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Acute Myeloid Leukaemia with Gene Mutation: A Correlation with Haematological and Immunophenotypic Characteristics and Our Experience in a Tertiary Care Cancer Center in South India

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ABSTRACT

Objective: Molecular genetic analysis of FLT3, NPM1, and CEBPA is already the standard of care in patients with acute myeloid leukaemia (AML) and represents the most frequent genetic alterations and important diagnostic and prognostic indicators. This study was undertaken to determine the frequency of FLT3 and NPM1 gene mutations in our institution and to characterize the association between gene mutations and haematological parameters as well as immunophenotypic features.

Material and Method: Morphological, haematological and immunophenotypic characteristics of NPM1 and FLT3 mutations in 126 patients of de novo AML including adults and children were studied. Apart from the French American British (FAB) method for classification, blasts were assessed for cuplike morphology as per strict definition for cuplike nuclei, ≥10% blasts with nuclear invaginations ≥25% of the nuclear area.

Results: FLT3 mutation in 31/126 (25%) and NPM1 mutation was found in 17/126 (13.4%) of the AML patients. 6 (5%) samples were positive for both NPM1 and FLT3/ITD mutations. Associations between the FLT3 and NPM1 gene mutations with haematological and immunophenotypic characteristics are reported.

Conclusion: The results suggest that presence of distinct morphology and haematological and immunophenotypic characteristics together may serve as important indicators and surrogate for NPM1 and FLT3/ITD mutations. Further, comprehensive studies on the biological effects of NPM1 and FLT3/ITD mutations and their interactions with other genetic alterations are needed to gain insight into the molecular mechanism of these mutations involved in the pathogenesis of AML.

Key Words: Acute myeloid leukemia, FLT3, NPM1, South India

INTRODUCTION

Acute Myeloid Leukemia (AML) develops from malignant transformation of immature haematopoietic cells through a complex multistep process that requires co-operation of different types of genetic alterations (1). Several molecular abnormalities have been shown to have prognostic importance in patients with AML. Genetic testing for FLT3, NPM1, and CEBPA is already the standard of care in patients with Acute Myeloid Leukemia (AML) and represents the most frequent genetic alterations and important diagnostic and prognostic indicators. The study presented here was conducted to determine the frequencies of FLT3, NPM1 gene mutations in patients of de novo AML. Associations of these two gene mutations with haematological parameters and immunophenotypic markers were also evaluated.

MATERIAL and METHODS

Retrospective observational study carried out in the Department of Laboratory Medicine, Basavatarakam Indo-American Cancer Hospital and Research Institute (BIACH&RI), Hyderabad. 126 cases (113 adults, 13 children) with confirmed diagnosis of AML on morphology, cytochemistry, immunophenotyping and tested for FLT3 and NPM1 gene mutations during Jan 2008 - July 2014 were included in the study. Cases diagnosed as AML on morphology, cytochemistry and/or immunophenotyping but not worked up further for genetic abnormalities were excluded from the study.

Morphology and Cytochemical Stains

Peripheral blood and bone marrow smears were stained with Leishman and Giemsa stain and cytochemistry was performed by standard methods (Myeloperoxidase-MPO;

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Periodic Acid Schiff-PAS; non specific esterase-NSE). Apart from the French American British (FAB) method for classification, blasts were assessed for cuplike morphology as per strict definition for cuplike nuclei, ≥10% blasts with nuclear invaginations ≥25% of the nuclear area.

Immunophenotyping

Flow cytometry was performed on P.B/B.M on a 4 colour Flowcytometer (FACSCalibur, Becton Dickinson, San Jose, CA,USA) using a standard protocol. Monoclonal antibodies used in this study included fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll-protein (Per-CP) or allophycocyanin (APC) labelled CD45, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD14, CD19, CD22, CD33, CD34, CD38, CD56, CD117, HLA-DR, Tdt, Cyto MPO and isotype control IgGs.

Molecular Tests

PCR for FLT3 and NPM1 mutations was carried out at an NABL accredited lab. FLT3/ITD mutations were assessed by using specific primers for exon 11, 12 and D835 mutation by Restriction Fragment Length Polymorphism (RFLP) mediated assay using primers flanking the mutation site. For NPM1 mutation, amplification of exon 12 of the NPM1 gene was carried out followed by analysis of PCR products using automated DNA sequencing.

Statistical Analysis

The data was subjected to statistical analysis using unpaired student's t-test, Chi square test and linear regression analysis.

RESULTS

A total of 126 AML cases were tested for gene mutations of FLT3 and NPM1 by PCR. The male-to-female ratio was 1.5:1 (76 males: 50 females). The age of diagnosis ranged from 12 to 62 years with a mean of 37.5 years. 113 (89.6%) patients were adults (18 years or older) while the remaining 13 (10.4%) patients were children (18 years or younger). A distinct cuplike morphology of the blasts (Figure 1) was seen in 81% of the cases positive for FLT3 and/or NPM1 mutation. FLT3 mutation was found in 31/126 (25%) and NPM1 mutation in 17/126 (13.4%) of the AML patients. 6 (5%) samples were positive for both NPM1 and FLT3/ITD mutations. FLT3/ITD mutation was associated with higher white blood count (P= 0.008) and higher blast count in the peripheral blood (P= 0.003) (Table I). Cuplike morphology of the blasts was seen in 81% of the cases positive for FLT3 and/or NPM1 mutation. Immunophenotypically, the NPM1 mutation was associated with the lack of CD34 (P= 0.009), while the FLT3/ITD mutation was positively associated with the expression of CD117 and CD7 (P= 0.04). CD34 and CD14 were found to be most important markers for diagnosis in NPM1 cases while CD117 and CD7 co-expression was found to be predictive for FLT3 mutations by regression analysis. Immunophenotypic expression of markers in FLT3 and NPM1 group has been summarized in Table II.

DISCUSSION

Genetic alterations in AML are known to be major determinants of the patients' response to therapy and outcome besides their role in the pathogenesis of the disease. FLT3 and NPM1 mutations have been shown to be the most prevalent somatic alterations in AML, particularly in cytogenetically normal AML. In the current study, the frequency of NPM1 and FLT3/ITD mutations was 25% (31/126) and 13.4% (17/126) in adults, respectively. Frequency of FLT3/ITD mutation is similar to that reported in Japanese (22.6%) (2) and in German adult AML population (21%) (3). However, NPM1 mutations seem to occur at much lower frequency in our setup as compared to Japanese (24.9%) and German cohort (27.4%). The frequency of both FLT3/ITD and NPM1 mutations was higher in Southeast Asian region as compared to our study (33% vs. 25% and 26% vs. 13.4% respectively) (4). Chauhan et al. from North India reported the same frequency of FLT3/ITD mutation as in our study, however NPM1 mutation was found to be higher (21% vs. 13.4%) (5). Patients with FLT3/ITD mutation had significantly higher white blood counts compared to patients without FLT3/ITD

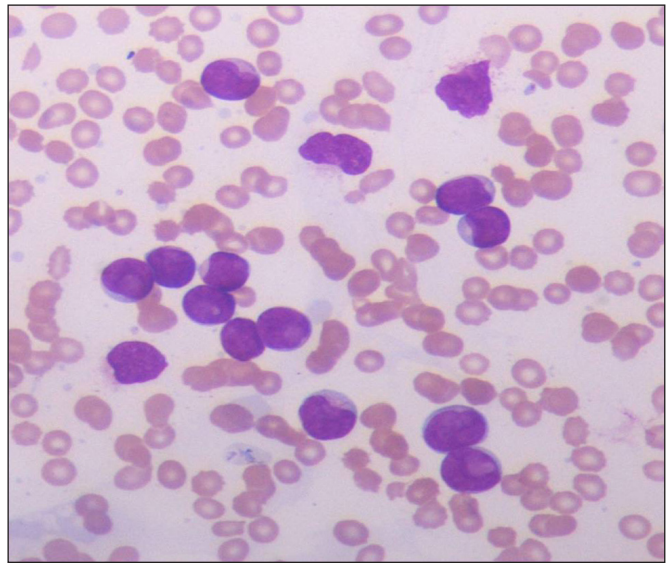

Figure 1: Peripheral smear showing blasts with cup like morphology (Leishman stain; x400) .

Table I: Comparison between FLT3/NPM1 positive and negative cases

	FLT3 (n=31)	NPM1 (n=17)
Age	32.9/31.6	48.3/40.6
Hb	8.5/8.2	9.1/8.2
WBC count	**74.2/35.4***	39.2/42
Blast count	**73.7/51.3***	36/48
PLT count	78.4/64.1	95.8/62.5

* indicates statistically significant difference between two groups

Table II: Immunophenotypic characteristics of FLT3 and NPM1 positive groups

	FLT3 (31/126) (25%)	NPM1 (17/126) (13.4%)
CD34	5/17 (29.4%)	**4/17 (23.5%)***
HLA.DR	4/17 (23.5%)	4/17 (23.5%)
CD13	31/31 (100%)	17/17 (100%)
CD33	31/31 (100%)	17/17 (100%)
CD117	**16/31 (52.9%)***	13/17 (76.4%)
CD14	9/31 (29%)	9/17 (52.9%)
CD19	0/17 (0%)	0/17 (0%)
CD22	1/17 (%5.8)	1/17 (5.8%)
CD7	**7/31 (22.5%)***	1/17 (5.8%)
CD56	0/17 (0%)	4/17 (23.5%)

* indicates statistically significant association of markers with gene

mutations (*P*= 0.008) in our study, similar to that reported by Chauhan et al. FLT3/ITD mutations were also associated with significantly higher blast counts as compared to patients without FLT3/ITD mutations (*P*=0.003). Higher WBC counts in patients with FLT3/ITD mutations can be attributed to ligand independent constitutive activation of FLT3 induced by ITD mutation, thus activating downstream signal molecules that contribute to cell proliferation and survival advantages (6). This may be the reason for the higher WBC and blast count in these cases.

Cuplike morphology of blasts was seen in 81% of the cases positive of FLT3 and/or NPM1 mutation. This distinct morphology has been described in the literature to be associated with FLT3 and NPM1 mutations (7), however significant conclusions in this regard could not be drawn in the present study because of the lower number of cases.

Immunophenotypically, combination detection of CD117/CD7 was found to be significantly associated with FLT3 mutations in AML cases. Recent studies have found CD7 as an important marker and predictor of FLT3 mutations in AML cases (8). High frequency of FLT3 mutations had been described in CD117 positive T-ALL cases (9). We suggest that expression of CD7 along with CD117 may be taken as a surrogate profile for FLT3 mutation.

NPM1 mutation was significantly associated with the lack of CD34 (23.5% vs. 73%) and expression of CD14 (52.9% vs. 14.8%), compared to the group without NPM1 mutation. On regression analysis, CD14 and CD34 were found to be most important indicators of NPM1 mutation. The NPM1 mutation was inversely associated with the expression of CD34 in several European studies (3,10) and those from South Asian countries on AML (4). Chauhan et al. reported the similar lack of CD34 expression in AML cases with NPM1 mutation (5).

In conclusion, the results suggest that presence of distinct morphology together with haematological and immunophenotypic characteristics may serve as important indicators and surrogate for NPM1 and FLT3/ITD mutations. Further, comprehensive studies on the biological effects of NPM1 and FLT3/ITD mutations and their interactions with other genetic alterations are needed to gain insight into the molecular mechanisms of these mutations involved in the pathogenesis of AML.

CONFLICT of INTEREST

The authors declare no conflict of interest

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