 2018 to January 2020. The study also included a control group of 20 healthy subjects who were donors for bone marrow transplant (aged between 18 and 44, in good health, free from any autoimmune or rheumatologic diseases, never had any type of cancers, not on any regular treatment and negative for human immunodeficiency virus (HIV) and hepatitis viruses B and C). AML patient diagnosis, management, and follow-up were performed according to the World Health Organization (WHO) classification of Tumours of Haematopoietic and Lymphoid Tissues [3] and European Society for Medical Oncology (ESMO) Clinical Practice Guidelines for diagnosis, treatment, and follow-up [4].

Informed consent was obtained from all participating individuals. The study was conducted in accordance with the stipulations of the local ethical and scientific committees, and the procedures conformed to the ethical standards in the Helsinki Declaration and its later amendments.

After diagnosis, all included patients received the same induction chemotherapy protocol (induction therapy with Standard "7+3" regimen). This regimen combines a 7-day continuous intravenous (IV) infusion of cytarabine (200 mg/m^2 per day) with a bolus of an anthracycline given on days 1 through 3 (doxorubicin "Adriamycin" 60 mg/m^2 for 3 days). Acute promyelocytic leukaemia (APL) patients were treated with all-trans-retinoic acid (ATRA), and arsenic trioxide (ATO) was added for a few patients.

Follow-up bone marrow aspiration was performed for all patients on day (D) 28 after induction to assess response to chemotherapy. Remission (responder group) was diagnosed by a D28 bone marrow aspirate showing < 5% blast cells. A patient with a blast count ≥ 5% was considered a non-responder in this study (resistant or partial responder).

AML patient characteristics were evaluated at diagnosis by the following:

Thorough history and clinical examination

Bone marrow sampling Approximately 5–6 ml BM aspirate was obtained. The sample was divided into 0.5 ml for Leishman-stained smears, and 3–4 ml was placed into 2 ethylene di-amine tetra-acetic acid potassium salt (K2-EDTA) vacutainer tubes for immunophenotyping and molecular studies; approximately 1 ml was added to a sterile preservative-free lithium heparin-coated vacutainer tube and used for culture before application of FISH probes.

Multi-parameter flow cytometric (FCM) analysis Immunophenotyping of bone marrow or peripheral blood samples was carried out using a Navios Flow cytometer (Beckman Coulter, Electronics, Hialeah, FL, USA) with a panel of monoclonal antibodies. Samples were considered positive for a certain marker when ≥ 20% of the cells expressed it, except for CD34, for which expression by 10% of cells was sufficient to be considered positivity [5].

Fluorescence in situ hybridization (FISH) analysis FISH was performed according to Campbell [6] following the practice cytogenetic guidelines 2007 and 2010 and Quality Assurance Guidelines 2013 [7] and analysed by at least two trained specialists using a Cyto Vision Leica Microsystem (Leica Biosystems, Richmond, USA). The analysis was performed using at least 100 interphase nuclei after culture for 24–48 h using the following probes: the locus-specific identifier (LSI) dual-colour double fusion (DF) RUNX1/RUNX1T1 probe for t(8; 21)(q22; q22) (Abbott), LSI dual-colour PML/RARA probe for t(15;17)(q24;q21) (Abbott), LSI core-binding factor beta (CBFB) break-apart rearrangement probes (Abbott), and LSI mixed-lineage leukaemia (MLL) dual-colour, break-apart rearrangement probe designed to detect the 11q23 rearrangement associated with various translocations involving region 2 band 3 of chromosome 11. Thresholds and the confidence limit were established for all FISH probes and probe sets in the cytogenetic lab. A cut-off value for the diagnosis of positive results was > 10% for single fusion probes and > 3% for double fusion probes. Interpretation of FISH probes was performed according to the manufacturer's recommendations. The limits of detection are documented in the manufacturer's report.

Molecular analysis of *BCEN1* expression Total ribonucleic acid (RNA) was isolated from whole blood samples by using QIAamp RNA Blood Mini Kit (Qiagen, Germany) following the manufacturer's instructions. Then, *BECN1* mRNA was reverse transcribed into complementary deoxyribonucleic acid (cDNA) using a QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). The cDNA was used for real-time PCR quantification of mature mRNA expression using sequence-specific primers and TaqMan probes (FAM dye-labelled) from the TaqMan

Gene Expression Assay mix. *BECN1* gene expression levels were amplified from mRNA using TaqMan Gene Expression Assays of *BECN1* (Cat. No: 4448892, I.D.: Hs01007018_ml), and ABL1 (Cat. No: 4453320, I.D.: Hs01104728_ml) (Qiagen, Germany) was used as the housekeeping gene. All samples were analysed using a 5-plex Rotor-Gene PCR Analyser (Qiagen, Germany).

The results are reported as relative quantification (target gene versus control gene) after determining the cycle threshold (CT) for each sample. The ΔCT value for each sample was evaluated by calculating the difference between the CT value of the target gene and the CT value of the endogenous reference gene, as determined for each unknown sample as well as for the calibrator sample. Next, the ΔΔCT value for each sample was calculated by subtracting the ΔCT value of the calibrator sample from the ΔCT value of the experimental sample: ΔΔCT = ΔCT (sample) − ΔCT (calibrator). Finally, the normalized level of target gene expression was calculated by using the formula 2−ΔΔCT.

Statistical analysis

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 23. A suitable analysis was performed according to the type of data obtained for each parameter.

Descriptive statistics

Quantitative data are presented as the mean and standard deviation for parametric data and as the median and range for non-parametric data. Qualitative data are presented as numbers and percentages.

Analytical statistics

Comparisons of qualitative variables were conducted between groups using the chi-square test. Comparisons of quantitative variables were conducted between groups using the Mann-Whitney test for non-parametric data and Student's *t* test for parametric data. A post hoc test was applied for comparisons of all possible pairs of group means or medians. Logistic regression analysis was used to predict the presence or absence of an outcome based on a set of independent variables; $P < 0.05$ and < 0.001 were set as statistically significant and highly significant, respectively.

Results

Clinical cohort

The baseline characteristics of the studied patient groups are shown in Tables 1 and 2. AML patients were classified according to the French–American–British (FAB) Cooperative Group Criteria [8], WHO cytogenetic/molecular classification 2016 [3], and cytogenetic risk group classification. Cases were classified as good, poor, and intermediate cytogenetic prognostic groups according to cytogenetic and molecular analyses, where t(8;21) (q22;q22), inv16 (p13;q22), and t(15;17) (q22;q21) were

Table 1 Demographic, clinical, and laboratory characteristics of the studied AML patients

Parameter	AML patients (total no. = 50)
Sex [*n* (%)]	
Females	28 (56.0%)
Males	22 (44.0%)
Age (years)	
Mean ± SD	(51.56 ± 20.17)
Clinical data [*n* (%)]	
Fever	11(22.0%)
Hepatomegaly	19(38.0%)
Splenomegaly	19(38.0%)
Lymphadenopathy	4(8.0%)
TLC ($x10^9$/L) (Median—IQR)	54.8 (22.3–76.4)
Hb (g/dl) (Mean ± SD)	(8.09 ± 2.01)
PLT ($x10^9$/L) (Median—IQR)	33 (24–68)
PB blasts (%) (Mean ± SD)	(56.96 ± 23.43)
BM blasts (%) (Mean ± SD)	(75.68 ± 20.83)
Immunophenotyping [*n* (%)]	
CD34 +	33 (66.0%)
CD117 +	32 (64.0%)
HLA-DR +	43 (86.0%)
FAB subtypes [*n* (%)]	
M0	5 (10.0%)
M1	10 (20.0%)
M2	20 (40.0%)
M3	5 (10.0%)
M4	7 (14.0%)
M5	3(6.0%)
Molecular abnormalities [*n* (%)]	
Wild type NPM1	5 (10.0%)
Mutated NPM1 and FLT3-ITD	2 (4.0%)
FLT3-ITD mutation	14 (28.0%)
c-KIT mutation	8 (16.0%)
Cytogenetic risk groups [*n* (%)]	
Favourable	11 (22.0%)
Intermediate	10 (20.0%)
Adverse	29 (58.0%)
Response to induction therapy (D28) [*n* (%)]	
Responders	15 (30.0%)
Non- responders	35 (70.0%)

TLC total leukocytic count, *Hb* haemoglobin, *PLT* platelets, *BM* bone marrow, *PB* peripheral blood, *FAB* French American British, *SD* standard deviation, *IQR* interquartile range, *NPM1* Nucleophosmin, *ITD* internal tandem duplication

Table 2 Results of karyotyping, FISH analysis, mutational screening and BECN1 expression

Case number	Conventional karyotyping	FISH results	FLT3-ITD/c-Kit/Nucleophosmin mutational status	***BECN1 expression***
1	Normal karyotype	t(8;21) Negative	FLT3-ITD Positive	0.37(RE)
2	Trisomy 8	t(9;22)Negative		0.13(RE)
3	Normal karyotype	11q23 Negative	WT NPM1	0.62(RE)
4	Normal karyotype	t(9;22)Negative	Mutated NPM1and FLT3-ITD	0.58(RE)
5	t(8;21) Positive	t(8;21) Positive	cKIT Positive	0.65(RE)
6	Normal karyotype	t(8;21) Negative	FLT3-ITD Positive	0.18(RE)
7	t(8;21) Positive	t(8;21) Positive		1.03(NE)
8	Variable rearrangement	t(8;21) Positive	cKIT Positive	0.51(RE)
9	17 p deletion	t(9;22)Negative		0.33(RE)
10	Normal karyotype	t(8;21) Negative	FLT3-ITD Positive	0.62(RE)
11	Normal karyotype	t(9;22)Negative	Mutated NPM1and FLT3-ITD	0.52(RE)
12	Normal karyotype	t(9;22)Negative	WT NPM1	0.35(RE)
13	t(15;17) Positive	t(15;17) Positive		1.04(NE)
14	t(15;17) Positive	t(15;17) Positive		1.07(NE)
15	t(9;22) Positive	t(9;22) Positive		0.27(RE)
16	Trisomy 8	t(8;21) Negative		0.54(RE)
17	Variable rearrangement	t(8;21) Positive	cKIT Positive	0.26(RE)
18	t(9;22) Positive	t(9;22) Positive	FLT3-ITD Positive	0.34(RE)
19	Normal karyotype	Inv16 Positive		0.64(RE)
20	t(9;22) Positive	t(9;22) Positive		1.05(NE)
21	t(8;21) Positive	t(8;21) Positive		1.07(NE)
22	Normal karyotype	t(8;21) Negative	FLT3-ITD Positive	0.31(RE)
23	t(15;17) Positive	t(15;17) Positive		0.29(RE)
24	Trisomy 8	t(8;21) Negative		0.28(RE)
25	Normal karyotype	t(9;22) Negative	WT NPM1	0.29(RE)
26	Normal karyotype	t(9;22) Negative	FLT3-ITD Positive	0.31(RE)
27	t(8;21) Positive	t(8;21) Positive	cKIT Positive	1.02(NE)
28	Normal karyotype	t(8;21) Negative	FLT3-ITD Positive	0.36(RE)
29	11q23 Positive	11q23 Positive		0.27(RE)
30	Variable rearrangement	t(8;21) Positive	FLT3-ITD Positive	0.33(RE)
31	Monosomy 7	t(9;22) Negative		1.04(NE)
32	Inv16 Positive	Inv16 Positive	cKIT Positive	0.26(RE)
33	11q23 Positive	11q23 Positive		0.17(RE)
34	Normal karyotype	t(9;22) Negative	FLT3-ITD Positive	0.34(RE)
35	Normal karyotype	Inv16 Positive		1.04(NE)
36	t(8;21) Positive	t(8;21) Positive		1.06(NE)
37	Double Philadelphia	t(9;22) Positive		0.29(RE)
38	Normal karyotype	t(9;22) Negative	FLT3-ITD Positive	0.26(RE)
39	Inv16 Positive	Inv16 Positive	cKIT Positive	0.32(RE)
40	Normal karyotype	Inv16 Negative	WT NPM	1.08(NE)
41	Inv16 Positive	Inv16 Positive		1.07(NE)
42	t(15;17) Positive	t(15;17) Positive		1.03(NE)
43	Normal karyotype	t(8;21) Negative	FLT3-ITD Positive	0.45(RE)
44	Normal karyotype	t(8;21) Negative	FLT3-ITD Positive	0.16(RE)

Table 2 Results of karyotyping, FISH analysis, mutational screening and BECN1 expression *(Continued)*

Case number	Conventional karyotyping	FISH results	FLT3-ITD/c-Kit/Nucleophosmin mutational status	***BECN1 expression***
45	t(8;21) Positive	t(8;21) Positive	cKIT Positive	0.38(RE)
46	t(8;21) Positive	t(8;21) Positive	cKIT Positive	1.06(NE)
47	Normal karyotype	t(8;21) Negative	FLT3-ITD Positive	0.43(RE)
48	Normal karyotype	Inv16 Negative	FLT3-ITD Positive	0.61(RE)
49	t(15;17) Positive	t(15;17) Positive		1.04(NE)
50	Normal karyotype	t(9;22) Negative	WT NPM	0.36(RE)

Data on conventional karyotyping and FLT3-ITD/c-Kit/Nucleophosmin mutational status (in some patients) were obtained from patients medical records
NE normal expression, *RE* reduced expression, *WT* wild type, *NPM1* Nucleophosmin1, *ITD* internal tandem duplication.

considered to have a good prognosis, normal karyotype and trisomy 8, mutated nucleophosmin (NPM1), and fms-like tyrosine kinase3-internal tandem duplication (FLT3-ITD^{high}); wild-type NPM1 without FLT3-ITD or with FLT3-ITD^{low} (without adverse-risk genetic lesions) were considered to have an intermediate prognosis; and 11q23 rearrangement, monosomy 7, t(9,22) (q34;q11) were considered to have a poor prognosis [9]. In addition, patients were classified regarding their response to induction therapy on day 28 as responders and non-responders.

BECN1 gene expression in the studied patients versus the control group

Our patients exhibited a highly statistically significant reduction in expression of *BECN1* compared to the control group [median 0.41 (IQR 0.29–1.03) vs. 1.12 (IQR 0.93–1.26), respectively, $P = 0.000$]. Upon further analysis, we found that 36 (72.0%) patients and 14 (28.0%) patients showed reduced and similar *BECN1* gene expression compared to the control group (Fig. 1).

When comparing each cytogenetic risk group with the control group, we found that *BECN1* gene expression was significantly reduced in patients with intermediate and adverse cytogenetic risks, though the favourable cytogenetic risk group did not show this reduction (Table 3).

AML patients were classified into two groups according to *BECN1* gene expression status (reduced versus normal expression) using the IQR range of the control group as the cut-off. The reduced *BECN1* gene expression group was associated with older age, higher total leukocyte count (TLC), and higher percentages of peripheral blood (PB) and bone marrow (BM) blasts. In addition, the reduced expression group contained a higher number of patients expressing CD34 and CD117 based on flow cytometry. Regarding molecular abnormalities, the FLT3-ITD mutation was detected in 14 (38.9%) patients, and all of them had reduced *BECN1* gene expression. Other molecular

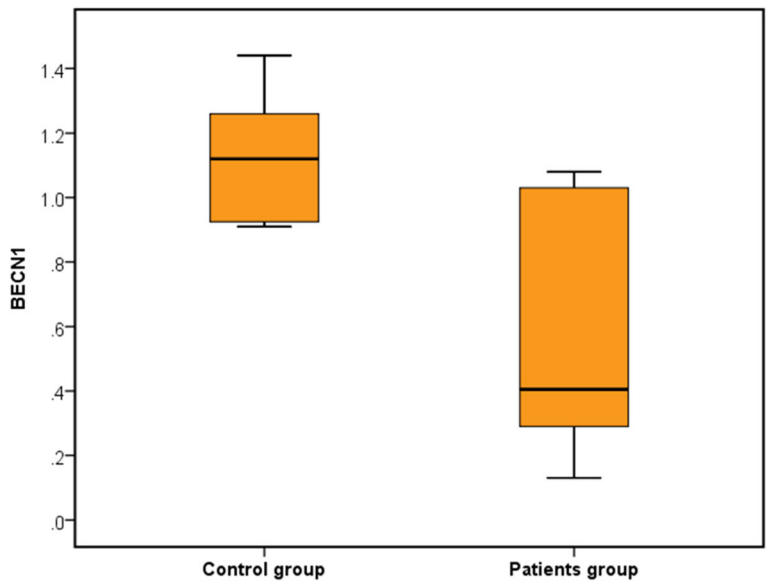

Fig. 1 Comparing the median of *BECN1* gene expression of AML patients and the control group

Table 3 Comparison between the control group and different cytogenetic risk groups regarding *BECN1* gene expression

	Control group	Cytogenetic risk group			*P* value	Sig.
		Favourable	Intermediate	Adverse		
BECN1 Median (IQR)	1.12 (0.93–1.26)	1.04 (1.03–1.07)	0.44 (0.29–0.58)	0.34 (0.27–0.51)	0.000	HS
Post hoc analysis						
	Control vs favourable	**Control vs intermediate**	**Control vs adverse**	**Favourable vs intermediate**	**Favourable vs adverse**	**Intermediate vs adverse**
BECN1	0.052	0.000	0.000	0.008	0.000	0.469

P value > 0.05: non-significant; *P* value < 0.05: significant; *P* value < 0.01: highly significant

abnormalities did not show significant differences regarding *BECN1* gene expression. The majority of patients with reduced *BECN1* gene expression were in the non-responders' group at D28 (Table 4).

Non-responder AML patients displayed highly significantly reduced levels of *BECN1* gene expression (P = 0.002). The median *BECN1* gene expression in responders and non-responders was 1.04 (0.58–1.07) and 0.35 (0.28–0.54), respectively (Fig. 2).

Univariate logistic regression analysis for predictors of response to induction therapy (D28)

A statistically significant association between age ≤ 51 years, TLC ≤ 25.9 × 10^3/μl, and a normal karyotype and complete remission was found. In contrast, PB blasts > 35%, expression of CD34, CD117, HLA-DR, and reduced *BECN1* gene expression showed a statistically significant association with a poor response to induction therapy (non-responders) (Table 5).

Discussion

Acute myeloid leukaemia (AML) is a malignant disease of haematopoietic stem cells characterized by uncontrolled clonal expansion of myeloid progenitors with subsequent bone marrow failure and impaired normal haematopoiesis. Despite advances in diagnosis and treatment, AML still has a high mortality rate [10].

Autophagy is a cellular process by which waste macromolecules and excess or damaged organelles are engulfed within autophagosomes. These autophagosomes then fuse with cytoplasmic lysosomes for degradation and recycling. Thus, efficient and strict regulation of autophagy is crucial for safeguarding cell homeostasis. Nonetheless, aberrant autophagy may underlie different human pathological conditions, as demonstrated by several studies [11–14].

The *BECN1* gene encodes the Beclin-1 protein, which is essential for autophagy initiation and regulation [15, 16]. Additionally, Beclin-1 is implicated in autophagic programmed cell death. In different types of human cancers, a reduction in *BECN1* has been associated with accelerated tumour growth, metastasis, and poor prognosis [2].

This work aimed to assess the expression status of the *BECN1* gene in newly diagnosed adult AML patients in comparison to a healthy control group and to determine its relationship to various haematological parameters and clinical outcomes, especially response to induction chemotherapy. In our study, we measured expression of *BECN1* in 50 de novo AML patients and 20 healthy controls.

The AML patients showed a highly significantly reduction in *BECN1* expression compared to the control group. Upon detailed analysis, we found that 72.0% of the patients had reduced *BECN1* gene expression compared to the control group and that 28.0% of the patients had normal *BECN1* gene expression. This result is in accordance with Zare-Abdollahi et al. [17], who analysed the expression status of the *BECN1* gene in a series of 128 de novo AML patients using real-time quantitative polymerase chain reaction, in which *BECN1* reduced expression was detected in 57 of the 128 cases (45%). In another study performed by Mohamadimaram et al. [18], expression of other autophagy genes (namely, *ATG7* and *light chain 3 (LC3)*) was investigated, and the authors found a significant decrease in expression levels of both genes in most AML patients (81.81% and 75.55%, respectively). However, *BECN1* was not examined.

In contrast, Keyvan et al. [19] assessed changes in *BECN1* gene expression in blood samples from 30 AML patients compared with samples from 15 healthy persons and found no significant differences in *BECN1* gene expression between patients with AML and normal controls (P > 0.05). This may be due to the smaller sample size in their study or to genetic differences. In addition, Tandel et al. [20] reported the reverse of our finding: *BECN1* gene expression levels were significantly higher in AML patients than in controls at a rate of 5/3-fold, with a significant P value (P < 0.0001). Thus, more effort is needed to investigate the expression status of the *BECN1* gene.

In AML patients, reduced *BECN1* gene expression was associated with older age and higher TLC. Our values were very close to the findings of Zare-Abdollahi et al. [17], who reported a mean age of 52.9 years and a mean

Table 4 Comparison between normal and reduced BECN1 gene expression groups

Parameter	***BECN1***gene expression		Test value	*P* value	Sig.
	Normal (*n* = 14)	Reduced (*n* = 36)			
Sex [*n* (%)]					
Females	6 (42.9%)	22 (61.1%)	1.363*	0.243	NS
Males	8 (57.1%)	14 (38.9%)			
Age (years)					
Mean ± SD	33.86 ± 15.92	58.44 ± 17.37	− 4.596˙	0.000	**HS**
Range	(18–70)	(19–78)			
Clinical data [*n* (%)]					
Fever	3(21.4%)	8(22.2%)	0.004*	0.951	NS
Hepatomegaly	4(28.6%)	15(41.7%)	0.734*	0.392	
Splenomegaly	5(35.7%)	14(38.9%)	0.043*	0.836	
Lymphadenopathy	0(0.0%)	4(11.1%)	1.691*	0.193	
TLC ($\times 10^9$/L) Median (IQR)	20.3 (13.3–35.4)	65.15 (37.3–83.05)	3.824ǂ	0.000	**HS**
Hb (g/dl) Mean ± SD	8.09 ± 2.31	8.09 ± 1.92	0.002˙	0.999	NS
PLT ($\times 10^9$/L) Median(IQR)	41 (28–68)	30.5 (20–67.5)	− 0.908ǂ	0.364	NS
PB blasts (%)Mean ± SD	40.71 ± 20.33	63.28 ± 21.64	− 3.364˙	0.002	**HS**
BM blasts (%)Mean ± SD	63.07 ± 22.17	80.58 ± 18.34	− 2.858˙	0.006	**HS**
Immunophenotyping [*n* (%)]					
CD34 +	5 (35.7%)	28 (77.8%)	7.948*	0.005	**HS**
CD117 +	5 (35.7%)	27 (75.0%)	6.752*	0.009	**HS**
HLA-DR +	11 (78.6%)	32 (88.9%)	0.891*	0.345	NS
FAB subtypes [*n* (%)]					
M0	0 (0.0%)	5 (13.9%)	9.106*	0.105	NS
M1	2 (14.3%)	8 (22.2%)			
M2	5 (35.7%)	15 (41.7%)			
M3	4 (28.6%)	1 (2.8%)			
M4	3 (21.4%)	4 (11.1%)			
M5	0 (0.0%)	3 (8.3%)			
Molecular abnormalities [*n* (%)]					
Wild type NPM1	1 (7.1%)	4 (11.1%)	0.176*	0.675	NS
Mutated NPM1 and FLT3-ITD	0 (0.0%)	2 (5.6%)	0.81*	0.368	NS
FLT3-ITD mutation	0 (0.0%)	14 (38.9%)	7.562*	0.006	**HS**
c-KIT mutation	2 (14.3%)	6 (16.7%)	0.043*	0.837	NS
Cytogenetic risk groups [*n* (%)]					
Favourable	9 (81.8%)	2 (18.2%)	20.314*	0.000	**HS**
Intermediate	1 (10.0%)	9 (90.0%)			
Adverse	4 (13.8%)	25 (86.2%)			
Response to induction therapy (D28) [*n* (%)]					
Non- responders	4 (28.6%)	31 (86.1%)	15.892*	0.000	**HS**
Responders	10(71.4%)	5 (13.9%)			

P value > 0.05: non-significant (NS); *P* value < 0.05: significant (S); *P* value < 0.01: highly significant (HS), *n* number
*Chi-square test
˙Independent *t* test
ǂMann-Whitney test

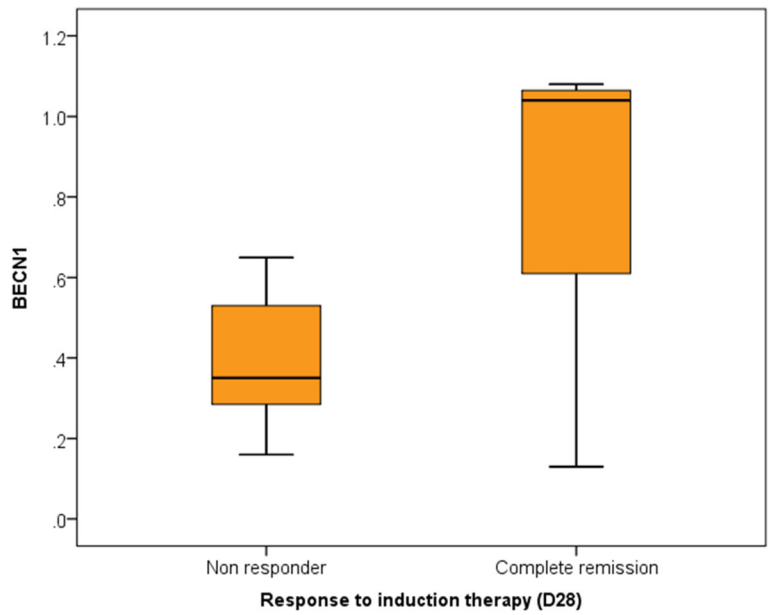

Fig. 2 Comparison between responders (complete remission) and non-responders regarding BECN1 gene expression

TLC of 52.6 × 10^3/μl in AML patients with reduced *BECN1* gene expression. However, we did not observe a significant relationship between the level of *BECN1* gene expression and sex, haemoglobin level, or platelet count. This was in accordance with Lian et al. [21] and Zare-Abdollahi et al. [17].

Furthermore, we noted that a higher percentage of AML patients in the FAB groups (M1 and M2) also showed reduced *BECN1* gene expression, though this association did not reach significance. In parallel, Folkerts and colleagues [22] found no significant difference in expression levels of autophagy genes (including *BECN1*) among AML patients in different FAB subtype groups.

Interestingly, in our study, the FLT3-ITD mutation was detected in 38.9% of patients, and all of them had significantly reduced *BECN1* gene expression. Similarly, Tao et al. [23] reported a remarkable association between reduced *BECN1* gene expression and FLT3-ITD mutation. The same finding was also reported by Zare-Abdollahi et al. [17], whereby reduced expression of *BECN1* coincided with the FLT3-ITD mutation in 27 of the 128 cases (21%) investigated.

Table 5 Univariate logistic regression analysis for predictors of response to induction therapy (D28)

Parameter	*P* value	Odds ratio (OR)	95% C.I. for OR	
			Lower	Upper
Age ≤ 51 years	0.000	56.000	6.259	501.026
TLC ≤ 25.9 × 10^3/ul	0.000	50.375	8.189	309.892
PB blasts > 35%	0.019	0.190	0.048	0.763
Normal Karyotype	0.010	6.781	1.585	29.016
CD34 +	0.003	0.125	0.032	0.485
CD117+	0.005	0.148	0.039	0.561
HLADR+	0.021	0.121	0.020	0.723
Reduced *BECN1*expression	0.000	0.065	0.014	0.288

Our results, along with other reports, suggest an inverse correlation between *BECN1* gene expression and the presence of the FLT3-ITD mutation. A recent study showed that FLT3-ITD protects leukaemic cells treated with PI3K/Akt/mTORC1 pathway inhibitors, a common pathway dysregulated in AML [24, 25], from apoptosis by STAT5 activation and subsequent MCL1 expression induction [26]. As mTORC1 acts as an autophagy repressor, it is speculated that major regulators/initiators of autophagy, such as *BECN1*, can also be regarded as a potential target of FLT3-ITD or STAT5 or other yet-to-be-identified mediators [17].

In another study, FLT3-ITD mutations were found to induce an increase in basal autophagy in leukaemic cells through a previously uncharacterized signalling cascade involving the transcription factor ATF4. Moreover, inhibiting autophagy or ATF4 significantly impaired FLT3-ITD leukaemic cell proliferation as well as tumour burden in murine xenograft models. Importantly, autophagy inhibition also overcame FLT3 inhibitor resistance due to the FLT3-TKD mutation both in vitro and in vivo. These results suggest that targeting ATF4 or autophagy in AML patients carrying FLT3 mutations may represent a promising alternative therapeutic strategy [27].

A higher percentage of c-KIT mutations was found in AML patients with reduced *BECN1* gene expression in our study. However, this was statistically insignificant.

Similarly, the group with reduced *BECN1* gene expression had a significantly higher number of patients expressing CD117, as based on flow cytometry. Considering its value in predicting a poor prognosis, the association of c-Kit mutations adds to the value of reduced *BECN1* expression.

Lian et al. [21] studied the mRNA levels of BECLIN1 and ATG5 in 101 newly diagnosed leukaemia patients and reported that AML samples with CEBPα or c-KIT mutations showed lower BECLIN1 expression levels than those without mutations. Additionally, patients with c-KIT mutation exhibited lower ATG5 expression.

In our study, *BECN1* gene expression was reduced in patients with intermediate and adverse cytogenetic risks compared to both favourable risk and control groups. These findings were similar to those reported by Zare-Abdollahi et al. [15]. Similarly, Marconi et al. [28] reported reduced expression levels of key autophagy regulatory genes, including *BECN1*, in an adverse cytogenetic risk group.

Patients showing reduced *BECN1* gene expression in our study also displayed significantly higher CD34 expression by flow cytometry. These findings are similar to those reported by Watson et al. [26], who examined the status of the autophagy pathway in human AML using bone marrow (BM) samples. They sorted the populations identified by CD34 (CD34-positive blasts) and examined expression of key autophagy genes, including *BECN1*. They reported decreased expression of autophagy genes, including *BECN1*, in the CD34-positive AML blast cell population compared with the blast marker-negative population.

Interestingly, low *BECN1* gene expression was highly associated with resistance to therapy in our patient cohort. Similar results were reported by Marconi et al. [28], who found that reduced expression levels of key autophagy regulatory genes (including the *BECN1* gene) are linked with resistance to therapy.

Conclusion

In our study, reduced *BECN1* gene expression was a well-recognized finding in adult Egyptian patients with AML. Furthermore, reduced *BECN1* gene expression was associated with older age and increased total leukocyte count (TLC) and increased percentages of PB and BM blasts. Reduced *BECN1* gene expression was also associated with intermediate/adverse cytogenetic risk groups and with a poor response to induction chemotherapy. Our results suggest that autophagy may have an important role in the pathogenesis of AML in Egyptian patients. Furthermore, reduced expression of the *BECN1* gene might correlate with a poor prognosis. Indeed, it was associated with many well-established poor prognostic factors, especially FLT3 mutation. Thus, focusing on the autophagy pathway might be beneficial. Overall, larger studies with longer follow-up periods are required to confirm our findings.

Abbreviations

AML: Acute myeloid leukaemia; HSC: Haematopoietic stem cells; qRT-PCR: Real-time quantitative polymerase chain reaction; PI3K: Phosphatidylinositol-3-kinase; FCM: Flow cytometry; BM: Bone marrow; PB: Peripheral blood; TLC: Total leukocytic count; WHO: World Health Organization; ESMO: European Society for Medical Oncology; K2-EDTA: Ethylene di-amine tetra-acetic acid potassium salt; FISH: Fluorescence in situ hybridization; LSI: Locus-specific identifier; DF: Dual-colour double fusion; cDNA: Complementary deoxyribonucleic acid; RNA: Total ribonucleic acid; MLL: Mixed-lineage leukaemia; CBFB: Core-binding factor beta; CT: Cycle threshold

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Authors' contributions

MFG: contributed to the concept and design of the study, revised statistics and the results, and revised the manuscript. BATF: contributed to the design of the study and to the practical work done in the study (bone marrow sampling; gene expression by PCR), revised statistics and the results, and revised the manuscript. WAE: contributed to the acquisition of data, collecting the related clinical data, drafted the work, revised statistics and the results, write the manuscript, and revised the manuscript. NAS: contributed to the design of the study and to the practical work done in the study (bone marrow sampling; gene expression by PCR), revised statistics and the results, and revised the manuscript. NBH: contributed to the design of the study and to the practical work done in the study (bone marrow sampling; gene expression by PCR), revised statistics and the results, and revised the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

Data and materials are available.

Ethics approval and consent to participate

All procedures performed in our study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.
-Informed verbal consent was obtained from all individuals who participated in the study; this was approved by the ethics committee.
-Research Ethics Committee (REC) number is FWA 000017585.

Consent for publication

We give you consent for publication.

Competing interests

No competing interests

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