Prevalence and Diagnosis of Acute Leukaemia - Experience in 50 Cases in AFIP

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Introduction

Cancer is set to become a major cause of morbidity and mortality in the coming decades in every region of the world. Today cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries.¹ Malignant disorders in haematopoietic system include different conditions and of which some of them are related to bone marrow and others may be related to the lymphatic system.² Three main types of blood cancers are leukaemia, lymphoma, and myeloma or plasma cell disorders.³ Leukemias are the 10th most common cancer in men and 12th most common in women and constitute 3% of the global cancer burden. Developing countries bear more than half of the global cancer burden because 75% of the world population lives in these...
countries.\textsuperscript{4-6} The incidence of leukaemia is highest in North America and Australia and lowest in sub-Saharan Africa.\textsuperscript{7-9} In India, lympho-haematopoietic malignancies constitute 9.5% of all cancer in men and 5.5% in women.\textsuperscript{10,11} These figures are comparatively lower than the rest of the world, but under-diagnosis and under-reporting cannot be ruled out.

**Materials and method**

This cross-sectional study was conducted in the Department of Haematology, Armed forces Institute of Pathology (AFIP), Dhaka, Bangladesh from January 2010 to December 2011. Total of 50 patients were included after fulfilling the inclusion and exclusion criteria. Verbal consent was taken from every patient and clearance was done by the ethical committee. The data were processed and analysed by computer software SPSS (Statistical Package of Social Science). Peripheral blood and bone marrow samples of 50 patients were evaluated to diagnose the different types of acute leukaemia.

**Inclusion criteria**

1. Cases from both the Armed Forces personnel and civilian population from different parts of the country who were referred to AFIP for bone marrow and immunophenotyping study for acute leukaemia.
2. Age limitation was between 06m-80yrs.

**Exclusion criteria**

1. All chronic leukaemia & lymphoma cases.
2. All relapse cases of acute leukaemia.

An informed verbal consent of the patient or legal guardian (in cases of children) was taken. Detail clinical information was obtained meticulously by history taking 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 the patient. Lidocaine hydrochloride 2% injection was used as local anaesthesia. Bone marrow aspirates were collected in Ethylenediaminetetraacetic acid (EDTA) tubes.

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

**Results**

A total of 50 patients were evaluated in the present study. Among them 29 (58%) were male and 21 (42%) were female. The age range was from 06 months to 80 years.

**Figure 1:** Age distribution of patients (n=50)

**Figure 2:** Sex distribution of patients (n=50)
It was observed that age groups are the most vulnerable for the various leukaemia. These were as follows- leukaemia was the commonest in the age group of 1-15 years (Figure 1), ALL were between 1-15 years of age group, and peak incidence found in the age group of 1-5 years followed by 6-10 years.

Male:Female =1.3:1. Median age: 08 yrs (Figure 2).

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

Discussion:

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

Among the 50 patients, the majority were in the age group of 06 months to 15 years which were 38 (76%) cases, followed by 12 (24%) patients belonging to the age group of 16 to 80 years. Amongst respondents 58% were male and 42% were female. Morphology of 50 bone marrow samples revealed that 19 (38%) cases were acute myeloid leukemia, 29 (58%) were found to be acute lymphoblastic leukaemia and 02 (04%) cases were remained indistinguishable on morphological examination.

Immunophenotypically out of 50 cases, 14 (28%) were acute myeloid leukaemia and 32 (64%) were acute lymphoblastic leukaemia, bi-phenotypic leukaemia was 02 (04%) and acute undifferentiated leukaemia were 02 (04%).

In our study, male predominance was seen, which is similar to most studies conducted in India and other countries.10-11

Figure 3: Prevalence of types of acute leukaemia by bone marrow study (n=50)

A 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 acute leukaemia of which 19 (38%) cases were found to be acute myeloid leukaemia, 29 (58%) were acute lymphoblastic leukaemia and 02 (04%) cases were remained indistinguishable on morphological examination.

Figure 3: Prevalence of acute leukaemia by immunophenotyping (n=50)
This study found that acute lymphoblastic leukaemia (ALL) was the second most common leukaemia subtype which was similar to the studies reported by Shome et al, Modak H et al and Chen et al. However, our observation varies from the studies conducted by Vasavada et al and Kushawaha et al which might be due to underdiagnosis or under reporting. It is also observed that maximum cases are seen in the first and second decade of life, that is comparable with D’Costa GG et al, Parkin DM et al, Meighan et al, and Alrudainy et al. Male predominance for ALL was found in North India (Delhi), rural areas of Maharashtra like Barshi, and South India (Chennai and Bangalore), which is in accordance with the present study.

Acute myeloid leukaemia (AML) was the third most common leukaemia in the present study which was in accordance with a study carried out by D’Costa GG et al. Incidence of lower percentage was reported by Vasavada et al and Menzes and Malik which vary from the present study. A large percentage of cases found in adults were comparable with most of the studies in India, eastern and western countries. In this study, male predominance was seen, which was similar to most studies mentioned.

In our study, out of 50 cases of acute leukaemia, morphological examination revealed AML in 19 (38%) and ALL in 29 (58%) cases; and 02 (04%) cases remained indistinguishable. However, immunophenotypically among acute leukaemia ALL was the commonest type 32(64%) followed by AML 14 (28%), biphenotypic acute leukaemia 02(04%), and acute undifferentiated leukaemia 02 (4%). Among 32 cases of ALL 23 (46%) were B-cell ALL and 09 (18%) were T-cell ALL. The difference between morphological and immunophenotypic disease pattern was significant (p< 0.01).

In the present study, out of 29 cases classified morphologically as acute lymphoblastic leukaemia (ALL), 25 (86.1 %) 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 (100%) were found ALL in immunophenotyping. Both morphological diagnosis of AML and ALL and immunophenotypic diagnosis was significantly different (p< 0.01).

Therefore, up to 86% of the cases of acute leukaemia could be classified according to their respective lineages by morphology. Whereas Immunophenotyping provided a 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.

A study was carried out in the haematology department of Dr. Ziauddin Hospital, Karachi from September 2004 to August 2006. Among 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 43 cases classified as Acute Lymphoblastic Leukaemia (ALL), 35 (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. Khalil et al in King Faisal Specialized Hospital and Research Centre also found ALL to be the commonest (63.2%) of all leukaemia by immunophenotyping followed by AML (21 %) and biphenotypic leukaemia (2%).

DOI: https://doi.org/10.37545/haematoljbd202281
study at Tata Memorial Hospital, AML was found to constitute (39.8%) of all leukaemia. In an American study, Scherr et al, however, reported that AML to be the most predominant type (78.2%) of acute leukaemia followed by ALL (19.1%) on the basis of immunophenotyping. 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, Yusuf et al found AML (27.4%), ALL (39.3%), CLL (5.9%), and Lymphoreticular malignancy (lymphoma) (11.9%). This morphological method of diagnosis of haematological malignancies closely confirms with the immunophenotypic method to classify haematological malignancies conducted at the same institute.

Conclusion

Morphological diagnosis of acute leukaemia by light microscopy remains the mainstay of diagnosis of acute leukaemia throughout the world especially in the developing countries. It is difficult at times to differentiate between different types of acute leukaemia 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 leukaemia 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 regimen (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 have some following recommendations.

1. In all divisional level government hospitals should have bone marrow study, immunophenotyping & RT-PCR test facilities.
2. We must establish 2/3 referral laboratory in major cities like Dhaka & Chattogram.
3. Need constant communication & liaison with government authorities so that they can give due attention to this burring field.

References:

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