Original Research Article

Clinical and morphological study of the acute leukemia with special cytochemical stains myeloperoxidase (MPO) and periodic acid schiff (PAS) – A prospective study in tertiary care hospital

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Abstract

Introduction: Acute leukemia is a hematological disorder defined by presence of 20% or more blasts in peripheral blood, bone marrow or other tissue[4-6]. It is divided into Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL) and Acute Leukemia of Ambiguous Lineage on the basis of morphological, cytochemical & antigenic characteristics. Further sub classification of AML & ALL has been done on the basis of morphology, cytochemistry, cytogenetics, immunophenotyping and molecular studies[4,5]. **Aims and Objectives:**

1. To find out incidence of leukemia in J.A. Group of Hospitals Gwalior (M.P)
2. To classify leukemias into different subtypes based on French–American-British (FAB) morphology.
3. To perform cytochemical staining of all cases and establishing role of cytochemistry in diagnosis of leukemia.
4. To study various clinical & hematological parameters in leukemias.
5. Flow cytometric evaluation of various types of leukemia will be done.

Material and method: This present study was conducted in department of pathology Gajra Raja Medical College and J.A. group of Hospitals Gwalior from January 2020 to June 2021 and total 71 cases of leukemia were studied, cytochemistry were done in 49 cases and FCM were done in 15 cases. **Results**: In our study on the basis of morphology alone 28 (39.43%) cases were diagnosed and sub classified as ALL- 10 cases (14.08%), AML-18 cases (25.35%) and 43 cases (60.56 %) were not sub classified. With combined use of cytochemistry along with morphology 44 cases (96%) were diagnosed and sub classified, 5 cases (4%) were not sub classified. **Conclusion:** In a setting of lack of facilities for immunophenotyping as in majority of centers in the underdeveloped and developing countries, morphology combined with cytochemical staining still serves the best purpose in diagnosis of acute leukemias.

Introduction

Leukemia is a disease resulting from the neoplastic proliferation of hemopoietic or lymphoid cells. It results from a mutation in a single stem cell, the progeny of which form a clone of leukemic cells (blast cells)[1].

These cells compromise normal hematopoiesis, causing anemia, neutropenia and thrombocytopenia, with abrupt outbreak of and signs and symptoms of weakness, fever/infection and hemorrhage[2]. Frequently these blasts are present in the peripheral blood, and may also infiltrate other tissues and organs such as liver, spleen and lymph nodes[3].

Acute leukemia is a hematological disorder defined by presence of 20% or more blasts in peripheral blood, bone marrow or other tissue[4-6]. It is divided into Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL) and Acute Leukemia of Ambiguous Lineage on the basis of morphological, cytochemical & antigenic characteristics. Further sub classification of AML & ALL has been done on the basis of morphology, cytochemistry, cytogenetics, immunophenotyping and molecular studies[4,5]. Acute Leukemia accounts for approx 0.15-0.60% of total medical admissions in hospitals in India[7].

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AML accounts for approx 79% cases in adults as compared to children (21%) while ALL is commoner in children (72%) as compared to 28% in adults[8]. A minority (about 5% of the cases) present characteristic of both the lineages (myeloid and lymphoid) and are defined as Acute leukemia of ambiguous lineage[9].

In morphological classification (FRENCH- AMERICAN- BRITISH classification) ALL is classified to L1, L2 and L3[10]. AML is classified to M0,M1, M2, M3, M4, M5, M6 and M7[11]. While the WHO classification uses all available information(morphology, cytochemistry, immunophenotyping, genetics and clinical features) to define clinically significant disease entities and to provide a classification that can be used in daily clinical practice as well as to serve as a common language for clinical trials and laboratory investigations[12].

Cytochemistry is worth in the diagnosis and classification of acute leukemia. Concordance rate as high as 86% between morpho/cytochemical diagnosis and flow cytometry has been found[13]. Of these, complete concordance was seen in 58% of cases and partial concordance in 22% cases. A study showed that cytochemical staining should be available for those cases in which flowcytometry fail to yield a definite diagnosis[14]. Currently, flow cytometry is considered the gold standard for ascertain the lineage of leukemic cells.

The combination of myeloperoxidase, and Periodic acid schiff stain are said to provide the desired information in most cases[11].

As per World Health Organization (WHO) 2008 classification on haematopoietic and lymphoid malignancies ,Myeloperoxidase (MPO)

detection either by enzyme cytochemistry or flow cytometry (FCM) plays a significant role for this sub-classification[4]. The cut-off of 3% for cytochemical MPO (cMPO) as positive was recommended by the French–American–British (FAB) classification and has been retained in WHO 2008 classification[15].

Immunophenotyping is used to determine lineage involvement of a newly diagnosed acute leukemia[6]. Combination of cytochemical stains and immunophenotyping data always give more appropriate results.

Material and method

After approval from institutional ethical committee study was done in the Department of Pathology, G. R. Medical College, Gwalior from Jan.2020 to June 2021. All the hematological cases suspicious for leukemias were considered. Peripheral smears and complete blood count was done using Mindrays 6 part analyser.

Routine Leishman Geimsa stain along with special stain Myeloperoxidase (MPO) and Periodic acid schiff (PAS) was done in 49 cases.

Flowcytometry evaluation of diagnosed cases was done in 15 cases. Clinical history was taken and various hematological parameters were co- related.

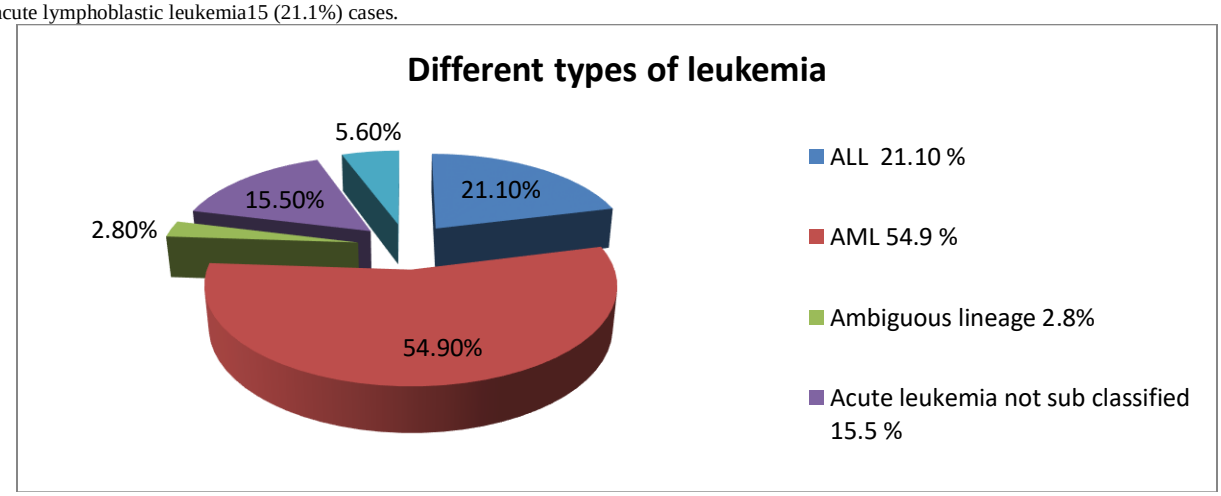
Observations &results

Table1

		ALL		AML		Ambiguous lineage		Acute leukemia not sub classified		Total cases of Acute leukemia		Others		Total cases	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Frequency		15	21.1	39	54.9	2	2.8	11	15.5	67	94.3	4	5.6	71	100
Age in years	0-15	10	66.7	9	23.1	2	100	1	9.1	22	32.8	0	0.0	22	31.0
	16-30	1	6.7	9	23.1	0	0.0	1	9.1	11	16.4	0	0.0	11	15.5
	31-45	2	13.3	10	25.6	0	0.0	6	54.5	18	25.4	0	0.0	18	25.4
	>45	2	13.3	11	28.2	0	0.0	3	27.3	16	26.8	4	100	20	28.2
Sex	Male	9	60	21	53.8	1	50	7	63.6	38	56.7	4	100	42	59.2
	Female	6	40	18	46.2	1	50	4	36.4	29	43.2	0	0.0	29	40.8
Hb in gm% (Anemia)	<6 (Severe)	8	53.3	17	43.6	2	100	6	54.5	33	49.2	0	0.0	33	46.5
	6.1-9 (Moderate)	4	26.7	16	41.0	0	0.0	4	36.4	24	35.8	1	25	25	35.2
	9.1-12 (Mild)	2	13.3	5	12.8	0	0.0	0	0.0	7	10.4	3	75	10	14.1
	>12	1	6.7	1	2.6	0	0.0	1	9.1	3	4.47	0	0.0	3	4.2
Total leucocytes count/cumm	<4000	1	6.7	0	0	0	0	0	0	1	1.4	0	0	1	1.4
	4000-11000	1	6.7	0	0	0	0	0	0	1	1.4	0	0	1	1.4
	11000-50000	4	26.7	14	35.9	2	100	4	36.4	24	35.8	1	25	25	35.2
	50000-1lac	8	53.3	19	48.7	0	0	3	27.3	30	44.7	3	75	33	46.5
	>1lac	1	6.7	6	15.4	0	0	4	36.4	11	16.4	0	0	11	15.5
Platelets count/cumm	<50000 (mild)	6	40	23	59	2	100	6	54.5	37	55.2	0	0	37	52.1
	50,000-1lac (moderate)	8	53.3	10	25.6	0	0	3	27.3	21	31.3	2	50	23	32.4
	1lac-1.5lac (severe)	1	6.7	3	7.7	0	0	1	9.1	5	7.4	1	25	6	8.5
	>1.5lac (normal)	0	0	3	7.7	0	0	1	9.1	4	5.9	1	25	5	7.0

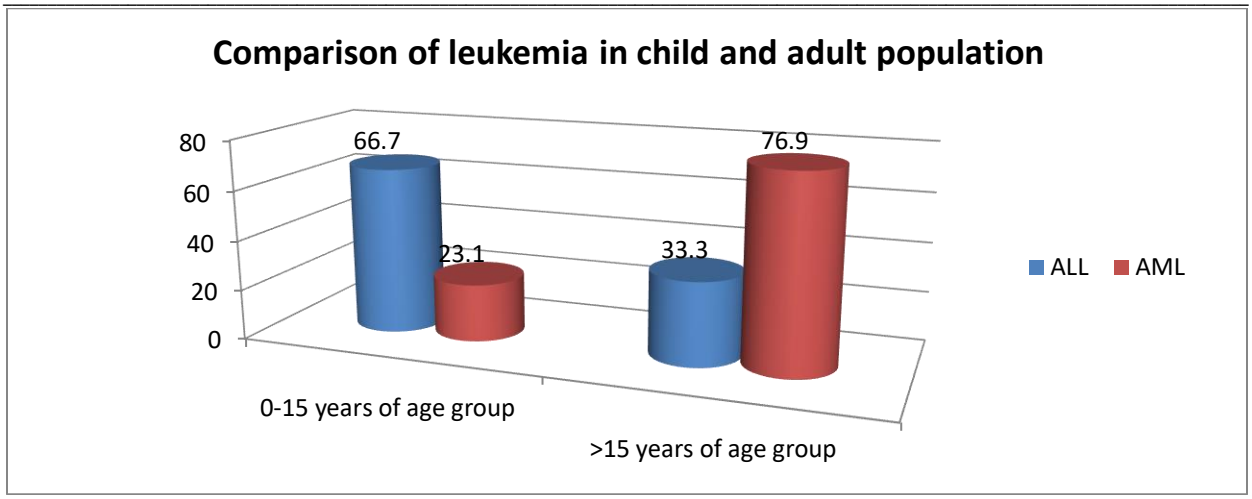
1. Incidence of acute leukemia in present study is 0.0354%

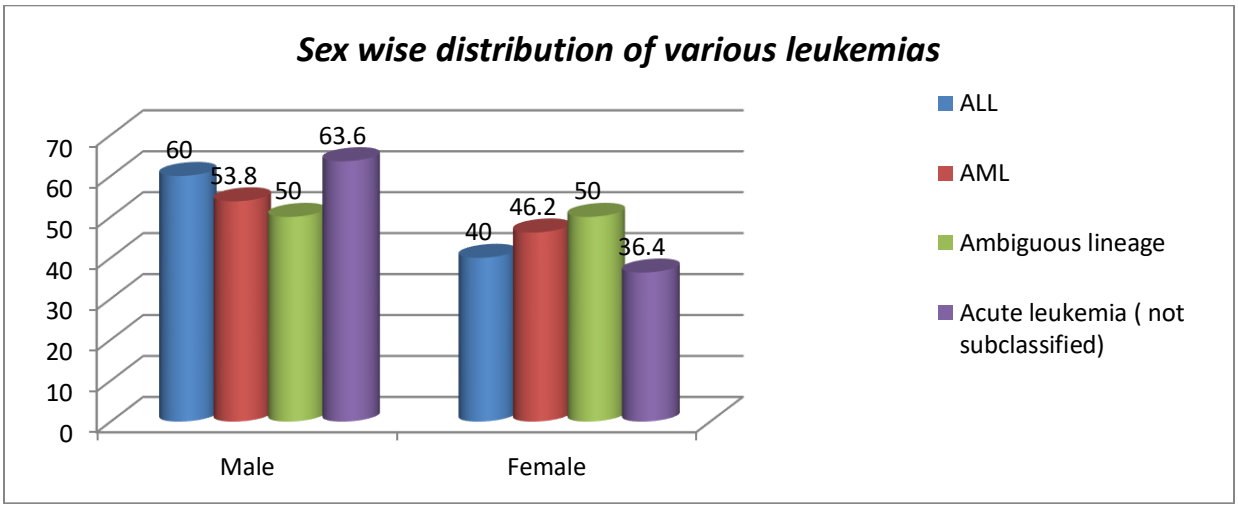
2. Total 71 cases of leukemia were studied, out of which most common acute leukemia was acute myeloid leukemia 39 (54.9%) followed by acute lymphoblastic leukemia15 (21.1%) cases.



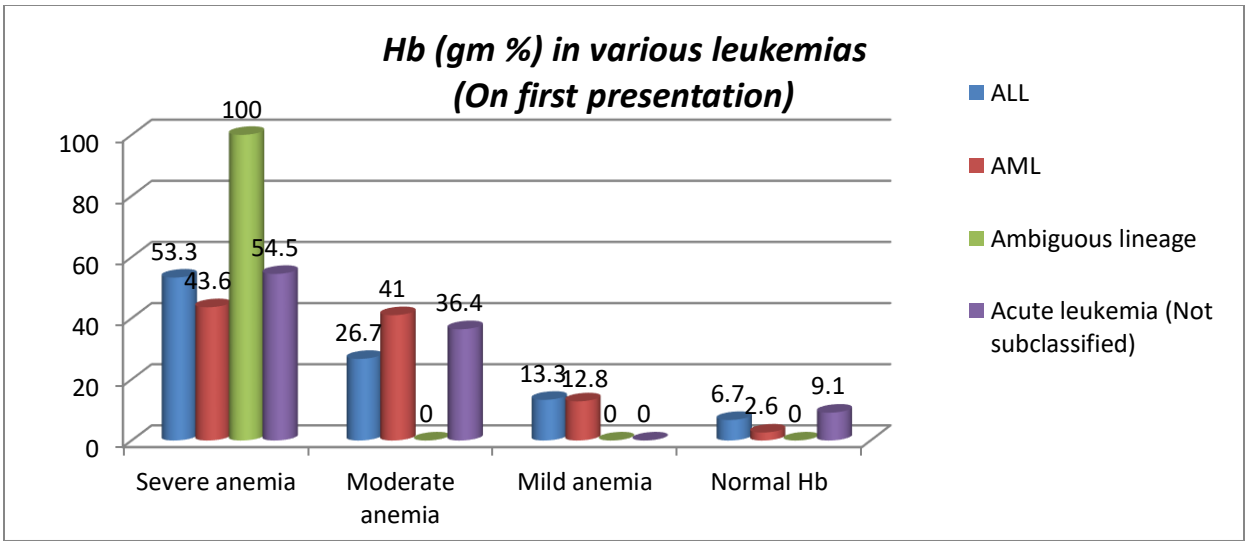
3. Acute myeloblastic leukemias (AML) cases were 3.9 times more common than acute lymphoblastic leukemias (ALL) in all age group and 6 times more common in adult population.

4. Acute Lymphoblastic Leukemia was more common (66.7%) in children (<15 years of age) than Acute Myeloid Leukemias (23.1%) and in Acute Myeloblastic Leukemias an adult predominance (76.9%) was observed.

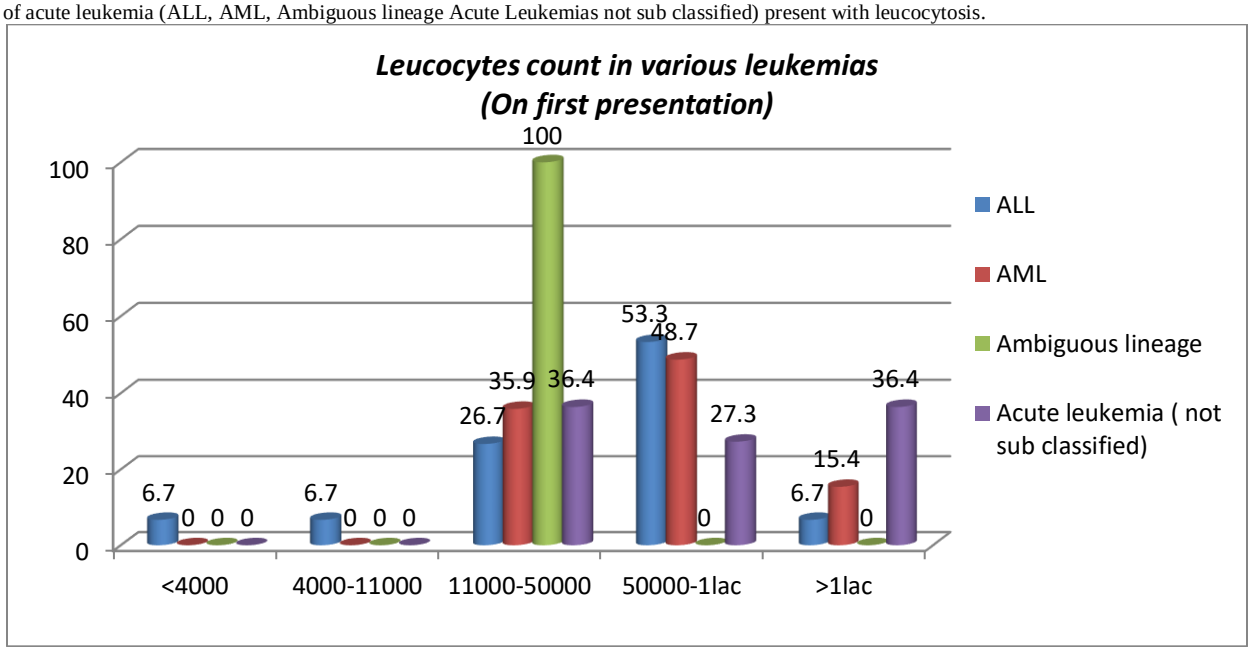




5. Acute leukemia shows male preponderance; male to female ratio were 1.5:1 in ALL and 1.17:1 in AML.
6. Acute Lymphoblastic Leukemia (53.3%) and Acute Myeloblastic Leukemia (43.6%) had severe anemia less than 6 gm% Hb. Therefore Patients of acute leukemia (ALL, AML, Ambiguous leukemia and Acute Leukemias not sub classified) present with features of anemia like weakness, pallor, dyspnoea, palpitation and tachycardia.



7. Acute Lymphoblastic Leukemia (60%) and Acute Myeloblastic Leukemia (64.1%) had total leucocyte count more than 50000/cumm. Patients of acute leukemia (ALL, AML, Ambiguous lineage Acute Leukemias not sub classified) present with leucocytosis.



8. Acute Lymphoblastic Leukemia (100%) and Acute Myeloblastic Leukemia (94.8%) had neutropenia. Therefore presenting features in patients of acute leukemia (ALL, AML, Ambiguous leukemia and Acute Leukemias not sub classified) was related to neutropenia e.g. fever, infection.

9. Acute Lymphoblastic Leukemia (93.3%) and Acute Myeloblastic Leukemia (84.6%) had thrombocytopenia. Therefore Patients of acute leukemia (ALL, AML, Ambiguous lineage and Acute Leukemias not sub classified) present with features of thrombocytopenia that is easy bruising, petechiae, epistaxis, gingival bleeding, conjunctival hemorrhages, and prolonged bleeding from skin injuries reflect thrombocytopenia and are frequent early manifestations of the disease. Rarely, gastrointestinal, genitourinary, bronchopulmonary, or CNS bleeding occurs at the onset of disease.

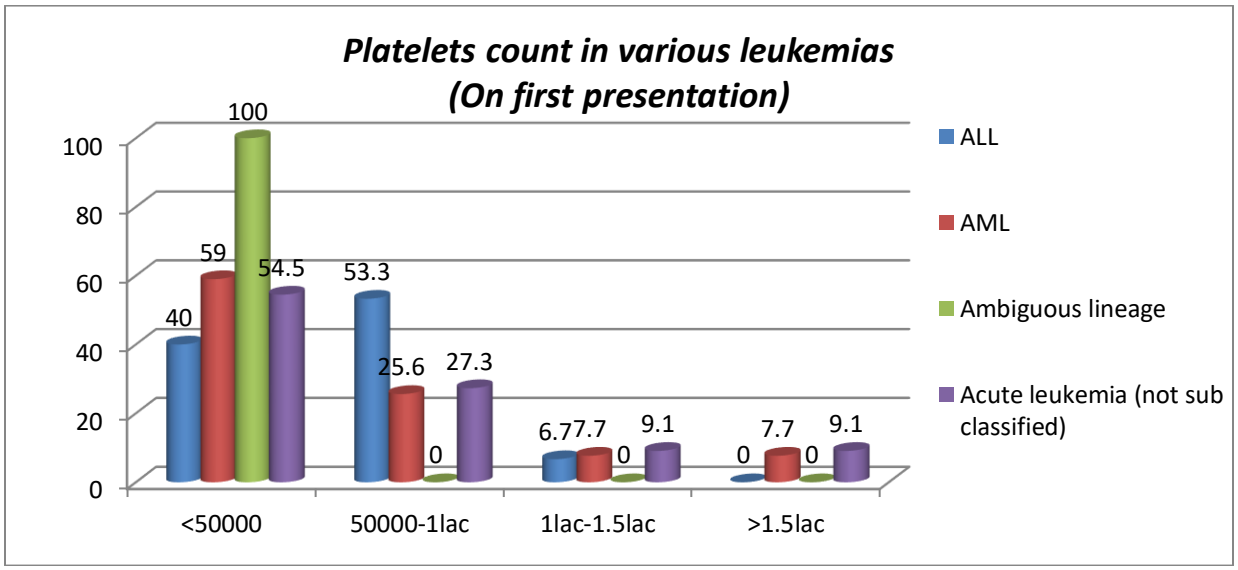


Table 2: Sub typing of acute myeloblastic leukemias (FAB Morphology)

S. No.	Sub type	Female		Male		Total	
		No.	%	No.	%	No.	%
1.	M0	0	0	0	0	0	0
2.	M1	0	0	1	12.5	1	6.3
3.	M2	2	25	5	62.5	7	43.8
4.	M3	3	37.5	0	0.0	3	18.8
5.	M4	2	25	2	25	4	25.0
6.	M5	1	12.5	0	0	1	6.3

7.	M6	0	0	0	0	0	0
8.	M7	0	0	0	0	0	0
Total		8	100	8	100	16	100

10. Highest number of AML cases (43.8%) was of M2 FAB subtype

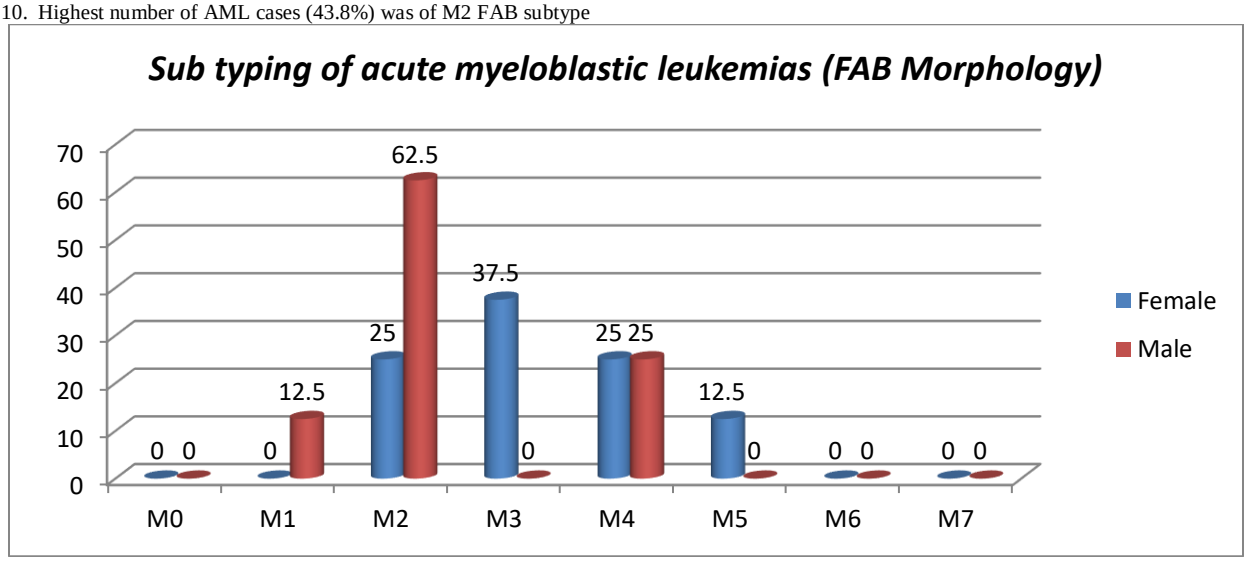


Table 3: Sub typing of acute lymphoblastic leukemias (FAB Morphology)

S. No.	Sub type	Total No. of cases	%	Female		Male	
				No.	%	No.	%
1.	L1	0	0	0	0	0	0
2.	L2	1	100	0	0	1	100
3.	L3	0	0	0	0	0	0
Total		1	100	0	0	1	100

Table 4: The results of the cytochemical staining (MPO &PAS) in patients with acute leukemia

Stain	ALL		AML		Ambiguous lineage		Others		Total	
MPO	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**
positive	0	0.0	31	93.93	2	100	0	0.0	33	67.34
Negative	11	100	2	6.06	0	0.0	3	100	16	32.65
Total	11	100	33	100	2	100	3	100	49	100
PAS	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**
positive	5	45.45	5	15.15	1	50	0	0.0	11	22.44
Negative	6	54.54	28	84.84	1	50	3	100	38	77.55
Total	11	100	33	100	2	100	3	100	49	100

11. Cytochemistry (MPO and PAS) done in 49 cases.33 cases (67.34%) were MPO positive and 16 cases (32.65%) were MPO negative. 11 cases (22.44%) were PAS positive and 38 cases (77.55%) were PAS negative. MPO is positive in AML and negative in ALL, while PAS is positive in both cases of AML as well as ALL. Therefore MPO is specific for myeloblast cells.

Table 5: Sensitivity and specificity testes of cytochemistry stains in patients with AML and ALL

Stains	Sensitivity test %	Specificity test %
MPO in AML	66.67%	100
PAS in ALL	0	88.89%

12. The sensitivity and specificity of the MPO stain alone for myeloblastic leukemia was 66.67% (4 true positives out of 6) and 100% (6 true negative out of 6), respectively. While the sensitivity and specificity of the PAS stain alone for lymphoblastic leukemia was 0.0% (0 true positives out of 3) and 88.89% (8 true negative out of 9), respectively.

Table 6: Diagnosis on the basis of different method

Diagnosis on the basis of	ALL		AML		Ambiguous lineage		Acute leukemia not sub classified		Total cases of Acute leukemia		Others		Total	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Morphology alone	10	14.08	18	25.35	0	0	43*	60.56	28	39.43	0	0	71	100
Cytochemistry	10	20.4	32	65.30	2	4.08	5	10.2	49	100	0	0	49	100
FCM	4	26.67	7	6.67	0	0	0	0	11	71.42	4	26.67	15	100

*1 case of subleukemia included

13. In our study on the basis of morphology alone 43 cases (60.56 %) were not sub classified and 28 (39.43%) cases were diagnosed and sub classified as ALL- 10 cases (14.08%) and AML- 18 cases (25.35%). With combined use of cytochemistry along with morphology 44 cases (96%) were diagnosed and sub classified, 5 cases (4%) were not sub classified.

Thus in a setting of lack of facilities for immunophenotyping as in majority of centers in the underdeveloped and developing countries, morphology combined with cytochemical staining still serves the best purpose in diagnosis of acute leukemias.

14. Cytochemical stains used in the present study included myeloperoxidase and Periodic acid-Schiff. Definite diagnoses were made for 32 cases (65.30%) of AML, whereas diagnoses were possible in only 10 cases (20.4%) patients with ALL when only morphology and cytochemical staining was used. In the rest of the cases, cytochemistry did not aid in diagnosis and therefore we opted for FCM to render a definitive diagnosis.

Hence, in our study, "Although cytochemical stains are essential to recognize the subtypes of AML, they are of limited use in differentiating the subtypes of ALL and therefore the FCM has become a standard tool for the assessment and management of patients with leukemia.

Flow-cytometry was done in 15 cases. The following were the results of FCA in these 15cases:-

a. ALL 4/15

• B-ALL (B lineage acute lymphoblastic leukemia): 2cases (50%) → CD 10 + and CD 19 +

• T-ALL: 2 cases (50%) →CD3 + and CD 7 +

• b. AML 7/15

• Only CD13 +1case (14.28%)

• Only CD33 + 2 cases (28.57%)

• Both CD13 and 33 + 2 cases (28.57%)

• CD13, 33 and 14 + 2 cases (28.57%)

c. Others 4/15

• B cell neoplasm→

Only CD 5+ 1case

CD 19and CD 20 + but CD 5and CD 10 negative 2 cases

• CLL→CD 5, CD23, CD19, CD20 and CD22positive.

Table 7: Concordance of FCM on morphology and cytochemistry, No.= 13 cases

	FCM				Total	
	Concordance		Discordance			
	No.	%	No.	%	No.	%
Morphology alone	2	15.3	11	84.7	13	100
Cytochemistry	7	53.9	6	46.1	13	100

The correlation between FCM and morphology showed complete concordance in 15.3% of the cases and discordance in 84.7% cases, and the correlation between FCM and cytochemistry showed complete concordance in 53.9% of the cases and discordance in 46.1% cases.

Hence FCM not only helps in confirming morphologic diagnosis in acute leukemia but also helps in assigning specific lineage to the blasts, particularly in acute lymphoid leukemia. Immunophenotyping is of utmost importance in classifying acute leukemia as it greatly influences the treatment and the prognosis.

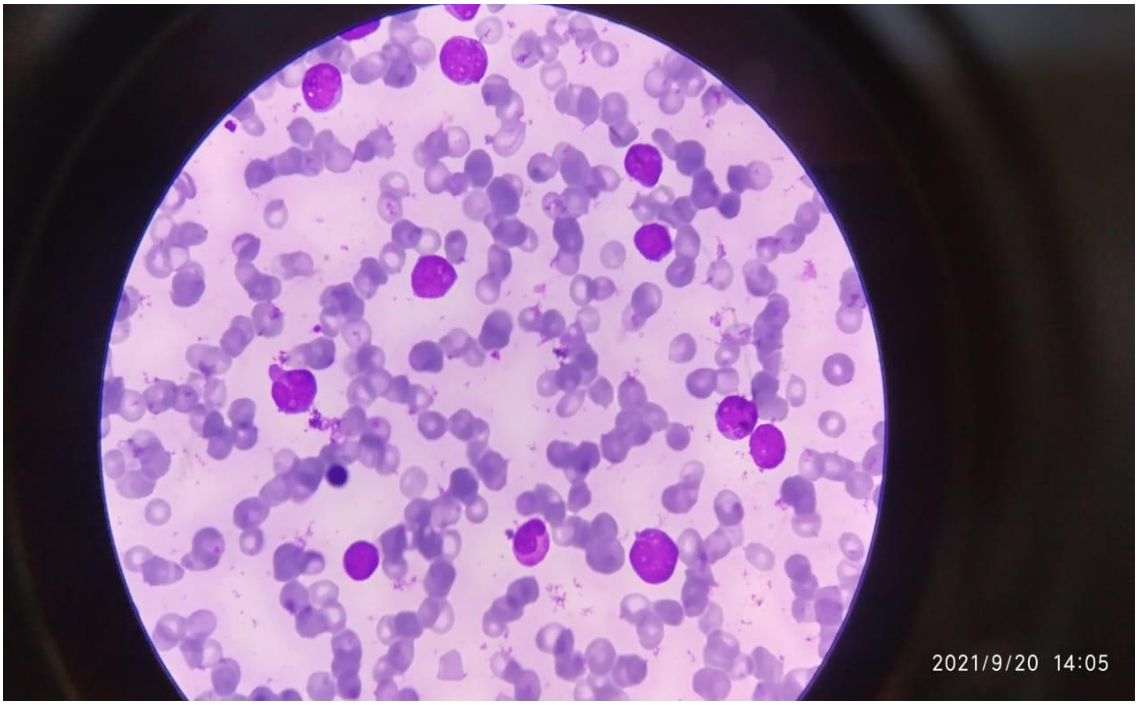


Figure 1- Peripheral smear of ALL- showing lymphoblasts (Leishman giemsa stain Oil immersion-100x)

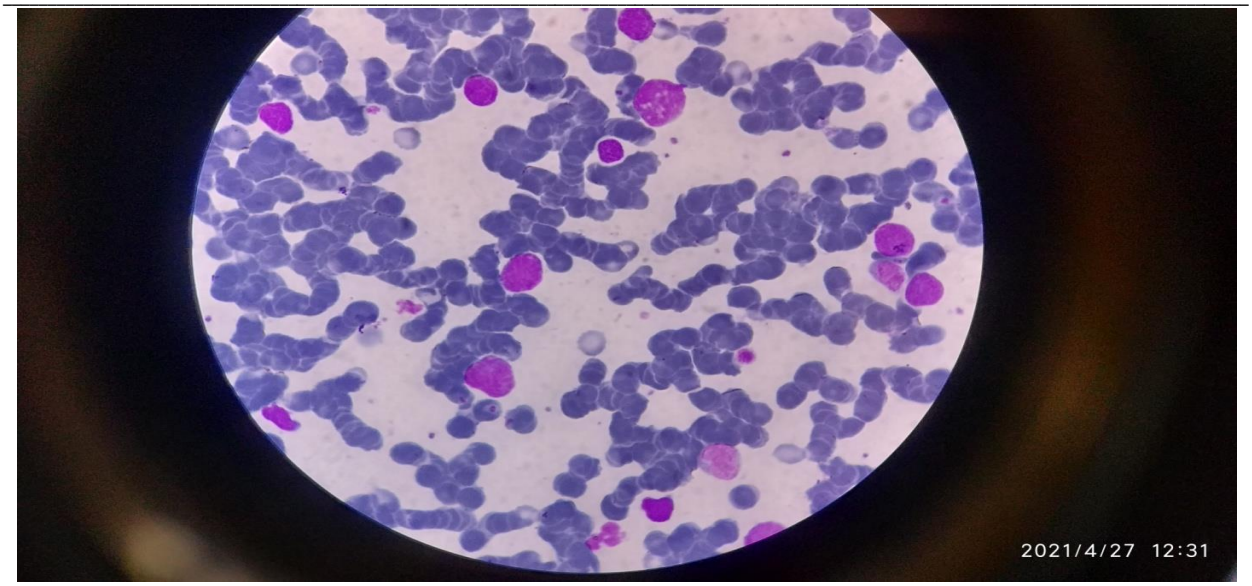


Figure 2- Peripheral smear of AL- showing blasts (Leishman giemsa stain Oil immersion100x)

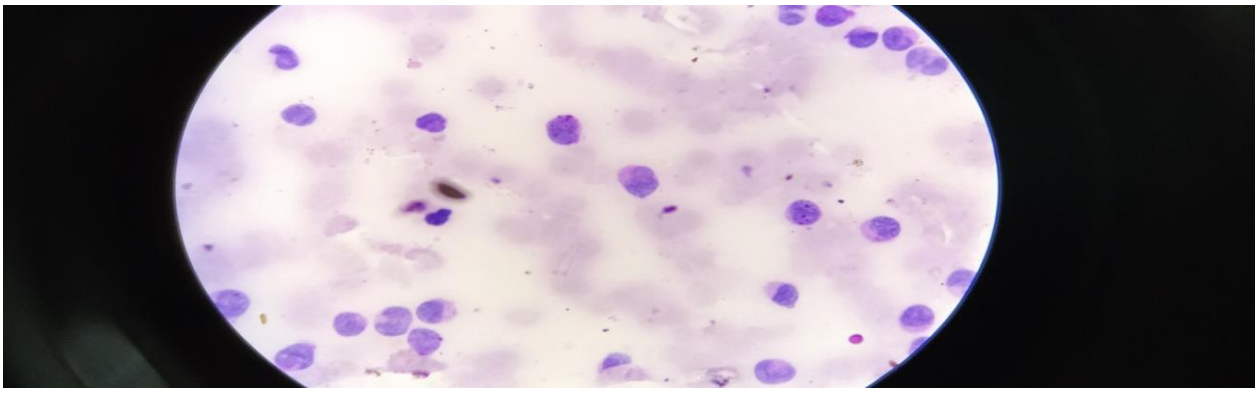


Figure 3- Peripheral smear of ALL showing PAS positive lymphoblast (PAS stain Oil immersion-100x) Block and Dot positivity

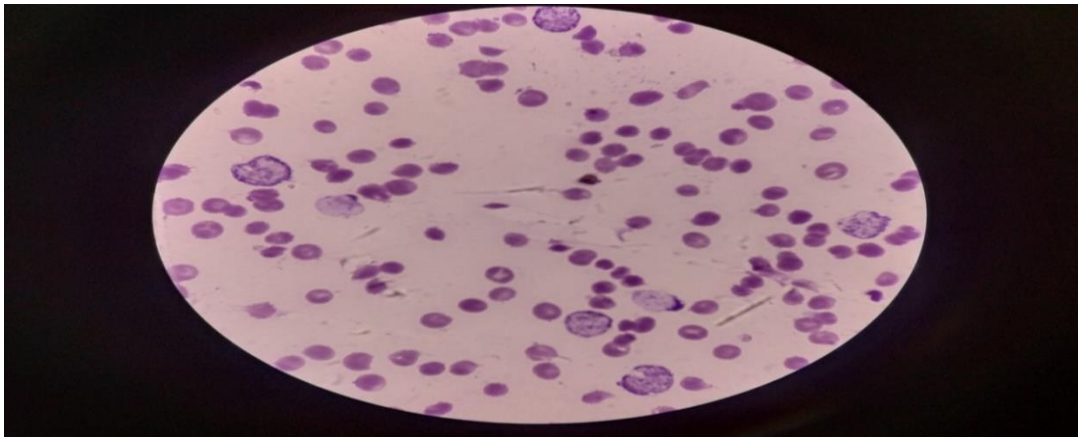


Figure 4- Peripheral smear of AML showing MPO positive coarse brown black granules (MPO stain Oil immersion-100x)

Discussion

Acute leukemia is a hematological disorder defined by presence of 20% or more blasts in peripheral blood, bone marrow or other tissue[4-6]. It is divided into Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL) and Acute Leukemia of Ambiguous Lineage on the basis of morphological, cytochemical & antigenic characteristics. Further sub classification of AML & ALL has been done on the basis of immunophenotyping and molecular studies[4,5].

In morphological classification (FRENCH- AMERICAN- BRITISH classification) ALL is classified into L1, L2 and L3[10] and AML is classified into M0, M1, M2, M3, M4, M5, M6 and M7[11].

In our study, incidence of acute leukemia is 0.0354 %**.** However**,** according to Kaushansky Kenneth, et al, the incidence rate of AML is

approximately 1.5 per 100,000 in infants younger than 1 year of age, decreases to approximately 0.4 per 100,000 children ages 5 to 9 years, increases gradually to approximately 1.0 persons per 100,000 population until age 25 years, and thereafter increases exponentially until the rate reaches approximately 25 per 100,000 persons[16]. Acute Leukemia accounts for approx 0.15-0.60% of total medical admissions in hospitals in India[7].

In present study total 71 cases of leukemia were studied. Out of which 67 cases (94.3%) were diagnosed as Acute leukemias, which included 15 cases (21.1%) of Acute lymphoblastic leukemias, 39 cases (54.9%) of Acute myeloid Leukemias, 2 cases (2.8 %) of Acute leukemia of ambiguous lineage and 11 cases (15.5 %) of Acute leukemia not sub classified, and 4 cases of others which included CLL (Chronic lymphocytic leukemia) and B cell neoplasm.

According to Boros, L. and Bennett, J.M et al, AML occurs twice as often as ALL[17], but in our study predominant cases were of AML (54.9%) which correlates with previous studies of Anil Meena et al[18], Ratanmala et al[19], and Ahmad et al[20]. However, studies of Hamid et al[21], Belurkar et al[13], Rajnikant Ahirwar et al[22] show ALL predominance which does not correlates with our study probably due to small population size.

ALL is commoner in children (72%) as compared to adults (28%)[8]. In present study Acute lymphoblastic leukemia is also more common (66.7%) than Acute myeloid leukemias (23.1%) in children that is similar to findings observed by Hamid et al[21], Anil Meena et al[18] and Rajnikant Ahirwar et al[22].

According to Kaushansky Kenneth, et al, AML accounts for 80 percent of the acute leukemias in adults[16]. In our study also Acute myeloblastic leukemias has an adult preponderance (76.9%) which correlates with studies of Hamid et al[21], Anil Meena et al[18] and Rajnikant Ahirwar et al[22].

In present study AML: ALL ratios for all ages are 3.9:1 and in adults the AML: ALL ratios are 6: 1. This shows that all age groups AML cases are more as compared to ALL cases and this pattern is similar to the ratio of AML and ALL (2.5:1) cases as reported by Anil Meena et al[18]. However Rajnikant Ahirwar et al[22] reported AML: ALL ratios for all ages are 0.47:1 which does not correlate with our study and this difference could be due to small population size.

In present study AML and ALL ratios in children are 0.9: 1, it means in children, ALL cases are slightly more than AML cases and in this reference findings of present study are similar to findings of Anil Meena et al[18] 0.3:1and Rajnikant Ahirwar et al[22] 0.15:1.

ALL[24] and AML[16] both are more common in males. In present study ALL and AML both show males preponderance 1.5:1 and 1.17:1 respectively, which correlates with the study of Anil Meena et al[18] who found ALL (1.6:1) and AML (2:1) was more common in males, Rajnikant Ahirwar et al[22] who found ALL (1.87:1) was more common in males and AML (0.57:1) was more common in females, Ahmad et al[20] found ALL (1.16:1) was more common in males and AML (1:1) was equally seen in females, Ratanmala et al[19] reported that there was male preponderance in the patients studied, with 55% males and 45% females. Male: Female ratio is 1.2:1.

Signs and symptoms that signal the onset of AML include pallor, fatigue, weakness, palpitations and dyspnea on exertion. These signs and symptoms reflect the development of anemia. Anemia is an almost constant feature[16]. Similar results were observed in present study in which Acute lymphoblastic leukemia (53.3%) and Acute myeloblastic leukemia (43.6%) had Hb less than 6 gm% (severe anemia) which correlates with the study of Anil Meena et al[18] who reported that in Acute lymphoblastic leukemia (56.25%) and Acute myeloblastic leukemia (41.66%) most of the cases had less than 6 gm% Hb (severe anemia). Rajnikant Ahirwar et al[22] reported that in Acute lymphoblastic leukemia (56.52%) and Acute myeloblastic leukemia (72.73%) most of the cases had less than 6 gm% Hb (severe anemia). Ratanmala et al [19] reported that in acute leukemia 66% had severe anemia.

White blood cell (WBC) counts are elevated in 58% of patients at diagnosis, low in 27%, and within the normal range in 15%[24]. Similar results were observed in present study in which 60% of Acute lymphoblastic leukemia and 64.1% of Acute myeloblastic leukemia had total leucocyte count more than 50000/cumm (leucocytosis), which correlates with the study of Anil Meena et al[18] who reported that 75% of Acute lymphoblastic leukemia and 72.21% of Acute myeloblastic leukemia cases had total leucocyte count more than 50000/cumm (leucocytosis). Ratanmala et al[19] reported that 49% of the patients had leukocytosis at presentation, 32% had leukopenia while 19% presented with a normal total count. However Rajnikant Ahirwar et al[22] reported that 8.70% of Acute lymphoblastic leukemia and 18.18% of Acute myeloblastic leukemia had total leucocyte count more than 50000/cumm (leucocytosis) which does not correlate with our study. The variations in results could be due to low population size.

Easy bruising, petechiae, epistaxis, gingival bleeding, conjunctival hemorrhages, and prolonged bleeding from skin injuries reflect thrombocytopenia and are frequently reported as early manifestations of the disease[16]. Similar results were observed in present study in which 40% of Acute lymphoblastic leukemia and 59%of Acute myeloblastic leukemia had severe thrombocytopaenia which correlates with the study of Anil Meena et al[18] who reported that 56.25% of Acute lymphoblastic leukemia and 52.77% of Acute myeloblastic leukemia cases had severe thrombocytopaenia. Rajnikant Ahirwar et al[22] reported that 69.57% of Acute lymphoblastic leukemia and 100% of Acute myeloblastic leukemia had severe thrombocytopaenia. Ratanmala et al[19] reported that anemia was the most common haematological abnormality (98%) followed by thrombocytopenia in 93% cases.

In many cases of myeloblastic leukemia, more prominent granulocytic maturation is evident (FAB type M2 or WHO designation AML with maturation). This variant is present in approximately 15 percent of AML cases[16]. In our study, among patients with AML, M2 subtype was the most common, similar findings were observed by Hamid et al[21], Belurkar et al[13], Anil Meena et al[18] while Rajnikant Ahirwar et al[22] Ahmad et al[20], Ratanmala et al[19] showed AML M3 was most common which does not correlate with our study.

In our study all AML cases were found to be MPO positive (100%) and ALL cases were found to be consistently MPO negative (0.0%). These findings were similar to previous study by Hamid et al[21], Belurkar et al[13] which also reported no MPO positive cases in ALL, and 95% and 91.6 % MPO positive cases in AML respectively.

In the present study, PAS stain was positive in 45.45% of ALL cases. Hamid et al[21] reported 80.6%, Belurkar et al[13] reported 66.7% and Biren Parikh et al[23] reported 27.9% of ALL cases as PAS stain positive.

In the present study, PAS stain was positive in 15.15% of AML cases. Hamid et al[21] reported 15 % and Biren Parikh et al[23] reported 3% of AML cases as PAS stain positive.

In this study, the sensitivity and specificity of the MPO stain alone for myeloblastic leukemia was 66.67% (4 true positives of 6) and 100% (no false positives), respectively. In Hamid et al[21] study the sensitivity and specificity of the MPO stain alone for myeloblastic leukemia was 95% (19 true positives of 20) and 100% (no false positives), respectively.

In this study, the sensitivity and specificity of the PAS stain alone for lymphoblastic leukemia was 0.0% (0 true positives of 4) and 88.89% (one false positives), respectively. 0.0% sensitivity can be explained by less percentage of confirmed cases of ALL on flow cytometry. Hamid et al[21] reported that the sensitivity and specificity of the PAS stain alone for lymphoblastic leukemia was 80.6% (25 true positives of 31) and 85% (three false positives) respectively.

In the current study the correlation between FCA and morphology showed complete concordance in 15.3% of the cases, and discordance in 84.7% cases, and the correlation between FCA and cytochemistry showed complete concordance in 53.9% of the cases, and discordance in 46.1% cases. A similar result was observed by Belurkar et al[13] who reported that the correlation between morphologic and FCA diagnosis showed complete concordance in 58% of the cases and discordance in 26% cases (partial concordance in 22% and non-

concordance in 4% cases) whereas in the remaining 16% FCA helped in establishing definite diagnosis where morphology had not helped.

Summary and conclusion

In a setting of lack of facilities for immunophenotyping as in majority of centers in the underdeveloped and developing countries, morphology combined with cytochemical staining still serves the best purpose in diagnosis of acute leukemias.

Although cytochemical stains are essential to recognize the subtypes of AML, they are of limited use in differentiating the subtypes of ALL and therefore the FCM has become a standard tool for the assessment and management of patients with leukemia.

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