Abstract

Background: Acute leukaemia (AL) are a heterogenous group of haematological malignancy characterized by uncontrolled clonal proliferation of haematopoietic progenitor cells. Objectives: The study was conducted to have a detailed understanding of immunophenotyping profile, the frequency of discrepancy between bone marrow morphology and immunophenotyping and importance of immunophenotyping in diagnosis of acute leukaemia. Methods: This prospective type of observational study was carried out with an aim to correlate the immunophenotype with bone marrow morphology and to see the discrepancy between this two in acute leukaemia. A total of 38 untreated acute leukemia patients attending in the Department of Haematology, Bangabandhu Sheikh Mujib Medical University, Dhaka, during the period from October 2016 to September 2017 were included in this study. At first the morphological diagnosis was done. Then the immunophenotypic profile was compared. Result: Around eighty-two cases of acute leukaemia did find similarity with immunophenotyping and remaining 18.4% shows discrepancy. Diagnosis in this 18.4% changes after immunophenotyping. Aberrant phenotypes were detected in 20 (52.6%) samples among them 13 (34.21%) cases were AML, 3 (7.8%) cases were B-ALL and 4 (10.52%) cases were T-ALL. Significant relation was not found between aberrant marker and FAB subtypes. Conclusion: In acute leukaemia morphological appearance of bone marrow does not always match with immunophenotyping. It is therefore imperative and absolutely essential to ascertain the lineage of leukaemia by immunophenotyping before starting treatment.

Key Words: Acute leukaemia, Discrepancy, Bone Marrow Morphology, Immunophenotype.

Introduction

Acute leukaemia (AL) are a heterogenous group of haematological malignancy characterized by uncontrolled clonal proliferation of haematopoietic progenitor cells. Leukaemia occurs about 10 times more often in adults than in children. Morphologic evaluation of bone marrow is an important tool for diagnosis of acute leukaemia. Blast percentage plays a central role in the diagnosis and classification of acute leukemias.

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Immunophenotyping is the identification and quantification of cellular antigens through fluorochrome labeled monoclonal antibodies. Immunophenotyping improves both accuracy and reproducibility of AL classification. For this reason, immunophenotype analysis become critical part of initial diagnosis and classification of acute leukaemia over the last decades. In addition, immunophenotyping provides prognostic information to monitor the progress of patients after chemotherapy and aids in detection of minimal residual disease.

In 2015, a study was done in NSCB Medical College, Jabalpur, Madhya Pradesh, India where Ashish Gupta et al. showed 73% cases of acute leukemia did find similarity in morphological appearance and immunophenotyping. Remaining 27% cases shows discrepancy between morphological and immunological diagnosis. That is morphological findings did not correlate with immunophenotyping expression. Diagnosis in these 27% patients changed after immunophenotyping. The present study was conducted to have a detailed understanding of immunophenotyping profile, the frequency of discrepancy between bone marrow morphology and
immunophenotyping and importance of immunophenotyping in diagnosis of acute leukaeemia.

Methodology

This observational study was carried out with an aim to correlate the immunophenotype with bone marrow morphology and to see the discrepancy between this two in acute leukaemia. A total of 38 untreated acute leukaemia patients attending in the Department of Haematology, Bangabandhu Sheikh Mujib Medical University, Dhaka, during the period from October 2016 to September 2017 were included in this study. At first the morphological diagnosis was done. Then the immunophenotypic profile was compared and correlated. The study was performed after getting official clearance for the protocol from Institutional Review Board (IRB) of BSMMU. Only newly diagnosed patients of acute leukaemia of ≥ 10 years of either sex was included in this study. Patients who did not gave informed written consent, patients already received chemotherapy, patients of acute promyelocytic leukaemia and patients of acute leukaemia transformed from Chronic Myeloid leukaemia (CML) and Myelodysplastic Syndrome (MDS) were excluded from the study. A pre-designed structured data collection sheet was used. For each and every subject separate data collection sheet was prepared. After selection of study subject’s data was collected by details history taking and clinical examination. The purpose and procedure of the study had been discussed with the subjects. Written consent and assent were taken from those who agreed to participate in the study. All patient’s data including demographical, clinical and laboratory data had been collected to evaluate basic hemogram. Complete blood count was done by automated cell counter machine. Morphological diagnosis of AL had been done initially by bone marrow study with or without trephine biopsy. Then immunophenotyping had been done by flow cytometer. In our laboratory, we used Beckman Coulter Cytocoms FC 500 for immunophenotyping analyzing the expression of cell surface and intracellular molecules using fluorescent-labeled antibodies detecting proteins. The staining procedure involved making a single-cell suspension from cell culture or tissue samples. The cells were then incubated in tubes or micro titer plates with unlabeled or fluorochrome-labeled antibodies and analyzed on the flow cytometer. Light scattered from the cells or particles was detected as they go through the laser beam. A detector in front of the light beam measures forward scatter (FS) and several detectors to the side measure side scatter (SS). Fluorescence detectors measured the fluorescence emitted from positively stained cells or particles.

Data analysis

The mean values were calculated for continuous variables. The quantitative observations were indicated by frequencies and percentages and mean with standard deviation measured from continuous data. Chi-square test was used for categorical variables. P values <0.05 was considered as statistically significant. Statistical analyses were carried out by using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

A total 38 patients were included in this study and almost one third (34.2%) patients belonged to age ≤ 20 years. The mean age was found 29.3±13.2 years with range from 14 to 55 years. More than half (52.6%) patients were male, 35 (92.1%) and male female ratio was 1.11. (Table 1)

Table 1: Age and sex distribution of the study population (n=38)

<table>
<thead>
<tr>
<th>Age (in year)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20</td>
<td>13 (34.2)</td>
</tr>
<tr>
<td>21-30</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>31-40</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>&gt;40</td>
<td>12 (31.6)</td>
</tr>
</tbody>
</table>

Mean ±SD 30.7 ±13.5
Range (min-max) 14-55

Sex
Male 20 (52.6)
Female 18 (47.4)

Among the study cases, 25 (around 66%) were diagnosed as Acute Myeloid Leukaemia (AML) and 13 were diagnosed as Acute Lymphoblastic Leukaemia (ALL) morphologically. (Figure 1)

Morphological Diagnosis

![Morphological Diagnosis](image)

Figure 1: Distribution of the study population according to morphological diagnosis (n:38)

Different immune marker used to identify the phenotypes acute leukemia. Among the lymphoid marker, CD3 was found in 9 (23.7%) cases, CD5 in 6 (15.8%), CD7 in 8 (21.1%), CD19 in 10 (26.3%), CD22 in 4 (10.5%) and
CD79a in 12 (31.6%). In case of myeloid marker, CD13 was found in 22 (57.9%) cases, CD33 in 25 (65.8%), CD117 in 15 (39.5%) and MPO in 25 (65.8%). In case of other marker, CD45 was found in 34 (89.5%) cases, HLA-DR in 27 (71.1%), TdT in 6 (15.8%), CD10 in 9 (23.7%) and CD34 in 2 (5.3%) cases. (Table 2)

Table 2: Distribution of study cases according to positivity to different immune markers. (n:38)

<table>
<thead>
<tr>
<th>Lymphoid Marker</th>
<th>n (%)</th>
<th>Myeloid Marker</th>
<th>n (%)</th>
<th>Others Marker</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>9 (23.7)</td>
<td>CD13</td>
<td>22 (57.9)</td>
<td>CD45</td>
<td>34 (89.5)</td>
</tr>
<tr>
<td>CD5</td>
<td>6 (15.8)</td>
<td>CD33</td>
<td>25 (65.8)</td>
<td>HLA-DR</td>
<td>27 (71.1)</td>
</tr>
<tr>
<td>CD7</td>
<td>8 (21.1)</td>
<td>CD117</td>
<td>15 (39.5)</td>
<td>TdT</td>
<td>6 (15.8)</td>
</tr>
<tr>
<td>CD19</td>
<td>10 (26.3)</td>
<td>MPO</td>
<td>25 (65.8)</td>
<td>CD10</td>
<td>9 (15.8)</td>
</tr>
<tr>
<td>CD22</td>
<td>4 (10.5)</td>
<td>CD34</td>
<td>2 (5.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD79a</td>
<td>12 (31.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total in 7 (18.4%) cases morphological diagnosis changed after immunohistochemistry. Among morphologically diagnosed 25 AML cases, in 4 cases (10.5%) diagnosis was changed to ALL (2 :5.3%) and Mixed Phenotypic Acute Leukaemia (MPAL) (2 :5.3%) and among 13 ALL cases, 3 (7.9%) diagnosis were changed to AML finally after immunohistochemistry. (Table 3)

Figure 2: Pie chart showing discrepancy of study patients

Table 3: Association of FAB subtype with immunophenotype and discrepancy (n=38)

<table>
<thead>
<tr>
<th>Morphological Type</th>
<th>Immunophenotype</th>
<th>Discrepancy of total; n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALL</td>
<td>AML</td>
</tr>
<tr>
<td>AML</td>
<td>25 (65.8)</td>
<td>02 (5.3)</td>
</tr>
<tr>
<td>ALL</td>
<td>13 (34.2)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>Total</td>
<td>38 (100.0)</td>
<td>12 (31.6)</td>
</tr>
</tbody>
</table>

Discussion

This is an observational study was carried out with an aim to correlate the immunophenotype with bone marrow morphology and to see the discrepancy between this two in acute leukaemia. A total of 38 untreated acute leukaemia patients attending in the Department of Haematology, Bangabandhu Sheikh Mujib Medical University, Dhaka, during the period from October 2016 to September 2017 were included in this study.

In this study it was observed that more than one third (34.2%) patients belonged to age ≤ 20 years. The mean age was found 29.3±13.2 years with range from 14 to 55 years. Similar observation was found by Venkateswaran et al. (2012) where they showed the maximum number of patients belonged to 1st & 2nd and 3rd & 4th decade of age group (37 & 35 cases respectively). The average age of the patients was 29.58 years. In the study done by Venkateswaran et al. (2012) where they reported the majority were males constituting 58 % and the male to female ratio was 1.38:1. Another study conducted by Seegmiller et al. (2009) where they observed the overall male/female ratio was 1.4:1.

In our study, CD79a is the most frequently detected lymphoid marker it was found in 12 (31.6%) cases. Next to CD79a CD19 and CD3 which was found in 10 (26.3%) and 9 (23.7%) cases respectively. Among myeloid markers, CD33 and MPO were most frequently expressed marker. These were found in 25 (65.8%) cases. Similarly, Gupta et al. (2015), in their study, found CD3 was the most commonly expressed T-cell antigen expressed in7 (78%) cases. Venkateswaran et al. (2012) analyzed the immunophenotype of 38 cases of Acute Myeloid leukaemia. They also showed that CD33, CD13, CD117 and CD64 were the most commonly expressed myeloid antigens.3 Kaleem et al. (2003) and Auewarakul et al. (2005) demonstrated that CD33, CD13, CD117 and CD64 were the most commonly expressed myeloid antigens in their studies of acute myeloid leukaemia.5-6 All cases of B-acute lymphoblastic leukaemia showed expression of pan B-cell markers (CD19, CD22 and cytoplasmic CD79a) and 117 (90%) cases expressed CD10. Cytoplasmic CD3 and CD5 were the most sensitive markers for diagnosis of T-acute lymphoblastic leukaemia.7 With respect to myeloid antigens expression in lymphoid leukaemia, the most frequent was CD13, CD33.

In this study discrepancy between bone marrow morphology and immunophenotype were found in 7 (18.4%) patients among them 3 (42.85%) samples have been found AML, 1 (14.28%) sample B-ALL, 1 (14.28%) sample T-ALL and 2 (28.57%) samples shows Mixed Phenotype Acute Leukaemia (MPAL). Similar study was done by Gupta et al. (2015) where in 73% cases of acute leukaemia found similarity in morphological appearance and immunophenotyping and remaining 27% cases shows discrepancy. Diagnosis in these 27% patients changed after immunophenotyping.
Limitations of the study

The sample size was small. Samples were collected from only one center; hence it may not represent the whole population of the community.

Conclusions

In acute leukaemia, morphological appearance of bone marrow does not always match with immunophenotyping. In our study 81.6% cases of acute leukaemia did find similarity with immunophenotyping and remaining 18.4% shows discrepancy. It is therefore, imperative and absolutely essential to ascertain the lineage of leukaemia by immunophenotyping before starting treatment. Further studies with larger numbers of patients are recommended to confirm our results.

Recommendations

Immunophenotyping should be performed routinely in all cases of acute leukaemia. Study period may be extended. Further multi-centered study with larger sample size is recommended.

References

Published Erratum


   In
   
   a. Cover and Content page -
      Khan MR$_6$
   b. Page 16-
      Khan MR$_6$ and
   6. Md. Rafiquil Islam Khan, Department of Haematology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

Will be replaced by-

   a. Khan MR$_6$
   b. Khan MR$_6$ and
   6. Md. Rafiquzzaman Khan, Department of Haematology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.