

An Observational Analysis on Clinico-hematological profile of Acute Myeloid Leukemia: At a Tertiary care facility

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Abstract

Background: Acute myeloid leukemia (AML) is characterized by clonal neoplastic proliferation of myeloid precursor cells in the marrow arrest of their maturation. Its annual incidence is 2-3 per 10,000 and increases with age. In adults this accounts for 80-90% of cases. The morphology {by Bone marrow aspiration, bone marrow biopsy}, cytogenetics and molecular abnormalities {by Immunophenotyping} of leukemic cells are currently used to classify AML into various risk categories. **Material and Methods:** This is an observational study performed over a period of six months from September 2022 to February 2023 in the department of Pathology, PGIMS, Rohtak. Bone marrow trephine biopsy, Flow cytometry and cytogenetics were also used for correlation wherever possible. **Results:** Out of total 243 bone marrow aspirates, 27 cases were diagnosed as AML in different age groups, with youngest being in 4 years male child diagnosed as acute promyelocytic leukemia. Most common clinical presentation was fever, generalized weakness, petechiae, lymphadenopathy and hepatosplenomegaly. On the rare note, a 11 year old male presented with right orbital malignant mass. In some cases, further morphological subcategorization was done and correlated with IPT and/or cytogenetics. **Conclusion:** Bone marrow aspiration provides cytomorphological details and biopsy reveals the pattern of distribution of cells. IPT allows for the objective and reproducible distinction of AML, also these can be further classified into a number of different subtypes. Cytogenetics is the most important prognostic factor for predicting remission rate, relapse and overall survival. Thus, all these hematological profiles should be correlated in the diagnosis and further management.

Keywords: Acute myeloid leukemia, Cytogenetics, Acute lymphoblastic leukemia.

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INTRODUCTION

Acute myeloid leukemia (AML) is described as an increase in myeloid cells in the bone marrow coupled with a stop in their maturation, which leads to hemopoietic deficiency, with or without associated leucocytosis and myeloblast invasion of the bone marrow and other tissues.^[1] Acute myeloid leukemia is complicated and has a great deal of genotypic and phenotypic variability.

In 2017, AML constituted 23.1% of all leukemia cases globally.^[2] According to the Global Burden of Disease 2017 database, from 1990 to 2017, the number of AML-related deaths rose significantly by 93%, while the number of AML incidence cases climbed by 87.3%.^[3] In 2019, AML was responsible for 28.11% of leukemia-related deaths worldwide.^[4] Most cases of acute myeloid leukemia occur in adults, with ages 15 to 39 being the peak for occurrence.^[5] Within the realm of AML, researchers have identified over 100 recurrent cytogenetic abnormalities alongside numerous point mutations.^[6]

Clinical manifestations associated with pancytopenia, encompassing indications such as infectious episodes, pyrexia, asthenia, fatigue, and hemorrhagic manifestations including petechiae, menorrhagia, and epistaxis, are evident among individuals afflicted with AML. These presentations arise from the progressive depletion of normal hematopoietic elements within the bone marrow owing to

the proliferation of malignant cellular populations. Additionally, clinical assessment may reveal pallor, lymphadenopathy, hepatomegaly, and splenomegaly.^[7] While the precise etiology of AML remains elusive, the majority of affected individuals exhibit chromosomal aberrations. Cytogenetic translocations give rise to fusion proteins, constituting a prevalent mechanism underlying the pathogenesis of leukemia.^[8]

The increasing incidence of acute leukemias in developing nations like India underscores the importance of delineating the clinical profile for facilitating early detection, diagnosis, and appropriate therapeutic interventions. Consequently, our objective was to find out the proportion of acute myeloid leukemia among the patients referred for bone marrow biopsy at Department of Pathology, at a tertiary care facility, India, and to explore the clinical and laboratory attributes of acute leukemia, and study cytogenetic properties of the patients. The discoveries

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from this study will hold significance in shaping management strategies for numerous low and middle-income nations facing constraints in resources for both diagnosis and treatment.

MATERIALS AND METHODS

This descriptive observational study, conducted at the Department of Pathology within a tertiary care facility located in Rohtak, was initiated in September 2022 subsequent to obtaining clearance from the Institutional Ethical Committee. Data collection was done from September 2022 to February 2023, with an additional two-month period allocated for subsequent data analysis.

Our study encompassed all patients exhibiting pertinent clinical manifestations and hematological findings indicative of acute myeloid leukemia / undifferentiated leukemia. Cases diagnosed as Acute Lymphoblastic Leukemia (ALL) were not included in this study and those who had undergone prior radiation therapy or chemotherapy were also excluded from the study cohort.

A comprehensive hematological analysis, including a Complete Blood Count (CBC), was conducted for all participants. Peripheral blood smears were stained using Leishman stain and meticulously examined. Additionally, bone marrow aspiration and evaluation were performed for each case, with examination of Leishman's stain smears. Furthermore, cytochemical stains, namely MPO (Myeloperoxidase), SBB (Sudan Black B), PAS (Periodic Acid Schiff Stain), and NSE (Non-specific esterase), were utilized for the diagnosis and subtyping of leukemias wherever required. Acute myeloid leukemias were categorized according to the FAB (French-American-British) criteria.⁹ Standard Hematoxylin and Eosin (H&E) staining, along with a range of histochemical and immunohistochemical stains, were employed on biopsy sections as part of routine diagnostic procedures. Additionally, cytogenetic analysis was utilized for further subtyping, wherever feasible.

Statistical Analysis: The investigator collected and entered the data into a Microsoft Excel Sheet on the same day to minimize potential bias associated with data entry.

Categorical data were presented as proportions, and Continuous data were summarized using mean and standard deviation.

RESULTS

In our study, a total of 243 bone marrow aspirates were received during the study period, and out of them 27 (11.1%) were identified with AML. [Table 1] More than one third (37%) of cases had more than 45 years of age, followed by 31-40 years age group of seven (25.9%) cases, 16-30 years age group of six (22.2%) and least (14.8%) cases belonged to 6 months to 15 years of age. More than half (55.6%) of cases were male, and rest (44.4%) were female. Male to female ratio was 1.25:1.

[Figure 1] Majority of the cases (88.9%) had generalized weakness, followed by hepatosplenomegaly in 55.6% cases, lymphadenopathy in 51.9% cases, fever in 40.7% cases and one (3.7%) case reported vomiting.

[Table 2] In Bone marrow aspiration more than half of cases were given impression of AML, followed by AL in eight (29.6%) cases, and least three (11.1%) cases of Acute promyelocytic leukemia (APML) each one of them being in pediatric age group. Biopsy obtained in 26 cases, out of which 2 were of APML and rest were diagnosed as AML. Two of 24 cases with AML have monocyte differentiation. In IPT, 24(88.9%) cases diagnosed with AML, and three (11.1%) were diagnosed with APML, two of 24 cases with AML have monocyte differentiation. One (3.7%) case has pancytopenia, with less than 4000 count of total leucocyte. And six (22.2%) cases have normal total leucocyte count. Majority of cases 19(70.4%) presented with leukocytosis, and five (18.5%) cases were with aleukemic presentation.

[Table 3] for five cases cytogenetic study was done, and their findings are presented. One of 27 cases (60 years male) presented with fever and shortness of breath, with the suspected clinical diagnosis of AML, on ultrasonography no organomegaly was seen. Diagnosis of AML was confirmed by Biopsy. In PCR followed by automated DNA sequencing, CEBPA mutation was not detected. And in RT-PCR+ gel electrophoresis, NPM 1 and FLT3-ITD mutation were detected. Out of 4 child cases, cytogenetics done in 3 cases including one case of APML revealing PML-RARA mutation.

Table 1: Sociodemographic profile of AML cases

Variable	Number (n=27)	Percentage
Age group		
6 months-15 years	4	14.8
16-30 years	6	22.2
31-45 years	7	25.9
>45 years	10	37.0
Sex		
Male	15	55.6
Female	12	44.4

Table 2: Diagnosis using BMA, BMB, and IPT

Diagnosis		Number	Percentage
Bone Marrow Aspiration (27)	AL	8	29.6
	AML	16	59.3
	APML	3	11.1
Bone Marrow Biopsy(26)	AML	24	88.9
	APML	2	7.4

IPT (27)	AML	24	88.9
	APML	3	11.1

Table 3: Cytogenetics study in five cases

Age (Years)	Gender	Diagnosis	Methodology	Mutation	Detection (Yes/No)
5	M	AL	Karyotyping	46,XY,t(5;9)9q22;p22)	
10	M	APML	RT-PCR	PML-RARA	Yes
11	M	AML	RT-PCR and Gel electrophoresis	BCR/ALB1, ETV6/RUNX1, E2A/PBX1, MLL/AF4	No
60	M	AML	RT-PCR and Gel electrophoresis	FLT3-ITD, NPM1	Yes
61	M	AML	PCR sequencing	CALR, MPL, JAK2	No

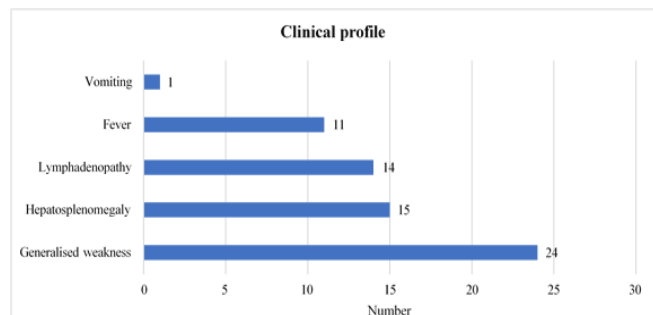


Figure 1: Clinical profile of AML cases

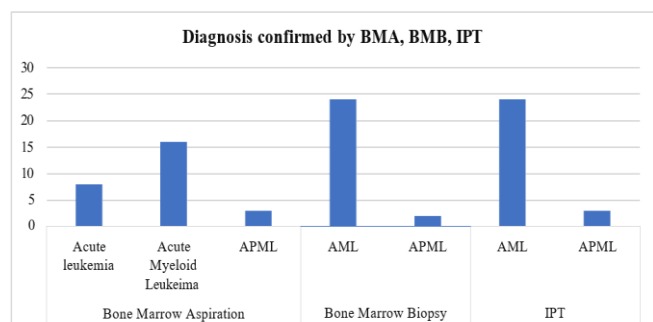


Figure 2: Diagnosis confirmed by BMA, BMB, IPT

DISCUSSION

Acute Myeloid Leukemia comprises a group of disorders distinguished by various clinical, morphological, immunophenotypic, and chromosomal irregularities. These 27 cases underwent thorough clinical history and examination, followed by a comprehensive hematological analysis, including special stains and bone marrow examination.

In our study, proportion of male patients were slightly higher than female, and male to female ratio was 1.25:1. Similar sex distribution in AML cases was reported by Asif N et al,^[10] in their study that proportion of male and female was 51% and 49% respectively and male to female ratio was 1.4:1. Similar male to female ratio was also reported by another study conducted by Rathod KB et al,^[11] In current study, almost half of cases were of 16-45 years of age group, around one third of cases were of more than 45 years of age, and 14.8% cases belonged to 6 months to 15 years of age. Similar trends in distribution of age was also reported by N Braham-Jmili et al, they concluded more than three fourth of patients were less than 60 years of age.^[12]

In our study, main presenting symptoms was generalized weakness in 88.9% of cases, this finding is in concordance with Shome DK et al,^[13] In current study lymphadenopathy was seen in 51.9% of cases similarly Shome DK et al also reported proportion of lymphadenopathy among more than 30% of AML cases. We reported hepatosplenomegaly in 55.6% cases, and Shome DK et al reported hepatomegaly in 73% of patients and splenomegaly among 52% of patients. In another study by Mathur et al,^[14] proportion of hepatomegaly and splenomegaly was 76% and 73% respectively among AML cases. We noted that fever was present in 40.7% cases and one (3.7%) case reported vomiting. This outcome is corroborated by the observation that the invasion of leukemic cells into the bone marrow and their heightened generation lead to neutropenia (manifesting as fever), anemia, and thrombocytopenia (manifesting as bleeding symptoms).^[15]

Although most of cases had leucocytosis, but one (3.7%) case has pancytopenia, with less than 4000 count of total leucocyte and six (22.2%) cases have 4000-12000 total leucocyte count. This may be because of the accumulation of immature cells in the bone marrow called myeloblasts, which displace normal cells and interfere with the normal process of blood cell generation.^[16] In most of the cases, diagnosis of AML as well as APML (19/27) could be made on bone marrow aspirate. Eight cases revealed undifferentiated morphology and could be typed as AML on IPT/ Bone marrow biopsy only. Cytogenetic analysis is the most important diagnostic tool for determining prognosis in acute myeloid leukemia (AML).

CONCLUSION

We concluded that detecting signs and symptoms indicative of leukemia early on is beneficial for promptly diagnosing hematological malignancies. This study is to emphasize that the light microscopic examination of peripheral smear and bone marrow continues to be essential for diagnosing acute leukemias, with cytogenetics serving as complementary procedures typically conducted at specialized centers. These findings will aid in the design of future prospective studies to investigate their correlation with treatment outcomes.

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Conflicts of interest

There are no conflicts of interest.

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