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Clinical Outcomes for Patients with Acute Myeloid Leukemia Harboring IDH1 Mutation After Intensive Chemotherapy

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ABSTRACT

Background: Acute myeloid leukemia (AML) is heterogeneous myeloid disorder with multifactorial pathogenic mechanisms and a broad range of prognosis. AML is characterized by clonal proliferation of poorly differentiated cells of the myeloid lineage.

Objective: This study aimed to detect Isocitrate Dehydrogenase 1 (IDH1) mutation in adult Egyptian AML patients and to find correlation between the mutation and prognosis & survival in those patients after intensive chemotherapy.

Patients and Methods: Our study included 98 subjects with newly diagnosed AML. They all presented to Ain Shams University Hospital (Hematology Unit). All Patients included in the study were subjected to: History taking and clinical examination, laboratory investigations including routine investigations and cytogenetic studies for detection of IDH 1 mutation. All patients were followed up for their response to treatment for a period of 6 months.

Results: IDH1 mutation occurs in a considerable percentage of Egyptian AML patients that shows independent bad prognostic impact on the clinical outcome.

Conclusion: IDH1 mutation occurred in a considerable percentage of Egyptian AML patients that showed independent bad prognostic impact on the clinical outcome.

Key words: Myeloid leukemia, IDH1 mutation, Intensive chemotherapy.

INTRODUCTION

Acute myeloid leukemia (AML) is heterogeneous myeloid disorder with multifactorial pathogenic mechanisms and a broad range of prognosis. AML is characterized by clonal proliferation of poorly differentiated cells of the myeloid lineage [(1)]. The pathogenesis involves recurrent genomic alterations, including somatic gene mutations and/or chromosomal abnormalities that can define biologically distinct clinical subtypes [(2)]. Comprehensive genomic profiling at the time of diagnosis can inform disease classification, risk stratification and prognosis and ultimately allow for more selective therapeutic interventions. Alterations to cellular metabolism, as well as somatic mutations of genes essential to epigenetic regulation, are implicated in the pathogenesis of several human malignancies [(3)].

Isocitrate dehydrogenases (IDHs) are homodimeric enzymes involved in diverse cellular processes, including adaptation to hypoxia, histone demethylation and DNA modification. IDH1 enzymes are localized to the cytoplasm and peroxisomes [(4)]. IDH1 protein catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) to produce reduced nicotinamide adenine dinucleotide phosphate. Diverse dioxygenases depend on sufficient levels of α-KG for multiple cellular processes, as well as for epigenetic regulation [(5)].

Somatic mutations in IDH1 (mIDH1) genes have been described in both solid and hematological malignancies [(6)]. IDH1mutations are heterozygous, retaining one wild-type, suggestive of an oncogenic gain of function. IDH proteins are encoded by the IDH1 gene located at chromosome 2q33. Recurrent IDH1 mutations are missense variants leading to a single amino-acid substitution of arginine residues at codon 132 in exon 4 of the IDH1 gene. Additionally, a germline-synonymous single-nucleotide polymorphism (rs11554137) located in codon 105 in exon 4 of the IDH1 gene has been reported to have prognostic relevance in AML[(7)]. The aim of the present study was to detect IDH1 mutation in adult Egyptian AML patients and to find correlation between the mutation and prognosis & survival in those patients after intensive chemotherapy.

PATIENTS AND METHODS

The study included 98 subjects with newly diagnosed acute myeloid leukemia. They all presented to Ain Shams University Hospital (Hematology Unit).

Inclusion criteria: The study included patients with age between 18 to 60 years who are eligible to receive intensive chemotherapy newly diagnosed AML not secondary to another malignancy or autoimmune disease.

Exclusion criteria: Patients with age under 18 years or older than 60 years, patients who are not eligible to receive induction chemotherapy with curative intent thanks to their old age, poor performance status and/or comorbidities and acute Promyelocytic Leukemia.

Patients included in the study were subjected to the following:

History taking and clinical examination, laboratory investigations including complete blood picture, coagulation profile, kidney & liver function tests, electrolytes and LDH. Bone marrow aspirate examination at diagnosis for immunophenotyping by flow cytometry, cytogenetic studies on bone marrow aspirate and molecular study when possible. Detection of IDH 1 mutation by High Resolution Melting- (HRM-PCR). All patients were followed up for their response

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to treatment. Follow up of the patients was done for a period of 6 months, complete remission (CR) in our patients is defined as morphological recovery of the BM and blood counts i.e. neutrophils ≥1, 500/ μL and platelets ≥100, 000/ μL, no circulating leukemic blasts, BM cellularity more than 20% with maturation of all cell lines, no Auer rods, less than 5% BM blasts and no evidence of extra medullary leukemia. Relapse was defined by ≥ 5% BM blasts, reappearance of circulating leukemic blasts or development of extra medullary leukemia and occurrence of CR is observed at day 28 of starting the chemotherapy protocol. The overall survival (OS) for our patients was measured from the date of admission until the date of death, except for patients alive at last follow up.

Molecular detection of IDH1 gene mutation by HRM:

Patients were subjected to the assessment for the presence of IDH1 gene mutation by High Resolution Melting HRM-PCR by using Eco™ Real-Time PCR System with IDHPrimer sequences:

IDH1 -exon4:
IDH1F: 5' ccatttgtctgaaaaactttgcttct 3' - IDH1R: 5' tcacattattgccaacatgactt 3'

The samples were taken at presentation, i.e. before receiving any medication. Bone marrow was the sample of choice. A sample of 1 to 2 ml of bone marrow was aspirated. Whole bone marrow was collected in the presence of an anticoagulant (on EDTA vacutainers).

Principles of the test:

Twenty nanograms of DNA were amplified in a final volume of 10 ul containing 1X High Resolution Melting PCR Master Mix (Type it, Qiagen) with a saturating fluorescent DNA-binding dye, 0.2 mM of each primer and 2.5 mM $MgCl_2$. Primer sequences were (forward IDH1: 5'-ccatttgtctgaaaaactttgcttct-3', reverse IDH1: 5'-tcacattattgccaacatgactt-3', forward IDH2: 5'-tctggttgaaagatggcggc-3' and reversed IDH2: 5'-caagaggatggctaggcgag-3'). One positive control and one non- template control were included in each experiment. All samples were tested in duplicate. Cyclic parameters were as follows: initial denaturation at 95°C for 10 min; 45 cycles of 95°C for 10 s, 58°C for 10 s and 72°C for 20 s. Final melting program was denaturation at 95°C for 1 min, renaturation at 45°C for 1 min and melting from 60°C to 95°C with a ramp of 0.02°C/sec and 25 fluorescence acquisitions/°C [8]. All reactions were performed in duplicate. Wild-type and mutated samples were defined as positive and negative controls in the software. All HRM results were analyzed as fluorescence versus temperature graphs by Eco Illumina software (San Diego, CA) with normalized, temperature-shifted melting curves displayed as difference plot.

Ethical consent:

Ethical approval was waived by the local Ethics Committee of Ain Shams University in view of the prospective nature of the study and all the procedures performed were part of the routine care. Written informed consent was obtained from every participant included in the study. All procedures performed in our study were in accordance with the ethical standards of our institution, national and with the 1975 Helsinki declaration as revised in 2008.

Statistical analysis

The collected data were revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 25). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Mean, standard deviation (± SD) and range were used for parametric numeric data, while median and interquartile range (IQR) for non-parametric numerical data. While, frequency and percentage were used for non-numerical data. Mann Whitney Test (U test) was used to assess the statistical significance of the difference of anon parametric variable between two study groups. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. P≤ 0.05 for significance.

RESULTS

In this study, 61 (62.2%) of the studied cases were males and 37 (37.8%) were females. The mean age was 40.93 ± 12.75 with range of 19-60 years. In this study the mean Initial Blasts percentage in BMA was 63.76 ± 13.81 with range of 21-99. The mean TLC was 50.8 ± 11.23. The mean HB was 7.92 ± 1.59. The mean PLTS was 45.63 ± 10.54 as shown in table (1).

Table (1): Sex, age and clinical laboratory characteristics of the studied patients

		N	%
Sex	Male	61	62.2%
	Female	37	37.8%
	Mean ± SD	**Median (IQR)**	**Range**
Age (years)	40.93 ± 12.75	41 (30 - 54)	19 – 60
Initial Blasts percentage in BMA	63.76%±13.81	70 (50-80)	21-99
TLC	50.8±11.23	29.5 (4.6-70)	-
HB (g/dL)	7.92±1.59	7.95 (6.8-9)	-
PLTS (mcL)	45.63 ± 10.54	30 (18-60)	-

In this study, according to risk classification according to cytogenetics, there were 13 (13.3%) of the studied cases had low risk, 64 (65.3%) had intermediate risk and 21 (21.4%) had high risk. And according to IDH1, there were 92 (93.9%) had wild IDH1 and 6 (6.1%) had mutated IDH1 as shown in table (2).

Table (2): Risk classification according to cytogenetics and IDH1 mutation in all studied patients

		N	%
Risk classification	Low risk	13	13.3%
	Intermediate risk	64	65.3%
	High risk	21	21.4%
IDH1	Wild	92	93.9%
	mutated	6	6.1%

In this study according to BMA results after induction, there were 44 (44.9%) had complete remission, 48 (49.0%) had refractory and 6 (6.1%) had partial response. There was statistically insignificant difference between IDH1 mutation as regards sex and age. There was statistically insignificant difference between IDH1 mutation as regards Initial Blasts percentage in BMA, TLC, HB and PLTS. There was statistically insignificant difference between IDH1 mutations as regards risk classification according to cytogenetics. In this study there was statistically insignificant difference between IDH1 mutations as regards BMA results after induction as shown in table (3).

Table (3): Association between IDH1 mutation and chemotherapy protocols & response and 6 months outcome in all studied patients

		IDH1		Fisher exact test	
		Wild (n =92)	Mutated (n =6)		
		n (%)	n (%)	p value	Sig.
BMA results after induction	Complete remission	41 (44.57%)	3 (50%)	1.000	NS
	Refractory	45 (48.91%)	3 (50%)		
	Partial Response	6 (6.52%)	0 (0%)		

Table (4) showed that there was highly statistically significant difference between mean survivals as regards time to complete remission. But, there was statistically insignificant difference between mean survivals as regards time to death as shown in table (5).

Table (4): Mean survival as regard time to complete remission and lines of chemotherapy

Time to complete remission	Mean survival (95%CI)	Log rank test		
		X2	p value	Sig.
One line	1.08 (0.99 - 1.16)	18.98	<0.001	S
Multiple lines	1.67 (1.39 - 1.94)			
Overall	1.26 (1.14 - 1.38)			

Table (5): Mean survival as regard time to death and lines of chemotherapy

Time to death	Mean survival (95%CI)	Log rank test		
		X2	p value	sig.
One line	3.15 (2.58 - 3.72)	0.96	0.328	NS
Multiple lines	4.68 (4.08 - 5.28)			
Overall	3.54 (3.07 - 4.01)			

There was statistically insignificant difference between mean survivals as regards time to complete remission (Table 6). But, there was statistically significant difference between mean survivals as regards time to death as shown in table (7).

Table (6): Mean survival as regard time to complete remission and IDH1 mutation

Time to complete remission	Mean survival (95%CI)	Log rank test		
		X2	p value	Sig.
Mutated	1.26 (1.13 - 1.39)	0.00	0.967	NS
Wild	1 (1 - 1)			
Overall	1.26 (1.14 - 1.38)			

Table (7): Mean survival as regard time to death and IDH1 mutation.

Time to death	Mean survival (95%CI)	Log rank test		
		X2	P value	Sig.
Mutated	3.71 (3.23 - 4.19)	9.17	0.002	S
Wild	1 (1 - 1)			
Overall	3.54 (3.07 - 4.01)			

DISCUSSION

Acute myeloid leukemia (AML) is a heterogeneous disease in adult with bad prognosis and short overall survival (OS). In spite of advanced chemotherapy protocols, more intensive research provides critical insights on the pathogenesis of AML. Cytogenetically normal AML (CNAML) represents 40–50 % of all AML cases with separate entities in WHO classification [(9)].

Identification of new gene mutations provides useful markers for diagnosis, prognosis assessment, and making therapeutic decision with monitoring therapy. The most frequent of these aberrations are mutations in the nucleophosmin (NPM1) gene, which are present in approximately 50% of these patients. Other common genetic lesions are internal tandem duplications (ITDs) in the Fms-like tyrosine kinase 3 (FLT3) gene that occur in nearly 20–30 % of CN-AML cases. Furthermore, loss-of-function mutations in both Wilms-tumor (WT1) and the CCAAT/enhancer-binding protein alpha (CEBPA) genes are present in about 10 % of CN-AML cases [(10)].

In this study, we demonstrated that there was statistically insignificant difference between IDH1 mutations as regards age and sex. In study to assess prognostic value of IDH1 mutations identified with

PCR-RFLP assay in acute myeloid leukemia patients, **Elsayed *et al.*** [11] found that IDH1-mutated patients showed no significant difference in age and sex as compared to IDH1-wild type group ($p > 0.05$). **Salem *et al.*** [12] found that there was no significant difference in demographic and clinical features between both patients' groups apart from age that was significantly higher within mutated group than in wild group ($p = 0.042$). **DiNardo *et al.*** [13] found that compared with IDH wild-type patients, IDH1 mutated patients were older (median age 67 years vs. 61 years, ($P < 0.0005$) while there was no significant difference in sex between both studied groups. In study to evaluate frequency and clinicopathologic features in acute myeloid leukemia with IDH1 or IDH2 mutation, **Patel *et al.*** [14] found that IDH1-mutated patients showed no significant difference in age and sex as compared to IDH1-wild type group ($p > 0.05$). **Schnittger *et al.*** [15] found that there was no significant difference in age between the IDH1-mutated cases compared to the wild (67.2 vs 65.7 years, $P = .134$).

In this thesis we illustrated that there was statistically insignificant difference between IDH1 mutations as regards FAB classification. **Elsayed *et al.*** [11] found that IDH1-mutated patients showed no significant difference in FAB Classification as compared to IDH1-wild type group ($p > 0.05$). **Patel *et al.*** [14] found that there was no significant difference in FAB classification between both studied groups. **Salem *et al.*** [12] studied the morphological pattern of AML according to French–American–British (FAB) classification in both patients' groups. The most common FAB subtypes noticed in the mutated IDH1 patients were M2 (5/9, 55.6%) followed by M4 (3/9, 33.3%) while M1 subtype was found only in one patient. None of this patient's group had M0, M5, or M6. On the other hand, the most frequent FAB subtypes found in the wild IDH1 group were M4 (14/41, 34.1%) followed by M5 (12/41, 29.3%). This could be attributed to their different patient group; as they included AML patients regardless the cytogenetic state.

In this study, we cleared that there was statistically insignificant difference between IDH1 mutation as regards Initial Blasts percentage in BMA, TlC, HB and PLTS. **Salem *et al.*** [12] found that as regards CBC, there was statistically significant lower platelet count noticed in wild IDH1 than in mutant patients' groups ($p = 0.01$). Both WBCs count and percentage of BM blasts were higher in wild IDH1 than in mutant IDH1 groups but without statistical significance. **Elsayed *et al.*** [11] found that IDH1-mutated patients showed no significant difference in TLC, platelets counts, and percentage of BM blasts as compared to IDH1-wild type group. **Guan *et al.*** [16] found that there were no statistically significant differences between the two IDH1 mutation groups and wild type group with regards to leucocyte count (n.s.), hemoglobin (n.s.), platelet count (n.s.) and the ratio of blast cells (n.s.). **Schnittger *et al.*** [15] found that there were no further parameters like white blood cell count, platelet count, blast count, or history (de novo, preceding MDS or preceding malignancy), detected to be associated with IDH1 mutations. **ElNahass *et al.*** [17] found that Median PB blasts % of mutant IDH was 67.5% (25-96) vs 44 % (0-98) for wild type IDH. Eight/10 (80%) mutant IDH patients had BM blasts ≥ 50% vs only 2/10 (20%) wild type patients.

In study in our hands, we found that there was statistically insignificant difference between IDH1 mutation as regards extramedullary infiltration (hepatomegaly, splenomegaly and lymphadenopathy). **Salem *et al.*** [12] found that IDH1-mutated patients showed no significant difference in hepatomegaly, splenomegaly and lymphadenopathy as compared to IDH1-wild type group.

Our results showed that there was statistically insignificant difference between IDH1 mutations as regards risk classification according to cytogenetics. **ElNahass *et al.*** [17] found that 80% of IDH mutant AML belonged to the intermediate risk group, which was significant to us as only 20% mutant IDH belonged to high risk category. However, when looking at the intermediate risk category 8/33 (24%) of patients were mutant for IDH vs 0/24 (0%) in the low risk category and 2/13 (15%) only in the high risk cytogenetic group. This shows that there is a higher association between IDH mutation and the intermediate risk cytogenetics AML. **DiNardo *et al.*** [13] found that Additionally, IDH-mutated patients were more likely to have intermediate-risk cytogenetics (77% vs 53%, $P < 0.0005$).

In this work, we demonstrated that there was statistically insignificant difference between IDH1 mutations as regards BMA results after induction. **Elsayed *et al.*** [11] found that within 28 of induction chemotherapy 48/100 (48%) of the whole group and 43/93 (46.2%) of the non-APL group achieved CR. There was no association with achievement of CR. Response to induction therapy for IDH1-mutant and IDH1-wild type AML, respectively, was as follows: CR, 12.5% and 87.5% in total AML group and 11.6%, and 88.4% for the non-APL group. **Salem *et al.*** [12] found that among our CN-AML patients, 26/50 (52%) achieve CR. CR rate was significantly higher in patients with wild IDH1 (24/41, 59 %) than in patients with mutated IDH1 (2/9, 22 %) with p value = 0.06.

In this study, we found that that there was statistically insignificant difference between mean survivals as regards time to complete remission. **Salem *et al.*** [12] aimed in his study to assess the prognostic impact of IDH1R132 mutation on patients' outcome. He found that within the nine patients with IDH1R132 mutation, only two responded to therapy and achieved CR. This finding implicates IDH1 mutation as bad prognostic factor on patients' outcome that agreed with some earlier studies. On other hand, other study showed that IDH1R132 mutation does not affect CR rate on such patients. This discrepancy might be explained by variable effect of different therapeutic protocols across the studies. For example, it has been proposed that repeated courses of high doses cytarabine improve the prognosis of patients with RAS mutations and WT1

mutations. The same might be true for IDH1R132 mutation. In addition the presence of other gene mutations in our study was not excluded (NPM1, FLT3, WT1 and other mutations) that may affect the patients' response to therapy. **Elsayed *et al.* (11)** found that IDH1-mutant patients showed no significant difference when compared to IDH1 negative patients in response to therapy, relapse risk or OS when the total AML group was analyzed.

In this study, we found that there was statistically significant difference between mean survivals as regards time to death. **Elsayed *et al.* (11)** found that there was a trend for worse cumulative OS in the IDH1-mutant group compared to IDH1-wild type group, especially when analysis was focused on non-APL AML (16.6% versus 39.6%, p = 0.08). **Salem *et al.* (12)** found that the median OS was significantly longer in cases with wild IDH (18 months; 95 % CI 11–22 months) than mutated cases (7 months; 95 % CI 1–20 months); p = 0.009. In univariate analysis of different prognostic variables, IDH1R132 found to be a bad prognostic indicator for OS as well as old age, low PS and higher BM blasts (p = 0.01, 0.04, 0.02, 0.01 respectively). Moreover, in multivariate analysis, only IDH1 mutation and PS found to have independent significant prognostic impact on the OS of our studied patients (p = 0.05 and 0.04 respectively). **Guan *et al.* (16)** found that the OS in patients with IDH1 non-R132 mutations was not statistically significant from that of patients with wild type IDH1. When the patients with IDH1 non-R132 mutations were grouped on the basis of having received a transplant, the OS in the transplanted group was higher than in the untransplanted group, but they were no statistical difference (*n.s.*).

This study had some limitations, from which small sample size that may affect our results, so further studies with larger sample size and longer follow up duration is needed to establish our results.

CONCLUSION

In conclusion, our study revealed that IDH1 mutation occurred in a considerable percentage of Egyptian AML patients that showed independent bad prognostic impact on the clinical outcome. Further studies on larger CN-AML patient cohort with more detailed molecular profile data are needed to confirm both its prevalence and prognostic impact in the presence of other gene mutations. This will help in stratifying patients for more intensive therapy. Furthermore, more studies are required to clarify the exact pathogenic role that may be used to generate a targeted therapy for patients with this mutation.

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