

RESEARCH ARTICLE

IMMUNOPHENOTYPIC ANALYSIS OF ACUTE PROMYELOCYTIC LEUKEMIA AT A TERTIARY CARE INSTITUTE.

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Introduction:-

Acute promyelocytic leukaemia (APL or AML with PML-RAR α is an AML in which abnormal promyelocytes predominate. Both hypergranular' or "typical" APL and microgranular (hypogranular) types exist. Acute promyelocytic leukaemia comprises 5-8% of AML^[1]. Typical and microgranular APL are frequently associated with disseminated intravascular coagulation(DIC).

Prompt diagnosis and proper use of all trans retinoic acid (ATRA) are needed to prevent death and improve overall prognosis. Most diagnostic tests for APL, including chromosome examination, polymerase chain reaction, and fluorescent in situ hybridization, are time consuming. Flow cytometric immunophenotypic analysis has gained attention as an effective and rapid diagnostic tool for APL. APL has a characteristic immunophenotype that facilitates its identification by flow cytometry. Nevertheless, not all APL-associated features are present in every case^[2]

The purpose of this study was to see the immunophenotype of APL and compare the Immunophenotypic features of hypergranular and microgranular APL using a large panel of monoclonal antibodies and to test if the immunophenotypic profile suggested by previous studies as highly characteristic of the PML-RAR α gene rearrangement was consistently present in our series^[3,4]

Materials And Methods:-

In this retrospective study a total of 23 patients with de novo APL, who were hospitalized at the SKIMS Hospital (Srinagar, J & K, India) from November 2015 to January 2018, were enrolled. Only patients who where cytogenetically confirmed as APL were taken into consideration.

Bone marrow or peripheral blood samples (when feasible) from all patients were collected in Heparin or EDTA tubes. Immunophenotyping was performed using 8 colour Navios flowcytometer by Beckman coulter. Each tube contained 1×10^6 nucleated cells in suspension in 100 µL of phosphate-buffered saline after adjustment. Cells were stained with 5 µL labeled monoclonal antibodies against the antigens listed below. Results were considered positive if 20% or more of the cells expressed a particular antigen. A cutoff of >10% was used to quantify the presence of a subpopulation of CD34+ and CD56+ cells, A total of 20 Antibodies were used of which only 12 were taken into consideration for this study and Antibody used in less than 7 (30%) cases were not taken into consideration. Monoclonal antibodies purchased from beckman coulter directed against CD2, CD3, CD7, CD13, CD15, CD19, CD33, CD34, CD56, CD117, HLA-DR and MPO were used and and flow cytometric analyses were performed with a Navaios software. CD45 vs Side Scatter dot plot was used for gating populations.

Results:-

A total of 23 samples were received of which 20 were bone marrow samples and rest 3 were peripheral blood samples. 14 of the samples were in EDTA tubes and 9 were in heparin tubes. The age of 23 patients ranged from 08 to 90 years. The median age was 34 years. 13 of the patients were males and 10 were females. Morphologically 5 were hypogranular varients and 18 were typical or hypergranular varients. 4 Of 5 Hypogranular varients presented with leucocytosis with TLC ranging from 37.6 to 52.3 X 10^3 , while only 1 of 18 cases of hypergranular varients presented with leucocytosis others having either normal TLC or Leucopenia. All patients were t (15; 17) or PML-RAR α positive. The percentages of patients, who were positive for each tested antigen, are listed in Table 1.

Antigens associated with hemopoietic stem cell-like HLA-DR and CD34 were not frequently expressed. HLA-DR was expressed in 1 of 5(20%) hypogranular variants and in none of the hypergranular variants, while CD34 was expressed in 2 of 5(40%) of hypogranular variants and 2 of 18(11%) of hypergranular variants. MPO positivity was seen in all cases while as CD33, CD13 and CD117 were expressed in 95.7%, 91.3% and 90.9% cases respectively. Prognostic markers like CD2 and CD56 were positive in 11% and 14.3% cases respectively. 2 of the 3 cases positive for CD56 were also positive for CD34. Data for CD15 was collected for 7 patients, of which 57.1% (4 patients) were CD15 positive and 42.9% were negative. B cell marker CD19 and T cell markers CD3 and CD7 were characteristically negative in all cases both in hypergranular and hypogranular variants in contrast to Non-APL AML were aberrant expression of T cell and B cell markers is quite frequent.

MPO showed moderate to bright expression in all cases in contrast to dim to moderate expression of CD13(74%), CD15(57.2%), CD33(78.2%) and CD117(86.3%) in most of the cases. Expression levels of different antigens are shown in table 2.

Antigens	Number(n)	Positive rate						
		0-10%	11-20%	21-40%	41-60%	61-80%	81-100%	
CD2	9	8 (88.9%)	0	1(11.1%)	0	0	0	
CD3	11	11(100%)	0	0	0	0	0	
CD7	7	7(100%)	0	0	0	0	0	
CD13	23	2(8.7%)	0	8(34.8%)	8(34.8%)	2(8.7%)	3(13.0%)	
CD15	7	3(42.9%)	0	4(57.1%)	0	0	0	
CD19	11	11(100%)	0	0	0	0	0	
CD33	23	1(4.3%)	0	2(8.7%)	1(4.4%)	0	19(82.6%)	
CD34	23	19(82.6%)	3(13.0%)	0	1(4.3%)	0	0	
CD56	21	18(85.7%)	2(9.5%)	0	1(4.8%)	0	0	
CD117	22	2(9.1%)	0	3(13.6%)	2(9.1%)	6(27.3%)	9(40.9%)	
HLA-DR	22	21(95.5%)	0	1(4.5%)	0	0	0	
MPO	23	0	0	4(17.4%)	1(4.4%)	3(13.0%)	15(65.2%)	

Table 1:- Immunophenotypic analysis of APML patients [n (%)].

 Table 2:- Expression levels of different antigens [n (%)].

Antigens	Number(n)		Positive rate					
		Negative	Dim	Dim	Moderate	Moderate	Bright	
				moderate		bright		
CD2	9	8	1(11.1%)	0	0	0	0	

CD3	11	11	0	0	0	0	0
CD7	7	7	0	0	0	0	0
CD13	23	2	14(60.9%)	2(8.7%)	1(4.4%)	3(13.0%)	1(4.4%)
CD15	7	3	3(42.9%)	0	1(14.3%)	0	0
CD19	11	11	0	0	0	0	0
CD33	23	1	11(47.8%)	5(21.7%)	2(8.7%)	4(17.4%)	0
CD34	23	19	2(8.7%)	0	0	0	2(8.7%)
CD56	21	18	2(9.5%)	0	0	1(4.8%)	0
CD117	22	2	16(72.7%)	2(9.1%)	1(4.5%)	0	1(4.5%)
HLA-DR	22	21	1(4.5%)	0	0	0	0
MPO	23	0	0	0	4(17.4%)	6(26.1%)	13(56.5%)

Discussion:-

The present study involved a flow cytometric immunophenotypic analysis on 23 patients with de novo APL to determine if any immunemarkers could be used as diagnostic tools for APL and to compare the Immunophenotypic features of classical and microgranular APL.

Our patients ranged from 08 to 90 years of age with median age of 34 years which was lower than the median age of 53 years (range, 22-77 years) as in study by Pedro Horna et al.^[5]

13 of our patients were males and 10 were females with Male:Female ratio of 1.3:1.0 similar to male to female ratio of 1.2:1.in study by Pedro Horna et al[5]Morphologically 21.7% were hypogranular varients compared to 10.3% in study by Pedro Horna et al^[5] And 32% in study by Edgar G Rizzatti et al.^[4]

80% Hypogranular varients presented with leucocytosis with TLC ranging from 37.6 to 52.3 X 10^3 , while only 1 of 18 cases of hypergranular varients presented with leucocytosis others having either normal TLC or Leucopenia. This study is consistent with previous reports, demonstrating that CD13+CD33+HLA-DR-CD34- is a classic immune pattern for APL ^[6,7,8,9,10,11]

Antigens associated with hematopoietic stem cell-like HLA-DR and CD34 were not frequently expressed. HLA-DR was expressed in 20% hypogranular variants and in none of the hypergranular variants, while CD34 was expressed in 40% of hypogranular variants compared to 11% of hypergranular variants which is in accordance with previous studies by Lee JJ et al and Foley R et al who reported a CD34 expression of 20% to 40% in APLs, particularly in the microgranular variant.^[12,13]

MPO positivity was seen in all cases while as CD33, CD13 and CD117 were expressed in 95.7%, 91.3% and 90.9% cases respectively which is similar to previous studies. $^{[4,8,10,19]}$ except for CD117 which was expressed by only $26\%^{[19]}$ to $51\%^{[10]}$ cases in some studies.

Prognostic markers like CD2 were positive in 11%. Previous research has shown that CD2+ immunophenotypes in patients with APL are associated with leucocytosis and the hypogranular M3v phenotype, as well as a higher probability of thrombosis.^[7,10,14,15]

14.3% positivity of CD56, a poor prognostic marker[16] was similar to published literature[1].2 of the 3 cases positive for CD56 were also positive for CD34. both the research findings and available literature have shown an association between leukocytosis and CD34, and CD56 expression. $^{[6,17]}$

57.1% (4 patients) were CD15 positive and 42.9% were negative. The reason for higher CD15 positivity in our study might be smaller sample size. B cell marker CD19 and T cell markers CD3 and CD7 were characteristically negative in all cases both in hypergranular and hypogranular variants in contrast to Non-APL AML were aberrant expression of T cell and B cell markers is quite frequent. ^[18]

MPO showed moderate to bright expression in all cases in contrast to dim to moderate expression of CD13(74%), CD15(57.2%), CD33(78.2%) and CD117(86.3%) in most of the cases (Table 2). In contrast a study by Fang Xu et al

showed bright CD33 expression in all patients with dim to bright CD13 expression, dim to moderate cMPO expression and generally dim CD117 expression [19]

In conclusion APL has a characteristic immunophenotypic profile, and on combining with the analysis of bone marrow aspiration morphology flow cytometric immunophenotyping may be considered as a useful tool for rapid recognition of APL and its varients. Also the immunophenotypic features of hypergranular and microgranular(hypogranular) APL with the PML-RAR α gene rearrangement are very similar, except for higher expression of CD34 and HLA-DR in microgranular variant.

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