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Molecular Alterations in *IDH* 1/2 Genes among Iraqi Adult Acute Myeloid Leukemia Patients: Their Response to Treatment

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Abstract:

BACKGROUND: The recurrent somatic variations in *IDH1/2* genes in AML play imperative roles in epigenetic dysregulation and the pathogenesis of AML, which could be useful prognostic markers for risk stratification.

AIM: The aim of the study was to detect the frequency of R132 mutations in the *IDH1* gene and R140Q mutation in the *IDH2* gene with their treatment outcomes.

PATIENTS, MATERIALS AND METHODS: *IDH* molecular alterations were detected by high-resolution-melting (HRM)-based real-time PCR assay in 56 newly diagnosed AML patients.

RESULTS: *IDH* molecular alterations were identified in 39.3% of AML patients; *IDH1* R132 and *IDH2* R140Q mutations were present in 32.1% and 12.5% of patients, respectively. The mean age of patients with mutant *IDH* (52±14.87 years) is higher than in wild type (41.68±20.4 years), $P = 0.041$. Females were seen in 53% of mutant *IDH* patients while in the wild-type 73.3% were males ($P = 0.038$). There were significantly lower mean levels of hemoglobin, absolute neutrophil count, and platelet count in mutant IDH than in wild-type ($P = 0.015$, 0,.03 and 0.01, respectively). After induction remission therapy, 68.2% of mutated IDH and 64.7% of unmutated *IDH* patients didn't achieve complete remission ($P > 0.05$). After 6 months; 59.1% of mutated *IDH* and 64.7% of unmutated IDH had unfavorable outcomes ($P > 0.05$).

CONCLUSIONS: *IDH* mutations are common in Iraqi adult AML patients and present in older age and females predominance with lower Hb level, WBC count, absolute neutrophil count, platelet count, and less extramedullary involvement. There is an insignificant association with treatment outcomes.

Keywords:

Acute myeloid leukemia, high-resolution-melting-polymerase chain reaction, *IDH*1/2

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Introduction

Point mutations in exon 4 of *IDH1* gene occur consistently at an arginine residue in codon 132 (IDH^{R132}) in the frequency of 2%–14% of acute myeloid leukemia (AML) patients.[1] The point mutations of exon 4 in *IDH2* have been reported in 1%–19% of AML cases, predominantly $IDH2^{R140}$ rather than $IDH2^{R172}$ alterations in the prevalence of 80% and 20% of *IDH2* mutations, respectively.[2-4] *IDH* genetic alterations could be present in secondary AML from myeloproliferative neoplasms or myelodysplastic syndrome with frequency about 9%.[5]

The data about the prognostic impact of *IDH1*/2 mutations in AML have been conflicting. Different studies have shown

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that *IDH1*/2 mutations are associated with a worse prognosis, a better prognosis, or have no association at all. It appears likely that the impact of *IDH1*/2 mutations on clinical outcomes may depend on the specific patient population.[6,7]

Patients and Methods

Fifty-six newly diagnosed adult AML patients were included in this cross-sectional study. There were 49 *de novo* AML patients and 7 AML cases secondary to chronic myeloid leukemia or myelodysplastic syndrome. Patients with AML-M3 were excluded. Informed consents had been obtained from all of participants. This study was approved by the Research Ethics Committee, College of Medicine and in accordance to the Declaration of Helsinki. The diagnosis of AML was based on the cytomorphological features, French – American – British (FAB) criteria, and immunophenotyping.

Standard induction chemotherapy (7 + 3 regimen); daunorubicin IV infusion in a dose of 60–90 mg/m^2 on days 1–3, and Cytarabine continuous IV infusion of 100–200 mg/m^2 per a day on days 1–7. Consolidation therapy (high-dose cytarabine [HiDAC] or IDAC consolidation courses) 4 weeks apart had been given for included patients. Seventeen patients were older than 60 years or with comorbidity; 10 patients had received low dose cytarabine 20 mg/m^2/12 h for 10 days every month; and seven patients received azacitidine 75 mg/m^2, SC, day 1–7.[8]

Patients were followedup after induction therapy by examining complete blood picture, blood film, and bone marrow [BM] to assess response status as complete remission (CR): Absolute neutrophil count (ANC) >1.0 × 10^9/L, platelet count >100 × 10^9/L, and no blast cells in blood film and blast cells <5% of nucleated cells in BM with no extra medullary involvement or not in CR (NR) or induction death.[9] Patients were further followed-up for 6 months, but unfortunately, data about some patients were lost.

DNA was extracted from whole peripheral blood (PB) by using WizPrep™ gDNA Mini Kit, Korea; the DNA was examined for the goodness and quantity by Quantus Fluorometer (Promega, USA).

Molecular alterations in *IDH1*/2 genes were analyzed by high-resolution-melting (HRM) real-time polymerase chain reaction (PCR) assay, two primers (F and R) were used for each gene (*IDH1* and *IDH2*) provided by (Macrogene company, Korea), the sequences of the primers and the reaction conditions adopted from the study of Berenstein *et al*. 2014.[10] The mixture of the reaction per run for the detection of either *IDH1* R132 or *IDH2* R140Q mutations was carried out on (Mic quantitative PCR [qPCR] Cycler Bio Molecular System, Australia) and composed of; 5 µL of GoTag qPCR Master Mix (Promega, USA), 0.5 µL of each primer; sequences of primers were: *IDH1*-hrmF 5`-GTCAAATGTGCCACTATCACTC-3`, *IDH1*-hrmR 5`-GCCAACATGACTTACTTGATCC-3` both with 219 bp length and *IDH2*-hrmF 5`-GCTTGGGGTTCAAATTCTGG-3`, *IDH2*-hrmR 5`-CTCTCCACCCTGGCCTAC-3` with product length 248 bp and all with annealing temperature (60°C), 1 µL of DNA template completed with 3 µL nuclease free water to final volume of 10 µL for aliquot per a single run. The conditions of cycling were initial denaturation in 95°C hold for 5 min for one cycle followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. Melting on green by holding at 95°C for 15 s then hold at 45°C for 60 s and lastly melt from 50°C to 95°C at 0.3°C/s. Positive control and negative nontemplate controls were included in each run. The interpretation of results was carried out on software of Mic qPCR Cycler Bio Molecular System, Australia and was based on the graphs of fluorescence against temperature, normalized fluorescence to normal, shifted melting temperature curves. Fifteen samples (9 for *IDH1* and 6 for *IDH2*) were verified for the presence or absence of mutations through sequencing technique performed in Macrogene company, Korea. Patient samples showing *IDH* mutations by Sanger sequencing were used as positive controls in PCR run.

Statistical analysis

Description of numerical values expressed as mean ± standard deviation (SD), median, ranges, and qualitative data was expressed as frequency and percentages. The association between categorical data was done by using Person Chi-square or Fisher exact test and the statistical difference between two independent means of quantitative data was examined by Student's *t*-test. $P \leq 0.05$ considered statistically significant.

Results

The mean (±SD) age of AML patients was 45.73 ± 18.9 years, and a median of 46 years, with a range of 15–85 years. There were 30 (53.6%) males and 26 (46.4%) females.

The mean of PB blast percentage was 59.5 ± 29.2% and for BM blast percentage was 68.1 ± 21.2%. The range of the blast percentage in PB was (5%–99%) and for BM blast was (26%–95%), the median of PB blast was 64% and for BM blast was 75%.

The frequency of combined *IDH*1/2 mutations was 39.3% (22/56). The clinical and hematological parameters

Table 1: Clinical and laboratory characteristics of acute myeloid leukemia patients at diagnosis and comparison between patients with and without various *IDH1/2* mutations

Patient's characteristics	All patients	*IDH* gene		*P*
		Mutated (*n*=22; 39.3%)	Wild type (*n*=34; 60.7%)	
Age (years), mean±SD/median (range)	45.73±18.9/46 (15-85)	52.0±14.87	41.68±20.4	0.041*
Gender, *n* (%)				
Male	30	8 (26.7)	22 (73.3)	0.037**
Female	26	14 (53.8)	12 (46.2)	
Hb (g/dL), mean±SD/median (range)	7.846±1.6184/7.5 (4.4-12)	7.5±1.2	8.03±1.8	0.015*
WBC count (×10^9/L), mean±SD/median (range)	49.896±55.0/32 (0.6-252)	42.3±49.41	54.7±58.57	0.365*
ANC (×10^9/L), mean±SD/median (range)	2.8±3.08/2 (0.5-15)	1.9±2	3.4±3.5	0.030*
Platelet count (×10^9/L), mean±SD/median (range)	68.98±51.3/49 (11-213)	61.32±35.91	73.94±59.23	0.01*
PB blast (%), mean±SD/median (range)	62.2±27/71 (10-99)	55.18±28.11	66.76±25.74	0.609*
AML (*de novo*, secondary AML), (*n*)	(49, 7)	(19, 3)	(30, 4)	1.000**
FAB subtypes, (*n*) (M0, M1, M2, M4, M5, undefined)	(8, 7, 15, 14, 8, 4)	(4, 4, 5, 5, 2, 2)	(4, 3, 10, 9, 6, 2)	0.789**
Extramedullary disease (yes), *n* (%)	36 (64.3)	10 (45.5)	26 (76.5)	0.018**
Fever (yes), *n* (%)	40 (71.4)	15 (68.2)	25 (73.5)	0.665**
Pallor (yes), *n* (%)	43 (76.8)	19 (86.4)	24 (70.6)	0.172**
Bleeding tendency (yes), *n* (%)	16 (28.6)	5 (22.7)	11 (32.4)	0.436**
Outcome after induction, *n* (%)				
Good (CR)	16 (28.6)	6 (27.3)	10 (29.4)	0.956**
Poor (NR or deceased)	37 (66.1)	15 (68.2)	22 (64.7)	
Data lost	3 (5.4)	1 (4.5)	2 (5.9)	
Outcome after 6 months, *n* (%)				
Favorable (CR)	11 (19.6)	5 (22.7)	6 (17.6)	0.943**
Unfavorable (relapsed or deceased)	35 (62.5)	13 (59.1)	22 (64.7)	
Data lost	10 (17.9)	4 (18.2)	6 (17.6)	
Combined *IDH1* R132 and *IDH2* R140Q mutations, *n* (%)				
Positive				
Negative				

Patient's characteristics	*IDH1* R132 mutation[‡]		*P*	*IDH2* R140Q mutation		*P*
	Positive (*n*=18; 32.1%)	Negative (*n*=38; 67.9%)		Positive (*n*=7; 12.5%)	Negative (*n*=49; 87.5%)	
Age (years), mean±SD/median (range)	49.33±14.487	44.03±20.751	0.020*	57.1±14.74	44.1±19.1	0.069*
Gender, *n* (%)						
Male	6 (33.3)	24 (63.2)	0.037**	3 (42.9)	27 (55.1)	0.693**
Female	12 (66.7)	14 (36.8)		4 (57.1)	22 (44.9)	
Hb (g/dL), mean±SD/median (range)	7.56±1.36	7.98±1.7	0.151*	7.58±0.33	7.88±1.72	0.002*
WBC count (×10^9/L), mean±SD/median (range)	47.5±52.8	51.03±56.73	0.898*	26.714±27.8	53.208±57.3	0.077*
ANC (×10^9/L), mean±SD/median (range)	2.1±1.98	3.2±3.5	0.058*	1.85±2.1	2.98±3.2	0.515*
Platelet count (×10^9/L), mean±SD/median (range)	59.72±36.5	73.37±56.96	0.042*	63.71±29.87	69.73±53.9	0.101*
PB blast (%), mean±SD/median (range)	60.1±27.02	63.2±27.4	0.904*	64.75±32.11	44.42±25.6	0.383*
AML (*de novo*, secondary AML), (*n*)	(16, 2)	(33, 5)	0.829**	(5, 2)	(44, 5)	0.169**
FAB subtypes, (*n*) (M0, M1, M2, M4, M5, undefined)	(4, 3, 2, 5, 2, 2)	(4, 4, 13, 9, 6, 2)	0.389**	(0, 2, 3, 1, 0, 1)	(8, 5, 12, 13, 8, 3)	0.314**
Extramedullary disease (yes), *n* (%)	9 (50)	27 (71.1)	0.125**	2 (28.6)	34 (69.4)	0.084**
Fever (yes), *n* (%)	12 (66.7)	28 (73.7)	0.587**	5 (71.4)	35 (71.4)	1.000**
Pallor (yes), *n* (%)	15 (83.3)	28 (73.7)	0.424**	6 (85.7)	37 (35.5)	0.550**
Bleeding tendency (yes), *n* (%)	4 (22.2)	12 (31.6)	0.469**	2 (28.6)	14 (28.6)	1.000**
Outcome after induction, *n* (%)						
Good (CR)	6 (33.3)	10 (26.3)	0.856**	1 (14.3)	15 (30.6)	0.774**
Poor (NR or deceased)	11 (61.1)	26 (68.4)		6 (85.7)	31 (63.3)	
Data lost	1 (5.6)	2 (5.3)		0 (0)	3 (6.1)	
Outcome after 6 months, *n* (%)						
Favorable (CR)	5 (27.8)	6 (15.8)	0.571**	1 (14.3)	10 (20.4)	0.943**
Unfavorable (relapsed or deceased)	10 (55.6)	25 (65.8)		5 (71.4)	30 (61.2)	

Contd...

Table 1: Contd...

Patient's characteristics	*IDH1* R132 mutation[†]		*P*	*IDH2* R140Q mutation		*P*
	Positive (*n*=18; 32.1%)	Negative (*n*=38; 67.9%)		Positive (*n*=7; 12.5%)	Negative (*n*=49; 87.5%)	
Data lost	3 (16.7)	7 (18.4)		1 (14.3)	9 (18.4)	
Combined *IDH1* R132 and *IDH2* R140Q mutations, *n* (%)						
Positive	3 (16.7)	4 (10.5)	0.669**	3 (42.9)	15 (30.6)	0.669**
Negative	15 (83.3)	34 (89.5)		4 (57.1)	34 (69.4)	

*Student's *t*-test; **Pearson Chi-square test with application of Fisher's exact test whenever needed; [†]*IDH1* R132G was found in 7 patients and *IDH1* R132C in 11 patients. WBC=White blood cells; ANC=Absolute neutrophil count; CR=Complete remission; NR=Not in complete remission and early death; SD=Standard deviation; Hb=Hemoglobin; PB=Peripheral blood; AML=Acute myeloid leukemia; FAB=French-American-British

at the diagnosis of AML patients in mutant- and wild-type *IDH* are shown in Table 1. The mean age of mutant *IDH* patients is significantly higher than patients with normal *IDH* (P = 0,041). *IDH* mutation in males is less frequent than that in females (P = 0.038). The ANC, hemoglobin level, and platelet count are lower in patients with *IDH* mutation than those with wild-type *IDH* (P = 0.03, 0.015, and 0.01, respectively). Furthermore, extramedullary involvement, namely lymphadenopathy, splenomegaly, and hepatomegaly is less frequently encountered in patients with mutated *IDH* (P = 0.018). There were no significant differences in the outcomes between mutated and unmutated *IDH* patients whether after induction remission therapy or after follow-up for 6 months (P > 0.05).

IDH1 R132 mutations were detected in 32.1% of patients (18/56) showing two types of missense mutations; 7 with *IDH1* R132G mutation and 11 with *IDH1* R132C mutation, *IDH1* R132H mutation was not detected. The mean age of patients positive for *IDH1* mutations was significantly higher than that of patients negative for the mutations (P = 0.02) and there was female predominance in patients having *IDH1* mutations (P = 0.037). Platelet count was significantly lower in patients with *IDH1* mutations (P = 0.042). There were insignificant associations between mutated forms and those negative for this mutation with outcomes after induction chemotherapy or after 6 months of follow-up. Ten out of 18 patients with *IDH1* R132 mutations deceased so they had unfavorable outcomes.

IDH2 R140Q mutation was determined in 7 of 56 patients (12.5%). Only hemoglobin level was significantly lower in *IDH* R140Q-mutated patients than those negative for the mutation (P = 0.002), the other clinical and laboratory characteristics and outcomes to treatment showed insignificant associations (P > 0.05).

Three patients harbored both mutations *IDH1* R132G and *IDH2* R140Q, but there was no significant association between *IDH1* and *IDH2* mutations (P > 0.05).

Discussion

The frequency and the effect of *IDH1*/2 molecular alterations on response to the treatment and disease outcomes in AML varied in different studies. Sample size, environmental effects on specific population, selection of patients according to the AML subtypes, the presence of cytogenetic abnormalities, and the methods used for detection of the molecular abnormalities of *IDH1*/2 genes, all of these factors may contribute to these variations.

The incidence of combined *IDH1*/2 mutations (39.3%) was lower than that reported in a French study, 64.6% reported by Janin *et al*. 2014[11] and higher than 19% of Chotirat *et al*. in 2012,[1] 18.2% of Chou *et al*. in 2011,[12] 16% of Paschka *et al*. in 2010,[13] and 14.3% in an Egyptian study reported by ElNahass *et al*. in 2020.[14]

The frequency of *IDH1* R132 mutations (32.1%) is higher than that reported by Janin *et al*. 2014[11] (24.3%), Salem *et al*. 2017[15] (18%), Paschka *et al*. 2010[13] (7.6%), Chou *et al*. 2011[12] (6.1%), Patel *et al*. 2011[16] (6%), ElNahass *et al*. 2020[14] (2.9%), and Elsayed *et al*. 2014[17] (*IDH1* R132C mutation was not detected).

The frequency of *IDH2* R140Q mutation (12.5%) is close to 11.4% of ElNahass *et al*.[14] and 10.2% in an Iranian study of Saadi *et al*.,[18] but it is higher than other studies; Paschka *et al*. 2010,[13] a Sweden study of Willander *et al*. 2014,[3] and Olarte *et al*. 2019,[19] (8.7%, 7.9%, and 5.9%, respectively).

In contrasts to our results where mutations in *IDH1* gene is higher than *IDH2* gene, Chou *et al*. 2011[12] reported a higher incidence of *IDH2* R140Q mutation (9.2%) than *IDH1* (6.1%) and Paschka *et al*. 2010[13] IDH1 (7.6%), and IDH2 (8.7%). However, Berenstein *et al*. 2014[10] reported comparable results: *IDH1* R132 mutations 15.3% more frequent than *IDH2* R140Q mutation (6.7%); these findings might be related to differences in population characteristics, sample size, and different methods used for the detection of *IDH* molecular alterations. The comparison of the frequencies of IDH1/2 mutations in various studies is shown in [Table 2].

Table 2: Comparative demonstration of the frequency of *IDH1/2* mutations in various studies

Study	No. of AML patients	Method	*IDH1/2* Mutation	*IDH1* mutation	*IDH1* R132	*IDH1* R132G	*IDH1* R132S	*IDH1* R132C	*IDH1* R132H	*IDH2* mutation	*IDH2* R140Q	*IDH2* R172
Mardis *et al.*, 2009[20]	187	Sequencing	-	8% of all AML; 16% (13 of 80 CN-AML)	-	-		-		-	-	-
Paschka *et al.*, 2010[13]	805	-	16.0%*	7.58%	7.45%					8.7%	6%	2.7%
Marcucci *et al.*, 2010[21]	358	PCR amplification/ sequencing	33% of *de novo* CN-AML	13.7%	13.1%	-		-		19.3%	15.6%	3.6%
Thol *et al.*, 2010[22]	272 CN-AML	-	-	-	-	-		-		12.1%	10.9%	1.2%
Thol *et al.*, 2010[22]	130 AML	-	-	-	-	-		-		3.8%	-	-
Wagner *et al.*, 2010[23]	275 CN-AML	-	-	-	10.9%	-		-		-	-	-
Chou *et al.*, 2011[12]			18.2%	6.1%	6.1%						9.2%	
Patel *et al.*, 2011[16]	199	Sequencing	11.8% (94% were CN-AML)	-	6.0%	-		-		-	-	2.0%
Chotirat *et al.*, 2012[1]			19%									
Rakheja *et al.*, 2012[24]	-	-	17% in AML, (27% in CN-AML)	-	-	-		-		-	-	-
Willander *et al.*, 2014[3]											7.9%	
Berenstein *et al.*, 2014[10]	230	-		16%	15.3%						6.7%	
Janin *et al.*, 2014[11]			64.6%		24.3%							
Elsayed *et al.*, 2014[17]								0%				
DiNardo *et al.*, 2015[25]	826	-	20%	-	7.14%						10%	2.78%
Papaemmanuil *et al.*, 2016[26]	1540	-	-	-	-	-		-		-	-	1%
Salem *et al.*, 2017[15]					18%							
Saadi *et al.*, 2018[18]											10.2%	
Olarte *et al.*, 2019[19]											5.9%	
ElNahass *et al.*, 2020[14]			14.3%		2.9%						11.4%	
Zarnegar-Lumley *et al.*, 2020[27]	3210 all ages		8.6%		3.7%					4.9%		
Kattih *et al.*, 2021[28]	363	-	17.9%	7.2%						10.7%		
Duchmann *et al.*, 2021[29]	319 (67% CN-AML)	-	-	-	40%	3.5%	3.1%	18.8%	13.8%	-	42%	18%
The present study 2019	56	HRM-RT-PCR	39.3%		32.1%	12.5%		19.6%		12.5%	12.5%	

CN-AML, cytogenetically normal acute myeloid leukemia. *Two patients had both IDH1 and IDH2 mutations

The absence of significant association between *IDH1* and *IDH2* mutations suggests that these molecular alterations are mutually exclusive.[13,14]

IDH1 R132 or *IDH2* R140Q mutations had a tendency to increase with patient's age that was consistent with Willander *et al*. 2014[3] and Paschka *et al*. 2010.[13] Female predominance in the studied Iraqi AML patients with *IDH* mutations is also noticed in Chotirat *et al*. 2012[1] and Willander *et al*. 2014[3] studies.

In relation to hematological parameters, the association of *IDH2* R140Q mutation and *IDH1* R132 mutations with decreased white blood count (WBC) count and ANC was comparable to other studies. In this study, platelet count was significantly lower in patients having *IDH* mutations than in patients without these mutations which was in accordance with Saadi *et al*. in 2018[18] and Salem *et al*. in 2017[15] but in contrast to Chou *et al*. in 2011 study.[12]

Most of patients with *IDH1*/2 mutations categorized in (M0, M1, and M2) FAB subtypes and these finding consistent with many previous studies Janin *et al*. in 2014[11] and Salem *et al*. in 2017.[15]

Prognosis of Iraqi AML patients with *IDH* mutations was one of the important goals of the current study as there were no available Iraqi studies about the effect of positivity of *IDH1* R132 and *IDH2* R140Q mutations on induction therapy and 6 months' follow-up outcomes, 11/18 patients with *IDH1* R132 mutations and 6/7 of patients with *IDH2* R140Q mutation were insignificantly observed to have poor outcomes on induction therapy, while 6/18 and 1/6 patients positive for *IDH1* R132 and *IDH2* R140Q mutations, respectively, did not achieve CR with unfavorable outcomes after 6 months of patients monitoring, these observations were comparable to previous studies who showed bad effect of *IDH1*/2 mutations on disease outcomes as in Salem *et al*. in 2017,[15] Abd El Maksoud *et al*. in 2019,[23] and Wagner *et al*. in 2010.[30]

However, the data about the prognostic impact of *IDH1*/2 mutations in AML have been conflicting. Different studies have shown that *IDH1*/2 mutations are associated with a worse prognosis, a better prognosis, or have no association at all. It appears likely that the impact of *IDH1*/2 mutations on clinical outcomes may depend on the specific patient population.[6,7]

HRM analysis is the best screening method to determine the heterogeneity of *IDH1* mutations. Furthermore, for the identification of mutations in *IDH2 hM* analysis showed approximately 98% concordance with Sanger sequencing.[10] Another study compared HRM to Sanger sequencing on 146 AML BM samples for validation and showed near-perfect concordance for all positive and negative results for *IDH*1 (98%) and *IDH*2 (94%).[31]

IDH1 and *IDH2 R140* are usually found in combination with *NPM1* mutations, while *IDH2 R172* is mutually exclusive with NPM1 mutations. In addition, *IDH1* and *IDH2* mutations co-occur with *FLT3-ITD* in 15%–27% and 8%–30% of AML patients, respectively.[32] The prognostic genetic risk stratification in AML includes a long list of abnormalities but the associations between the abnormalities as for FLT3-ITD with NPM1 should be studied which may locate the patient in low-, intermediate-, or high-risk category.

Various subtypes of *IDH* mutations might contribute to different prognosis and be allowed to stratify intermediate-risk AML further.[7]

Increasing evidence of clinical role of DNMT3A and *IDH*1/2 mutations highlights the need for a robust and inexpensive test to identify these mutations in routine diagnostic workup. Herein, we compared routinely used direct sequencing method with HRM assay for screening DNMT3A and *IDH*1/2 mutations in patients with AML. Very high concordance between HRM and Sanger sequencing was shown.[33]

Unfortunately, at the time of the study, only some patients had done genetic studies for FLT3-ITD, NPM1, t (8;21), inv16, t (16;16), and t (15;17). All our patients had *DNMT3A* R882H mutation tested by HRM-PCR and only five patients were positive.[34]

There is no current evidence that DNMT3A and *IDH*1/*IDH*2 mutations warrant their assignment to a distinct ELN prognostic group.[35] The presence of *DNMT3A*, *IDH*1, or *IDH*2 mutations may confer sensitivity to novel therapeutic approaches, including the use of demethylating agents.[6]

Conclusion

In conclusion, *IDH1*/2 molecular alterations observed as common finding in Iraqi adult newly diagnosed AML patients and were higher than many other populations. *IDH* alterations are associated with older age, female predominance, lower WBC count, ANC, hemoglobin level, and platelet count, and less extramedullary involvement at the time of diagnosis. There is no association between *IDH1* R132 mutations and *IDH2* R140Q mutation and there is insignificant association with treatment outcome. Assessment of *IDH1*/2 mutations in cytogenetically normal AML is recommended. Association of NPM1 and FLT3 should be excluded to unravel clearly the prognostic significance of these alterations.

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Conflicts of interest
There are no conflicts of interest.

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