Research Article

Frequency of Nucleophosmin1 Gene Mutation (NPM1) in Acute Myeloid Leukemia - A Single Center Experience

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Abstract: Introduction: NPM1 mutation is considered to be an important event in the process of leukemogenesisas it affects the p53 tumor suppressor pathway in the form of frame shift mutation. It is thought to provide a favorable outcome to the disease especially in the absence of *FLT3* mutation.This study was conducted to find out the frequency of NPM1 mutations in patients with AML in Pakistani population.

Materials & Methods: This was a descriptive cross sectional study conducted for a time period at the National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi. Study subjects' demographics including age, gender, presence and duration of the symptoms. Diagnosis was made on the morphology of blood and/or bone marrow samples in accordance to the revised WHO classification of myeloid neoplasms, 2016. All the patients were treatment naive at the time ofenrollment into the study. Sanger sequencing was performed to detect NPM1 mutation.

Results: Out of 100 patients who were enrolled in the study, 60% were males. The mean age of patients was 38.9 years (range: 8yrs - 60yrs). NPM1mutation was detected in 14(14%) patients of the total AML cases with equal presence in both genders. According to the WHO classification 2016, NPM1 was found in 3(3%) in AML without maturation, 4(4%) in AML with maturation, 3(3%) in acute promyelocytic leukemia and 3(3%) in acute myelomonocytic leukemia.

Conclusion: NPM1 was detected in 14% cases of AML in our study. The presence of the NPM1 mutationhas a considerable impact on the prognosis of the disease as it may help in the tailoring of the future treatment of the AML patients particularly those with normal cytogenetics.

Keywords: Acute Myeloid Leukemia, NPM1, Prognosis, Bone marrow morphology, Immunophenotyping.

INTRODUCTION

The Acute Myeloid Leukemia (AML) is an uncontrolled proliferation of hematopoietic precursor cells of myeloid lineage. Its diagnosis is accomplished on the basis of clinical symptoms, peripheral and bone marrow morphology, cytochemical stains, immunophenotyping and cytogenetic analysis. It has a heterogeneous nature with regard to its clinical signs and symptoms, cytogenetic and molecular findings [1].

NPM1 mutations found in approximately 35% of AML patients [2, 3] involving exon 12 of the gene resulting in a frame shift during the process of translation [4]. NPM is a polypeptide that moves between the nucleus and cytoplasm [5] with its important activities being focused within the nucleolus [6] where playing the role of a chaperone [7]. It stops protein aggregation and also controls the assemblage and transport of ribosome related proteins through the nuclear membrane [5]. It has also been found to play a central role for the CDK2-cyclin E complexes during centrosome duplication [8] as well as controlling the alternate-reading-frame protein adenosine diphosphateribosylation factor (ARF)-P53 tumor suppressor pathway [8, 9]. The genome sequencing led to the discovery of six (06) sequence variants (named A,B,C, D, E and F), all resulting in a frame shift in the C-terminal region of the NPM1 protein that is crucial for nucleolar localization of NPM1 [2].

Several studies demonstrated better prognostic effect of NPM1 mutations [10, 11], whereas some studies found no effect of the mutation on the prognosis of the AML patients [12, 13]. A meta-analysis showed the 67% disease free survival (DFS) and 63% overall survival (OS) in AML patients with

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NPM1 mutations [14]. Studies showed thatthe coexistence of NPM1 mutation with *FLT3*-ITD found in approximately 40% of AML patients [15] had poorer DFS, OS and a higher relapse rate as compared to those who only had NPM1 mutation.

MATERIALS AND METHODS

The study was conducted at the National Institute of Blood Diseases and Bone Marrow Transplantation. This was adescriptive cross sectional study approved by the institutional ethics committee. Patients presenting in our out patient department and diagnosed with AML on the morphological basis in accordance to the revised WHO classification of myeloid neoplasms 2016 were enrolled. All study participants were treatment naive at the time of enrollment in the study. The non-probability consecutive sampling technique was employed to enroll the study participants.

Study participants comprised of adults patients aged 18 to 60 years of either gender. Patients presenting with symptoms like high grade unretractable fever, loss of appetite, documented weight loss of >10% in last 4 months or had complains of bleeding for >4 weeks were enrolled into the study. The main criteria for the selection of the patients was the diagnosis of acute myeloid leukemia (AML) on the basis of peripheral morphology examination as well as bone marrow biopsy seconded by immunohistochemistry and/or flow cytometry according to revised WHO classification 2016. Patients with the diagnosis of Chronic Myeloid Leukemia (CML) in lymphoid blast crises, B-Cell/T-Cell Acute Lymphoblastic Leukemia (ALL), Myelodysplastic Syndrome (MDS) or those who had received prior conventional chemotherapy or any other tyrosine kinase inhibitor (TKI) were excluded from the study.

The study participants had given their voluntary informed consent before their enrolment into the study. A performa containing details i.e. age, gender, presence and duration of the symptoms was then filled. Three milliliters of venous blood was collected in K3 EDTA (Becton & Dickinson, USA) vacutainers for the analysis; bone marrow samples were sent for morphological diagnosis and cytogenetic analysis. The peripheral or bone marrow samples were sent to the molecular laboratory of the institute. Sanger sequencing method was used to detect NPM1 mutation on the peripheral or the bone marrow sample.

Sanger Sequencing

Genomic DNA was isolated from the whole blood using the QIAampDNA mini kit (GmbH Hilden, Germany). Sanger sequencing of NPM1 gene was carried out by amplification of exon 12 using primers NPM – F(5'- TTA ACT CTC TGG TGG TAG AAT GAA-3') and NPM – R(5'- CAA GAC TAT TTG CCA TTC CTA ACA-3'). The total reaction volume of 20µl contained approximately 50-100ng of DNA, 20 picomoles of each primers, deoxynucleotide triphosphates (dNTPs, 10 Mm each), 10µl Taq DNA polymerase. Samples were amplified using the following PCR conditions: 96°C for 5 minutes; 30 cycles of 96°C for 30 seconds; 60°C for 1 minute; 72°C for 30 seconds; 72°C for 10 minutes. PCR products were analyzed using 2% agarose gel electrophoresis. PCR product was purified by ethanol/sodium acetate method and directly sequenced with Primer NPM1-R2 (5'-GG-CATTTTGGACAACACA-3') using the ABI Ready Reaction Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA). Sequences were compared with wild type NPM1gene for variation in DNA sequences checked by databases on Ensemble and 1000 genome browser for mutation detection of NPM1 gene.

Data Analysis Procedure

The collected data was analyzed using SPSS Version 17.0. Quantitative variables like age and duration of symptoms were calculated employing mean and standard deviation (95% CI) while qualitative variables like gender and presence of NPM1 mutation were quantified using frequency and percentage. To minimize the effect of modifiers like age and gender for the observation of *FLT3*/ITD, stratification was done. Chi-Square test was applied to the results obtained and p-value of ≤ 0.05 was considered significant.

RESULTS

Out of 100 patients who were enrolled into the study, 60% were males while 40% were females. The mean age of patients was 38.9 ± 13.5yrs. NPM1 mutation was detected in 14(14%) patients (Fig. **1**).

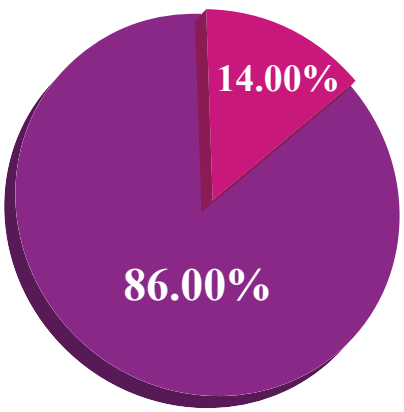

Fig. (1). Frequency of NPM1 in AML Patients.

The mutation was observed in 7(11.6%) out of 60 males and 7(17.5%) out of 40 females (Fig. **2**). According to the revised

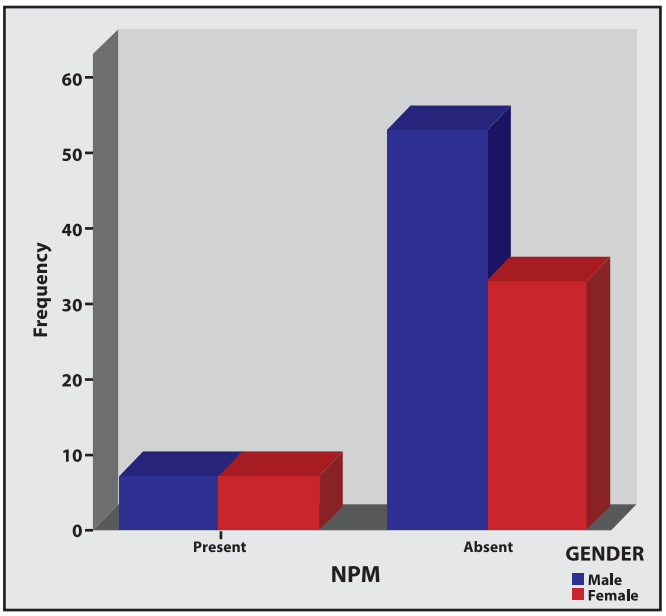

Fig. (2). Frequency of NPM1 in AML Patients with Respect to Gender.

WHO classification 2016 NPM1 was found in 3(3%) in AML without maturation, 4(4%) AML with maturation, 3(3%) in acute promyelocytic leukemia, and 4(4%) in acute myelo-monocytic leukemia (Table **1**).

Table 1. Frequency of Patients with Respect to AML Subtypes and NPM Status.

AML SUBTYPES (WHO 2008)	NPM1 MUTATION STATUS		TOTAL
	POSITIVE	NEGATIVE	
AML with minimal differentiation	0	2 (2%)	2 (2%)
AML without maturation	3 (3%)	27 (27%)	30 (30%)
AML with maturation	4 (4%)	27 (27%)	31 (31%)
Acute Promyelocytic leukemia	3 (3%)	13 (13%)	16 (16%)
Acute Myelomonocytic leukemia	4 (4%)	12 (12%)	16 (16%)
Acute Monoblastic leukemia	0	0	0
Acute Erythroblastic leukemia	0	2 (2%)	2 (2%)
Acute Megakaryoblastic leukemia	0	0	0
AML with dysplasia related changes	0	3 (3%)	3 (3%)
	14(14%)	**86(86%)**	**100(100%)**

The results showed that the frequency of NPM1 mutations was 14%. We identified the most frequent NPM1 mutant type was type A mutation which is TCTG 4 base pair mutation in exon 12 as shown in Fig. (**3**).

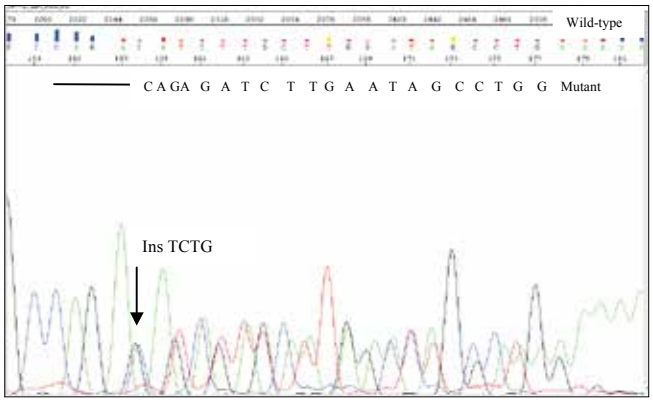

Fig. (3). Electropherogram of NPM1 Type A Mutation is Duplication of TCTG (Nucleotides 956-959), Creating an Insertion at Position 960.

DISCUSSION

An extensive array of mutations, including NPM and *FLT3* has been studied for their effect on the prognosis of AML by a number of European leukemia groups [16-19]. Whitman *et al.* from the Cancer and Leukemia Group B(CALGB) study, reported that in the absence of the wild-type allele led to a poorer prognosis in adult *de novo* AML with normal karyotype and internal tandem duplication of *FLT3* (*FLT3*-ITD) [19]. Similar conclusions in relation to NPM1 mutations were observed in all Eurpoean studies keeping in mind the limitations that included different time spans of follow-up periods, age, gender ratio, treatment regimens and outcomes [16-18].

A meta-analysis of nine studies including 4509 participants was performed by Liu *et al.* reported a wide range of frequency from 6.45% to 56.08% of NPM1 mutations. It was observed that the patients carrying NPM1-mutation type had two times greater chances of achieving complete remission in comparison to those who had NPM1-wild-type [20]. It was also reported that the Hazard Ratio (HR) of NPM1-mt/NPM1-wt for disease-free survival (DFS) and overall survival (OS) was 0.67 and 0.63 respectively. Isolated NPM1 with no FLT3-ITD mutations i.e. NPM1+*FLT3*- were found to be associated with better OS, relapse-free survival and event-free survival (EFS) [16, 18] in AML with normal karyotype. Subgroups including NPM1+*FLT3*+, NPM1-*FLT3*+, and NPM1-*FLT3* had similar survival outcomes. Additional findings included no association of NPM1 mutation with age. A female preponderance was observed including an association with raised white cell count, myelomonocytic subgroup, low CD34+ count but reduced incidence with concurrent partial tandem duplication

of MLL [16, 17, 19]. Schnittger *et al*. and Verhaak *et al*. also observed a similar finding of isolated NPM1 mutation rendering a survival advantage and also a reduced concurrent occurrence of NPM1 and *CEBPA* mutations [4, 16]. Studies have proven that NPM1 mutations although have frequent association with FLT3 mutation but has greater stability compared to it and thus can prove as a better indicator for the monitoring of residual disease in AML [4].

In the current study, we analyzed the frequency of NPM1 mutation in 100 AML patients with normal karyotype (CN-AML). Our study reported 14% of type A NPM1 exon 12 mutation in AML patients. Type A that involves duplication of TCTG (nucleotides 956-959) leading to an insertion at position 960 is the most frequent type and accounts for approx. 80% of the cases followed by Types B and D both creating a 4 bp insertion at position 960. Any other mutations are rare and account for <1% of cases [21]. Our results are consistent with the frequency of NPM1 in (14.3%) as reported by Ahmed *et al*. in which NPM1was found in 19.5% of all population and 34.2% in those with normal karyotype [20]. In addition, Falini *et al*. showed that the frequency of NPM1 mutation was 61.7% [2], while different mutation rate was reported by Dohner *et al*. (48.3%) and Boissel *et al*. (47%). The discrepancy in the frequencies of NPM1 mutation between our study and other group's studies may be due to different population groups as well as number of cases in above mentioned studies.

NPM1 mutations have been related with various pretreatment characteristics of AML patients with normal karyotype and include female preponderance, extensive array of morphological findings, raised percentage of blasts in the peripheral blood and bone marrow, raised levels of lactate dehydrogenase, increased CD33- antigen but low or absent CD34-antigen expression, increased frequency of FLT3-ITD and a distinct gene-expression profile [22].

CONCLUSION

At present NPM mutations are most frequently occurring submicroscopicaberration in AML with normal karyotype and play an important role in the clinical outcome prediction model due to its major effect on the prognosis. The application of cytogenetic and molecular studies in AML has helped in the detection of NPM1 mutation immunohistochemically. NPM1 mutationis quickly becoming an important tool for the purpose of monitoring of MRD in AML patients both because of its frequent occurrence as well as its stability as a marker.

CONFLICT OF INTEREST

Declared None.

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