IDH1 and IDH2 Gene Mutations in Omani Patients with Acute Myeloid Leukemia: Prognostic Significance and Clinic-pathologic Features

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Abstract

Objectives: We aim to define the prevalence of isocitrate dehydrogenase (IDH) mutation, evaluate the clinico-pathologic impact of IDH mutations, asses the effect of IDH mutation on response to the currently offered treatment for acute myeloid leukemia (AML) cases, and to determine the impact of other common concurrent mutations with IDH.

Methods: A single-center retrospective cohort study was conducted at Sultan Qaboos University Hospital (SQUH) from October 2009 to October 2019. We included all Omani patients (pediatric and adult) treated at SQUH with the standard therapy, for whom DNA extraction was performed at diagnosis. The target mutations in both genes IDH1 & IDH2 were screened by direct PCR product sequencing method. Statistical analysis was conducted using SPSS software. Survival differences were estimated using log-rank test. Continuous variables were presented as median and interquartile ranges while categorical variables were presented as frequency.

Results: A total of 61 patients treated from 2010 to 2019 whose DNA extracted at diagnosis were evaluated. Median age was 40 (range = 25.5–65.5). The prevalence of IDH1 R132, IDH2 R140, and IDH2 R172 mutations among the study group were 6.6%, 3.3%, and 1.6%, respectively. Clinic- pathologic characteristics associated with IDH mutations at diagnosis included older age, lower white blood cell (WBC) count, higher median platelet counts, normal karyotype AML, and cytogenetics intermediate-risk group. The overall survival (OS) in patients harboring IDH mutations was poor with median OS was 9 months. This analysis confirms that response rate and OS for both IDH-mutated and IDH wild-type AML patients were comparable. This will provide contemporary data to be used for comparison with results of novel investigational (e.g., selective IDH inhibitor) strategies.

Conclusions: The current study results are consistent with the other international studies of IDH mutations in AML and demonstrated the poor prognosis associated with IDH mutations. Clinicopathologic features associated with IDH mutations included older age, lower WBC count, higher median platelet counts, normal karyotype AML, and cytogenetics intermediate-risk group.

Keywords: Acute Myeloid Leukemia; Isocitrate Dehydrogenase 1; Isocitrate Dehydrogenase 2.

Introduction

The myeloid leukaemia is a heterogeneous group of diseases characterized by infiltration of the blood, bone marrow, and other tissues by neoplastic cells of the hematopoietic system.[1] In 2022, 20,050 new cases of acute

myeloid leukaemia (AML) were estimated in the United States.[2] Data demonstrated an association of myeloid leukaemia with irradiation, smoking, some rare congenital abnormalities, chemical exposure, and obesity.[3]

According to the WHO classification of haematological malignancies, recurrent genetic abnormalities were identified in acute myeloid leukaemia (AML). Those genetic abnormalities are associated with distinctive clinicopathological features and have prognostic significance. The Cancer Genome Atlas (TCGA) Research Network evaluation of 200 AML cases found an average of 13 mutations per case of AML, with at least 23 recurrent mutations identified. The most common identified mutations are FLT3, NPM1, CEBPA, cKIT, NRAS, MLL, WT1, IDH1/2, TET2, DNMT3A, and ASXL1.

In recent years, the analysis of whole genome leads to the identification of two mutually exclusive mutations in isocitrate dehydrogenase genes; IDH1 in the cytoplasm and IDH2 in the mitochondria.[4,5]

After the discovery of IDH1 & IDH2, various studies were conducted to evaluate the prevalence of these mutations, clinical & prognostic impact. Three main locus were studied, IDH1R132, IDH2R140 and IDH2R172.

Studies reported IDH mutations in around 33% of the patients, including 6–16% for IDH1 and 8–19% for IDH2 mutations.[6-8] Five missense mutations were found in IDH1, including R132H, R132C, R132S, R132G and R132V. IDH1R132 mutation was associated with other concurrent mutations including NPM1, FLT3, CEBPA, and NRAS.[9] Moreover, IDH2 mutations were found in 10.4%, with 2 missense mutations detected: R140Q and R172K.[10]

IDH mutation has significant association with old age and higher median platelet counts, normal karyotype AML, cytogenetics intermediate-risk group, and NPM1, FLT3-ITD mutations.[6,7,11,12] IDH1 mutation status is an unfavourable prognostic factor[11] and patient harbouring IDH mutations have significantly lower rate of Five-year overall survival (OS) of 15.6% than patients who lack the IDH mutation (32.0%).[7]

The remission rates by AML treatment status were reported as 68% during induction, 42% in Salvage-1 (S1), and 27% in Salvage-2 and beyond (S21).[12] In addition, they found no difference in response identified by IDH mutation status.[12]

All the previous studies have addressed the impact and clinicopathological features of IDH mutations in AML patients conducted internationally and no local studies are available. In addition to the common gene mutations detected frequently in AML patient such as NPM1, FLT3 & others, analysis at haematology department at SQUH, the *IDH* gene mutation testing will serve as an additional important diagnostic molecular marker in Omani patients with AML. To fill the gap, the present study aims to estimate the prevalence of IDH mutations among Omani patients diagnosed to have AML and the common types of *IDH* mutation will be identified and correlated with the clinical and laboratory findings.

Methods

Study design

This is a single centre retrospective cohort study conducted in Sultan Qaboos University Hospital (SQUH). Archived DNA collected from October 2009 to October 2019 were used in this study. The clinical data of study population was obtained from the hospital information system (HIS). This research was ethically approved be Institutional Review Board (IRB) at SQU. (MREC ♯2048) and cconfidentiality protected.

In this study we included all Omani patients (pediatric and adult) treated at Sultan Qaboos University Hospital who were diagnosed with AML and diagnostic DNA was available for testing. All other patients who have missing data or their DNA extraction done at relapse or for other diagnosis (i.e myelodysplastic syndrome) were excluded from the study.

Variables

The following hematological parameters have been assessed in all patients; full Blood Count including hemoglobin (Hb), hematocrit (HCT), white blood cells (WBC) and platelets count. Additionally, blood film smear, bone marrow aspirate, karyotyping, and DNA-based genetic studies were evaluated.

IDH1 and IDH2 mutations detection

Genomic DNA was extracted from EDTA-anticoagulated whole blood using the QIAamp DNA blood mini kit (Qiagen Inc, Hilden, Germany). The concentration and quality of the sample DNA was checked by NanoDrop ND-1000 (Nano-Drop Technologies, Wilmington, USA).The target regions for IDH1 and IDH2 genes were PCR amplified and directly sequenced using 3500 Genetic Analyzer. FLT3-ITD and NPM1 mutations were screened by capillary electrophoresis as described before.

Statistical analysis

Statistical analysis was conducted using SPSS software. The Kaplan- Meier method and the log-rank test were utilized to estimate the distribution of overall survival (OS), with a p-value of less than 0.05 was considered statistically significant. Continuous variables were presented as median and interquartile ranges. While categorical variables such as FAB classification and cytogenetics were presented as frequency.

Results

We analysed a total of 61 patients with AML, median age of the total population was 40 years. The baseline characteristics of study population are summarized in Table 1.

Total number of patient	**N= 61**
Age at diagnosis, years, median (IQR)	40 (25.5 – 65.5)
Gender:	
Male, n(%)	31 (50.8%)
Female, n(%)	30 (49.2%)
Hemoglobin, g/dL, median (IQR)	8.3 (7.4 – 9.8)
WBC, 10^9/L, median (IQR)	9.8 (2.9 – 53.8)
PLT count, 10^9/L, median (IQR)	35 (23 – 99)
Blast %, median (IQR)	77 (52.3 – 87.2)
LDH, median (IQR)	394 (266 – 857)
Flowcytometry results	
APML, n(%)	10 (16.4%)
AML, n(%)	46 (75.4%)
Mixed phenotype, n(%)	5 (8.2%)
Risk stratification*:	
Favourable prognosis, n(%)	8 (31.1%)
Intermediate risk, n(%)	17 (27.9%)
High risk, n(%)	35 (57.4%)

* One patient died before bone marrow and cytogenetics testing.

Frequency and type of IDH1 and IDH2 mutations

Out of all tested AML cases, whereby 54 (88.52%) had no IDH mutations (IDH1/IDH2^{wildtype}AML), and 7 (11.48%) harbouring either an IDH1 or an IDH2 mutation (Figure 1). Among all tested AML patients, an IDH1 mutation was detected in 4 (6.56%) cases and an IDH2 mutation in 3 cases (4.92%). The main loci were identified in IDH1 mutation are p.R132C in 3 (75%) and p.R132H in 1(25%) (Figure 2). While for IDH2 mutation, p.R140Q and p.R172K were detected in 3.28% & 1.64% (respectively. No mutated cases had both *IDH1* and *IDH2* mutations, suggesting that these mutations are mutually exclusive.

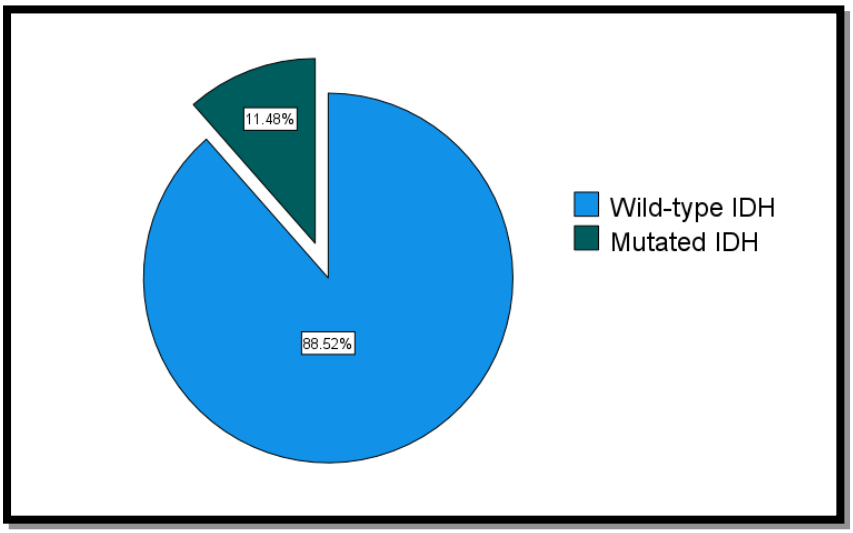

Figure 1: *Percentage of IDH mutation*

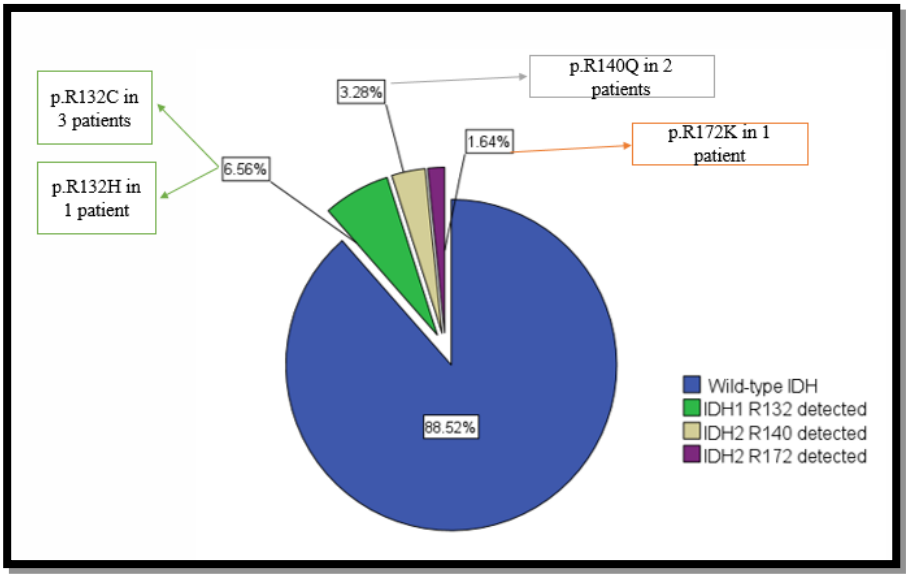

Figure 2: *Loci of IDH 1/IDH2 mutations detected*

Clinicopathological features of patients with IDH1 or IDH2 mutations

1- Clinical features

The median age of wild type group was 37 years whereas the median age for mutated IDH group was 56 years. The male: female ratio is 1:2.5. Mutated IDH group tend to have lower white cell count with median $4.4x10^9$/L (1- 37 $x10^9$/L) and higher platelet count with median of $47x10^9$/L (32- 138 $x10^9$/L) at diagnosis. There was no difference in haemoglobin level between both groups, median was 8.3g/dl for wild type IDH and 7.9g/dl for mutated IDH group. Patient characteristics of both wild and mutated IDH are displayed in Table 2.

Parameter	Wild-type IDH (N= 55)	Mutated IDH (N= 7)
Age at diagnosis, years, median (IQR)	37 (18 – 61)	56 (42 – 70)
Gender:		
Male, n(%)	29 (52.7%)	2 (33.2%)
Female, n(%)	26 (47.3%)	5 (71.4%)
Hemoglobin, g/dL, median (IQR)	8.3 (7.5 – 9.9)	7.9 (6.9 – 8.3)
WBC, 10^9/L, median (IQR)	10 (3 – 59)	4.4 (1 – 37)
PLT count, 10^9/L, median (IQR)	32 (23 – 94)	47 (32 – 138)
Blast %, median (IQR)	77 (51.5 – 85.7)	81 (63 – 90)
Flowcytometry results		
APML, n(%)	10 (18.2%)	0
APML variant, n(%)	0	1 (14.28%)
AML, n(%)	40 (72.7%)	6 (85.7%)
Mixed phenotype, n(%)	5 (9.1%)	0
Risk stratification *:		
Favourable prognosis, n(%)	8 (14.8%)	0
Intermediate risk, n(%)	12 (22.2%)	7 (100%)
High risk, n(%)	34 (63%)	0

* One patient died before bone marrow and cytogenetics testing.

2- Morphological and immunophenotypic features

The median blast count in mutated IDH group was 81% (63- 90%) whereas in wild type IDH was 77% (51.5-85.7%). All the cases in mutated group were classified as AML, not otherwise specified (AML- NOS) based on WHO AML classification. These cases were further classified using French American-British classification (FAB) classifications into AML without maturation (M1) in 1 patient (14.28%), AML with maturation (M2) in 1 patient (14.28%), acute promyelocytic leukemia variant (M3-v) in 1 patient (14.28%), acute myelomonocytic leukemia (M4) in 2 patients (28.5%) and acute monocytic leukemia (M5) in 2 patients (28.5%). Dysplastic features were described in 4 patients (57.1%) of mutated IDH. The dysplastic features were identified mainly in granulocytes 1 patient (14.28%), megakaryocytes 2 patients (28.5%) and erythroid 1 patient (14.28%) lineages Table 3.

Table 3: Hematologic and morphologic features of AML with mutated *IDH1*^{R132}, *IDH2*^{R140} and *IDH2*^{R172}

Pt. No	Mutation	Nucleotide Change	A.A Change	Age/Sex	FAB	WBC	Hb	PLT	Blast %	Auer Rods	Dysplastic features
1	IDH1	CGT-TGT	p.R132C	74/F	M4	3	10.1	138	31	++	Myeloid, Megas
2	IDH1	CGT-TGT	p.R132C	73/F	M1	91	8.3	338	81	-	Megas
3	IDH1	CGT-TGT	p.R132C	56/M	M5	1	7.2	32	79	?	?
4	IDH1	CGT-TGT	p.R132H	36/M	M5	8	5.9	14	63	-	Absent
5	IDH2	CCG-CAG	p.R140Q	70/F	M3v	4	7.9	47	90	-	Absent
6	IDH2	CCG-CAG	p.R140Q	48/F	M2	37	6.9	35	95	++	Absent
7	IDH2	AGG-AAG	p.R172K	43/F	M4	1	8.3	132	88	-	Erythroid

FAB: French-American-British classification of AML, WBC: White cell count, Hb: hemoglobin, PLT: platelet
Case 3: BMA not done as patient refused

3- Molecular and Cytogenetics features

Of the studied AML patients, favourable, intermediate, and adverse cytogenetics risk were found in 8(31.1%), 17(27.9%), 35(57.4%) respectively. Two (28.5%) of AML cases with mutated IDH, has normal karyotype, in both groups. Whereas, five (71.4%) had various cytogenetics abnormalities including trisomy 4, trisomy 11, trisomy 8, del (Y) and t.[10,17] All IDH mutated cases (100%) were in the intermediate-risk cytogenetics group. 14.28% (1 patient) of IDH mutated group had concurrent mutations of IDH1 and NPM1 mutations. Table 4. The association with other molecular abnormalities couldn`t be assessed because of small sample size.

Table 4: Cytogenetic and molecular features of AML with *IDH1*^{R132}, *IDH2*^{R140} and *IDH2*^{R172} mutations

Pt No	Age/Sex	FAB	Karyotype	Cyto-Risk group	Mutation	Nucleotide Change	A.A Change	Other mutations (FLT3-ITD, NPM1)
1	74/F	M4	N. karyotype	Intermediate	IDH1	CGT-TGT	p.R132C	NPM1-, FLT3ITD-
2	73/F	M1	+4	Intermediate	IDH1	CGT-TGT	p.R132C	**NPM1+**, FLT3ITD-
3	56/M	M5	+11	Intermediate	IDH1	CGT-TGT	p.R132C	NPM1-, FLT3ITD-
4	36/M	M5	-Y	Intermediate	IDH1	CGT-TGT	p.R132H	NPM1-, FLT3ITD-
5	70/F	M3v	t(10;17)	Intermediate	IDH2	CCG-CAG	p.R140Q	NPM1-, FLT3ITD-
6	48/F	M2	N. karyotype	Intermediate	IDH2	CCG-CAG	p.R140Q	NPM1-, FLT3ITD-
7	43/F	M4	+8	Intermediate	IDH2	AGG-AAG	p.R172K	NPM1-, FLT3ITD-

FAB: French-American-British classification of AML

Associations of IDH Mutations with Clinical Outcome

The median overall survival (OS) was 9 months for of IDH1 and IDH2 mutated groups (P-value: 0.593). The estimated OS in the study cohort did not significantly differ between IDH wildtype and mutated IDH (Figure 3).

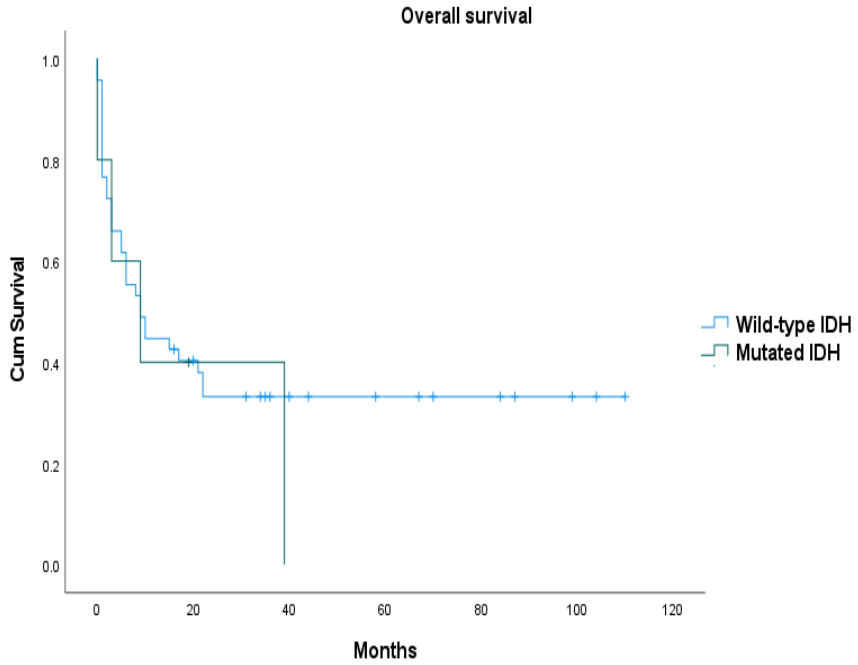

Figure 3: *Overall survival between wild type IDH and mutated IDH.*

Discussion

IDH mutations were described in variety of malignancies such as gliomas,[13] chondrosarcomas,[14] cholangiocarcinomas,[6] and breast carcinomas.[7] Moreover, it has been identified in numbers of myeloid neoplasms such as AML, MDS, and myeloproliferative neoplasm.[3] IDH enzymes encoded by IDH genes are normally functioned to convert isocitrate to a-ketoglutarate. Whereas mutant IDH enzymes, reduce a-ketoglutarate to its oncometabolite 2-hydroxyglutarate. The later inhibits a-ketoglutarate dependent enzymes, such as TET2, resulting in aberrant epigenetic modifications that blocks cell differentiation.[8]

In this retrospective study we found that the overall frequency of IDH mutations among AML patients was 11.48%. This frequency was lower than the reported incidence ranging from 16%-33%.[4,5,15-17] This could be explained by extremely small sample size compared with other published studies. In subgroup analysis of IDH mutations, IDH1mutation was detected in four patients (6.56%) with the two main loci IDH1R132C (4.92%) and IDH1R132H (1.64%). Whereas IDH2 mutation was found in 4.92% with the two main loci IDH2R140Q in (3.28%) and IDH2R172K in (1.64%). The reported incidence of IDH1 mutation ranging from 6-14% of cases while IDH2 found in 8-19%.[4,5,15-17] Hence, the incidence of IDH1 and IDH2 mutation were consistent with international studies.

In our study, IDH mutant patients tended to be older in age with female predominance at ratio of 1:2.5. The reported median age of patients with mutated IDH ranging from 49- 67 years.[11,15,16,18] Keyur P. Patel and college[18] reported a male to female ratio of 1:3 whereas Claire L et al,[11] reported a ratio of 1:2. A lower median white cell count and higher median platelet count and blast percentage were observed in IDH mutated patients at diagnosis. These findings were consistent with other published international studies.[5,15,16] In agreement with other studies,[5,18,19] all mutated IDH cases were associated with intermediate cytogenetics risk group. Sadudee Chotirat et al[16]; identified 55% (11 cases) and 50% (12 cases) normal karyotype in mutated IDH1 and IDH2 respectively. In addition, they reported various cytogenetics abnormalities in aberrant karyotype including del(9q), trisomy 8, trisomy 11, del(12) (p12.1p13.1), t(15,17) and t(8;21). However, in this study, only 28.5% (2 patients) has normal karyotype, one in each group. The identified cytogenetics abnormalities include trisomy 4, trisomy 11, trisomy 8, del (Y) and t(10,17). Furthermore, multiple studies conducted to assess the concurrent presence of other gene mutations along with IDH mutation. Keyur P. Patel et al[18]; reported the following additional gene mutations including *NPM1, FLT3-ITD, CEBPA, NRAS, KIT,* and *FLT3-D835 in IDH1*-mutated cases. Significant association between NPM1 and mutated IDH1 74%; $P < 0.001$) and IDH2 (60%; $P < 0.001$) was demonstrated by Claire L. Green.[11] We found concurrent presence of NPM1 mutation in one case with mutated IDH1. Hence, the association couldn`t be assessed because of small sample size. Table 5 is comparing our results with other international studies.

Table 5: Comparison of results between our study and other international results for patients with IDH1

	Our Study (N=4)	Mardis et al, 2009 (N=16)	Chou et al, 2010 (N=27)	Marcucci et al, 2010 (N=49)	Wagner et al, 2010 N=30
No. of cases	4/61	16/188	27/493	49/358	30/275 (11)
Median age in years	61.5	48.9 ±	52.5	62	50
(Mean, Range)	(43.5 – 70.1)	15.4	(25–75)	(21–82)	(33–80)
M:F	1: 1	1.3:1	1.1:1	1:1.1	1:1.7
% Blast (Mean, Range)	80 (55 – 91.5)	76.7 ± 16.4	NA	73 (33–99)	80 (20–99)
Risk stratification				NA	NA
Favorable	0	0	0		
Intermediate	4 (100%)	16 (100%)	26/26 (100%)		
High risk	0	0	0		
IDH1 mutation					
R132H	1 (25%)	7 (44%)	7 (26%)	24 (49%)	21 (70%)
R132C	3 (75%)	8 (50%)	10 (37%)	15 (31%)	5 (17%)
R132S	-	1 (6%)	5 (19%)	5 (10%)	3 (10%)
R132L	-	-	1 (4%)	-	-
R132G	-	-	4 (15%)	-	1 (3%)
Coexisting Mutations					
NPM1	1 (25%)	7 (44%)	15 (56%)	34 (71%)	17 (57%)
FLT3-ITD	-	4 (25%)	10 (37%)	10 (20%)	4 (13%)
CEBPA	-	NA	1(4%)	2 (6%)	8 (27%)

*** Meta-analysis data extracted from Acute Myeloid Leukemia with IDH1 or IDH2 Mutations: Frequency and Clinicopathologic Features study, Am J Clin Pathol. 2011 Jan; 135 (1): 35-45*

The impact of IDH mutations on overall survival was statistically insignificant in comparison with wild type IDH because of small sample size.

This is the first study conducted to study prognostic significance of IDH mutation in AML patients in Oman. Some limitations of our study need to be emphasized: First, this is a "real-world" observational, retrospective, single centre study with a limited number of AML patients harbouring IDH mutations. The effectiveness of the current therapy to clear such mutation could not be assessed due to short survival of patients. We are aiming to increase the sample size of our study by including patients from Royal Hospital and national genetic center in Oman.

Conclusion

In conclusion; the overall frequency of IDH mutations among AML patients was 11.48% (with an IDH1 frequency of 6.56% and an IDH2 frequency of 4.92%). Accordingly, in our study cohort IDH mutant patients also tended to be older age, lower median white cell count and higher median platelet count and blast percentage. In addition, mutated IDH associated with intermediate cytogenetics risk group. We didn`t find prognostic impact of IDH mutation between the study groups. Based on these results and in view of the availability of new targeted therapies for IDH mutations; testing for it to be implemented as a primary diagnostic test is recommended for all newly diagnosed AML cases.

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