Original Article

Morphological and Immunophenotypic Analysis in Diagnosis of Acute Leukaemia

Md. Nurunnabi[1], Mosammath Khadiza Mamdu[2], Ayesha Siddika[3], Farzana Zafreen[4], Md Abdul Wahab[5], Shahana Shermin[6]

Abstract

Background: *Leukaemias are neoplastic proliferations of haematopoietic stem cells and form a major proportion of haematopoietic neoplasms that are diagnosed worldwide.* ***Objective:*** *To differentiate between morphological and immunophenotypic analysis in the diagnosis of acute leukemia.* ***Materials and method:*** *This cross sectional study was conducted in the department of Haematology, Armed Forces Institute of Pathology (AFIP), Dhaka, Bangladesh from January 2008 to December 2008. Total 50 patients were included after fulfilling inclusion and exclusion criteria.* ***Results:*** *The total of 50 bone marrow samples from suspected cases of acute leukaemia were included in the study. Out of 50 samples, 48 cases were diagnosed as either acute myeloid leukaemia (19 or 38%) or acute lymphoblastic leukaemia (29 or 58%) and 02 (04%) cases were morphologically indistinguishable. All 50 cases were subjected to immunophenotypic study. Out of 50 cases immunophenotypically 14(28%) were acute myeloid leukaemia (AML), 32(64%) were acute lymphoblastic leukaemia (ALL), and bi-phenotypic leukaemia and acute undifferentiated leukaemia were 02(04%) each. In this study Male: Female ratio was 1.3:1. Out of 19(38%) cases of AML, 29(58%) cases of ALL and 02(04%) cases of indistinguishable diagnosed morphologically, 14(28%) were found to be AML, 32(64%) ALL, 02(04%) bi-phenotypic and 02(04%) were acute undifferentiated leukaemias on immunophenotyping respectively. Out of 29 cases identified as ALL on morphology 25(86.2%) were confirmed as ALL, 02(07%) turned out to be AML, 01(3.4%) was bi-phenotypic and 01(3.4%) was undifferentiated.* ***Conclusion:*** *In this study, acute lymphoblastic leukaemia was the commonest type of leukemia followed by acute myeloid leukaemia with male predominance seen in all types of leukaemia.*

Keywords: *Incidence; Leukaemia; Immunophenotyping.*

Delta Med Col J. Jan 2020;8(1):15 – 20

1. Clinical Specialist in Pathology, CMH Sylhet, Bangladesh.
2. Senior Consultant Gynaecology & Obstetrics, Mahbubur Rahman Memorial Hospital, Brahmanbaria, Bangladesh.
3. Assistant Professor, Dept. of Oral & Maxillo Facial Surgery, Sir Salimullah Medical College, Dhaka, Bangladesh.
4. Associate Professor and Head, Dept. of Community Medicine, Medical College for Women & Hospital, Uttara, Dhaka, Bangladesh.
5. Associate Professor, Dept. of Biochemistry, Armed Forces Medical College, Dhaka, Bangladesh.
6. Senior Medical Officer, Dept. of Gynaecology & Obstetrics, BIRDEM General Hospital, Dhaka, Bangladesh.

Correspondence: Col. Md. Nurunnabi. e-mail: nabi2742@gmail.com

Introduction

The acute leukaemias are heterogeneous group of neoplasms arising from transformation of uncommitted or partially committed hematopoietic stem cells and it is broadly divided into myeloid and lymphoid leukemia. Acute myeloid leukaemia (AML) has an incidence of 2-3 per 100,000 per annum in children arising to 15 per 10,000 in older adults. On the other hand acute lymphoblastic leukaemia (ALL) in 75% of cases occurring in children under the age of 10 years at diagnosis.[1,2] Obtaining an accurate diagnosis i.e. microscopy, immunophenotyping, cytogenetic and molecular genetic testing must be done before initiation of definitive therapy for a patient with leukaemia. But in our country in most of the cases we need to rely only on morphology due to lack of facility of doing immunophenotyping, cytogenetic and molecular genetic analysis. Even most experienced morphologists can accurately classify 70 to 80% of acute leukaemia as ALL or AML on Leishman stained smear.[3-5]

The aim of the study was to find out the subtypes of acute leukaemia which are morphologically indistinguishable and to demonstrate the usefulness and advantages of immunophenotyping technique over the traditional method of leukaemia diagnosis.

Materials and method

This cross sectional study was conducted in the department of Haematology, Armed Forces Institute of Pathology (AFIP), Dhaka, Bangladesh from January 2008 to December 2008. A total of 50 patients were included after fulfilling inclusion and exclusion criteria. Peripheral blood and bone marrow samples of 50 patients were evaluated to diagnose different types of acute leukaemia.

An informed verbal consent of the patient or legal guardian (in cases of children) was taken. Detailed clinical information were obtained by meticulous history and thorough physical examination. Relevant investigations were also carried out. Aspiration was done by Salah marrow puncture needle. Site of aspiration varied according to the age of patient. Lignocaine 2% was used as local anaesthesia. Bone marrow aspirates were collected in EDTA tubes. Slides were prepared directly at bedside and from anticoagulated (EDTA) aspirates by squash method in the laboratory. In selected cases cytochemical stains like myeloperoxidase (MPO), periodic acid-Schiff (PAS) and alpha naphthyl acetate esterase (ANAE) were used.

For immunophenotyping, peripheral blood and bone marrow aspirates collected in EDTA tubes were used. Bone marrow samples were filtered and cell suspensions were prepared before reagent is mixed. One hundred microlitre (µl) of the sample was taken and mixed with 10 µI of monoclonal antibodies (Mcab). The mixture was incubated in dark at room temperature for 15 min. Then 100 µI of leucocyte fixative reagent was added and incubated at room temperature for 10 min. To the mixture 2.5 ml erythrocyte lysing agent was added for 20 min. The prepared sample was then ready for run in flow cytometer (Partec, Germany).

Monoclonal antibodies from Partec, GmbH, Munster Germany for imrnunophenotyping included the fluorescein isothiocyanate (FITC) and phycoerythrin (PE) conjugated monoclonal antibodies (Mcab). Following panel of monoclonal antibodies (Mcab) were used at AFIP for immunophenotyping.

Table I: Panel of monoclonal antibodies used for immunophenotyping analysis

Types of leukaemia		Monoclonal antibodies
AML		Anti MPO, CD_{13}, CD_{33}, CD_{117}, CD_{34}, CD_{14}
ALL	B-lineage	CD_{19}, CD_{79a}, CD_{22}, CD_{10}, CD_{20}, CD_{79b}
	T-lineage	CD_2, CD_3, CD_5, CD_7, CD_{23}

Bone marrow samples were analyzed with Partec flow cytometer equipped with argon laser emitting a 488 nm green (FITC=FL1) and orange phycoerythrin (Rhodamine=FL2) fluorescence with forward (FSC) and side scatter (SSC) were collected on all sample. FSC and SSC were collected linearly and displayed in a two-parameter histogram. FL1 and FL2 were collected, logged and displayed in a four-decade log/log two parameter histogram. The FSC/SSC histogram was used to determine the cell population of interest for gating. Gating was performed to include the blast population bone marrow cells determined by their size (FSC) characteristics.

The morphologic characteristics of the blast cell population were determined by light microscopy prior to flow cytometric analysis and at least 20% blast cells were required for processing of bone marrow samples. FL1 and FL2 were then collected for cells within the determined gate. A minimum of 2000 gated cells were analyzed and saved as list mode data. Positivity of any given antibody stain was determined by quadrant analysis as compared to the isotypic negative controls. Results excess of 30% positivity were considered to be positive for a given antibody. If a single color staining was used, FL1 and FL2 were displayed on a single four-decade long axis. Positivity was determined by placing a cursor or the isotype negative control peak such that it defined the region positivity and negative fluorescence intensity. The same 30% cutoff value for differentiation of positivity applied.

Results

A total of 50 bone marrow samples of morphologically diagnosed cases of different types of acute leukaemia were included in this study.

Table II: Age distribution of patients (N=50)

Age range (years)	Frequency	Percentage (%)
01 - 18	38	76
19 - 80	12	24
Total	50	100

Among the 50 patients majority were in the age group of 01 to 18 years of age which were 38(76%) cases and 12(24%) were in the age group of 19 to 80 years. Age range was from 01 year to 80 years. Median age was 08 years. (Table II)

Table III: Sex distribution of patients (N=50)

Sex	Frequency	Percentage (%)
Male	29	58
Female	21	42
Total	50	100

Amongst respondents 58% were male and 42% were female. Male: Female was 1.3:1. (Table III)

Table IV: Proportion of acute leukaemia by morphology (N=50)

Acute leukaemias	Frequency	Percentage (%)
AML	19	38
ALL	29	58
Indistinguishable	02	04
Total	50	100

Out of 50 samples, 48 cases were morphologically diagnosed as acute leukaemias, of which 19(38%) cases were found to be acute myeloid leukaemia, 29(58%) were acute lymphoblastic leukaemia and remaining 02(04%) cases were indistinguishable on morpholological examination. (Table IV)

Table V: Proportion of acute leukaemia by immunophenotypic study (N=50)

Acute Leukaemia	Frequency	Percentage (%)
AML	14	38
ALL	32	64
Bi-phenotypic acute leukaemia	02	04
Acute undifferentiated leukaemia	02	04
Total	50	100

All 50 cases were subjected to immunophenotypic study. Out of 50 cases immunophenotypically acute myeloid leukaemia (AML) were 14(28%), acute lymphoblastic leukaemia (ALL) were 32(64%), bi-phenotypic leukaemia were 02(04%) and acute undifferentiated leukaemia were 02(04%). (Table V)

Out of 29 cases identified as acute lymphoblastic leukaemia (ALL) on morphology, 25(86.2%) were confirmed as ALL, 02(07%) turned out to be acute myeloid leukemia (AML), 01(3.4%) was bi-phenotypic and 01(3.4%) was undifferentiated. Finally, out of 02 indistinguishable cases, both were found to be ALL on immunophenotyping. (Table VI)

Table VI: Rate of changes in diagnosis of morphologically diagnosed cases after immunophenotyping

Morphology		Immunophenotyping Frequency (%)			
Types	Frequency (%)	AML	ALL	Bi-phenotypic	Acute undifferentiated
AML	19 (38)	12 (63.1)	05 (26.3)	01 (5.3)	01 (5.3)
ALL	29 (58)	02 (07)	25 (86.2)	01 (3.4)	01 (3.4)
Indistinguishable	02 (04)	-	02	-	-
Total	50 (100)	14	32	02	02

Discussion

Haematological malignancies cover a wide range of diseases ranging from acute leukaemia to different type of lymphoproliferative disorders among which acute leukaemia is a major concern all over the world. Many a time, making a precise diagnosis using traditional morphological method including cytochemistry is difficult. Immunophenotyping is a method with improved sensitivity and precision which in acute leukaemia can not only detect cases indistinguishable on traditional morphological examination but also detects the subtypes of each category especially in ALL.[6-8] Immunophenotyping has been offered as a regular diagnostic facility at AFIP, Dhaka Cantonment.

In the present study out of 50 cases of acute leukaemia, morphological examination revealed AML 19(38%), ALL 29(58%) and 02(04%) cases remained indistinguishable. However, immunophenotypicalIy among acute leukaemia ALL was the commonest type 32(64%) followed by AML 14(28%), bi-phenotypic acute leukaemia 02(04%) and acute undifferentiated leukaemia 02(04%). Among 32 cases of ALL 23(46%) were B-cell ALL and 09(18%) were T-cell ALL.

In the present study out of 29 cases classified morphologically as acute lymphoblastic leukaemia (ALL) 25(86.2 %) were ALL, 02(07%) turned out to be acute myeloid leukaemia (AML), 01(3.4%) was bi-phenotypic and 01(3.4%) was acute undifferentiated leukaemia on immunophenotyping. Similarly, among 19 cases classified morphologically as AML, 12(63.1%) were AML, 05(26.3%) turned out to be ALL, 01(5.3%) was bi-phenotypic and 01(5.3%) was undifferentiated. Finally, out of 02 morphologically indistinguishable cases both were found to be ALL in immunophenotyping. Both morphological diagnosis of AML and ALL and immunophenotyical diagnosis was significantly different ($p< 0.01$).

Therefore, up to 74% of the cases of acute leukaemia could be classified according to their respective lineages by morphology. Whereas immunophenotyping provided correct diagnosis in 99% of cases establishing a superior diagnostic efficacy in cases of acute leukaemia which would otherwise be misdiagnosed on morphology and cytochemistry.[9]

A study was carried out in the Haematology department of Dr. Ziauddin Hospital, Karachi during September, 2004 to August, 2006. Of 100 cases of acute leukaemia aged between 2 to 50 years, inducted from various hospitals and laboratories of Karachi, examined morphologically and on cytochemistry, among the 53 cases classified as acute lymphoblastic leukaemia (ALL), 45(81%) were ALL, 4(9%) turned out to be AML and 4(9%) were bi-phenotypic on immunophenotyping. Similarly, among 46 cases classified as AML on morphology and cytochemistry, 38(83%) were AML, 5(11%) turned out to be ALL, 2(4%) were bi-phenotypic, while 1(2%) was still unclassified on immunophenotyping.[10-12]

Khalil et al. in King Faisal Specialized Hospital and Research Center also found ALL to be the commonest (63.2%) of al leukaemias by immunophenotyping followed by AML (21%) and biphenotypic leukaemia (12%).[13]

In another study at Tata Memorial Hospital, India, AML was found to constitute 39.8% of all leukaemias.[14] In one American study, Thalhammer-Scherr et al. however reported AML to be the predominant type (78.2%) of acute leukaemia followed by ALL (19.1%) on the basis of immunophenotyping.[15] One case of AML in present study presented with aberrant expression of T-lineage antigen.

In a study conducted at AFIP on morphological types of haematological malignancy Mahfuz et al. found ALL 39.3%, AML 30.8%, CML 11.5%, and lymphoreticular malignancy (lymphoma) 4.7%.[16] This morphological method of diagnosing haematological malignancies closely conforms with the immunophenotypic method to classify haematological malignancies conducted at the same institute.

The limitation of this study was inability to compare with other standard studies carried out in our country and abroad because of non-availability of flow cytometer in most of the centers in our country as well as a few such study carried out in abroad. There are wide variation in the results of immunophenotypic findings, yet it has been used as a major tool for diagnosis of haematological malignancies. The results can be used to select specific regimen of chemotherapy and assessing the prognosis of the patient. In Bangladesh no other immunophenotypic study is carried out so far and as such the exact pattern of acute leukaemia in our country is not known.

Conclusion

Morphological diagnosis of acute eukaemia by light microscopy remain the mainstay of management of acute leukaemia throughout the world especially in the developing countries. It is difficult at times to differentiate between different types of acute leukaemias on the basis of morphology or even cytochemistry. Immunophenotyping has been introduced in association with above mentioned methods to differentiate and classify different types of acute leukaemia. Besides acute leukemia immunophenotyping has already opened wider avenues at diagnosis of different haematological malignancies in the recent years. It is now also used to select different types of chemotherapy regime (monoclonal antibodies) and to assess prognosis of different types of haematological malignancies. Considering the diagnostic efficacy immunophenotyping will soon be established as a basic diagnostic tool for acute leukaemia. To explore the proven benefit in the management of haematological malignancies, we will have to make immunophenotyping facilities available throughout the country.

References

1. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Jhons N, Lozano R, et al. A Systematic Analysis of Global Anaemia Burden from 1990 to 2010. Blood. 2014;5:615-24.
2. Rodak BF, Fristma GA, Keohane EM. Haematology: Clinical Principles and Applications. Philadelphia: Elsevier WB Saunders; 2011. p.135-36.
3. Martin A, Thompson AA. Thalassemias. Pediatr Clinic North Am. 2013;60(6):1383-91.
4. Cao A, Galanello R. Beta Thalassaemia. Genet Med. 2010;2:61-76.
5. Burnett AK. Acute Myeloid Leukemia. In: Hoffbrand AV, Catovsky D, Edward G, Tuddenham D, editors. Postgraduate Haematology. 5th ed. Oxford: Blackwell; 2005. p.509.
6. Boon NA, Colledge NR, Walker BR, Hunter J. Davidson's Principle and Practice of Medicine. 20th ed. London: Churchill Livingstone; 2006. p.1016.
7. Bhatia S, Neglia JP. Epidemiology of Acute Leukaemia. 3rd ed. New York: Oxford University Press; 2005. p.98-100.
8. Hoffman R, Benz EJ Jr., Silberstein LE, Heslop HE, Weitz JI, Anastasi J, et al. Haematology Basic Principles and Practice. 4th ed. St Louis: Elsevier Churchill Livingstone; 2005. p.1071.

9. Abeloff MD, Armitage JO, Niederhuber JE, Kastan MB, McKenna WG. Clinical Oncology. 3rd ed. St. Louis: Elsevier Churchill Livingstone; 2004. p.2831-32.

10. Pui C, Robinson L, Look AT. Acute Lymphoblastic Leukaemia. The Lancet. 2008;371:9617.

11. Estey E, Donner H. Acute Myeloid Leukaemia. The Lancet. 2006;368:1894.

12. Hoffbrand AV, Pettit JE, Moss PAH. Essential Haematology. 4th ed. London: Blackwell Science Ud; 2001. p.174.

13. Khalil HS, Jackson MJ, Pyle HR, Robichaud M. Immunophenotyping of Acute Leukemia at King Faisal Specialist Hospital and Research Centre. Ann Saudi Med. 1995;15(2):137-39.

14. Ghosh S, Sinde SC, Kumaran GS, Sapre RS, Dhond SR, Badrinath Y, et al. Haematologic and Immunophenotypic Profile of Acute Myeloblastic Leukaemia: An Experience of Tata Memorial Hospital. Indian Journal of Cancer. 2003;40(2):71-76.

15. Thalhammer-Scherrer R, Mitterbauer G, Simonitsch I, Jaeger U, Lechner K, Schneider B, et al. The Immunophenotype of 325 Adult Acute Leukaemias: Relationship to Morphologic and Molecular Classification and Proposal for a Minimal Screening Program Highly Predictive for Lineage Discrimination. American J Clin Path. 2002;117(3):380-89.

16. Mahfuz H, Rahman MM, Bhyuian MN, Shaha D. Bone Marrow Morphological Examination - an Analysis of 500 Cases. JAFMC Bangladesh. 2013;9(2):59-63.