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Original Research Article

Mutation Analysis and Clinicopathological Implications of Isocitrate Dehydrogenase 1/2 Mutation in Acute Myeloid Leukemia Patients in North India: A Tertiary Centre Based Study

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Abstract

Acute myeloid leukemia has been classified on the basis of morphology, cytochemistry and genetic profile. IDH gene is an epigenetic modifier whose mutation is involved in pathogenesis of various malignancies. This study was planned to study the prevalence of IDH1/2 mutation in denovo AML cases at a tertiary care Centre in North India and also to study its clinicopathological effect in these cases. We evaluated 60 patients registered at our Centre for diagnosis and treatment of AML. Routine investigations, bone marrow examination, flow cytometry were done followed by PCR and sequencing. Out of the total 60 patients of AML 4(6.7%) patients had IDH1R312 mutation and 5(8.3%) Patients had IDH2R172 mutation. IDH2 R140 mutation was not detected in any sample. IDH mutation was significantly associated with high risk AML group. No significant clinicopathological correlation was seen. In this study significant association was observed between IDH mutation and high risk AML cases. There was no paediatric case with IDH mutation. IDH mutation and AML might have an age related association.

Keywords: IDH1/2 mutation, Acute myeloid leukemia.

INTRODUCTION

Acute myeloid leukemia (AML) is a hematological malignancy with poor prognosis. Myeloid progenitor cells acquire certain genetic alterations which affects proliferation and terminal differentiation capacity of these cells. This leads to clonal proliferation of myeloid blasts in peripheral blood, bone marrow or other tissue. Worldwide prevalence is 2.5-3 cases per 100,00 population per year [1]. A hospital based study from north India revealed the prevalence of AML to be 36.5% amongst all leukemias [3]. As per ICMR annual incidence of AML is 2-3 per 100,000 [2]. Incidence of AML increases with age. It accounts for < 10% of all leukemias in children less than 10 years of age and 80-90% of all leukemias in adult population (>60 years). The incidence is higher in males than in females.

Initially classified on the basis of morphology and Cytochemistry; cytogenetics and molecular evaluation is necessary for the more accurate classification of the disease. Around 50% of AML cases show recurrent translocation [4]. Large subpopulation of cytogenetically normal AML (intermediate prognosis group) needs further molecular evaluation as these effects treatment outcome and prognosis. The inclusion of FLT3, NPM1, CEBPA, RUNX1 as entities in WHO 2016 classification emphasizes the impact of molecular alterations in such cases [5].

Many of the studied mutations have defined prognostic significance and treatment options for these cases however there are still large number of mutations that are under study for any coexisting significant consequences. IDH mutation is one of the epigenetic

modifier aberrations whose pathogenetic profile is being studies in various malignancies. IDH mutation in cytogenetically normal AML cases was first identified by Madris *et al*., in 2009 [6]. IDH1/2 are one of these new mutations and researches are ongoing to study its effect on prognosis and treatment response.

In this study, we plan to evaluate the prevalence of IDH1/2 mutation in de novo AML cases, its correlation with various clinicopathological parameters and early treatment response.

MATERIAL AND METHODS

Sample and Study Design

Sixty cases of Acute Myeloid Leukemia registered in Leukemia Lymphoma lab, Department of Pathology, King Georg's Medical University, Lucknow, India, were included in this prospective study. Peripheral blood examination, Bone marrow aspiration, bone biopsy (or clot section in paediatric cases), flow cytometric evaluation, karyotyping and FISH was routinely done on all the cases. The study was approved by the Institutional Ethical Committee board, King George's Medical University, Lucknow. Informed consent was obtained from all patients prior to sample collection.

Direct Sanger Sequencing For IDH

Genomic DNA was extracted from peripheral blood or bone marrow samples using Pure Link genomic DNA mini kit, Invitrogen. Sanger sequencing was performed as previously described. Exon 4 of the *IDH 1 and IDH 2* genes were amplified by polymerase chain reaction (PCR) using the primers pairs [IDH1F (5'- AGCTCTATATGCCATCACTGC-3'), IDH1R (5'-AACATGCAAAATCACATTATTGCC-3'), IDH2F-(5'- AATTTTAGGACCCCCGTCTG-3'), IDH2R (5'-CTGCAGAGACAAGAGGATGG-3')]. For every PCR cycle 30 µl reaction consisted of 100 ng genomic DNA, 12.5 pmol of each oligonucleotide primer, 15µl of master mix and 12µl of DNAse free water .The amplification protocol for IDH1 comprised initial denaturation at 98°C for 30 seconds; 35 cycles of denaturation at 98°C for 10 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 5 minutes using platinum hot start PCR master mix (thermo fisher). For IDH2 initial denaturation at 98°C for 30 seconds; 35 cycles of denaturation at 98°C for 10 seconds, annealing at 61°C for 30 seconds, and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 5 minutes. PCR products were electrophoresed on 2.5% (wt./vol) agarose gels and subsequently sequenced in both sense and antisense direction using the above-mentioned primers. DNA sequences and the *IDH* mutation were determined using a 3730XL DNA analyzer (thermo fisher Scientifics). The results were compared with wild type IDH1 and IDH2 cDNA (genbank accession number NG_023319.2 and NC_000015.10 respectively.

Statistical Analysis

Statistical analyses were performed using SPSS version 19. Categorical variables are presented as numbers with percentages. All *P* values were two-sided and *P* values <0.05 were considered statistically significant.

RESULTS

Cohort demographics

60 newly diagnosed AML cases 37 males (61.2%) and 23 females (38.3%) were included in the study. The median age was 29 years (6 -70). All the subjects belonged either to medium or low socioeconomic status. There was no significant association with dietary pattern or any addiction (tobacco, alcohol or analgesics).

Fever (88%) fatigue (15%) were the most common presenting features. On examination bleeding manifestation, abdominal fullness, respiratory symptoms were present in 30%, 15% and 28.3% cases respectively. Other comorbidities like neurological complains (3%), bone and joint pain (11.7%), UTI (3%), TB (5%), neurofibromatosis (1.7%), facial palsy (1.7%) and fungal pneumonia (33%) were also observed. Lymphadenopathy and hepato splenomegaly were seen in 23.3% and 43.3% cases. Median HB was 6.55g/dl (2.7 – 13.0), median TLC was $14x10^6$cumm (0.5 -199) and median PC was 0.4 x 10^9/L (0.1 – 85). Median protein was 6.4g/dl (3.5 – 12.0), creatinine 0.8 mg /dl (0.5 – 1.83) and LDH was 789.9 IU/L(110.3-7835.0).

Median PB blast were 36% (0 – 98) and BM blast were 70% (22 -98). 78% marrow were hypercellular with 38% having blasts with Auer rod and 53% having dysplastic features. 23% study cases were classified as M0 and 38% as M2.

40% cases belonged to ECOG risk group 4 and 36.7% in risk group 5. AML case distribution among all risk groups was- high risk group – 41.7%, intermediate risk – 33.3% and standard risk -25%.

65% of total AML cases were CN – AML cases. Induction mortality was 30% and complete remission was seen in 55% cases. Median overall survival was 2.24 years.

Result of sequencing for IDH mutation

IDH mutation occurred in 9/60 (15%) patients. Both IDH 1 and 2 were mutually exclusive. IDH1 mutation was observed in 4/60 cases (6.6%) and IDH2 mutation in 5/60 cases (8.3%). FLT3 mutation was see in the 18/60 (30%) cases and NPM1 in (10/60) 16.6%

cases. Both NPM1 and FLT3 mutation was seen in 5/60 cases (8.3%).

In this cohort 3 cases showed IDH1R132c mutation, 1 case had IDH1R132S mutation and 5 cases had IDH2R172K mutation. Both IDH1 and IDH2 mutations were mutually exclusive.

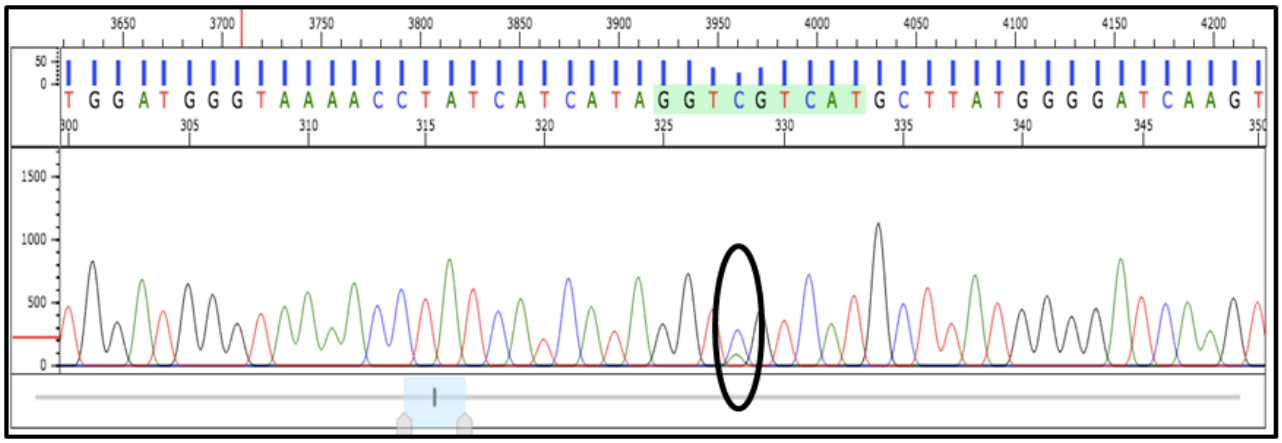

Fig 1: PCR sequence showing IDH1R132S mutation in forward sequence

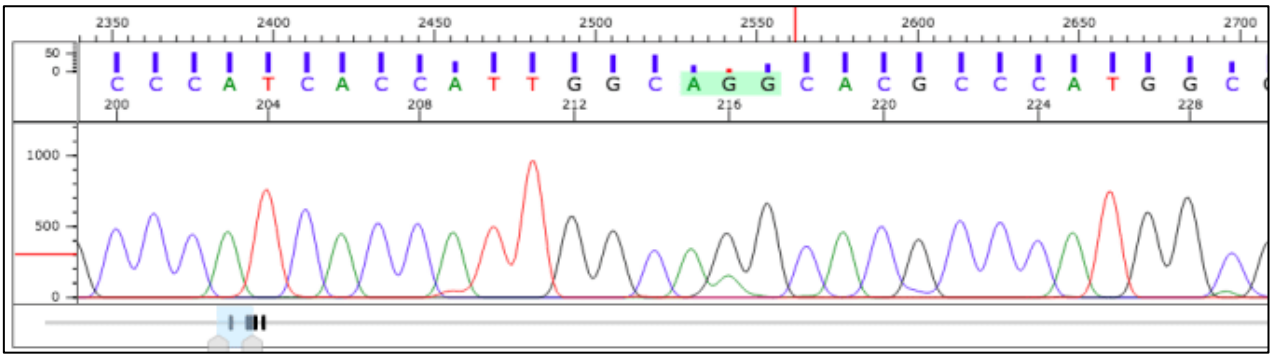

Fig 2: PCR sequence showing IDH2R172K mutation in forward sequence

	02	06	08	14	20	27	28	42	59
CN-AML									
T(8;21)									
T((15;17)									
T(14;22)									
T(9;22)									
T(3;4)									
MLL reg.									
Inv 16									
Del 20q									
Tris. 21									
FLT3-ITD									
FLT3-TKD									
NPM1									
IDH1									
IDH2									
SNP									

Fig 3: Bar Code Graph Depicting Observed Genetic Alteration in IDH Mutated AML Cases

Demographic data of all AML cases with and without IDH mutation has been represented in Table 1.

Table 1: Demographic data of the entire cohort

Sl. No.	Characteristic	IDH wild N %		IDH1 mutated N %	IDH2 mutated N %			P value
1.	Age Group							
	<9	5	9.8	0	0	0	0	
	10-19	8	15.7	0	0	1	20	0.909
	20-50	31	60.8	3	75	3	60	
	>50	7	13.7	1	25	1	20	
2.	Sex							
	Male	33	64.7	2	50	2	40	0.491
	Female	18	35.3	2	50	3	60	
3.	ECOG Score (%)							
	Fully active	0	0	0	0	0	0	
	Ambulatory+ restricted strenuous work	4	7.8	0	0	1	20	0.258
	Ambulatory+ self-care	6	11.8	1	25	2	40	
	Limited self-care	20	39.2	3	75	1	20	
	Completely disabled	21	41.2	0	0	1	20	
	dead	0	0	0	0	0	0	
4.	Cytogenetic aberration							
	Present	16	31.4	2	50	3	60	0.356
	Absent	35	60.6	2	50	2	40	
5.	Prognostic group							
	Favorable	12	23.5	2	50	3	60	
	Intermediate	19	37.2	0	0	2	40	0.359
	adverse	20	39.2	2	50	0	0	
6.	Hemoglobin Median(range) (gm/dl)	6.5(2.7-13.0)		5.3(3.1-7.9)	8.2(7.1-9.1)			0.083
7.	Total Leukocyte Count Median(range) (X 10^6/cumm)	15.0(0.5-199)		15.5(3.2-40.0)	5.4(1.4-84.2)			0.479
8.	Platelet count Median(range)(x 10^9/L)	0.4(0.1-85)		1.95(0.5-3.5)	0.37(0.25-3.64)			0.073
9.	Anemia Grade							
	Grade 1 (10- lower limit of normal gm/dl)	7	13.7	0	0	0	0	0.000
	Grade 2 (8-10 gm/dl)	1	1.96	0	0	3	60	
	Grade 3 (6.5-7.9 gm/dl)	18	35.3	1	25	2	40	
	Grade 4 (<6.5 gm/dl)	25	49.1	3	75	0	0	
10.	S.LDH Median (range) (IU/dl)	832.0(150-7835.0)		798.5(110.3-1008.0)	423.0(301.0-967.0)			0.323
11.	Peripheral blood blast Median (range) (%)	34.0(0-98.0)		69.5(8.0-89.0)	46.0(0-83.0)			0.451
12.	BM cellularity (%)							
	Hypercellular	40	78.4	3	75	4	80	
	Cellular	10	19.6	1	25	1	20	0.993
	Hypocellular	1	1.96	0	0	0	0	
13.	Bone marrow blast Median (range) (%)	66.0(22.0-98.0)		75.5(62.0-90.0)		75.0(41.0-90.0)		0.490
14.	Auer rod (%)							
	Present	21	41.2	0	0	2	40	.264
	Absent	30	58.8	4	100	3	60	
15.	dysplastic features (%)							
	Present	27	52.9	2	50	3	60	.946
	Absent	24	47.1	2	50	2	40	
16.	FAB classification							
	M0	13	25.5	0	0	1	20	
	M1	5	9.8	2	50	2	40	
	M2	20	39.2	1	25	2	40	
	M4	5	9.8	1	25	0	0	
	M5	5	9.8	0	0	0	0	
	M7	3	5.9	0	0	0	0	
17.	NPM1 wild	44	86.3	2	25	4	80	
	NPM1 mutant	7	13.7	2	75	1	20	.168
18.	FLT3ITD wild	35	68.6	3	75	4	100	
	FLT3 ITD mutant	16	31.4	1	25	1	0	.328
19.	Response to induction (%)							
	CR	29	56.8	3	75	3	60	
	Failure	4	7.8	0	0	1	20	.775
	Not applicable/ lost to follow up	18	35.3	1	25	1	20	
20.	Over all survival Median (95% CI) years	2.2(1.28-3.1)		2.6(.5-4.69		3.2(.83-5.56))		.727

Relationship of IDH mutation with various AML risk groups:

To evaluate whether there is any implication of presence of IDH mutation in different risk categories of AML; chi-square test for association was done. It was observed that there was no significant association of mutant type IDH in low risk AML patient, while mutant type IDH gene was significantly associated with moderate risk (P val=0.01) and high risk (P val=0.01) AML cases.

Relationship of IDH mutation with common genetic aberrations in AML cases

To understand whether there is any association between FLT3 ITD mutant and wild type genotypes with presence of IDH mutation, Chi square test was performed. A significant association between the two types was observed (P val <0.05). In cases carrying FLT3 mutant allele, there were significantly higher wild type IDH cases compared to IDH mutant type cases. Similar association was observed in CN AML cases and AML cases with wild IDH allele. Whereas there was no significant difference in IDH mutant or wild type cases numbers in NPM mutant gene cases and cases with t(8;21).

DISCUSSION

Overall frequency of IDH mutation in AML cases was 15%. This result is consistent with the findings of Figueroa *et al*., who studied IDH mutation in denovo AML cases in 2010 [7]. Similar results were later on reported by Koszarska *et al*., in 2013 [8], DiNardo CD *et al*., in 2015 [9], Salah Aref *et al*., in 2015 [10] and Yasser HE *et al*., in 2019 [11]. Marcucci *et al*., [12] had reported IDH mutation prevalence in AML cases as 32.9%. This result could be due to larger cohort size.

In our denovo AML cohort IDH1R132 mutations were found in 8.3% of total population. This is consistent with reported frequencies of 6.0-16.0% in previous studies [13, 14]. Prevalence of IDH1 mutation in CN-AML cases in our studies was 6.1% which is consistent with the reported prevalence. IDH1 R132C and H are the most commonly reported IDH1 mutation in both de novo AML cases and CN-AML. However our study showed IDHIR132C in 3/4 cases and IDH1R132S in 1/4 cases. There was no case with IDH1R132 H mutation in our study.

In Asian population the prevalence of IDH1R132 mutation between the range of 4.4% to 9.6% [13-17]. In 2014 and 2015 Ahmad *et al*., [14] and Raveendran *et al*., [17] studied IDH1 mutation prevalence in Indian population. It was found to be 4.4% and 5.5% respectively.

IDH2 mutation was detected in 6.6% of AML cases and 4.08% of CN-AML cases. No case of IDH2R140 mutation was detected in this cohort. Studies have reported IDH2R140 mutation between 8.3-15.6% and IDH2R172 between 2.5-9% [18-20].

Studies have shown IDH2R140>IDH2R172 in various population [12, 21] . However our study result varied as in our cohort only IDH2R172 mutation was detected. IDH2 R172K substitution is most common IDH2R172 mutation reported. Our study showed only IDH2 R172K mutation which is consistent with the results of Green *et al*., [23]. The paediatric cases in our cohort had no IDH mutation. childhood AML has 1.8% IDH2R140 and one case of reported IDH2R172 mutation.

Over all percentage IDH1 mutation was less than IDH2 mutation in this cohort. This incidence is in agreement with *Papaemmanuil et al*., 2016 [23], Montalban-Bravo *et al*., [24] and DiNardo *et al.*, in 2018 [9]. However other studies have established incidence of IDH2 more than IDH1 in AML cases (Raveendran *et al*., 2015 [17]). In this study IDH1 and 2 were mutually exclusive as reported previously [23, 24]

IDH mutation was correlated with patient characteristics, laboratory findings and prognostic markers of AML. All the patients belonged to North India and were from median to low economics group. IDH mutation was associated with middle age group (20 – 50years). There was a slight but insignificant female preponderance (4M/5F) which was also suggested by Raveendran *et al*., [17].

There was no significant association between Haemoglobin, Total leukocyte count and platelet count (p=0.083, 0.48 and 0.073 respectively). This is in Concordance with the findings Patel *et al*., 2011[19].

No significant association with biochemical parameters like S. protein, S. creatinine and S. LDH. Median BM blast % was equal in both IDH1 and 2 mutation cases. However the median blast % in IDH mutated case was marginally higher than the median blast % of all AML cases. Majority of cases with IDH1 and IDH2 mutation belonged to FAB classification M1 type which is equivalent to AML without maturation-WHO 2016, (percentage being 50% and 40% respectively).

Our study showed IDH mutation was not significantly associated with CN-AML or NMPM1 or FLT3-ITD mutation. However during risk grouping, IDH mutation cases were significantly associated with High risk group (p .015). This was due to significant number of FLT3 ITD positive cases in our cohort. This result is in agreement with others who found FLT3-ITD mutated AML not associated with IDH mutations (Marcucci *et al*., 2010 [12]; Virijevic *et al*., 2016 [25]). However this result also contradict the findings of other

studies who suggested that IDH mutation was associated with FLT3 mutation in AML cases (DiNardo *et al*., 2016 [9]; Papaemmanuil *et al*., 2016 [23], Boddu *et al*., 2017 [26]).

CONCLUSION

It is to be understood that deciphering the status of IDH mutation in AML cases may help to understand the epigenetic pathway. It may also help in developing a).novel genetic marker for MRD (minimal residual disease) evaluation, b). part of epigenetic gene panel to predict disease pathway and outcome; and c). to develop newer, targeted treatment modalities.

To the best of our knowledge this is the first report of IDH mutation study in AML cases in North India. Overall the frequency of IDH1 mutation in North Indian population is similar to those found in international studies. No IDH mutation has been described in children <3 year [27]; this shows a probable age related association between AML and IDH mutation.

Unfortunately, due to lack of available clinical data for the patients in this study cohort, no analysis of outcomes could be performed. As such, although the frequencies of IDH1 and IDH2 mutations could be determined, it was not possible to establish their prognostic impact whether alone, or in combination with NPM1 and FLT3-ITD mutations. IDH prognostic impacts having been based on observational studies and retrospective analysis, future prospective studies or randomized trials are needed. However, the studies to date demonstrate that IDH mutations have a likely unfavourable (or possibly favourable in the case of $IDH2^{R140}$mutations) prognosis impact, which warrants further investigations and implementation in a routine diagnostic set up.

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