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Prognostic implications of IDH1rs11554137 and IDH2R140Q SNPs mutations in cytogenetically normal acute myeloid leukemia

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Abstract

Background: Mutant isocitrate dehydrogenase (IDH) 1 and 2 alter the epigenetic landscape in acute myeloid leukemia (AML) cells through production of the oncometabolite (R)-2-hydroxyglutarate.

Methods: We aimed to determine the prevalence and clinical and prognostic effect of IDH1rs11554137 and IDH2R140Q SNPs mutations, in 80 newly diagnosed cytogenetically normal AML (CN-AML), using real-time polymerase chain reaction (PCR).

Results: Heterozygous mutations of IDH1 and IDH2 SNP were detected in 13.8% and 16.3% of patients, respectively. Both mutations were associated with older age, higher platelet count, and shorter overall survival. Survivors showed significantly younger age and lower mean platelet and blast counts, as well as negative IDH1 SNP ($p = 0.001$, 0.016, 0.002, and 0.003, respectively). Multivariate logistic regression analysis identified high bone marrow blast percentage as an independent prognostic predictor for 6-month mortality ($p = 0.014$, OR 1.049, 95% CI 1.010–1.090).

Conclusion: IDH1/2 SNPs mutations are recurrent events in CN-AML associated with negative prognostic impact, representing a new subgroup for risk stratification and may indicate new treatment options.

Background

Acute myeloid leukemia (AML) is a clonal hematopoietic stem cell disorder, characterized by enhanced proliferation and aberrant differentiation, resulting in accumulation of immature cells in bone marrow, with failure of hematopoiesis [1]. It is the most common form of acute leukemia, existing in 15 per 100,000 in adults, with reducing incidence to 2–3 per 100,000 in children [2]. AML pathogenesis involves an array of genetic and epigenetic alterations that disrupt all aspects of cell transformation [3].

Isocitrate dehydrogenases 1 and 2 (IDH 1/2) are enzymes in the citric acid cycle that catalyze the oxidative decarboxylation of isocitrate, producing α-ketoglutarate (α-KG) and NADPH [4]. Their mutations result in accumulation of the oncometabolite D-2 hydroxyglutarate (D-2HG) [5], which competitively inhibits α-KG-dependent deoxygenases, that are involved in both histone and DNA demethylation, as well as in hypoxia adaptation [6].

IDH mutations are one of the common genomic abnormalities in AML, detected in 15–20% of all AML. They are particularly common in cytogenetically normal AML patients (CN-AML), with an incidence of 10.9% and 12.1% of IDH1 and IDH2 mutations, respectively [7, 8].

Mutant IDH1/2 may contribute to AML pathogenesis through epigenetic alterations [9]. They are acquired early in the progression from normal hematopoietic stem/progenitor cells (HSPCs) to frank leukemia, with stabilization during disease evolution, indicating that a population of IDH1/2 mutant cells survive chemotherapy and contribute to relapse [10, 11]. Thus, identification of these mutations at diagnosis may be pivotal for better risk stratification of AML patients. Furthermore, a

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strong rationale for therapeutic targeting of mutant IDH proteins may be beneficial for those patients.

Although the poor prognostic impact of IDH1/2 mutations in AML has been validated and was reported by some authors, complete concordance regarding this topic was not found among all relevant published researches. Thus, our aim in this study was to estimate the effect of each in terms of response to therapy and clinical course of the disease.

Subjects and methods

Patients

This prospective cohort randomized study was conducted on 80 newly diagnosed AML patients, attending Haematology/Oncology unit of Ain Shams University Hospitals, during the period from July 2017 till July 2018. The selected patients were diagnosed and classified based on integrated morphological (the FAB Classification of AML) and immunophenotyping features. Patients diagnosed as biphenotypic leukemia or associated with any other hematopoietic or non-hematopoietic malignancies were excluded.

Eight age- and sex-matched healthy subjects were enrolled in the study as a control group in order to verify PCR technique results.

Informed written consents were obtained from all enrolled patients. The study was approved by the Scientific and Ethical Committee, Ain Shams University (FMASU MD 161/2017) and was in accordance with the Declaration of Helsinki. (Data and material are available with the corresponding author upon reasonable request.)

Initial assessment

All the included subjects were subjected to comprehensive history taking and thorough clinical examination, laying stress on the presence of extramedullary disease.

Two milliliters of EDTA anticoagulated venous blood were collected from each patient to perform complete blood count (CBC) using LH 750 cell counter (Coulter Electronics, Hialeah, FL, USA), with morphologic examination of Leishman stained smears.

Four to five milliliters bone marrow aspirate were obtained, where the first 0.5–1 ml was used to prepare Leishman and Myeloperoxidase stained smears. One milliliter was collected on lithium heparin for cytogenetic analysis. The rest was divided into 2 K2-EDTA tubes in order to perform immunophenotyping (IPT) and PCR. All tests were performed on the same day of collection except PCR samples which were frozen at − 80 °C till use.

IPT was performed on Coulter Navios flow cytometer (Coulter Electronics, Hialeah, FL, USA), using the standard panel for acute leukemia.

Further assessment

Cytogenetically normal samples (CN-AML) were based on both normal standard G-banding analysis of ≥ 20 metaphase cells subjected to short-term unstimulated cultures (24–48 h) and negative fluorescence in situ hybridization (FISH) for any of the following abnormalities t(8;21)(q22;q22), t(15;17)(q22;q12), inv(16)(p13q22), and 11q23 [12].

These CN-AML samples were further analyzed for the detection of IDH1/2 mutations expression levels by real-time PCR (Custom TaqMan SNP Genotyping, SNP IDrs11 554137 A105G, Chr.2: 208248468 on GRCh38, exon 4, Context Sequence[VIC/FAM]:AGATAATGGCTTCTCTG AAGACCGT[A/G]CCACCCAGAATATTTCGTATGGTG C and SNP IDrs121913502 Q140R, Chr.15: 90088702 on GRCh38,exon4,ContextSequence[VIC/FAM]:GAAGACAG TCCCCCCCAGGATGTTC[T/C]GGATAGTTCCATTGG GACTTTTCCA for IDH1 and IDH2, respectively). DNA extraction was performed using the Genomic DNA Extraction kit, according to the manufacturer's spin protocol (Gene Proof manufactured). The extracted DNA was subjected to PCR amplification and endpoint plate read analysis using a real-time PCR instrument Slan 96P Real Time PCR System (SANSURE BIOTECH, China).

Results were reported in relative quantification. Relative quantification is based on calculating the expression levels of a target gene versus a reference gene. Calculations were done based on the comparison of a distinct cycle in real-time PCR determined by cycle threshold (CT) values of thermal cyclers at a constant level of fluorescence. The @CT value for each sample was determined by calculating the difference between the CT value of the target gene and the CT value of the endogenous reference gene.

Treatment protocol

Induction therapy begins with Adriamycin 25 mg/m^2/ 15min, intravenous infusion for 3 days, then shift to Cytarabine 100 mg/m^2 /12 h for 7 days. Three courses of post remission consolidation therapy, with high-dose ara-C Cytarabine 3 g/m^2/12 h, were infused by continuous IV infusion over 3 h on days 1, 3, and 5. The courses were administered at monthly intervals.

Outcome measures and endpoint of the study

Patients were assessed at day 28 after induction therapy and re-evaluated at 6 months. They were classified according to the 2017 European LeukemiaNet (ELN) AML recommendations [13] (Table 1).

Statistical analysis

The sample size was calculated using STATA® version 11 programs, setting alpha error at 5% and power at 80%. Data were analyzed using the NCSS© 12 Statistical Software 2018 (NCSS, LLC, Kaysville, Utah, USA) and XLSTAT© version 19.5 (Addinsoft©, Paris, France). Qualitative data were presented as number and percentages while quantitative variables were described as mean and standard

Table 1 Response categories according to ELN AML recommendations guidelines

Category Response	Definition	Comment
CR without minimal residual disease (CR_{MRD-})	If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR or CR with negativity by MFC	Sensitivities vary by marker tested and by method used; therefore, test used and sensitivity of the assay should be reported; analyses should be done in experienced laboratories (centralized diagnostics)
Complete remission (CR)	Bone marrow blasts < 5%, absence of circulating blasts and blasts with Auer rods, absence of extramedullary disease, ANC ≥ 1×10^9/L; platelet count ≥ 100×10^9/L	MRD^+ or unknown
CR with incomplete hematologic recovery (CRi)	All CR criteria except for residual neutropenia (< 1×10^9/L) or thrombocytopenia (< 100×10^9/L)	
Morphologic leukemia-free state (MLFS)	Bone marrow blasts < 5%, absence of blasts with Auer rods, absence of extramedullary disease, no hematologic recovery required	Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%
Partial remission (PR)	All hematologic criteria of CR, decrease of bone marrow blast percentage to 5–25%, and decrease of pretreatment bone marrow blast percentage by at least 50%	Especially important in the context of phase 1–2 clinical trials

ANC absolute neutrophil count
Dohner et al. [13]

deviation in parametric data or median and interquartile range in non-parametric one (IQR; the difference between 25th and 75th centiles). Comparison between two groups with qualitative data was done by using the Chi-square test (X^2). Fisher exact test was used instead of the Chi-square test when the expected count in any cell was found at less than 5. A comparison between two independent groups regarding quantitative data with parametric distribution was done by using an independent *t* test while the comparison of quantitative data with non-parametric distribution among two groups was done using the Mann-Whitney test. A p value < 0.05 was considered the cut-off value for significance in all analyses.

Time to event analysis was done using the Kaplan-Meier method. The predictive value of continuous variables was examined using receiver-operating characteristic (ROC) curve analysis. Finally, the predictive value of IDH genotype was examined using 2-by-2 contingency of the binary outcome (e.g., death/survival) versus the genotype (heterozygous/normal).

Results

Eighty newly diagnosed adult AML patients, who had both normal karyotype and cytogenetic analysis, were enrolled in the study. Their age ranged from 18 to 73 years with a mean of 45 ± 16 and male/female ratio 2.2: 1. Baseline demographic, clinical, and laboratory characteristics of the studied patients are illustrated in Table 2.

Heterozygous IDH1 SNP rs11554137 and IDH2R140Q SNP mutations were detected in 11 (13.8%) and 13 (16.3%) patients, respectively. None of them presented with homozygous mutations. Only one patient had both IDH isoforms mutations (Fig. 1).

Table 2 Baseline demographic, clinical and laboratory characteristics of the studied patients

Age (years)	Mean ± SD	45 ± 16
Gender	*N* (%)	
Male		56 (70)
Female		24 (30)
Hepatomegaly	*N* (%)	
Negative		43 (53.8)
Positive		37 (46.2)
Splenomegaly	*N* (%)	
Negative		29 (36.3)
Positive		51 (63.7)
Lymphadenopathy	*N* (%)	
Negative		54 (67.5)
Positive		26 (32.5)
Hemoglobin (g/dl)	Mean ± SD	6.8 ± 1.6
TLC ($\times 10^9$/L)	Mean ± SD	69 ± 41.7
Platelets ($\times 10^9$/L)	Mean ± SD	30 ± 17
Blast cells (%)	Mean ± SD	72.2 ± 16.3
FAB Classification	*N* (%)	
M1		11 (13.8)
M2		44 (55)
M4		19 (23.8)
M5		6 (7)
Time from diagnosis (days)	Mean ± SD	94 ± 78

N number, *SD* standard deviation, *TLC* total leucocytic count

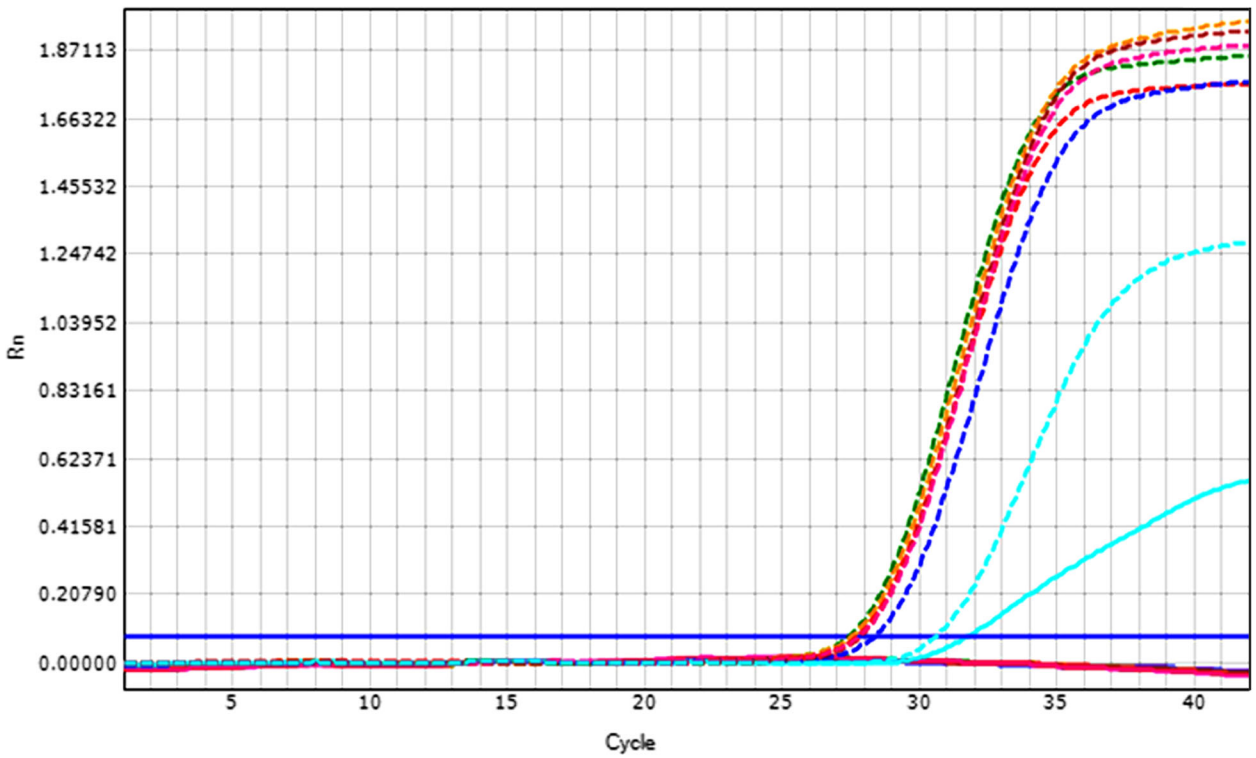

Fig. 1 AML patients examined for both IDH1rs11554137 and IDH2R140Q SNPs mutations (each in a separate well) by real-time PCR. Wild gene is detected by FAM filter (dotted line), whereas mutant gene is detected by HEX filter (straight line). Rn is the fluorescence of the reporter dye divided by the fluorescence of a passive reference dye. AML, acute myeloid leukemia

Unpaired *t* test revealed significant older age, higher platelet, and higher blast count among the positive group of patients with heterozygous mutant IDH1 SNP rs11554137 than those with wild type gene ($p < 0.001$). All patients with monocytic component (M4&M5) lacked IDH1SNP mutation (Table 3). On the other hand, the heterozygous mutant IDH2R140Q SNP group showed a significant statistical difference from the wild type as regards age, total leucocytic count (TLC), and platelet count ($p = 0.002$, < 0.001, and < 0.001, respectively) (Table 4).

Upon assessing the clinical outcome at day 28, none of the mutant IDH1 SNP rs11554137 or IDH2R140Q SNP groups achieved either complete remission (CR) or CR with incomplete hematologic recovery (CRi). On the other hand, at 6 months, all patients with IDH1 SNP rs11554137 mutation remained in partial remission and three out of thirteen patients with mutant IDH2R140Q achieved CR. Overall survival was measured from the date of diagnosis until the date of death or last date that the patient was known to be alive. Kaplan-Meier survival analysis showed lower median survival among patients with mutant IDH1 and 2 SNPs than those with wild type gene ($p < 0.0001$ and $= 0.027$, respectively) (Tables 3 and 4) (Figs. 2 and 3).

In order to assess variables with effect on patients' survival, comparison between survivors and non-survivors at 6 months was done. It revealed that the survivors showed significantly younger age and lower mean platelet and blast counts, as well as negative IDH1 SNP rs11554137 ($p = 0.004$, 0.016, 0.002, and 0.003, respectively). On the other hand, IDH2 mutation did not show significant difference as regards the overall survival ($p = 0.146$) (Table 5). Finally, multivariate logistic regression analysis identified high bone marrow (BM) blasts percentage as an independent prognostic predictor for 6-month mortality ($p = 0.014$, OR 1.049, 95% CI 1.010 to 1.090).

Discussion

The molecular pathogenesis of AML is complex; hence, the understanding of the link between the multiple genetic defects and their effect on the biological properties of the leukemic cells may be beneficial in developing more precise and specific therapies for AML [3]. In 2009, whole-genome sequencing of an AML sample identified a mutation in the IDH1 gene [14]. Later on, several studies confirmed the mutation of the IDH2 gene [15]. Furthermore, they denoted that ~ 15% of AML patients had mutations in either IDH1 or IDH2 [16]. Thus, the identification of these molecular markers, that may have a significant prognostic impact and further improvement in managing AML patients, was our study's aim.

Table 3 Comparison between normal and mutant IDH1 SNP rs11554137 patients

		Normal IDH1SNP (*N* = 69, 86.2%)	Heterozygous IDH1SNP mutation (*N* = 11, 13.8%)	*p* value*#
Age (years)	Mean ± SD	42.1 ± 14.2	62.3 ± 15.7	*< 0.001*
Gender	*N* (%)			
Male		50 (72.5)	6 (54.5)	0.228
Female		19 (27.5)	5 (45.5)	
Hepatomegaly	*N* (%)			
Negative		36 (52.2)	7 (63.6)	0.479
Positive		33 (47.8)	4 (36.4)	
Splenomegaly	*N* (%)			
Negative		23 (33.3)	6 (54.5)	0.174
Positive		46 (66.7)	5 (45.5)	
Lymphadenopathy	*N* (%)			
Negative		44 (63.8)	10 (90.9)	0.074
Positive		25 (36.2)	1 (9.1)	
Hemoglobin (g/dl)	Mean ± SD	6.7 ± 1.7	7.3 ± 1.0	0.260
TLC ($\times 10^9$/L)	Mean ± SD	68.4 ± 39	72.7 ± 58.7	0.754
Platelets ($\times 10^9$/L)	Mean ± SD	26.6 ± 16.2	48.1 ± 9.2	*< 0.001*
Blast cells (%)	Mean ± SD	69.6 ± 16.1	87.8 ± 6.3	*< 0.001*
FAB classification	*N* (%)			0.099
M1		10 (14.5)	4 (36.4)	
M2		37 (53.6)	7 (63.6)	
M4		16 (23.2)	0 (0)	
M5		6 (8.7)	0 (0)	
IDH2R140Q	*N* (%)			0.488
Normal		57 (82.6)	10 (90.9)	
Heterozygous		12 (17.4)	1 (9.1)	
Outcome at 28 days	*N* (%)			0.054
Favorable^{+}		18 (26.1)	0 (0)	
Unfavorable^{++}		51 (73.9)	11 (100)	
Outcome at 6 months	*N* (%)			*0.003*
Favorable^{+}		33 (47.8)	0 (0)	
Unfavorable^{+++}		36 (52.2)	11(100)	
Median survival (days)	Median (IQR)	170 (27–170)	12 (8–20)	*< 0.0001*

N number, *SD* standard deviation, *TLC* total leucocytic count, *IQR* interquartile range
*Unpaired *t* test for quantitative data with parametric distribution
#Chi-square test for qualitative data
Fisher's exact test when the expected count in any cell was found at less than 5
+Favorable outcome = CR (complete remission)
++Unfavorable outcome = D (dead) or PR (partial remission)
+++Unfavorable outcome = D (dead) or R (relapse)
A *p* value <0.05 was considered the cut-off value for significance in all analyses

Eighty newly diagnosed adult CN-AML patients were enrolled in the study; 11/80 (13.8%) were positive IDH1 SNP rs11554137 gene mutation. This was in agreement with Wagner and his fellows, Ho et al., and Ali and his colleagues, who detected mutant IDH1 SNP in AML cases in a percentage of 12%, 11%, and 11.8%, respectively [7, 17, 18]. On the other hand, the frequency of mutant IDH2R140Q was higher than that found by Ali et al. [18] (16.3% vs 3.9%), but near to Wagner et al.'s [7] finding (11%). These discrepancies can be attributed to variable inclusion criteria of the studied sample, ethnic variability, sample size, and variable detection assays sensitivity. As previously reported, all our patients with positive IDH1/2 SNP mutations were heterozygous [18, 19]. Moreover, only one patient harbored both

Table 4 Comparison between normal and mutant IDH2R140Q patients

		Normal IDH2R140Q (*N* = 67, 83.7%)	Heterozygous IDH2R140Q mutation (*N* = 13, 16.3%)	*p* value*#
Age (years)	Mean ± SD	42.6 ± 16.0	57.2 ± 8.5	*0.002*
Gender	*N* (%)			0.950
Male		47 (70.1)	9 (69.3)	
Female		20 (29.9)	4 (30.7)	
Hepatomegaly	*N* (%)			0.221
Negative		34 (50.7)	9 (69.3)	
Positive		33 (49.3)	4 (30.7)	
Splenomegaly	*N* (%)			0.149
Negative		22 (32.8)	7 (53.8)	
Positive		45 (67.2)	6 (46.2)	
Lymphadenopathy	*N* (%)			*0.037*
Negative		42 (62.7)	12 (92.3)	
Positive		25 (37.3)	1 (7.7)	
Hemoglobin (g/dl)	Mean ± SD	6.7 ± 1.5	7.3 ± 2.2	0.228
TLC ($\times 10^9$/L)	Mean ± SD	77.9.4 ± 39.1	22.2 ± 15.6	*< 0.001*
Platelets ($\times 10^9$/L)	Mean ± SD	25.9 ± 13.2	49.1 ± 22.7	*< 0.001*
Blast cells (%)	Mean ± SD	70.4 ± 21.0	81.3 ± 7.4	0.069
FAB classification	*N* (%)			0.439
M1		11 (16.4)	0 (0)	
M2		35 (52.2)	9 (69.2)	
M4		16 (23.8)	3 (23.1)	
M5		5 (7.6)	1 (7.7)	
IDH1SNPrs11554137	*N* (%)			0.488
Normal		57 (85.1)	12 (92.3)	
Heterozygous		10 (14.9)	1 (7.7)	
Outcome at 28 days	*N* (%)			*0.010*
Favorable[+]		18 (26.9)	0 (0)	
Unfavorable[++]		49 (73.1)	13 (100)	
Outcome at 6 months	*N* (%)			0.146
Favorable[+]		30 (44.8)	3 (23.1)	
Unfavorable[+++]		37 (55.2)	10 (76.9)	
Median survival (days)	Median (IQR)	160 (25–170)	14 (9–55)	*0.027*

N number, *SD* standard deviation, *TLC* total leucocytic count, *IQR* interquartile range
*Unpaired *t* test for quantitative data with parametric distribution
#Chi-square test for qualitative data
Fisher's exact test when the expected count in any cell was found at less than 5
+Favorable outcome = CR (complete remission)
++Unfavorable outcome = D (dead) or PR (partial remission)
+++Unfavorable outcome = D (dead) or R (relapsed)
A *p* value <0.05 was considered the cut-off value for significance in all analyses

mutations, in accordance with Saadi and his fellows, suggesting that these mutations are mutually exclusive [20].

In line with our findings, Aref et al. reported that AML patients with mutant IDH 1/2 SNP were associated with older age and higher platelet and blast counts, as well as lower TLC [21]. Also, Chotirat et al. found mutant IDH2 was associated with both older age and higher platelet count but not with low TLC [22]. Furthermore, Zhang and his colleagues [23] observed a shorter DFS in the high platelet count group among de novo non-M3 AML patients which could be attributed to either release of platelet growth factor that act on platelet-derived growth factor (PDGF) receptors on leukemia cells, affecting their proliferation [24] or

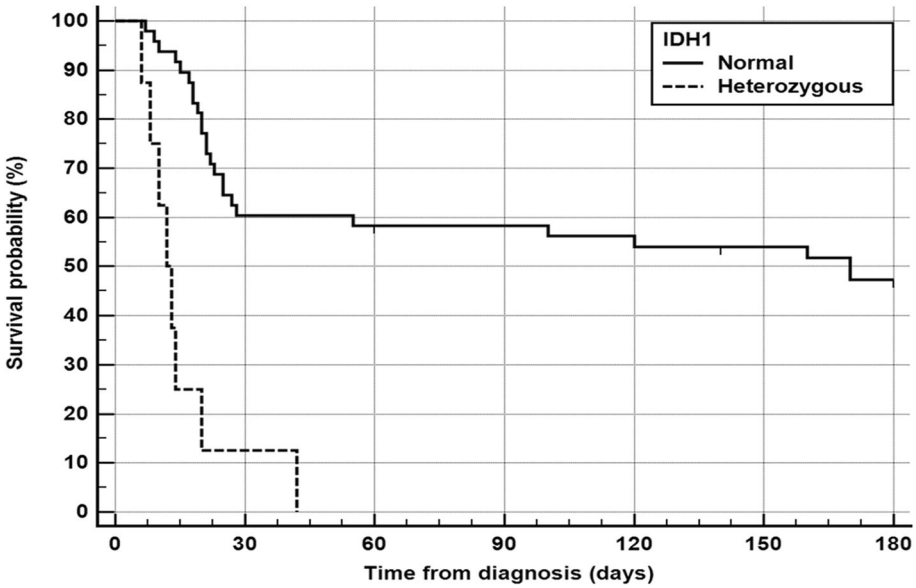

Fig. 2 Kaplan-Meier survival curves in patients with normal or heterozygous IDH1. There is a statistically significant difference between both curves (χ^2 [1] = 26.009, p value < 0.0001, hazard ratio = 5.937, 95% CI = 1.262–27.937)

cytogenetic abnormalities that may have an influence on the proliferation and differentiation of megakaryocytes, finally resulting in the increase of platelet count [25]. In contrast, Ali et al. showed no significant differences in demographic and clinical features between mutant IDH SNP and wild type gene [18].

Despite the fact that none of the patients who harbor the mutation has achieved CR, the difference in CR rates between patients with and without IDH1 SNP mutation was insignificant ($p = 0.054$). Still, IDH1 SNP was associated with shorter OS (HR 5.93, $p < 0.0001$), suggesting an adverse prognostic role in AML patients. These findings were in agreement with those reported by Wagner et al., Ali et al., and Xu et al., who declared that patients with mutant IDH1 SNP were significantly associated with inferior OS in CN-AML [7, 18, 26]. Potential mechanisms to explore how SNP rs11554137 may alter IDH1 activity include alterations in RNA stability, folding and splicing, differences in tRNA selection, or binding of non-coding RNAs [27]. This was supported by higher expression of IDH1 m RNA in patients

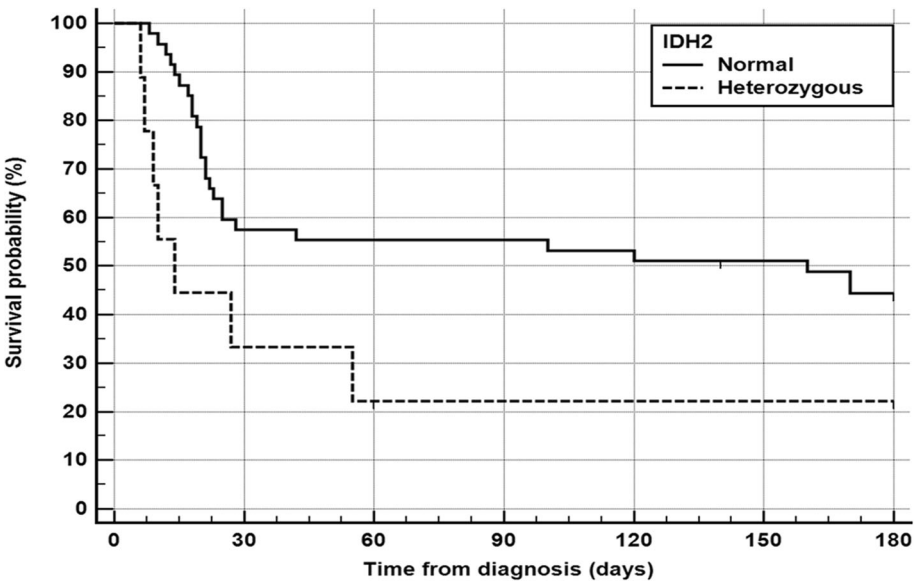

Fig. 3 Kaplan-Meier survival curves in patients with normal or heterozygous IDH2. There is a statistically significant difference between both curves (χ^2 [1] = 4.893, p value = 0.027, hazard ratio = 2.460, 95% CI = 0.784–7.725)

Table 5 Comparison between survivors and non-survivors at 6 months

		Survivors (*N* = 33, 41.3%)	Died (*N* = 47, 58.7%)	*p* value*#
Age (years)	Mean ± SD	38.8 ± 10.7	49.2 ± 17.7	*0.004*
Gender	*N* (%)			0.077
Male		24 (72.7)	25 (53.2)	
Female		9 (27.3)	22 (46.8)	
Hepatomegaly	*N* (%)			0.429
Negative		16 (48.5)	27 (57.4)	
Positive		17 (51.5)	20 (42.6)	
Splenomegaly	*N* (%)			
Negative		12 (36.4)	17 (36.2)	1.000
Positive		21 (63.6)	30 (63.8)	
Lymphadenopathy	*N* (%)			0.537
Negative		21 (63.6)	33 (70.2)	
Positive		12 (36.4)	14 (29.8)	
Hemoglobin (g/dl)	Mean ± SD	6.3 ± 2.5	7.1 ± 1.6	0.085
TLC ($\times 10^9$/L)	Mean ± SD	72.4. ± 34.5	66.6 ± 46.5	0.545
Platelets ($\times 10^9$/L)	Mean ± SD	24.2 ± 9.5	33.5 ± 20.1	*0.016*
Blast cells (%)	Mean ± SD	65.4 ± 17.8	76.9 ± 13.6	*0.002*
FAB classification	*N* (%)			0.082
M1		3 (9.1)	9 (19.1)	
M2		16 (48.5)	28 (59.6)	
M4		12 (36.4)	6 (12.8)	
M5		2 (6.1)	4 (8.5)	
IDH1SNPrs11554137	*N* (%)			*0.003*
Normal		33 (100)	36 (76.6)	
Heterozygous		0 (0)	11 (23.4)	
IDH2R140Q	*N* (%)			0.146
Normal		30 (91.3)	37 (78.7)	
Heterozygous		3 (9.1)	10 (21.3)	

N number, *SD* standard deviation, *TLC* total leucocytic count, *IQR* interquartile range
*Unpaired *t* test for quantitative data with parametric distribution
#Chi-square test for qualitative data
Fisher's exact test when the expected count in any cell was found at less than 5
A *p* value <0.05 was considered the cut-off value for significance in all analyses

with positive SNP rs11554137 mutation, which might affect IDH1 proteins and consecutively, intracellular NADPH-dependent redox reactions, with modulation of granulopoiesis and thereby chemosensitivity of leukemic cells [28].

Interestingly, the presence of IDH2R140Q mutation was associated with poor response to therapy assessed at day 28 ($p = 0.01$), as well as shorter OS (14 vs 160 days, HR 2.46, p = 0.027). Similarly, Willander and his colleagues declared that mutations in the IDH2 gene codon 140 revealed a significant increased risk for shorter OS in the whole patient group in relation to the wild type IDH2 codon 140 (HR = 1.94; $p = 0.03$), especially among the intermediate-risk group [19]. On the other hand, Patel et al. found that IDH2R140Q mutation could improve OS. This can be explained by lower patient median age in the study of Patel et al. than that in our cohort (48 vs 61 years), an assumption that is reinforced by the finding of Xu et al. that IDH2R140Q mutation was associated with prolonged OS among younger patients (mean/median <50 years; HR 0.64; $p = 0.0005$) [16, 26]. Green et al. revealed a favorable response to therapy and improved OS ($p = 0.008$), among IDH2R140Q-positive mutation, with larger impact in mutant NPM1 AML, which could contribute to the studies discrepancies [29].

Finally, our survivors showed a significantly younger age and lower mean platelet and blast counts, as well as negative IDH1 SNP rs11554137 ($p = 0.004$, 0.016, 0.002, and 0.003, respectively). However, multivariate analysis

identified high BM blasts percentage as an independent prognostic predictor for 6-month mortality, after adjusting for age, platelet count, and IDH1 SNP mutation. Similar results were obtained by Ali and his co-workers [18]. Those findings supported the importance of using the selective IDH-inhibitors as Enasidenib (oral inhibitors of IDH2R140 and IDH2R172 enzymes), which results in ≥ 90% decrease of 2HG serum levels, reduction in abnormal histone hypermethylation, and restoration of hematopoietic differentiation [30]. The oral inhibitor of mutant IDH1R132 enzyme is currently under several clinical trials [31]. Also, new areas of investigation for potential new therapeutic approaches aiming to inhibit the all-trans-retinoic acid differentiation pathway that is primed by mutant IDH AML cells [32].

Conclusion

Our results could not establish the primary and independent prognostic role of IDH mutations, this in addition to the difference found between the two mutations as regards the extent of their poor prognostic impact, as IDH2 surpassed IDH1 in early detection of unfavorable prognosis (at 28 days vs 6 months follow up, respectively). Yet, we still recommend their routine inclusion in the genetic work-up of CN-AML patients who harbor the mutations, as they can be candidates for the newly introduced selective IDH-inhibitors (i.e., to be used as molecular target therapies) which can bind the active catalytic sites of mutant IDH enzymes. Such therapies can benefit patients who cannot stand extensive chemotherapy or those with the least chances of maintaining the complete remission state.

Abbreviations

AML: Acute myeloid leukemia; BM: Bone marrow; CBC: Complete blood count; CN-AML: Cytogenetically normal acute myeloid leukemia; CR: Complete remission; CRi: CR with incomplete hematologic recovery; CT: Cycle threshold; DNA: Deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; ELN: European LeukemiaNet; FISH: Fluorescence in situ hybridization; HR: Hazard ratio; HSPCs: Hematopoietic stem/progenitor cells; IDH: Isocitrate dehydrogenase; inv: Inversion; IPT: Immunophenotyping; IQR: Interquartile range; NADPH: Nicotinamide adenine dinucleotide phosphate; NPM1: Nucleophosmin 1; OS: Overall survival; PCR: Polymerase chain reaction; RNA: Ribonucleic acid; ROC: Receiver-operating characteristic; SNP: Single nucleotide polymorphism; t: Translocation; TLC: Total leucocytic count; tRNA: Transfer ribonucleic acid; α-KG: α-Ketoglutarate

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Authors' contributions

All authors contributed to data interpretation and manuscript writing. SA conceptualized, designed the study, and supervised laboratory analysis. RE and SP contributed to study design and data interpretation. DS contributed to the conceptualization and the writing of the drafted manuscript. HA selected cases and clinical data collection. HA selected cases, collected clinical data, and performed technical work. All authors read and approved the final manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

Informed written consent was obtained from all enrolled patients. The study was approved by the Scientific and Ethical Committee, Ain Shams University (FMASU MD 161/2017), and was in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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