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A STUDY OF CYTOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN ACUTE LEUKEMIA PATIENTS IN A TERTIARY CARE HOSPITAL

Pathology

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ABSTRACT

Introduction: Leukemias are a heterogenous group of hematopoietic cells disorders, having varied clinical, morphological, molecular cytogenetic characteristics, and has prognostic and therapeutic implications on identifying them correctly. In limited resource centers, where the genetic and immunophenotyping are not available, the use of cytochemical stains is still have a value in the diagnosis of leukemia. **Aim:** To evaluate the role of cytochemistry in classifying the various types of Leukemia and its subtypes as well as correlate with the clinical and hematological findings of the cases. **Results:** In this study we analyzed 60 cases of acute leukemia and found predominance of lymphoid neoplasms over myeloid leukemia among acute leukemia case, seen more commonly in the childhood category. The gender distribution in both AML and ALL was M:F ratio 1.6:1. Acute leukemias were well studied, with the stains, SBB and PAS, and accurately classified as per FAB classification. Flow cytometry was done in only four doubtful cases to give definitive diagnosis. SBB stain shows 96.2% sensitivity and 100% specificity which helps to correctly differentiate myeloid series from lymphoid series. **Conclusion:** Cytochemical stains, being cheap, simple and require no special instruments or highly trained personnel are particularly important in developing countries for the diagnosis and treatment of acute leukemia and can be used in places that do not yet have the most advanced diagnostic resources and / or immunophenotyping, cytogenetics and molecular biology.

KEYWORDS

Acute leukemia, cytochemical stains, Flow cytometry

INTRODUCTION

Leukemias are neoplastic proliferation of hematopoietic cells, which form a major proportion of hematopoietic neoplasms, which are diagnosed worldwide. Correct classification permits optimal patient management.[1] Two widely used classifications are used, one by the French, American, and British group called the FAB Classification, and other by the World Health Organization (WHO Classification), based on the morphologic findings, genetic abnormalities, clinical and Immunophenotyping characteristics. In chronic leukemia, the identification of cell type, seldom poses a problem because of abundance of easily identifiable mature cells. However in acute leukemias, the cell identification often poses a problem because there is a marked similarity between earlier precursors of different cell series. In these cases the cytochemical stains are of great help in recognizing the type of precursor cells. Despite the widespread use of immunophenotyping in the diagnosis of hematopoietic neoplasms, cytochemical studies are still of diagnostic importance. This study attempts to evaluate the role of cytochemistry in classifying the various types of Leukemia and its subtypes, by a combination of morphology on the peripheral blood smear, as well as correlate with the clinical and hematological findings of the cases, with available means in our laboratory.

MATERIALS AND METHODS

The prospective study was done on 60 patients between June 2019 and June 2021 approved by The Ethical Committee of BJMC and Civil hospital, Ahmedabad. Those cases referred to the department of pathology, with clinical suspicion, were subjected to peripheral smear study by standard Romanowsky stains first, mainly by Giemsa stain and a provisional diagnosis was made following which special cytochemical stains were used Sudan Black B(SBB) and Periodic acid Schiff's reagent stain(PAS) prepared by conventional techniques. The percentage of blast cells were enumerated, and the cytomorphology studied based on their positive staining effects on the respective blood smears, were noted. Thereby the types and subtypes of leukemias were classified and reported as per FAB Classification. Relevant clinical history and hematological investigations was obtained from each case.

RESULTS

Based on FAB classification of Acute leukemia, results and observations deduced were as follows.

Table No. 1. Age Distribution of acute leukemia cases

AGE IN YEARS	NO. OF PATIENTS	
	ALL	AML
	NO.	%	NO.	%
1-20	26	76.5	3	11.5
21-40	6	17.6	12	46.2
41-60	2	5.9	9	34.6
61-80	0	0	2	7.7
>80	0	0	0	0
TOTAL	34	100	26	100

Table No. 2 Sex distribution of Acute leukemia cases

SEX	NO. OF PATIENTS	PERCENTAGE (%)
MALE	37	61.7
FEMALE	23	38.3
TOTAL	60	100

The Male to female ratio was 1.6:1.

Table No. 3. Sex distribution according to type of leukemia

SEX	ALL		AML	
	NO.	%	NO.	%
MALE	23	67.6	14	53.8
FEMALE	11	32.4	12	46.2
TOTAL	34	100	26	100

Table No. 4. Type of Acute leukemia cases

Type of leukemia	No. of patients	Percentage (%)
ALL	34	56.7
AML	26	43.3
TOTAL	60	100

Table No. 5 Distribution of patients according to subtypes of Acute leukemia (FAB classification) – Myeloid series

SUBTYPES OF AML	NO. OF CASES	PERCENGTAGE (%)
M0	1	3.8
M1	2	7.7
M2	12	46.1
M3	5	19.3
M4	5	19.3
M5	1	3.8
M6 M7	0	0
TOTAL	26	100

Table No. 6 Distribution of patients according to subtypes of Acute leukemia (FAB classification) – Lymphoid series

Types And Subtypes Of Leukemia	No. Of Cases	Percengtage (%)
ALL		

L1	16	47.05
L2	16	47.05
L3	2	5.9
TOTAL	34	100

Table No. 7. Distribution of Acute leukemia cases as per cytochemistry

STAINS	AML		ALL	
	NO.	%	NO.	%
SBB				
POSITIVE	25	96.2	0	0
NEGATIVE	1	3.8	34	100
TOTAL	26	100	34	100
PAS				
POSITIVE	6	23	30	88.2
NEGATIVE	20	77	4	11.8
TOTAL	26	100	34	100

Two special stains were used in making the diagnosis of acute leukemia as AML and ALL which were SUDAN and PAS. SBB and PAS reaction were observed in all the cases by counting two hundred blast cells at least on each slide. Results were recorded as a percentage of such cells showing block positivity for PAS stain. 5% was taken as a cut-off above which their action was considered positive.

For SBB, 3% blasts or above with positive Sudan positivity were taken as a cut off. Based on the morphological examination of the marrow and/or blood smear and cytochemical staining results, a morphological diagnosis was made and cases were classified according to FAB classification. Table 7 shows that the SBB positive cases were 96.2% of AML patients (25/26), while SBB negative cases in AML patients (1/26) were 3.8%. However, in the PAS stain, positive cases of ALL patients (30/34) were 88.2% and those with negative PAS stains cases were 11.8% of ALL patients (4/34)

6 cases of AML shows mixed staining pattern, SBB stain shows coarse granular positivity however PAS stain shows diffuse pink cytoplasmic positivity as compared to block positivity seen in lymphoblasts of ALL cases. So considering morphology together with cytochemistry and clinical correlation these cases were diagnosed as AML.

4 cases out 34 ALL cases shows mild positivity for PAS stain as compared to coarse positivity seen in lymphoblasts. But all these cases were SBB negative which was taken in account for labelling these cases as ALL along with clinical correlation.

Table No. 8. Sensitivity and specificity tests of cytochemistry stains in patients with AML and ALL cases

STAINS	SENSTIVITY TEST %	SPECIFICTY TEST %
SBB in AML	96.2	100
PAS in ALL	88.2	76.9

Table No. 9. Distribution of cases (diagnosed on basis of Routine hematological stain and cytochemical stain)

Diagnosis	Routine hematological stain Only	Routine hematological stain with cytochemical stain
AML	20	24
ALL	28	32
Undiagnosed	12	4
TOTAL	60	60

Table no. 10 Immunophenotyping results

Case no.	Cytochemistry	Immunophenotyping	Comment
15	SBB-, PAS-	POSITIVITY FOR CD13, CD33, ANTI MPO	Immunophenotyping revealed immature myeloblast suggestive of AML M0
24	SBB-, PAS-	POSITIVITY FOR CD19, CD22, CD79a	ALL blasts resemble AML M0 blasts so after immunophenotyping, classified as ALL L2
38	SBB-, PAS-	CALLA POSITIVITY WITH CD10 GATED BLASTS	ALL blasts confused with normal lymphocytes so after immunophenotyping, classified as ALL L1
44	SBB +, PAS DIFFUSE POSITVITY	POSITIVITY FOR CD13, CD33, CD117, CD14, CD64, CD11b	Since ANAE stain was not in our scope, so we depend on immunophenotyping for identification of monocytic differentiation. AML M5

Table 10 depicts immunophenotyping by flowcytometry results of 4 cases which remain undiagnosed after routine hematological stain and cytochemical evaluation. Flowcytometry using regular AML panel and ALL panel were done, results were analyzed and compared with their morphologic diagnosis for cases suspected and doubtful. This helps to render definitive diagnosis of the patients. Moreover, The FAB criteria for the recognition of monocytic differentiation is the presence of fluoride-sensitive naphthol AS acetate esterase (NASA) or NASDA activity, but in our condition, when these stains are not available, we depend on morphology and immunophenotyping for these cases.

Out of 4 cases, 2 cases were found to be AML with M0 and M5 subtype and 2 cases were found to be ALL with L1 and L2 subtype according to FAB classification after overall analysis.

Table No.11. Hemoglobin distribution among the cases

Hb in gram %	ALL		AML	
	NO.	%	NO.	%
<6	19	55.8	12	46.2
6.1-9	7	20.7	9	34.6
9.1-12	8	23.5	5	19.2
12 and above	0	0	0	0
TOTAL	34	100	26	100

Table No.12. Total leukocyte count in cases

TLC/cu mm	ALL		AML	
	NO.	%	NO.	%
<4000	4	11.8	2	7.7
4001-11000	3	8.8	3	11.5
11001-50000	20	58.8	7	27.1
50001-100000	2	5.9	6	23
100001-200000	3	8.9	5	19.2
>200000	2	5.8	3	11.5
TOTAL	34	100	26	100

Table No. 13. Platelet count in cases

PLATELET COUNT /CUMM	ALL		AML	
	NO.	%	NO.	%
<50000	24	70.6	15	57.7
50000-1 LAC	3	8.8	7	27.1
1 LAC-1.5 LAC	1	3	2	7.6
>1.5 LAC	6	17.6	2	7.6
TOTAL	34	100	26	100

Table No.14. Clinical features of acute leukemia cases

Clinical features	No.	Percentage(%)
Pallor	58	96.6
Splenomegaly	27	45
Hepatomegaly	23	38.3
Lymphadenopathy	26	43.3

Two cases of AML M3 had disseminated intravascular coagulopathy in the present study.

DISCUSSION

The observations and results of the patients in the present study were compared with various similar studies and findings are discussed below.

Comparison Of Acute Leukemia Cases- In our study, maximum cases were seen in the first and the second decade which is in accordance with studies carried out by D'Costa GG et al [2], Parkin DM et al [3] , Meighan et al 4] , Alrudainy et al.[5]

Table No. 15 Comparison Of Agewise Ditsribution Of All Cases

REFERENCE	STUDY OBSERVATIONS
Neglia, jp, and Robison. [6] (1988)	In children, acute lymphoblastic leukemia (80%) is more common than acute myeloid leukemia

Ribera JM, Oriol A. [7](2009)	Acute lymphoblastic leukemia (ALL) is the most frequently diagnosed malignancy in children, representing nearly one third of all pediatric cancers
Rajnikant et al [8] (2013-2014)	Acute Lymphoblastic Leukemia is more common (56.52%) in age group of 0 to 15 years
Abu et al [9] (2016)	Acute lymphoblastic leukemia is more common in the age group of 0 to 20 years (92%)
Hamid et al [10] (2018)	In children, the ALL was the commonest type of leukemia (86%)
Present study (2021)	ALL is more common in the age group 1-20 years (76.5%)

Table No. 16 Comparison Of Agewise Ditsribution Of Aml Cases

REFERENCE	STUDY
Boros, L. and Bennett, J. M (1984) [11]	AML occurs twice as often as ALL, the vast majority of cases occurring in adults
Khalid Hassan Nadeem Ikram, Sajid Hussain Shah [12] (1994)	AML was observed more commonly in adults (79%) as compared to children (21%), whereas ALL was commoner in children (72%) as Compared to in 28% amongst adults.
Greer John P, Baer Maria. R, Kinney Marsha. C (2009) [13]	AML accounts for less than 15% of cases of leukemia in children below 10years, 25-30% between 10-15 years, and in adults, it accounts for 80-90% of cases of acute leukemias.
Rajnikant et al (2014) [8]	Acute Myeloblastic Leukemias an adult predominance (81.81%)
Abu et al [9] (2016)	Acute myelod leukmia has an adult predominance (54%).
Hamid et al [10] (2018)	54.5% of adult patients had AML.
Present study (2021)	In AML, adult predominance was observed 46.12%in 21-40 years age group and as much as 34.6% in the 41-60 age group.

Comparison Of Sexwise Distribution According To The Type Of Acute Leukemia

In our study, sex distribution shows male preponderance as a whole in the study of 60 cases of acute leukemia. Both ALL and AML shows higher male preponderance of 67.6% and 53.8% against 32.4% and 46.2% in female respectively. Similar incidences were observed in studies conducted by Venkateswaran et al [14], whereas in contrast AML had a higher female preponderance than males in study conducted by Abu et al[9] which may be due to geographical difference.

Table No. 17 Comparison Of Frequency Of Various Leukemias In India (in Percentage)

REFRENCE	REGION OF STUDY CONDUCTED	ALL	AML	Total Cases
Chatterjee et al [15]	Calcutta (1949–1961)	22.5	32.5	544
Advani et al [16]	Mumbai (1960–1975)	30	13	1126
Prakash et al [17]	Pondicherry (1970–1979)	35	29.5	278
Rani et al [18]	Delhi (1970–1979)	15.5	30.8	490
Varghese et al [19]	Kerala (1980–1983)	39.2	19.6	1016
Kushawaha et al [20]	Lucknow (1971–1984)	9.3	38.7	970
Shome et al [21]	Chandigarh (1975–1983)	24	29.3	820
Dicosta et al [2]	Mumbai (1975–1984)	36	22	242
Rathee et al [22]	Haryana (2008-2012)	17.2	33.8	650
Rajnikant et al [8]	Bhopal (2013-2014)	31.51	15.07	73
Abu et al [9]	Tamil Nadu (2015-2016)	22	43	56
Hamid et al [10]	Aden (2018)	58.5	37.7	53
Present study	Ahmedabad (2019-2021)	56.7	43.3	60

Table No. 18 Comparison Of The Distribution Of Fab Types Of AML (in Percentage)

Reference studies (Total cases)	M0	M1	M2	M3	M4	M5	M6	M7
Sultan et al (250) 1981	-	21	32	16	16	12	3	0
Miguel et al (120) 1986	-	13	14	14	22	21	7	0
Chessells et al (112) 1986	-	10	26	6	22	24	9	0
Alvi et al (26) 1990	-	15.3	35	15.3	19.2	11.5	3.7	0
Chaudhry et al (54) 1993	-	13	44.4	11.1	24	3.7	3.7	0
Khalid Hassan (81) 1994	-	18.5	23.9	11.1	29.6	11.1	1.2	1.2
Hamid et al (53) 2011-2012	0	5	40	10	25	20	0	0
Rajnikant et al (73) 2013-2014	0	18.8	9.09	36.3	0	9.09	9.09	18.18
Abu et al (56) 2015-2016	8	25	54	5	8	0	0	0
Present study (60) 2019-2021	3.8	7.7	46.1	19.3	19.3	3.8	0	0

Comparison Of The Distribution Of Fab Types Of All

Present study shows L1 and L2 cases equally distributed among acute lymphoblastic leukemia constituting 47.05% each, which are more common than FAB L3 which constitutes 5.9% only. This finding is in correlation with similar other studies by Abu et al [9] whereas higher incidence of FAB L1 followed by L2 then L3 were seen in studies by Nakase et al [23], Muhammad et al [24], Hamid et al [10] , Keleti et al, Hann et al, Viana and Bennett et al[25].

Table No. 19 Comparison Of Positvity Of Pas In All And SBB In AML With Other Studies

REFERENCE STUDY	PAS positivity in ALL (%)	SBB positivity in AML (%)
Sushma et al	66.7	-
AAM Deghady	40.3	100
Liqaa M et al	60	78.2
A.gupta et al	62	66
Present study	88.2	96.2

Comparison Of Pas Reactivity In All Cases

Present study shows PAS positivity to be 88.2% in ALL cases which is similar to other studies as shown in above table. Sushma et al, Ashish Gupta et al [26] , Liqaa M et al showed PAS reactivity to be 66.7%, 62% and 60% respectively whereas AAM Deghady [27] showed PAS reactivity to be 40.3% only. Present study shows mild positivity for PAS stain in 4 cases out 34 ALL cases as compared to coarse positivity seen in lymphoblasts. But all these cases were SBB negative which was taken in account for labelling these cases as ALL along with clinical correlation. Our observation is in correlation with studies done by Samir et al, Flandrin , Scott and Hayhoe et al .

Comparison Of SBB Reactivity In Aml Cases

Present study shows SBB staining positive in 96.2% cases of AML while only 3.8% cases show negative staining pattern. A.gupta et al [26] , Liqaa M et al showed SBB reactivity to be 66%, 78.2% respectively while study by AAM Deghady [27] shows 100% reactivity for SBB.

In our study, PAS was granular and concomitant diffuse positive in 6 cases of AML-M4, M5 but with positivity for SBB which was taken in account to label them as AML. These observations were similar to studies conducted by Hayhoe, Flandrin.

Comparison Of SBB Sensitivity In Acute Leukemia Cases

Present study shows 96.2% sensitivity of SBB for AML cases which is similar to other studies by Hamid et al [10], AAM Deghady [27] where SBB shows good sensitivity.

In our study, no case of ALL was SBB positive thus most valuable in distinguishing AML from ALL. Sensitivity of up to 100% and specificity of 86.67% have been found in study conducted by AAM Deghady [27]. Another study by Liqua et al showed sensitivity of SBB to be around 78.2% in AML cases.

Comparison Of Pas Sensitivity In Acute Leukemia Cases

In our study, PAS sensitivity is found to be 88.2% in ALL cases. However, PAS sensitivity ranging from 40.3% to 66.7% has been found in ALL cases in previous other studies conducted by Belulkar et al [28] , AA Deghady [27] , SA kheri et al, Ashish G. et al [26] , Snower DP et al.

Table No. 20 Comparison Of Concordance Of Cytochemistry With Morphology In Leukemia Cases.

REFERENCE	OBSERVATIONS
Belurkar S et al. [28]	Cytochemical analysis of leukemia by (PAS, MPO, SBB), when coupled with morphology rendered the diagnosis in >80% of our acute leukemia cases

Mhawech et al.	Included Sudan-black, specific esterase (Alfa-naphthyl ASD chloroacetate esterase), non-specific esterase (Alfa-naphthyl butyrate esterase), Periodic acid-Schiff, and acid phosphatase. Definite diagnoses were made for all 10 of their AML cases, whereas diagnoses were possible in only 79.4% patients with ALL when only morphology and cytochemical staining were used
Rajnikant et al [8]	71% were diagnosed on the basis of morphology only, and with use of cytochemistry along with morphology 96% cases were diagnosed
Present study	48 cases (80%) were diagnosed on the basis of morphology only, but with combined use of cytochemistry along with morphology 56 cases (93.3%) were diagnosed.

Comparison Of Laboratory Hematological Parameters
Hemoglobin Level

Present study shows 55.8% cases having anemia in ALL cases and 46.2% cases in AML patients. Our observation is in correlation with similar other studies conducted by K.Das et al , Prajapati et al , Abu et al [9]. This clearly indicates, severe anemia as a co-existing disorder, in cases of acute leukemia. The main cause being inadequate production of red cells and shortened life span. Abnormalities of size and shape can also occur.

Total Leucocyte Count

Present study shows maximum cases have total WBC count between 11001- 50000/mm3. In this study, leucocytosis is more common than leucopenia. Similar observations were seen by studies conducted by K Das, Prajapati et al who found 42% cases and 62.06% cases in the same range group 11001-50000/mm3. This suggests that leucopenia is an uncommon feature. However this was in contrast to study by Rajnikant et al [8] who observed more cases having less than 4000/cumm count.

Platelet Count

Present study shows maximum cases of acute leukemia, both AML and ALL had platelet count less than 50000/cumm. Similar findings showing maximum cases with moderate to severe thrombocytopenia in acute leukemia cases is seen in study conducted by Rajnikant et al [8] . This signifies that acute leukemias, end up with less production of platelets with decreased survival, causing thrombocytopenia. A clear picture of the haematological values in assessing the Total count, platelet count and haemoglobin is essential in the diagnostic aid of leukemias.

Table No.21 Comparison Of Clinical Features In Acute Leukemia Cases

Clinical features	K.Das	Prajapati et al	Alpana et al	Present study
Pallor	92%	81%	100%	96.6 %
Splenomegaly	63%	82.7%	65.14%	45%
Hepatomegaly	70%	65.5%	73.8%	38.3%
Lymphadenopathy	72%	36.2%	73.9%	43.3%

CONCLUSION

With resource limitations and considering the fact, that at present, Flow cytometry is either not available, or beyond the capacity of poor patients in our country, this study was carried out, with the aim that routine morphology and cytochemistry would be a cost effective method, in diagnosing leukemias in localities that do not have advanced technique which justifies this study. Further these patients from PHC and CHC levels can be referred early to the higher centers for immunophenotyping and cytogenetics after which specific therapy can be started at the earliest as if not treated, such cases are rapidly fatal. So we can conclude that being cheap, simple and require no special instruments or highly trained personnel, cytochemical stains are particularly important in developing countries for the diagnosis of acute leukemia and can be used in places that do not yet have the most advanced diagnostic resources and / or immunophenotyping, cytogenetics and molecular biology.

Conflict of interest : None

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