Original Research Article

Aberrant phenotypes in acute myeloid leukemia in India

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ABSTRACT

Background: Acute myeloid leukemia (AML) is a heterogeneous disease, associated with a high diversity of phenotypes. The study was done with the aim to study the aberrant phenotypes in acute myeloid leukemia cases and the correlation among the aberrant phenotypes and poor prognostic factors in acute myeloid leukemia.

Methods: This cross sectional study was conducted on 35 cases of newly diagnosed AML according to the selection criteria at Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai for a period of 6 months. Immunophenotyping analysis by flow cytometry was done on fresh bone marrow aspirate or peripheral blood sample by applying Acute Leukemia Panel. The co-expression of different antigen markers on lymphocytes was analyzed.

Results: Aberrant lymphoid markers were seen in 17 (49%) cases. 5 (14%) cases had lymphoid associated antigen expression alone. 3 (8%) cases had asynchronous antigen expression alone. 9 (27%) cases had both asynchronous antigen expression and lymphoid associated antigen expression which is of cases . In total, lymphoid associated antigen expression is seen in 41% of cases and asynchronous antigen expression in 35% of cases. CD3, CD19 (lymphoid associated antigen) and CD34+ CD15+ (asynchronous aberrant phenotype) were the most common equally expressed aberrant phenotypes, each in 7 cases. CD 3 was significantly more common in males (P=0.021) but in general there were no statistically significant association between adverse prognostic factors and aberrant phenotypic AML.

Conclusions: CD19 and CD3 were the most commonly expressed lymphoid associated antigen. Most common asynchronous aberrant phenotype was CD34+CD15+. None of the aberrant phenotypic expression was not associated with poor risk factors in acute myeloid leukemia except for common expression of CD3 in males.

Keywords: Aberrant phenotypes, Acute myeloid leukemia, Flow cytometry

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease, presenting with a high diversity of phenotypes and characterized by immature myeloid cell proliferation and bone marrow failure.1,2

Immunophenotype in acute myeloid leukemia (AML) had remained elusive. In recent years, along with the wide application of AML immunophenotype testing, immunophenotype itself and its relationship with genetics and morphology became better understood.

Aberrant phenotype is a well-known phenomenon in acute myeloid leukemia. Currently, the aberrant phenotypes are classified into different types: co-expression of lymphoid-associated antigens or lineage infidelity; asynchronous antigen expression, in which early antigens are coexpressed with more mature ones; or antigen overexpression and existence of abnormal light scatter patterns.3
Flow cytometry (FCM) is the molecular method used for the identification minimal residual disease (MRD) especially in identification of immunophenotypical aberrancies in AML. The outcome of this procedure is strongly associated with treatment outcome and clinical remission.4,5

The present study was done with the objective to evaluate the frequency of aberrant phenotypes and its correlation with known prognostic factors such as gender, age, WBC count, platelet count and blast percentage.

METHODS

This cross-sectional study was conducted in Institute of internal medicine and Department of hematology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai for a period of 6 months, from February 2014 to July 2014.

35 cases of newly diagnosed AML were selected according to the selection criteria. Immunophenotyping analysis by multi parameter flow cytometry was done on fresh bone marrow aspirate or peripheral blood sample by applying Acute Leukemia Panel with the following CD markers: CD3, CD7, CD10, CD13, CD14, CD15, CD10, CD19, CD33, CD34, CD45, CD117. CD marker was considered to be positive when more than 20% blast cells are positive.

Selection criteria

Inclusion criteria were cases of newly diagnosed AML and exclusion criteria were patients under treatment and relapsed AML patients.

Flow cytometric analysis of acute leukemia cases

The reagents and capped polystyrene test tubes were provided by Beckman coulter. Ten microliters of fluorescein isothiocyanate (FITC) conjugated monoclonal antibody, 10μl of Phycoerythrin (PE) conjugated monoclonal antibody, 5μl of Allophycocyanin (APC) conjugated monoclonal antibody, and 5μl of Peridinin-chlorophyll protein complex (perCP) conjugated monoclonal antibody was added to the tubes, afterward 100μL of whole blood / bone marrow was in added each tube. Monoclonal antibodies (Abs) used in this study included fluorescein isothiocyanate (FITC), phycoerythrin (PE), or peridinin chlorophyll-protein (Per-CP), Allophycocyanin (APC) labeled CD3, CD7, CD10, CD13, CD14, CD15, CD10, CD19, CD33, CD34,CD45,CD117. The mixture was vortexed tenderly and incubated about 45 minutes to 1 hour in the dark area at room temperature (20-25°C). Two ml of 1X lysis buffer was added to incubated mixture. Then it was vortexed tenderly and incubated for 20 minutes in the dark area at room temperature again; after that Centrifuge at 500 g for 5 minutes was done. The supernatant was removed. Subsequently 2-3 ml of washing buffer was added and centrifuged at 500g for 5 minutes and the supernatant was removed. 1 ml of 1% cell fix (paraformaldehyde solution) was added and mixed completely, analysis can be done immediately or fixed cells can be stored at 2-8°C until analysing them. Analysis was done by Beckman coulter brand flow cytometer. Samples vortexed thoroughly prior to acquisition. Abnormal populations were recognized by CD45/SSC gating, which was the base of calculating the positive rate of leukemia-related antigens expressed on the abnormal cells. Antigen expression was considered to be positive when the percentage of positive blast cells was equal or greater than 20%. Similarly, aberrant phenotypes were defined when at least 20% of the blast cells expressed that particular phenotype.

Statistical analysis

Data were analyzed qualitatively and quantitatively by means of SPSS 14. Frequency and descriptive analysis were used in all statistical process. The statistical significance value was chosen to be below 0.05.

RESULTS

The study included 35 samples of newly diagnosed acute myeloid leukemia. Patients age ranged from 15-80 years with the mean age of 40.65 years. The male to female ratio was 1.9:1. Based on immunophenotyping analysis by multi parameter flow cytometry, we selected 35 cases. 17(48.5%) cases were found to be aberrant phenotype including 5 (14%) cases with lymphocytic antigen expression alone, 3 (8%) cases with asynchronous antigen expression and 9 (25.7%)cases showed expression of both antigens (Figure 1).

![Aberrant phenotype in AML (n=35)](image)

Of the total 35 cases, 14 samples showed lymphocyte antigen positive (Ly+). CD33, CD34 and CD117 was positive in majority of the samples (71.4%) followed by CD13 in 9 (64.3%) samples, CD3 positive in 7 (50%) in samples and CD7, CD10, CD14, CD15 positive in 4 (28.6%) samples each. CD3 (50%)was the most common T cell antigen and CD19 (50%)was the most common B cell antigen expressed as shown in Table 1.
Table 1: Distribution of aberrant markers in acute myeloid leukemia cases.

| Antigen | Total no. of cases (n=35) | No. of Ly+AML (n=14) | T cell markers Ly+AML | B cell markers Ly+AML | Both T & B Ly+AML |
|---------|--------------------------|----------------------|-----------------------|-----------------------|-------------------|
|         | N (%)                    | N (%)                | N (%)                 | N (%)                 | N (%)             |
| CD3     | 7 (20)                   | 0                    | 0                     | 7 (50)                | 0                 |
| CD7     | 4 (11.4)                 | 4 (28.6)             | 4 (28.6)              | 4 (28.6)              | 4 (28.6)          |
| CD10    | 4 (11.4)                 | 4 (28.6)             | 4 (28.6)              | 4 (28.6)              | 4 (28.6)          |
| CD13    | 23 (65.7)                | 9 (64.3)             | 9 (64.3)              | 9 (64.3)              | 9 (64.3)          |
| CD14    | 6 (17.1)                 | 4 (28.6)             | 4 (28.6)              | 4 (28.6)              | 4 (28.6)          |
| CD15    | 7 (20)                   | 7 (50)               | 7 (50)                | 7 (50)                | 7 (50)            |
| CD19    | 7 (20)                   | 7 (50)               | 7 (50)                | 7 (50)                | 7 (50)            |
| CD33    | 24 (68.6)                | 10 (71.4)            | 10 (71.4)             | 10 (71.4)             | 10 (71.4)         |
| CD34    | 24 (68.6)                | 10 (71.4)            | 10 (71.4)             | 10 (71.4)             | 10 (71.4)         |
| CD117   | 16 (45.7)                | 10 (71.4)            | 10 (71.4)             | 10 (71.4)             | 10 (71.4)         |
| CD3, CD19 | 3 (21.4)                |                      |                      |                      | 3 (21.4)          |
| CD3, CD10 | 1 (7.1)                |                      |                      |                      | 1 (7.1)           |
| CD7, CD19 | 1 (7.1)                |                      |                      |                      | 1 (7.1)           |
| CD7, CD10 | 2 (14.3)                |                      |                      |                      | 2 (14.3)          |
| CD3, CD7, CD19 | 1 (7.1) |                      |                      |                      | 1 (7.1)           |
| CD7, CD19, CD10 | 1 (7.1) |                      |                      |                      | 1 (7.1)           |
| CD3, CD19, CD10 | 1 (7.1) |                      |                      |                      | 1 (7.1)           |
| CD3, CD7, CD10, CD19 | 1 (7.1) |                      |                      |                      | 1 (7.1)           |

AML: Acute myeloid leukaemia; Ly+: Lymphocyte antigen positive.

As shown in Table 2, most common asynchronous antigen expression was CD34+CD15+ in 7 (20%) cases.

As shown in Table 3, CD3 antigen expression was more commonly seen in male patients which was statistically significant (p=0.021). CD13 and CD7 had 73.9% and 50% cases respectively with WBC count more than 50000/mm³ but it was not statistically significant. CD3 and CD19 were the most common antigens expressed incases with platelet count less than 30000/mm³ but were not statistically significant. Statistically significant association could be made out between CD117 expression and low blast%. CD19 was the most common aberrant antigen having the blast% more than 70%.

Table 2: Distribution of aberrant markers in acute myeloid leukemia cases (n=35).

| Asynchronous antigen expression | Total no. of cases (%) |
|---------------------------------|------------------------|
| CD34+CD15+                      | 7 (20)                 |
| CD34+CD14+                      | 4 (11.4)               |
| CD117+CD34+CD15+                | 4 (11.4)               |
| CD117+CD34+CD14+                | 4 (11.4)               |
| CD117+CD34-CD15+                | 0                      |
| CD117+CD34-CD14+                | 0                      |
| CD117-CD34+CD15+                | 3 (8.6)                |
| CD117-CD34+CD14+                | 1 (2.9)                |

Table 3: Correlation of immunophenotype and prognostic factors.

| Antigen | Age (>60 yrs) | Male | WBC count (>50,000/mm³) | Platelet count (<30,000/mm³) | Blast (%) (>70%) |
|---------|---------------|------|-------------------------|------------------------------|------------------|
|         | n | % | n | % | n | % | n | % | n | % |
| CD3     | 0/7 | 0 | 0 | 0 | 2/7 | 28.6 | 2/7 | 28.6 | 1/7 | 14.3 | 2/7 | 28.6 | 1/7 | 14.3 |
| CD7     | 0/4 | 0 | 0 | 0 | 2/4 | 50 | 2/4 | 50 | 2/4 | 50 | 2/4 | 50 | 2/4 | 50 |
| CD10    | 1/4 | 25 | 2/4 | 50 | 1/4 | 25 | 2/4 | 50 | 2/4 | 50 | 2/4 | 50 |
| CD13    | 2/23 | 8.7 | 17/23 | 73.9 | 17/23 | 73.9 | 8/23 | 34.7 | 13/23 | 56.5 |
| CD14    | 0/6 | 0 | 0 | 0 | 3/6 | 5 | 3/6 | 5 | 3/6 | 5 | 3/6 | 5 | 3/6 | 5 |
| CD15    | 0/7 | 0 | 0 | 0 | 4/7 | 57.1 | 2/7 | 28.6 | 3/7 | 42 | 5/7 | 71.4 |
| CD19    | 0/7 | 0 | 0 | 0 | 2/7 | 28.6 | 1/7 | 14.3 | 5/7 | 71.4 | 5/7 | 71.4 |
| CD33    | 2/24 | 8.3 | 15/24 | 62. | 6/24 | 25 | 13/24 | 54.2 | 10/24 | 41.7 |
| CD34    | 1/23 | 4.2 | 15/23 | 65.2 | 6/23 | 26.1 | 12/23 | 52.2 | 12/24 | 52.2 |
| CD117   | 2/17 | 11.8 | 10/27 | 58.8 | 1/17 | 5.9 | 5/17 | 29.4 | 5/17 | 29.4 |
As shown in Table 4, no statistically significant association was noticed between the Ly+ AML and prognostic factors (age >60 years, difference in sex (males), WBC count (>50,000/mm³), platelet count (<30,000/mm³) and percentage of blast cells (>20%).

**Table 5: Association of prognostic factors with asynchronous antigen expression.**

| Prognostic factors | CD34+ (n=7) | CD34+CD14+ (n=4) |
|--------------------|-------------|------------------|
| Age (>60 yrs)      | 4 (57)      | 2 (25)           |
| Gender (Male)      | 0 (0)       | 0 (0)            |
| WBC count (>50,000/mm³) | 3 (42.8) | 1 (25) |
| Platelet count (<30,000/mm³) | 4 (57) | 2 (50) |
| Blast % (>70%)     | 5 (71)      | 1 (25)           |

Table 5 presents the correlation of prognostic factors with asynchronous antigen expression. There was no statistically significant association between them.

**DISCUSSION**

In the present study, out of 35 samples of newly diagnosed acute myeloid leukemia studied, 17 (49%) cases were of aberrant phenotype. As reported in previous studies, the incidence rate was ranging from 20%-88%. In a recent Study in Saudi Arabia in 40 AML patients aberrant antigens were present in 67.5%. As high as 88% was reported by Bahia et al. Most of the recent studies have reported aberrant phenotype between 50%-60% of cases.

In total, lymphoid associated antigen expression is seen in 41% of cases and asynchronous antigen expression in 35 % of cases. But in majority of the studies asynchronous antigen expression was the most common like 82% in a study done by Bahia et al.

CD3, CD19 (lymphoid associated antigen) CD34+ CD15+ (asynchronous aberrant phenotype) were the most common equally expressed aberrant phenotypes, each in 7 cases. Similar findings were also noted by Bhushan et al. In his study, CD19 expression was the most common aberrant antigen. In contrast to this CD7 was most common lymphoid associated antigen expressed, in the studies of Bahia et al, Jha et al, and Chang et al.

In our study, CD34+CD15+ is common asynchronous antigens expressed in 20% AML cases. This incidence rate was lower when compared to the previous studies of Bahia et al, in which have of CD34+ CD15+ was reported in 61.5% AML cases. In majority of studies expression of early stem cell antigens, CD34 and CD117 with mature myeloid antigens was the most common aberrant changes, like in study by Haase et al and Wells et al.

In the present study, correlation between prognostic factors and Ly+ AML and Ly- AML groups were compared. Their association still remains controversial. Expression of aberrant phenotypes was more in case of males compared to females. This was comparable to the findings of Noronha et al. In the total study population average age was 40.65 years, average WBC count was about 33979/mm³, average platelet count was about 80114/mm³ and average blast % was 62.65%. In present study, no statistically significant association was found between adverse prognostic factors and aberrant phenotypes. This was in agreement with the findings of Putti et al and Pui et al.

**CONCLUSION**

The findings of the study conclude that CD19 and CD3 were the most commonly expressed lymphoid associated antigen markers. Lymphoid associated expressions were slightly more common than asynchronous antigen expression. Most common asynchronous aberrant phenotype was CD34+CD15+. Aberrant phenotypic expressions were not associated with prognostic factors in acute myeloid leukemia except for common expression of CD3 in males.

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