Original Research

INCIDENCE OF ACUTE LEUKAEMIAS IN ADULT POPULATION ATTENDING TERTIARY CARE CENTRE AS DIAGNOSED BY CYTOCHEMICAL ANALYSIS AND IMMUNOPHENOTYPING - A STUDY

Dr. Doyel Halder[1], Dr Victor Roy[2*], Dr. Ankur Ghosh[3], Mrs. Rebatee Roy[4]

[1]Demonstrator , MD (Pathology) , Department of Pathology , Institute of Post Graduate Medical Education & Research and Seth Sukhlal Karnani Memorial Hospital , Kolkata- 700020.

[2*]Assistant Professor , MBBS, MS(General Surgery) , Department of General Surgery , Rampurhat Government Medical College and Hospital, Rampurhat, Birbhum, West Bengal. Pin- 731224.

[3]Assitant Professor , MD(Dermatology, Venereology & Leprosy) , Dept of Dermatology , Gouri Devi Institute of Medical Sciences & Hospitla Rajbandh, West Bengal 713212.

[4]Assistant Teacher, MSc (Physics), Baghar Upper Primary Girls School, Baghar , Burdwan -713141.

Corrosponding Authors: Dr Victor Roy
Assistant Professor , MBBS, MS(General Surgery) , Department of General Surgery , Rampurhat Government Medical College and Hospital, Rampurhat, Birbhum, West Bengal. Pin- 731224.

Email: vroy.cmc@gmail.com

ABSTARCT

Introduction: Acute leukaemias are rapidly progressive haematological malignancies characterized by uncontrolled proliferation of immature hematopoietic cells. Early and accurate diagnosis is essential for prompt treatment and improved survival. Cytochemical analysis and immunophenotyping play a crucial role in classifying acute leukaemias into acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML).

Aims: To determine the incidence of acute leukaemias in the adult population attending RIMS and to classify the cases using cytochemical staining and immunophenotyping.

Materials and Methods: This study was a hospital-based observational cross-sectional study conducted over a period of 1 year in the Department of pathology, Rajendra Institute of Medical Sciences. Ranchi, Jharkhand, India. The study population comprised adult patients aged ≥18 years who presented with clinical suspicion of acute leukaemia, including symptoms such as anaemia, fever, bleeding tendencies, and abnormal haematological findings. A total of 52 adult patients fulfilling the inclusion criteria were enrolled based on clinical evaluation and laboratory confirmation during the study period.

Results: In the present study, a total of 52 patients were evaluated for acute leukaemia. Based on final diagnosis, acute myeloid leukaemia (AML) was found to be the most common type, observed in 34 patients (65.4%), whereas acute lymphoblastic leukaemia (ALL) was diagnosed in 18 patients (34.6%).

Conclusion: Acute myeloid leukaemia is more common than acute lymphoblastic leukaemia in adults attending RIMS. Combined use of cytochemical analysis and immunophenotyping significantly improves diagnostic accuracy and is essential for proper classification and management of acute leukaemias.

Keywords: Acute Leukaemia, Acute Myeloid Leukaemia, Acute Lymphoblastic Leukaemia, Cytochemical Analysis, Immunophenotyping, Flow Cytometry, Bone Marrow Examination, Incidence.

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INTRODUCTION

Acute leukaemias are a heterogeneous group of clonal haematopoietic stem cell disorders characterized by the rapid proliferation and accumulation of immature blast cells in the bone marrow, peripheral blood. And occasionally other tissues. These disorders lead to bone marrow failure, resulting in anaemia, thrombocytopenia, and neutropenia, which clinically manifest as fatigue, bleeding tendencies, and recurrent infections. Acute leukaemias are broadly classified into acute myeloid leukaemia (AML) and acute lymphoblastic

leukaemia (ALL) based on the lineage of the malignant cells. Although they can occur at any age, ALL is more common in children, whereas AML predominates in adults, making age-specific epidemiological evaluation essential for proper diagnosis and management [1].

The incidence of acute leukaemias varies geographically and is influenced by genetic, environmental, and occupational factors. In adult populations, AML constitutes the majority of acute leukaemia cases, accounting for approximately 60–70% of diagnoses, while ALL accounts for the remaining proportion. The increasing incidence of AML with advancing age is particularly notable, with a median age at diagnosis around 65 years. In contrast, ALL in adults is less common but often associated with a poorer prognosis compared to childhood ALL [2].

The pathogenesis of acute leukaemia involves a series of genetic mutations that disrupt normal haematopoiesis, leading to uncontrolled proliferation and impaired differentiation of blast cells. Common cytogenetic abnormalities in AML include translocations such as t(8;21), inv(16), and abnormalities involving chromosome 5 and 7, while ALL frequently shows translocations such as t(9;22) (Philadelphia chromosome), especially in adult cases. These molecular abnormalities not only aid in diagnosis but also have significant prognostic implications [3].

Accurate diagnosis of acute leukaemias requires a combination of morphological evaluation, cytochemical staining, immunophenotyping, cytogenetic, and molecular studies. Morphological assessment of peripheral blood and bone marrow smears remains the first step in identifying blast cells. However, morphology alone is often insufficient to distinguish between AML and ALL, especially in poorly differentiated or minimally differentiated cases [4].

Cytochemical staining plays a vital role in the initial categorization of acute leukaemias. Myeloperoxidase (MPO) and Sudan Black B (SBB) are positive in myeloid lineage cells, whereas Periodic Acid-Schiff (PAS) staining is often positive in lymphoid blasts, particularly in ALL. These stains help in rapid and cost-effective differentiation between AML and ALL, especially in resource-limited settings [5].

Immunophenotyping by flow cytometry has revolutionized the diagnosis of acute leukaemias by enabling precise lineage identification based on the expression of cluster of differentiation (CD) markers. AML is typically associated with markers such as CD13, CD33, and MPO, while ALL expresses markers like CD19, CD10, CD3, and TdT depending on B-cell or T-cell lineage. Immunophenotyping also helps in identifying mixed phenotype acute leukaemia (MPAL), which has both myeloid and lymphoid characteristics [6].

The integration of cytochemical analysis and immunophenotyping significantly enhances diagnostic accuracy, allowing for better classification and risk stratification. This is particularly important because treatment protocols differ markedly between AML and ALL, and accurate diagnosis directly influences therapeutic decisions and outcomes [7].

In developing countries and tertiary care centres such as RIMS, resource constraints often limit access to advanced molecular diagnostics. Therefore, cytochemical techniques combined with immunophenotyping remain the backbone of diagnostic workup for acute leukaemias. Studies from various regions have shown that such combined approaches are both cost-effective and reliable in routine clinical practice [8].

Despite advancements in diagnostic techniques, acute leukaemias continue to pose a significant health burden due to their aggressive nature and high mortality rates, especially in adults. Early detection and accurate classification are crucial for improving survival rates. Hence, studying the incidence and diagnostic profile of acute leukaemias in specific populations is essential for understanding disease patterns and improving healthcare strategies [9].

In this context, the present study aims to determine the incidence of acute leukaemias in the adult population attending RIMS and to classify them using cytochemical analysis and immunophenotyping. This will provide valuable epidemiological data and highlight the importance of integrated diagnostic approaches in routine haematological practice [10].

The aim of this study is to determine the incidence of acute leukaemias in the adult population attending RIMS and to evaluate the usefulness of cytochemical analysis and immunophenotyping in their diagnosis and classification. Acute leukaemias are rapidly progressive haematological malignancies that require early and accurate identification for appropriate management, and this study seeks to highlight their occurrence pattern in the study population.The primary objective is to assess the incidence of acute leukaemia among adult patients diagnosed at RIMS during the study period. The secondary objective is to classify acute leukaemias into acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) using cytochemical stains such as myeloperoxidase, Sudan Black B, and PAS, along with immunophenotyping by flow cytometry for precise lineage determination and subtyping. Another objective is to evaluate the diagnostic utility and complementarity of cytochemistry and immunophenotyping in improving accuracy of diagnosis in routine practice.

MATERIALS AND METHODS

Study Design: This study was a hospital-based observational cross-sectional study

Study Place: Department of pathology, Rajendra Institute of Medical Sciences. Ranchi, Jharkhand, India.

Study Duration: 1 year

Study Population : The study population included adult patients (≥18 years) attending RIMS with clinical suspicion of acute leukaemia, based on presenting symptoms such as anaemia, fever, bleeding tendencies, and abnormal haematological findings.

Sample Size:

A total of 52 adult patients suspected of acute leukaemia were included in the study based on laboratory and clinical evaluation during the study period.

Study Variables:

- Age Group (years)
- Sex
- Diagnosis
- Cytochemical Result
- Immunophenotype

Inclusion Criteria:

- Patients aged 18 years and above
- Patients with clinical suspicion of acute leukaemia
- Patients with peripheral blood smear showing blast cells
- Patients undergoing bone marrow examination and immunophenotyping

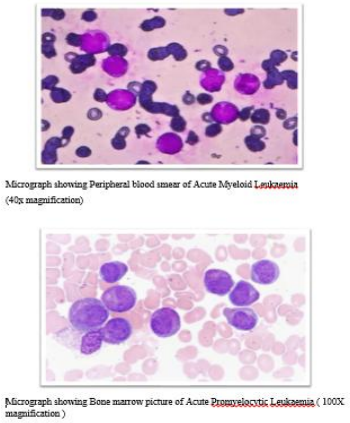
Exclusion Criteria:

- Patients already on chemotherapy for leukaemia
- Patients with chronic leukaemias or other haematological malignancies
- Inadequate or haemolysed blood/bone marrow samples
- Patients unwilling to give consent for participation in the study

BD FACS Canto II

Flourochrome tagged Monoclonal Antibodies used in FCM

PERM-2 and Lysing solution used in FCM



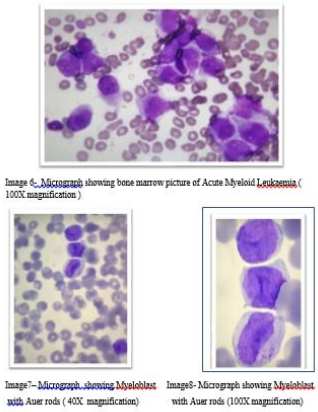


Image 6- Micrograph showing bone marrow picture of Acute Myeloid Leukaemia (100X magnification)

Image7– Micrograph showing Myeloblast with Auer rods (40X magnification)

Image8- Micrograph showing Myeloblast with Auer rods (100X magnification)

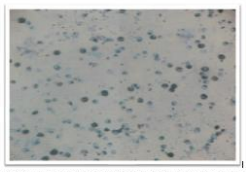


Image 9- Micrograph showing positive staining for MPO in bone marrow aspirate of Acute myeloid leukaemia (.100X magnification)

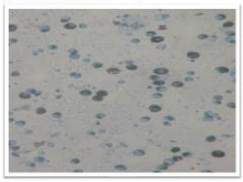


Image 10- Micrographs showing positive staining of MPO in bone marrow aspirate of Acute myeloid leukemia (40X magnification)

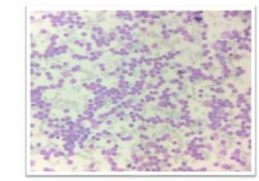


Image 11-Micrograph showing peripheral blood picture of Acute Lymphoblastic Leukaemia (.100X magnification)

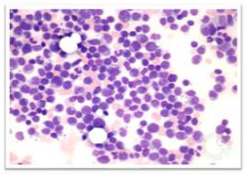


Image 12 – Micrographs showing peripheral blood picture of Acute Lymphoblastic Leukaemia (.40X magnification).

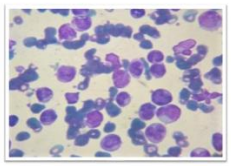


Image 13- Micrograph showing bone marrow picture of Acute lymphoblastic leukaemia (. 100X magnification)

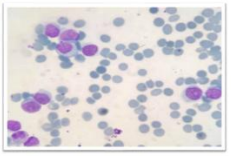
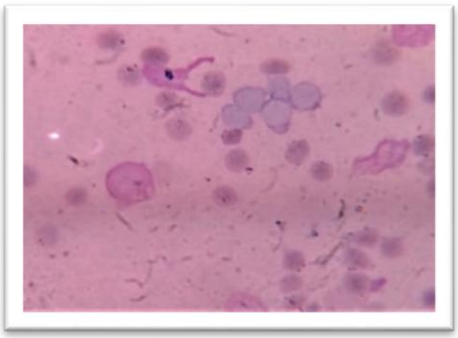
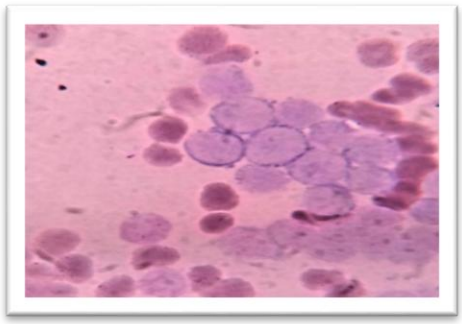


Image 14- Micrograph showing bone marrow picture of Acute lymphoblastic leukaemia (100X magnification).



Micrograph showing PAS staining in Acute lymphoblastic leukaemia. Block positivity seen. (40X magnification)



Micrograph showing PAS staining in Acute lymphoblastic leukaemia. Block positivity seen. (100X magnification)

Statistical Analysis: For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. Two-sample t-tests for a difference in mean involved independent samples or unpaired samples. Paired t-tests were a form of blocking and had greater power than unpaired tests. A chi-squared test ($\chi 2$ test) was any statistical hypothesis test wherein the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. Without other qualification, 'chi-squared test' often is used as short for Pearson's chi-squared test. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate.

Explicit expressions that can be used to carry out various t-tests are given below. In each case, the formula for a test statistic that either exactly follows or closely approximates a t-distribution under the null hypothesis is given. Also, the appropriate degrees of freedom are given in each case. Each of these statistics can be used to carry out either a one-tailed test or a two-tailed test.

Once a t value is determined, a p-value can be found using a table of values from Student's t-distribution

.If the calculated p-value is below the threshold chosen for statistical significance (usually the 0.10, the 0.05, or 0.01 level), then the null hypothesis is rejected in favour of the alternative hypothesis.

P-value ≤ 0.05 was considered for statistically significant.

RESULT

Table 1: Age Distribution of Patients (N = 52)

Age Group (years)	Frequency (n)	Percent (%)
18–30	12	23.1
31–50	20	38.5
51–70	15	28.8
>70	5	9.6
Total	52	100

Table 2: Sex Distribution of Patients (N = 52)

Sex	Frequency (n)	Percent (%)
Male	30	57.7
Female	22	42.3
Total	52	100

Table 3: Type of Acute Leukaemia (N = 52)

Diagnosis	Frequency (n)	Percent (%)
AML	34	65.4
ALL	18	34.6
Total	52	100

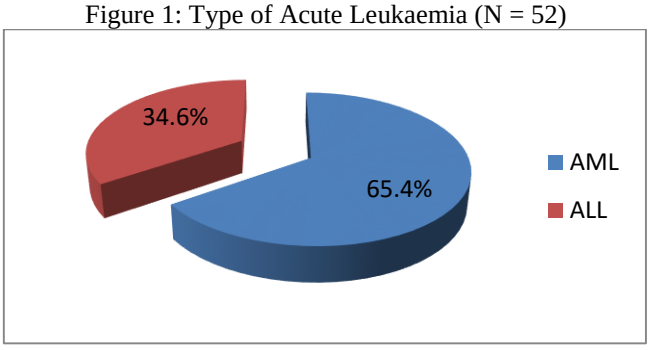
Table 4: Cytochemical Analysis (MPO/SBB/PAS) (N = 52)

Cytochemical Result	Frequency (n)	Percent (%)
Positive (Myeloid pattern)	34	65.4
Negative / Lymphoid pattern	18	34.6
Total	52	100

Table 5: Immunophenotyping Results (N = 52)

Immunophenotype	Frequency (n)	Percent (%)
AML (Myeloid markers CD13/CD33/MPO)	34	65.4
B-ALL (CD10/CD19/CD22)	14	26.9
T-ALL (CD3/CD7)	4	7.7
Total	52	100

Figure 1: Type of Acute Leukaemia (N = 52)



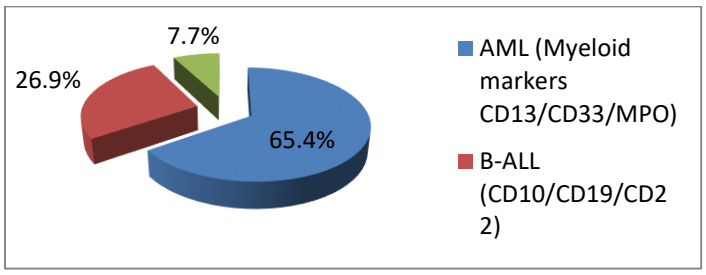


Figure 2: Immunophenotyping Results (N = 52)

In the present study, a total of 52 patients were included for analysis. The age distribution showed that the maximum number of patients belonged to the 31–50 years age group, for 20 patients (38.5%), followed by the 51–70 years age group with 15 patients (28.8%). The younger age group of 18–30 years comprised 12 patients (23.1%), while the least number of cases were observed in the age group of more than 70 years, with 5 patients (9.6%).

In the present study, a total of 52 patients were included. Regarding sex distribution, 30 patients (57.7%) were males and 22 patients (42.3%) were females.

In the present study, a total of 52 patients were evaluated for acute leukaemia. Based on final diagnosis, acute myeloid leukaemia (AML) was found to be the most common type, observed in 34 patients (65.4%), whereas acute lymphoblastic leukaemia (ALL) was diagnosed in 18 patients (34.6%).

In the present study, a total of 52 patients were evaluated using cytochemical analysis for acute leukaemia. Positive cytochemical staining showing myeloid pattern (Myeloperoxidase/Sudan Black B positive) was observed in 34 patients (65.4%), while negative staining or lymphoid pattern (PAS predominant or MPO/SBB negative) was seen in 18 patients (34.6%).

In the present study, a total of 52 patients were evaluated by immunophenotyping for classification of acute leukaemia. The most common immunophenotype observed was acute myeloid leukaemia (AML) characterized by myeloid markers such as CD13, CD33, and MPO, seen in 34 patients (65.4%). Among lymphoid malignancies, B-cell acute lymphoblastic leukaemia (B-ALL) expressing markers such as CD10, CD19, and CD22 was identified in 14 patients (26.9%), while T-cell acute lymphoblastic leukaemia (T-ALL) showing CD3 and CD7 positivity was observed in 4 patients (7.7%).

DISCUSSION

In the present study, a total of 52 adult patients with suspected acute leukaemia were evaluated using cytochemistry and immunophenotyping, and AML was found to be the most common subtype. Similar observations were reported by Kumar CC et al. [11], who described that acute myeloid leukaemia is the predominant acute leukaemia in adults and its incidence increases with age due to accumulation of genetic abnormalities.

In our study, AML accounted for 65.4% of cases, while ALL constituted 34.6%. This distribution is consistent with the findings of Voso MT et al. [12], who in the WHO classification emphasized that AML predominates in adult populations, whereas ALL is comparatively less frequent in this age group.

The male predominance observed in the present study is similar to the report by Parodi S et al. [13], who demonstrated a higher overall incidence of haematological malignancies in males, possibly due to occupational exposure, environmental factors, and lifestyle-related risks.

Cytochemical analysis in our study showed MPO and SBB positivity in the majority of cases, indicating myeloid lineage. A similar diagnostic utility of cytochemistry was highlighted by Samson EO et al [14], who stated that myeloperoxidase and Sudan Black B remain important and reliable stains for differentiating AML from ALL, especially in resource-limited settings.

Immunophenotyping revealed AML as the most common subtype followed by B-ALL and T-ALL. This finding is supported by Li W et al [15], who reported that flow cytometry plays a crucial role in identifying lineage-specific CD markers such as CD13 and CD33 for AML and CD19 and CD10 for B-ALL, thereby improving diagnostic accuracy.

The predominance of B-ALL over T-ALL among lymphoid cases observed in this study is comparable with the findings of Teachey DT et al. [16] , who reported that B-cell lineage ALL is more common in adults and carries distinct prognostic implications compared to T-cell ALL.

The combined use of cytochemistry and immunophenotyping in our study improved diagnostic precision, which is in agreement with Kansal R et al. [17], who emphasized that integrated diagnostic approaches are essential for accurate

classification of acute leukaemias in modern haematopathology practice.

The age distribution pattern observed in this study, with maximum cases in the 31–50 years age group, is similar to findings reported by Deschler B et al [18], who noted that AML incidence increases with age but may also occur in younger adults depending on genetic susceptibility and environmental exposure.

Overall, the findings of this study are consistent with the comprehensive review by De Shimony S [19], who concluded that AML remains the most common acute leukaemia in adults and requires integrated diagnostic techniques for accurate classification and management.

Finally, the importance of early diagnosis using combined cytochemical and immunophenotypic methods is strongly supported by Jarden M et al [20] , who emphasized that multimodal diagnostic strategies are essential for optimal treatment planning and improved clinical outcomes in acute leukaemia patients.

CONCLUSION

The present study included 52 adult patients with suspected acute leukaemia evaluated using cytochemical analysis and immunophenotyping at RIMS. Acute myeloid leukaemia (AML) was found to be more common than acute lymphoblastic leukaemia (ALL), with a higher prevalence observed in the 31–50 years age group and a male predominance. Cytochemical staining such as myeloperoxidase and Sudan Black B effectively identified myeloid lineage, while immunophenotyping provided definitive classification and accurate subtyping of acute leukaemias.

The study highlights that the combined use of cytochemistry and immunophenotyping significantly improves diagnostic accuracy, especially in distinguishing AML from ALL and identifying lymphoid subtypes. Thus, integration of these diagnostic modalities is essential for early and precise diagnosis, which is crucial for appropriate treatment planning and improved patient outcomes in acute leukaemia.

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