

## RESEARCH ARTICLE

## Mutation Analysis of *IDH1/2* Genes in Unselected *De novo* Acute Myeloid Leukaemia Patients in India - Identification of A Novel *IDH2* Mutation

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### Abstract

*IDH1/2* mutations which result in alternation in DNA methylation pattern are one of the most common methylation associated mutations in Acute myeloid leukaemia. *IDH1/2* mutations frequently associated with higher platelet level, normal cytogenetics and *NPM1* mutations. Here we analyzed *IDH1/2* mutations in 200 newly diagnosed unselected Indian adult AML patients and investigated their correlation with clinical, cytogenetic parameters along with cooperating *NPM1* mutation. We detected 5.5% and 4% mutations in *IDH1/2* genes, respectively. Except *IDH2* c.515\_516GG>AA mutation, all the other identified mutations were reported mutations. Similar to reported c.515G>A mutation, the novel c.515\_516GG>AA mutation replaces 172<sup>nd</sup> arginine to lysine in the active site of the enzyme. Even though there was a preponderance of *IDH1/2* mutations in NK-AML, cytogenetically abnormal patients also harboured *IDH1/2* mutations. *IDH1* mutations showed significant higher platelet count and *NPM1* mutations. *IDH2* mutated patients displayed infrequent *NPM1* mutations and lower WBC count. All the *NPM1* mutations in the *IDH1/2* mutated cases showed type A mutation. The present data suggest that *IDH1/2* mutations are associated with normal cytogenetics and type A *NPM1* mutations in adult Indian AML patients.

**Keywords:** Acute myeloid leukaemia - *IDH1* - *IDH2* - Type A *NPM1* mutation

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### Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease with poor outcome as a result of clonal transformation of hematopoietic precursors through gaining multiple gene mutations and chromosomal rearrangements. The cytogenetic analysis of AML cells is routinely used by the clinicians owing to its pivotal role in both diagnosis and predicting outcome in AML patients (Mardis et al., 2009). Recurrent chromosomal structural variations in AML are well established as diagnostic and prognostic markers, suggesting that acquired genetic abnormalities (i.e., somatic mutations) have an essential role in pathogenesis. However, approximately 50% of AML samples have a normal karyotype (NK-AML), and many of these genomes lack structural variations, even when analyzed with high density comparative genomic hybridization or SNP arrays (Ley et al., 2013). A fraction of these NK-AML patients are found to have mutations of genes that normally function in cell proliferation, differentiation and survival (e.g. *NPM1*, *CEBPA*, *FLT3*, *RAS*, *WT1* etc.) (Chotirat et al., 2012). As a result of the

advancement in whole genome sequencing techniques, a number of gene mutations that are related to epigenetic control of the genome have been found in AML patients (Conway et al., 2014). In 2009, using massively parallel DNA sequencing, Mardis et al. found that isocitrate dehydrogenase (*IDH*) which involved in cellular metabolism mutated in significant proportion of NK-AML patients (Mardis et al., 2009).

Among the three *IDH* isoforms (*IDH1*, *IDH2* and *IDH3*), *IDH1* and *IDH2* have 70% structural resemblance in humans (Xu et al., 2004). *IDH1* gene located on 2q33.3 and it produces cytoplasmic and peroxisomal protein. *IDH2* gene located on 15q26.1 and unlike *IDH1* it produces mitochondrial residing protein. *IDH1* and *IDH2* encoding enzymes interconverts isocitrate and  $\alpha$ -ketoglutarate ( $\alpha$ KG) by utilizing nicotinamide adenine dinucleotide (NAD) or NAD phosphate (NADP) as a cofactor (Dang et al., 2010). *IDH1* mutations were first reported in a genome-wide mutation analysis of 22 glioblastoma multiforme (GBM) [World Health Organization (WHO) grade IV astrocytoma] patients (Parsons et al., 2008). Later *IDH1* and *IDH2* mutations

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were reported in acute myeloid leukaemia notably in patients lacking chromosomal abnormalities (Marcucci et al., 2010). Recently IDH mutations have been reported in many cancers, including chondrosarcomas (Amary et al., 2011), acute lymphoblastic leukemia (ALL) (Zhang et al., 2012), MDS (Patnaik et al., 2012), AITL (Cairns et al., 2012), cholangiocarcinomas (Borger et al., 2012) and pancreatic cancer (Ghiam et al., 2012).

In all the reported cases, IDH mutations were heterozygous in nature and frequently seen at evolutionary conserved arginine residues in the substrate binding site of the enzyme (R132 in IDH1 and R140 and R172 in IDH2) (Losman et al., 2013). As a result of this amino acid substitution, it gains a neomorphic function to produce a oncometabolite 2-Hydroxy glutarate (2-HG) from isocitrate instead of  $\alpha$ -ketoglutarate ( $\alpha$ KG) (Dang et al., 2009). This elevated level of 2-HG competes with  $\alpha$ -KG for binding dioxygenases such as Jumonji family of histone demethylases (Xu et al., 2011) and Tet2 (Figueroa et al., 2010), which are involved in DNA demethylation process. As a result, cells with IDH mutation display hypermethylation as well as cellular differentiation blockage (Figueroa et al., 2010).

In previous studies from various countries, the frequency of IDH mutations in AML patients ranges from 2% to 14% for IDH1 and 1% to 19% for IDH2 (Chotirat et al., 2012; Ahmad et al., 2014). Due to these discrepant frequencies it is necessary to study the mutation status in different regions. In India, to date, there is only one study by Ahmad et al. in 2014 investigated IDH1 and IDH2 mutations in *de novo* acute myeloid leukaemia patients (Ahmad et al., 2014). They analyzed IDH1 and IDH2 mutations in a cohort of 45 *de novo* patients exclusively in patients with normal karyotype. To the best of our knowledge, there are no available data regarding IDH1 and IDH2 mutations in cytogenetically unselected adult *de novo* AML patients in India. In the present study, we aimed to characterize IDH1 and IDH2 mutations in large cohort of 200 newly diagnosed unselected adult AML patients and investigate their correlation with clinical and cytogenetic parameters. Based on the previous published data, we also analyzed the frequency and type of NPM1 mutation, one of the most concurrent mutated gene in IDH mutated cases (Chotirat et al., 2012; Im et al., 2014).

## Materials and Methods

### Patients

A total of 200 *de novo* adult AML patients treated at the Regional cancer centre, Trivandrum, India were studied. Informed consent for research purpose was obtained from all the patients and the study approval was obtained from the institutional review board. AML samples were classified according to FAB criteria. The study population consisted of 200 patients, of which 91 were males and 109 were females, with median age 39 years ranging from 18 to 72 years.

### Cytogenetic and FISH analysis

Bone marrow aspirate withdrawn at the time of disease diagnosis was used for classical cytogenetic analysis after

short-term culture. Harvesting and GTG banding were performed as per the standard procedure (Henegariu et al., 2001). Karyotypes were described according to ISCN 2013 (Shaffer et al., 2013). Twenty metaphases were karyotyped using Cytovision Software (Cytovision, USA). FISH analysis was performed to determine recurring gene fusions (*RUNX1-RUNX1T1*, *PML-RARA*, and *CBFB-MYH11*) associated with specific FAB subtypes M2, M3 and M4, respectively using Vysis probes (Abott Molecular/Vysis, Des Plains, IL). The slides were hybridized overnight following the Abott Molecular/Vysis protocol.

### IDH1 and IDH2 mutation detection

Genomic DNA was isolated from peripheral blood or bone marrow samples using standard phenol-chloroform method. Exon 4 of IDH1 and IDH2 were amplified by polymerase chain reaction using the primer pair; IDH1F (5'-AGCTCTATATGCCATCAC TGC-3'), IDH1R (5'-AACATGCAAATCACATTATTGCC-3'), and IDH2F (5'-AATTTT AGGACCCCGTCTG-3'), and IDH2R (5'-CTGCAGAGACAAGAGGATGG-3') (Chotirat et al., 2012). The PCR performed in a 25  $\mu$ l reaction containing 50 ng of genomic DNA, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 12.5 pmol of each oligonucleotide primers, and 0.5 units of Taq polymerase (Merck, Germany). The PCR condition consisted of an initial denaturation step at 98°C for 30 seconds, followed by 35 cycles at 98°C for 10 Sec, 62°C for 30 Sec, 72°C for 30 Sec and a final step at 72°C for 5 min for IDH1 and initial denaturation step at 98°C for 30 seconds, followed by 35 cycles at 98°C for 10 Sec, 61°C for 30 Sec, 72°C for 30 Sec and a final step at 72°C for 5 min for IDH2. Then the PCR products were analyzed for single-strand mobility patterns and the samples showing altered mobility were sequenced in both sense and antisense directions to confirming the mutations. The sequences were compared to the wild type IDH1 and IDH2 cDNA (GenBank Accession number, NM\_005896.2 and NM\_002168.2, respectively).

### NPM1 mutation detection

For the screening of the NPM1 mutation, we amplified genomic DNA corresponding to exon 12 of NPM1 by PCR using the primers NPM1F (5'-CTAGAGTAACTCTCTGGTGG-3') and NPM1R (5'-CCTGGACAACATTTATCAAAC-3') as previously reported (Ahmad et al., 2009). The Briefly PCR reaction was performed in a 25  $\mu$ l reaction containing 50 ng of genomic DNA, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 10 pmol of each oligonucleotide primers, and 0.5 units of Taq polymerase (Merck, Germany). The PCR condition consisted of an initial denaturation step at 95°C for 5 min followed by 35 cycles at 94°C for 30s, 57°C for 1 min, 72°C for 1 min and a final step at 72°C for 10 min. Then the PCR products were analyzed for single-strand mobility patterns and the samples showing altered mobility were sequenced for confirming the mutations. The sequences were compared to the wild type NPM1 cDNA (GenBank Accession number, NM\_002520).

### Statistical analysis

Data was analyzed using the SPSS software 21. Chi-

Square test and Fisher's exact test was used to find the association between the status of IDH and other covariates such as gender, FAB, NPM1 mutation and cytogenetics. The Mann Whitney U test used to find the difference in the distribution of covariates such as WBC, BLAST (%), HB (g/%) and PC (cmm) with status of IDH. A p value <0.05 was taken as statistically significant.

## Results

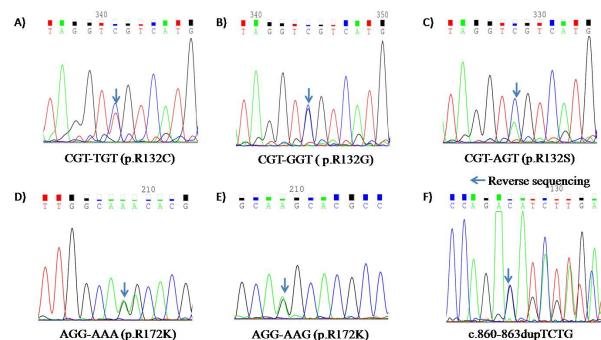
### Characterization of IDH1 and IDH2 mutations

In a total of 200 *de novo* AML patients, 19 (9.5%) patient samples showed aberrant band shifts in PCR-SSCP analysis. Further, it was confirmed as mutation by sequencing analysis. IDH mutations were predominantly found in IDH1 as eleven (5.5%) patients had IDH1 mutations. There were three types of missense IDH1 mutations, including the most reported c.394C>T; p.R132C in nine cases, c.394C>G; p.R132G in one case and c.394C>A; p.R132S in one case (Table 1) (Fig 1.A,B&C). All the mutated cases showed a change in 132<sup>nd</sup> arginine to cysteine/glycine/serine. Apart from these, three patients had a coexisting IDH1 mutation and previously reported prognostically relevant synonymous SNP rs11554137 (IDH1105<sup>GGT</sup>). Among these, two patients had both c.394C>T and heterozygous IDH1 SNP rs11554137 and one patient had concurrent c.394C>A and homozygous IDH1 SNP rs11554137. Regarding IDH2 mutations, only eight (4%) patients had an IDH2 missense mutation, included c.515G>A; p.R172K in seven cases and c.515\_516GG>AA; p.R172K in one case (Table 2) (Fig 1.D & E). Similar to the IDH1 mutations, these cases also showed a change in arginine amino acid. Among this c.515\_516GG>AA was a novel mutation that has not yet

been reported. No mutated cases harbored mutations both in IDH1 and IDH2 genes, indicating that these mutations are mutually exclusive. All the mutated cases were heterozygous in nature and retained the wild type allele.

### Correlation of IDH1 and IDH2 mutations with clinical parameters and morphological subtypes

IDH1 mutations were more predominant in female patients than in male patients (3M/8F). However, the difference was not significant. The median age was 52 in IDH1 mutated cases compared to 38 in unmutated cases. IDH1 mutated subjects had significantly higher platelet count and Hb level compared with the unmutated cases ( $P<0.001$  and  $P<0.05$ , respectively). There was no significant statistical difference in WBC counts and bone marrow blasts between IDH1 mutated and wildtype cases (Table 3). Unlike IDH1 cases, IDH2 mutated subjects had



**Figure 1. Representative electropherograms of the IDH1, IDH2 and NPM1 Mutations. (A) c.394C>T (p.R132C) (IDH1) (B) c.394C>G (p.R132G) (IDH1) (C) c.394C>A (p.R132S) (IDH1) (D) c.515\_516GG>AA (p.R172K) (IDH2) (E) c.515G>A (p.R172K) (IDH2) (F) c.860-863dupTCTG**

**Table 1. The Mutation Patterns and Type of NPM1 Mutations in AML Patients with IDH1 Mutations**

Sl.No.	Age/Sex	FAB	Karyotype	Mutation	Nucleotide change	Aminoacid change	Type of NPM1 mutation
1	24/F	M1	Normal	c.394C>T	CGT-TGT	p.R132C	None
2	57/F	M2	Normal	c.394C>G	CGT-GGT	p.R132G	c.860-863dupTCTG
3	52/F	M1	Abnormal	c.394C>T	CGT-TGT	p.R132C	c.860-863dupTCTG
4	40/F	M1	Abnormal	c.394C>T	CGT-TGT	p.R132C	c.860-863dupTCTG
5	32/M	M2	Normal	c.394C>T	CGT-TGT	p.R132C	c.860-863dupTCTG
6	39/F	M1	Normal	c.394C>T	CGT-TGT	p.R132C	None
7	52/M	M1	Normal	c.394C>T	CGT-TGT	p.R132C	c.860-863dupTCTG
8	62/F	M1	Normal	c.394C>T	CGT-TGT	p.R132C	c.860-863dupTCTG
9	54/F	M1	Normal	c.394C>A	CGT-AGT	p.R132S	c.860-863dupTCTG
10	54/F	M2	Normal	c.394C>T	CGT-TGT	p.R132C	None
11	47/M	M1	Normal	c.394C>T	CGT-TGT	p.R132C	c.860-863dupTCTG

**Table 2. The Mutation Patterns and Type of NPM1 Mutations in AML Patients with IDH2 Mutations**

Sl.No.	Age/Sex	FAB	Karyotype	Mutation	Nucleotide change	Aminoacid change	Type of NPM1 mutation
1	44/F	M1	Normal	c.515G>A	AGG-AAG	p.R172K	c.860-863dupTCTG
2	26/M	M5	Abnormal	c.515G>A	AGG-AAG	p.R172K	None
3	52/F	M5	Abnormal	c.515_516GG>AA	AGG-AAA	p.R172K	None
4	70/M	M5	Normal	c.515G>A	AGG-AAG	p.R172K	None
5	55/M	M5	Normal	c.515G>A	AGG-AAG	p.R172K	None
6	63/F	M2	Normal	c.515G>A	AGG-AAG	p.R172K	None
7	56/M	M5	Normal	c.515G>A	AGG-AAG	p.R172K	None
8	34/F	M1	Normal	c.515G>A	AGG-AAG	p.R172K	None

**Table 3. Clinical Details of AML with IDH1 Mutations**

	Total (%)	IDH1 <sup>m</sup>	IDH1 <sup>wt</sup>	p-value
All patients	200	5.5%(11/200)	94.5% (189/200)	
Male/Female Ratio	91/109	3/8	88/101	NS
Median age, Yrs(range)	39(18-72)	52(24-62)	38(18-72)	NS
Median WBC, X10 <sup>9</sup> /L(range)	16.7(0.7-404)	5.8(1.3-71.4)	17.1(0.7-404)	NS
Median platelet, X10 <sup>9</sup> /L( range)	38.5(0.7-367)	128(20-285)	36(0.7-367)	<0.001
Median Hb, gm%(range)	8.1(1.8-17)	9.4(7.7-10.6)	8(1.8-17)	<0.05
Median BM Blast, %(range)	72(6-99)	70(48-94)	72(6-99)	NS
FAB subtypes				
AML-M0	0.5%(1/200)	0	100%(1/1)	-
AML-M1	22.5%(45/200)	17.8%(8/45)	82.2%(37/45)	NS
AML-M2	22.5%(45/200)	6.7%(3/45)	93.3%(42/45)	NS
AML-M3	13.5%(27/200)	0	100%(27/27)	-
AML-M4	19%(38/200)	0	100%(38/38)	-
AML-M5	21%(42/200)	0	100%(42/42)	-
AML-M6	1%(2/200)	0	100%(2/2)	-
AML-M7	0	0	0	-
Cytogenetics				
Total	200			
Total cytogenetics	187			
Normal karyotype	55%(110/200)	8.2%(9/110)	91.8%(101/110)	NS
Abnormal				
t(8;21)	13.5%(27/200)	0	100%(27/27)	-
t(15;17)	11.5%(23/200)	0	100%(23/23)	-
inv(16)	4%(8/200)	0	100%(8/8)	-
Trisomy 8	2%(4/200)	0	100%(4/4)	-
Other trisomies	2.5%(5/200)	0	100%(5/5)	-
Monosomies or deletions	2%(4/200)	0	100%(4/4)	-
Complex	3%(6/200)	33.3%(2/6)	66.7%(4/6)	NS
Unknown	6.5% (13/200)	0	100%(13/13)	-
NPM1	21.5%(43/200)	72.7%(8/11)	18.5%(35/189)	<0.001

\*NS: not significant

significantly higher median age compared to unmutated cases (53.5 vs 38.5,  $p < 0.05$ ). However, except significant variation in WBC count ( $p < 0.05$ ) other clinical parameters does not show any statistical correlation between IDH2 mutated and wild type cases (Table 4).

Concerning FAB subtypes in IDH mutated cases, the frequency of IDH1 mutations was higher in AML M1 (8/11) followed by AML M2 (3/11). In IDH2 mutated cases, the majority were in AML M5 (5/8) followed by AML M1 (2/8) and AML M2 (1/8). None of the cases with Undifferentiated acute myeloblastic leukemia (AML M0), Acute promyelocytic leukemia (AML M3), Acute myelomonocytic leukemia (AML M4), Acute erythroid leukemia (AML M6), and Acute megakaryoblastic leukemia (AML M7) was found among IDH mutated cases (Table 3, Table 4).

#### *Correlation of IDH1 and IDH2 mutations with chromosomal pattern and NPM1 mutation*

Karyotypic analysis was available in 187 out of 200 samples. Among these, 110 patients had a normal and 77 had an abnormal karyotypic pattern. In eleven IDH1 mutated cases there were a higher frequency of patients in the normal karyotype AML group (9/110) compared to abnormal karyotype group (2/77). However, the difference was not significant ( $p > 0.05$ ). In IDH1 mutated abnormal karyotype patients, we found rare chromosomal translocations like t(X;2) and t(8;17). Similar to IDH1 mutated cases, in eight IDH2 mutated patients, six of them had a normal karyotype (6/110). The rest of the mutated

patients showed numerical chromosomal abnormalities (eg. +8). Noticeably, there were no IDH mutations in cases with recurrent chromosomal abnormalities (eg. t(15;17), t(8;21) and inv(16)). Analysis of frequency and type NPM1 mutation in the IDH mutated cases revealed an interesting result. Among eleven IDH1 mutated patients, we found 8 cases with NPM1 mutation with a statistical significance  $p = 0.001$ . Unlike IDH1 mutated cases, in eight IDH2 mutated patients only one case showed NPM1 mutation. Strikingly, all the NPM1 mutations were type A mutation (c.860\_863dupTCTG) (Figure 1F) that causes a change in the reading frame of the C-terminal portion of nucleophosmin.

## **Discussion**

Although IDH mutations were extensively studied in AML around the world, only one study by Ahmad et al. in a 45 normal karyotype AML (NK-AML) patients had published from India (Ahmad et al., 2014). In the present study, we have analyzed IDH1/2 mutations in a large cohort of 200 newly diagnosed unselected adult AML patients and investigated their correlation with clinical, cytogenetic parameters along with NPM1 mutation. In this study, IDH mutations were observed in 19 patients, which include eleven patients with IDH1 mutations and eight patients with IDH2 mutations.

Among IDH mutations, IDH1 mutations are one of the predominant mutations in glioma and the second most common in acute myeloid leukaemia. The frequency of



**Table 4. Clinical Details of AML with IDH2 Mutations**

	Total (%)	IDH1 <sup>m</sup>	IDH2	p-value
All patients	200	4%(8/200)	96%(192/200)	
Male/Female Ratio	91/109	4/4	87/105	NS
Median age, Yrs(range)	39(18-72)	53.5(26-70)	38.5(18-72)	<0.05
Median WBC, X10 <sup>9</sup> /L(range)	16.7(0.7-404)	2.2(0.7-116)	17.7(0.8-404)	<0.05
Median platelet, X10 <sup>9</sup> /L ( range)	38.5(0.7-367)	63(5-208)	37(0.7-367)	NS
Median Hb, gm%(range)	8.1(1.8-17)	8.1(7.3-10.3)	8.1(1.8-17)	NS
Median BM Blast. %(range)	72(6-99)	76(27-96)	71.5(6-99)	NS
FAB subtypes				
AML-M0	0.5%(1/200)	0	100%(1/1)	-
AML-M1	22.5%(45/200)	4.5%(2/45)	95.5%(43/45)	NS
AML-M2	22.5%(45/200)	2.2%(1/45)	97.8%(44/45)	NS
AML-M3	13.5%(27/200)	0	100%(27/27)	-
AML-M4	19%(38/200)	0	100%(38/38)	-
AML-M5	21%(42/200)	11.9%(5/42)	88.1%(37/42)	NS
AML-M6	1%(2/200)	0	100%(2/2)	-
AML-M7	0	0	0	-
Cytogenetics				
Total	200			
Total cytogenetics	187			
Normal karyotype	55%(110/200)	5.5%(6/110)	94.5%(104/110)	NS
Abnormal				
t(8;21)	13.5%(27/200)	0	100%(27/27)	-
t(15;17)	11.5%(23/200)	0	100%(23/23)	-
inv(16)	4%(8/200)	0	100%(8/8)	-
Trisomy 8	2%(4/200)	25%(1/4)	75%(3/4)	NS
Other trisomies	2.5%(5/200)	20%(1/5)	80%(4/5)	NS
Monosomies or deletions	2%(4/200)	0	100%(4/4)	-
Complex	3%(6/200)	0	100%(6/6)	-
Unknown	6.5%(13/200)	0	100%(13/13)	-
NPM1	21.5%(43/200)	12.5%(1/8)	21.9%(42/192)	NS

\*NS: not significant

IDH1 mutations in AML patients from various countries ranges from 2% to 14% (Chotirat et al., 2012; Ahmad et al., 2014). In the present study, we got 5.5 % IDH1 mutation which is comparable with most of the previous reports from other countries (Abbas et al., 2010; Figueroa et al., 2010; Patel et al., 2011). Considering the IDH1 mutation frequency in Asian countries, our mutation frequency is similar to Taiwan (5.5%), almost similar to Chinese (3.6%, 5.9% and 6.7%) and somewhat lesser to Thailand (8.7%) and Japanese (8.6%) reports (Chou et al., 2010; Zou et al., 2010; Lin et al., 2011; Zhang et al., 2011; Chotirat et al., 2012; Yamaguchi et al., 2014). This discrepancy in the incidence rate may be due to ethnic variation (Zou et al., 2010). The majority of the reported mutations in the IDH1 gene bring about an amino acid substitution at 132<sup>nd</sup> arginine residue in the substrate binding site of the enzyme (Paschka et al., 2010). Among the three IDH1 missense mutations (c.394C>T, p.R132C; c.394C>A, p.R132S and c.394C>G, p.R132G), c.394C>T was the predominant mutation (n=9, 82%), followed by c.394C>A (n=1, 9%) and c.394C>G (n=1, 9%). The predominance of c.394C>T, p.R132C mutation in our study is similar to most of the Asian studies (Chou et al., 2010; Zou et al., 2010; Yamaguchi et al., 2014). Apart from these mutations, three patients had both IDH1 mutation and previously reported adverse prognostic silent polymorphism, rs11554137 (IDH1105GGT) (Wagner et al., 2010; Ho et al., 2011).

The frequency of IDH2 mutations in AML patients

in earlier studies ranges from 1% to 19% (Chotirat et al., 2012; Ahmad et al., 2014). In the present study, we got 4 % IDH2 mutation. But the incidence rate of IDH2 mutations is significantly lower than all Asian reports [Thailand (10.4%) and Japan (8.2%)] and majority of the western reports (Paschka et al., 2010; Figueroa et al., 2010; Ward et al., 2010; Abbas et al., 2010; Chotirat et al., 2012; Yamaguchi et al., 2014). Among the reported IDH2 mutations in acute myeloid leukaemia, R140Q mutations is the predominant mutations followed by R172K; bring about a change in arginine residue of the enzyme (Abbas et al., 2010; Chou et al., 2010; Ward et al., 2010). Contradictory to the most of the Asian reports where the most predominant mutations are R140Q, all our identified mutations were R172K (c.515G>A, p.R172K; c.515\_516GG>AA, p.R172K) (Chotirat et al., 2012; Yamaguchi et al., 2014). Among these c.515G>A, the most reported IDH2 mutation in AML was the predominant mutation (n=7, 87.5%), followed by a previously unreported complex- compound substitution c.515\_516 GG>AA (n=1, 12.5%). Alike c.515G>A mutation, the novel c.515\_516GG>AA mutation replaces 172<sup>nd</sup> arginine to lysine in the active site of the enzyme. Owing to this similar change we hypothesized that the novel c.515\_516GG>AA might have a similar effect as c.515G>A in AML pathogenesis.

Out of nineteen mutated IDH subjects, fifteen (15/19, 79%) of them showed a normal karyotype pattern, which

included nine cases with IDH1 mutation (9/11, 82%) and eight patients with IDH2 mutation (6/8, 75%). Significant predominance of *IDH1/2* mutations in CN-AML in our study matched with previous reports (Mardis et al., 2009; Marcucci et al., 2010; Paschka et al., 2010; Yamaguchi et al., 2014). Concerning NK AML, among 110 cases, IDH, IDH1 and IDH2 mutations were observed in 13.6% (15/110), 8.2% (9/110) and 5.4% (6/110) of cases respectively. The frequency of IDH mutations in NK AML was somewhat similar to the previous Indian report by Ahmed et al, where IDH mutation frequency was 15.4% (Ahmad et al., 2014). On the other hand, in the present study AML patients showed a preponderance of IDH1 mutation (8.2% vs 4.4%) rather than IDH2 mutation (5.4% vs 11.1%) in previous study. The discrepancy might be as a result of difference in the size of the cohort. Noticeably similar to the report by Patel et al, we could not identify any IDH mutations in patients harbouring recurrent chromosomal abnormalities like t(8;21), t(15;17) and inv(16) (Patel et al., 2011).

Concerning the clinical and haematological parameters, unlike previous Indian report, IDH1 mutations in present study shows predominance in females rather than males (3M/8F). However, it was comparable with German and Thailand study (Schnittger et al., 2010; Chotirat et al., 2012). Higher platelet counts are commonly found with *IDH1/2* mutated subjects compared to wild type cases (Im et al., 2014). In the present study, only IDH2 mutated cases showed a significant higher platelet count among *IDH1/2* mutated cases ( $p=0.001$ ). With respect to FAB subtypes, we observed a higher frequency of IDH1 mutations in Acute myeloblastic leukemia with minimal maturation (AML M1) (8/11) which were similar to other reports (Mardis et al., 2009; Schnittger et al., 2010; Patel et al., 2011). Unlike majority of the previous reports, we observed a predominance of IDH2 mutations in monocytic leukemia (AML M5) (5/8) instead of AML M1/M2 (Patel et al., 2011; Chotirat et al., 2012).

Previously published data from various countries including India shows that IDH mutations, especially IDH1 mutations are significantly associated with NPM1 mutations (Chotirat et al., 2012; Yamaguchi et al., 2014; Ahmad et al., 2014). Based on the published data, we performed mutation analysis of NPM1 gene in our cohort. Strengthening the previous reports, our results showed concurrent IDH1 and NPM1 mutations in eight (8/11, 73%) patients with a statistical significance  $p=0.001$ . Noticeably all the NPM1 mutations were type A mutation (c.860\_863dupTCTG). Unlike IDH1 mutated subjects, IDH2 mutated cases showed an infrequent mutations in both NPM1 and IDH2 gene (1/8, 12.5%) which were different from most of the other reports (Abbas et al., 2010; Chotirat et al., 2012). To the best of our knowledge, this is the first report analyzing the type of NPM1 mutations in IDH mutated cases. The frequent association IDH1 and NPM1 mutation strengthen the 2-hit theory in the leukaemiogenesis (Chou et al., 2010).

The definite mechanism behind the involvement of IDH1 and IDH2 mutations in leukaemiogenesis are elusive. Alike our report, all the reported IDH1 or IDH2 mutations are heterozygous and mutually exclusive. The

majority of the reported IDH1 gene mutations brings about a change in the Arg-132 to other amino acids like Cys, His, Leu or Ser. Mutations in IDH2 are more frequently at R140 and less frequently at R172 (Marcucci et al., 2010). The mutations that occur at a single amino acid residue of the IDH1 and IDH2 active site not only reduce the enzyme's ability to catalyze conversion of isocitrate to  $\alpha$ -ketoglutarate but also acquire a new ability to catalyze the NADPH-dependent reduction of  $\alpha$ -ketoglutarate to R(-)-2-hydroxyglutarate (2HG) (Dang et al., 2009; Ward et al., 2010). The recent studies also suggest that the excess 2HG which accumulates *in vivo* contributes to the formation and malignant progression. Furthermore, the majority of the IDH mutations in AML is associated with mutations in NPM1 gene, one of the first hit mutated gene in AML (Im et al., 2014). So it was speculated that IDH mutations are likely to be associated with disease progression rather than disease initiation (Chotirat et al., 2012). Even though patients with mutated IDH1 have a better prognosis in glioma, the prognostic significance of IDH mutations in AML is still unclear (Van den Bent et al., 2010; Döhner et al., 2011; Shih et al., 2012). However, recent studies show that IDH1 mutations are associated with favourable prognosis in AML patients with normal karyotype and an NPM1 mutation (Patel et al., 2012; Yamaguchi et al., 2014).

To summarize, we identified 9.5% IDH mutations, predominantly IDH1 mutations in 200 unselected *de novo* adult Indian AML patients. Even though there was a preponderance of IDH mutations in NK-AML, cytogenetically abnormal patients also harboured IDH mutations. IDH1 mutations display significant higher platelet count and NPM1 mutations. IDH2 mutated patients showed infrequent NPM1 mutations and lower WBC count. Noticeably all the NPM1 mutations in the IDH mutated cases were type A mutation. Identified IDH2 novel c.515\_516GG>AA mutation replaces 172<sup>nd</sup> arginine to lysine in the active site of the enzyme. Further studies with survival analysis of patients are needed for the prognostic stratification of IDH mutations in AML.

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