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Acute Myeloid Leukemia: Correlation of NPM1 Mutation with Morphology and Immunophenotypical Findings

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Abstract

Objective: To correlate NPM1 mutation in acute myeloid leukemia (AML) with specific morphological and immunophenotypic findings in the Pakistani population.

Methodology: This descriptive cross-sectional study was conducted at the Chughtai Institute of Pathology from June 2022 to April 2024. Clinical history, along with peripheral blood and bone marrow aspirate samples, were collected from 50 newly diagnosed AML cases at the Chughtai Institute of Pathology after obtaining informed consent. NPM1 mutation was detected using the Imegen-NPM1 kit via Reverse Transcriptase-Polymerase Chain Reaction (PCR). Data normality was assessed using the Kolmogorov-Smirnov test. Associations between NPM1 mutation-positive and NPM1 mutation-negative groups and 1-year remission on follow-up were analyzed using the Chi-square test, with a p-value of <0.05 considered significant.

Results: NPM1 mutation was detected in 20% of AML cases, predominantly affecting females aged 40 years and above. Relatively high total leukocyte count (TLC), M4 FAB type morphology, and low CD34 expression ($p<0.05$) were observed in cases with NPM1 mutation. A 1-year remission rate of 60% was seen in NPM1-mutated cases, suggesting a significant correlation between NPM1 mutation and remission ($p<0.05$).

Conclusion: NPM1 mutation is less common in our population and is mainly associated with female gender, older age, specific hematologic and immunohistochemical markers, as well as improved overall remission.

Key Words: Acute myeloid leukemia (AML), Nucleophosmin 1 (NPM1) gene, Hematologic and immunophenotypic parameters.

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Introduction

NPM1 mutation is the most commonly observed leukemic mutation in acute myeloid leukemia (AML) and is generally associated with a favorable prognosis. It is important to identify associations between this mutation and clinical presentation, various hematological parameters, and immunohistochemistry to assist pathologists in stratifying AML cases into different prognostic subgroups, especially in low-resource settings where molecular analysis for NPM1 detection may not be readily available.

Acute myeloid leukemia (AML) is a heterogeneous group of hematological neoplasms arising from various genetic alterations in clonal hematopoietic myeloid progenitor cells in the bone marrow.[1] These mutated clonal hematopoietic stem cells undergo uncontrolled proliferation and differentiation, disrupting normal hematopoiesis and causing peripheral cytopenias with or without an elevated white blood cell count.[2,3] Although the global incidence of AML has risen in recent years, its association with poor disease prognosis and increased mortality has decreased.[4]

In the 2017 WHO classification of hematopoietic neoplasms, AML with NPM1 (Nucleophosmin1) gene mutation was recognized as a distinct entity due to its characteristic clinical, morphological, and immunophenotypic features.[5] The recent international consensus classification has adjusted the threshold for blast cells from >20% in the WHO revised 4th edition to ≥10% in NPM1-positive AML (WHO 2022).[6]

The NPM1 gene encodes a protein that primarily resides in the nucleolus but shuttles between the nucleus and cytoplasm.[7] Nucleophosmin1 protein performs several crucial functions, including assisting histones in chromatin organization, promoting cell division and growth by

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supporting ribosomal machinery in RNA translation, and protecting cells from damage by indirectly increasing TP53 levels.[8] Mutations typically involve exon 12 of the NPM1 gene, resulting in cytoplasmic displacement of the protein and overexpression of HOX/MEIS1.[9] NPM1-mutated AML is usually associated with a high white blood cell (WBC) count, distinctive blast morphology, and often shows negative CD34 expression.[10]

NPM1 mutations account for more than 30% of all AML cases detected annually, making NPM1 one of the most frequently altered genetic entities in AML.[11] These mutations are exclusively associated with AML and often co-occur with FLT3, DNMT3A, and TET2 mutations.[12] NPM1-mutated AML is generally associated with a good prognosis and improved survival rates, provided there are no exacerbating factors, due to the high sensitivity of these blast cells to intensive chemotherapy.[13]

Despite ongoing global research and molecular discoveries regarding NPM1 mutations in AML, extensive genetic studies and high-cost molecular diagnostics are challenging to implement in underdeveloped or developing countries. Few studies have explored the correlation between clinical, morphological, and immunophenotypic findings with NPM1-mutated AML. This study aims to identify associations between clinical presentation, morphology, and immunophenotypic characteristics of AML patients and self-funded NPM1 mutation detection. This approach will help physicians in developing countries initiate early targeted therapy for NPM1-mutated AML even in the absence of molecular NPM1 mutation diagnostics.

Methodology

This descriptive cross-sectional study was conducted at the Chughtai Institute of Pathology from June 2022 to April 2024, following approval from the Institutional Review Board (IRB). A total of 50 newly diagnosed cases of acute myeloid leukemia (AML), based on bone marrow morphology, cytochemical staining, and immunophenotyping (using either immunohistochemical stains or flow cytometry), were included in the study. Both male and female patients of all ages were studied. AML patients who had received chemotherapy were excluded from the study.

After obtaining informed consent, detailed history and physical examinations were performed for each patient. Peripheral blood and bone marrow aspirate samples were collected (using Salah trephine needle for bone marrow aspiration and trephine biopsy) under aseptic conditions. Peripheral blood samples were analyzed on the Sysmex XN 9000 for basic complete blood count parameters. A 3 ml bone marrow aspirate was collected in both EDTA and Sodium/Lithium Heparin vials for flow cytometry and molecular analysis. Trephine biopsy samples were processed, and immunohistochemical markers were applied to diagnose and subtype AML. For flow cytometry, a lysed bone marrow aspirate sample was checked for viability index. The sample was incubated with monoclonal antibodies and analyzed using the BD FACS Lyric. Sudan Black B staining was employed on bone marrow aspirates to assist in the diagnosis of AML.

DNA extracted from white blood cells was amplified using real-time polymerase chain reaction (RT-PCR). A total of 50 ng of DNA was required for analysis. The Imegen-NPM1 kit utilized a combination of mutation-specific primers and targeted fluorescence probes in a real-time PCR assay to simultaneously amplify three frameshift mutations (A, B, and D) and the endogenous β-globin gene as an internal positive control. FAM™-labeled probes were used for detecting amplified products of the A, B, and D forms of NPM1, while VIC™-labeled probes were used for specific detection of the β-globin gene.

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 23.0. Descriptive statistics were calculated as mean ± SD and median. The normality of data was assessed using the Kolmogorov-Smirnov test. Frequencies of different clinical presentations and FAB (French-British-American) types of all AML cases (NPM1 mutated and non-mutated) were evaluated. Comparisons of basic CBC parameters, blast percentage, and CD34 expression between NPM1 mutation-positive and mutation-negative groups were conducted using the Chi-square test. The association between NPM1 mutation status (positive vs. negative) and 1-year remission on follow-up was also evaluated using the Chi-square test, with a p-value of <0.05 considered significant.

Results

The study comprised of a total of 50 cancer patients in which 24 patients were females (48%) and 26 were males (52%). Mean age was 44.7±16 years ranging from 11

years to 78 years. NPM1 mutation was detected in 10 out of 50 patients (20%). Rest of 40 AML patients were not diagnosed with NPM1 mutation.

All the NPM1 mutated patients had symptoms of fever, generalized weakness and weight loss at the time of presentation. 1 patient had history of epistaxis; rest of 9 mutated patients did not suffer from any bleeding /bruising episode. 2 patients had lymphadenopathy, one with cervical lymphadenopathy and other with inguinal lymph nodes enlargement.

All 50 patients who were diagnosed with AML were further categorized into specific FAB type with 2 cases (4%) having morphology of M0, 13 cases (26%) having M1 morphology, 19 cases with M2 morphology (38%), 11 cases having M4 morphology (22%) and 5 cases with FAB type M5 (10%). Morphology of all the NPM1 mutated cases was studied separately. Out of 10 NPM1 mutated cases, 1 case was subtyped as M2 (10%), 6 cases as M4 (60%) and 3 cases as M5 (30%). Table I.

Table I: Distribution of FAB types in NPM1 mutated AML cases.

FAB type	Case numbers (n=10)	%
M4	6	60%
M5	3	30%
M2	1	10%

Normality of data was analyzed using Kolmogorov-Smimov test. Means were calculated for normally distributed data and median was calculated for data which was not normally distributed. Chi-square test was used to assess correlation of NPM1 mutation with age, hemoglobin levels, white blood cell count, platelet count, blast percentage and CD34 expression. Mean age was found to be almost same in both groups that was 47±13 years in mutation positive and 44±16 years in mutation negative group (p value= 0.82). Mean Hemoglobin and median platelet count was also similar in both groups. Mean Hemoglobin was 7±1.4g/dl in mutation positive group and 8±1.7g/dl in mutation negative group (p value= 0.25). Median platelet count was 47 x 109/L in mutation positive group and 33 x 109/L in mutation negative group (p value= 0.35). However, median total leucocyte count was found to be higher in NPM1 mutated group as compared to non-mutated group (p value= 0.09). Blast percentage was equally high in mutation positive and mutation negative AML groups (p value= 0.72). 8 out of 10 NPM1 mutated cases were negative for CD34 (p value <0.05). Table II.

Table II: Correlation of NPM1 mutation with hematologic and immunophenotypic parameters.

Parameters	NPM1 Positive (n=10)	NPM1 Negative (n=40)	P value
Mean age	47±13 years	44±16 years	0.82
Mean Hemoglobin	7±1.4 g/dl	8±1.7 g/dl	0.25
Median TLC	51 x 109	18 x 109	0.09
Median platelet	47 x 109/L	33 x 109/L	0.35
Mean blast percentage	72%	71%	0.72
CD34 positive expression	20%	90%	0.00

NPM1 mutation was not equally distributed in genders. Out of 10 NPM1 positive cases, 7 were female patients and 3 were male patients (2.3:1). Majority of NPM1 mutated cancer patients were females.

These patients were put on chemotherapy post diagnosis and were followed for 1 year for remission assessment. They were assessed on the basis of morphological and hematological criteria set by an international working group. 10 out of 50 patients underwent remission following chemotherapy (20%). 6 patients in remission were detected with NPM1 mutation (60%) and only 4 patients from non-mutated group went into remission (10%). Association between detection of mutation in 2 groups (1 group= mutation negative and 2 group= mutation positive) and 1 year remission was calculated by applying Chi-Square test and was found to be significant (p value < 0.05) Table III. Remission percentage was higher in NPM1 mutated group as compared to non-mutated group.

Table III: Association between NPM1 mutation and remission.

Mutation detected	Total population (n=50)	Number of cases with 1 year remission following chemotherapy
Yes	n=10	6/10 (60%)
No	n=40	4/40 (10%)
P value < 0.05 after applying Chi-square correlation		

Discussion

Mutation of NPM1 gene is established as an AML defining event but this mutation can also arise in patients of myelodysplastic syndrome (MDS) as well as chronic myelomonocytic leukemia (CMML) and is associated with normal karyotype and good response to chemotherapy with high allelic burden.[14]

NPM1 mutation was detected in only 20% of the newly diagnosed AML patients in our study with mean age of 47±13 and incidence was high in population with age

ranging in between 40s and 50s. The incidence of mutation is similar to a study conducted in Syria in 2021 where mutation was found in 22.7% of AML patients and majority of these patients fell in age ranging from 40-50 years.[15]

NPM1 mutation was not equally distributed in both genders with females (70%) being more affected as compared to males (30%). This association was also seen in a study conducted by Naeem S. et al. where NPM1 mutation was found more in female population.[16]

A recent study conducted in Pakistan detected NPM1 mutation in 34.3% of 108 patients and found its association with female predominance, high hemoglobin levels, high platelet count, low CD34 expression but high TLC was associated with FLT3 mutation.[17] In contrast, in our study, there was no significant difference in hemoglobin levels, platelet count and blast percentage between mutation positive and mutation negative group. However, NPM1 mutated blasts expressed CD34 less frequently (p value< 0.05). No more significant correlation with other immunophenotypic markers was identified.

Total leucocyte count was relatively higher in NPM1 mutated group in our study as compared to non-mutated group and NPM1 mutation is considered to be positively associated with increased WBC count along-with FLT3 mutation.[18]

In a study conducted by Mostafa H. et al comprising 89 patients with NPM1 mutation detected in 37.1% patients, 34.7% cases were FAB type M2 and 24.7% cases fell under M4 FAB category.[19] However, our study found M4 in majority of cases positive for NPM1 mutation (60%), followed by M5 (30%) and M1 (10%), M4 being the commonest in another study done by Naseem S. et al.[16]

NPM1 mutation in the absence of co-existing FLT3 mutation is strongly associated with complete remission and better overall survival rate (>70% and >30% respectively) following chemotherapy in younger age groups[20]. Our study compared remission of NPM1 mutation positive and negative groups after being followed for 1 year of starting chemotherapy, 60% cases with NPM1 mutation went into remission (p<0.05) as compared to only 10% cases (NPM1 negative) who went into remission.

5 year survival rate in NPM1 diagnosed elderly patients aged above 60 years with intensive chemotherapy is above 60% and OS decreases with increasing age.[14] A few studies in regard of targeted therapy towards NPM1 mutated AML in elderly supported use of combination therapy (hypomethylating agent and venetoclax) to achieve better prognosis.[21]

Conclusion

In our study, NPM1 mutation was detected in 20% of newly diagnosed AML patients. This mutation was correlated with female gender, age over 40 years, relatively high total leukocyte count, and negative CD34 expression. These findings are valuable for oncologists in initiating targeted combined chemotherapy in settings where molecular diagnosis of NPM1 mutation is either unavailable or unaffordable. The negative correlation of the mutation with certain clinical and hematologic parameters may be attributed to the smaller sample size. Future studies with larger populations are recommended to assist clinicians in making more informed decisions regarding treatment for both younger and older patients, whose prognosis can vary based on age and the type of intensive chemotherapy administered.

Limitations of the Study: The primary limitations of this study include the small sample size, lack of funding, and delays in the availability of Imegen-NPM1 kits, which also contributed to an unexpected extension of the study.

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