DISCUSSION

A total of 95 newly diagnosed cases of acute myeloid leukemia were evaluated in our study. Their peripheral blood samples and bone marrow aspirates were studied for the morphology, cytochemical staining and immunophenotypic profile of the blast cells.

In our study population (ranging 7 months to 80 yrs), 65.26% (62/95) patients were less than 16 yrs of age while the rest, 34.74% (33/95) patients were

> or = 16 yrs of age. Out of which, 21.05% were between 16 to 45 years of age and 13.68% were above 45 years. The mean age at the time of diagnosis was 18.76 yrs. This division holds importance because it has been seen that AML has a worse prognosis in adults aged >45 years. We know that AML is more common in adults, contrary to our findings here. Also, in other studies conducted such as Ansari et al (in 2003) it was found that seventy-six percent of their cases were adults. In their study, the age of the patients ranged from one year to 78 years with a median age of 27.2 years(46). The difference in age distribution may be attributed to the more paediatric cases visiting our hospital.

The commonest FAB subtype was Acute Myeloid Leukemia With Maturation (31/95; 32.63% cases) while the least common was Acute Myelomonocytic Leukemia (1/95;1.05% cases). The order was Acute Myeloid Leukemia with maturation□M1□ M3□M0□M6□M4□M7□M5. Acute Myeloid Leukemia Without Maturation and Acute Myeloid Leukemia with maturation both are the most common subtypes in the pediatric population while Acute Myelomonocytic Leukemia is the least common. Also, Acute Myeloid Leukemia with maturation is the most common subtype in the adult population while Acute Myelomonocytic Leukemia, AML M5 and AML M7 are the less common subtypes. Other studies have also observed a higher frequency of Acute Myeloid Leukemia with maturation in both pediatric and adult population from the same institution. (46) The proportion of Acute Monocytic Leukemia in our series was 4.21 % only. Roberts et al have reported a frequency of 40% in case ofAcute Monocytic Leukemia.[53] The incidence of

Acute Promyelocytic Leukemia in our study was 12.63%. Incidence of AML-M6 was 8.42%, more than 3-5% quoted in the literature. (77,78,79)

Most common morphological diagnosis was Acute Myeloid Leukemia with maturation (32.63%) followed by Acute Myeloid Leukemia without maturation (27.37%), Acute Promyelocytic Leukemia (12.63%),Acute Myeloid Leukemia with minimal differentiation (11.58%), AML-M6 (8.42%),Acute Monocytic Leukemia (4.21%), AML-M7 (2.11%) and least common was AML-M5 (1.05%).

Comparison of the percentage of subtypes of AML in different studies

Author	Total cases	M0	M1	M2	M3	M4	M5	M6	M7
Ansari et al (46)	260	--	16	32	5	20	19	1.6	0
Advani et al		--	--	34	5.4	3.5	20	1.6	--
Wintrobe (62)		---	10-20	---	7-8	5-10	2-10	2-4	0.6-10
Ihsan (55)	106	27.9	21.2	13.5	13	12.5	5.8	--	5.8
Our study	95	11.58	27.37	32.63	12.63	4.21	1.05	8.42	2.11

The minimum age was seen in Acute Megakaryoblastic Leukemia while the maximum was seen in Acute Myeloid Leukemia with maturation subtype. The highest mean is seen in the Acute Myelomonocytic Leukemia subtype.

There were 54 males and 41 females with a male to female ratio of 1.3:1. In a similar study by Ansari et al, the ratio obtained was 2.56:1.(46) Also, this ratio was 1.26:1 in a study by S Gujral et al(33). The most and least common FAB type in females are Acute Myeloid Leukemia Without Maturation and Acute Monocytic Leukemia respectively. While in males it is the Acute Myeloid Leukemia With Maturation subtype which is the most common and Acute Monocytic Leukemia and Acute Megakaryoblastic Leukemia are the less common types.

Amongst the clinical findings, the most common was fever (72.63%) followed by fatigue (55.79%), weight loss (48.42%) and infection, including diarrhea, respiratory tract infections(48.42%), the less common ones were bleeding(40%), lymphadenopathy(26.32%), bone pain(20.21%),

hepatomegaly(17.89%) and splenomegaly(14.74%) while the least common was abnormal mass manifesting as orbital swelling in all the cases (3.16%). However, a higher percentage (approximately 75%) has been reported in the literature(73,74) In a similar study by Ansari et al (46) the majority of their patients (82%) presented with pallor and weakness. Lymphadenopathy was fairly common and seen in 94 patients (36.2%). Cervical lymph nodes were most often involved followed by the axillary nodes. Hepatosplenomegaly was seen in 68 patients (26.2%). Fever was a prominent most common presenting symptom seen in 72.63% (69/95) of the patients.

In our study, the proportion of patients presenting with fever was higher in Acute Promyelocytic Leukemia (83.33%), Acute Myeloid Leukemia without Maturation (80.77%), Acute Myeloid Leukemia with Maturation (77.42%) and Acute Myelomonocytic Leukemia (75.00%) as compared to patients with other morphological diagnosis (0 to 54.55%).

Splenomegaly was most common in AML M1 subtype followed by AML M0 and least common in AML M4, M5 and M7. Hepatomegaly was most common in AML M2 and M3 subtype and least common in AML M4 and M5. Lymphadenopathy was most common in AML M0 subtype followed by AML M2 and M1 and least common in AML M5 and M7.

Of all subtypes, acute promyelocytic leukemia cases had a significantly (p=0.041%) higher proportion (83.33%) of patients with bleeding symptoms while the rest 16.66% cases did not manifest with bleeding. It was seen in the mucosa and skin. Ansari et al in 2003 reported that bleeding was present in 57 patients (21.9%) out of 260 cases. It was most common in the AML-M3 and M5b categories and seen in the mucosa and skin. AML M3 was followed by AML M1 for having bleeding manifestation. The mean platelet value was least in AML M3. However, it was low in other AML subtypes as well, but they showed much lesser bleeding manifestation. Thus, depicting the possibility of other causes for bleeding in AML M3 which is well supported by literature.(15)

Abnormal mass (i.e. orbital swelling) was found in only 3 (3.16%) patients. Abnormal mass was observed in 2 (6.45%) patients of Acute Myeloid Leukemia with maturation and 1 (25.0%) patients

of Acute Monocytic Leukemia. Thus, these cases presented with extramedullary leukemia with orbital masses.

Anemia was seen to be the most common finding at the time of presentation as 95.79% (91/95) of the patients had hemoglobin levels less than 10gm%. The Haemoglobin levels ranged from 2.6 to 12.6 gm% with a mean value of 6.03 gm% ± 1.89 (SD). The lowest as well as the highest values were found in Acute Myeloid Leukemia with maturation subtype. Acute Monocytic Leukemia had the lowest mean value while Acute Myeloid Leukemia with Minimal Differentiation had the highest mean value.

The total leukocyte count ranged from 1100 cells/cu.mm to 4,10,000 cells/cu.mm. The TLC had mean value 37,872 cell/cu.mm ± 57400 cells/cu.mm (SD). The lowest value was seen in acute myeloid leukemia with maturation while the highest value was found in acute myeloid leukemia with minimal differentiation subtype. Acute monocytic leukemia had the lowest mean value while the acute myeloid leukemia with minimal differentiation had the highest mean value. In the literature, however, AML M4 is quoted to have a higher TLC count commonly(62). Most of the patients (67.37%) presented with leukocytosis (Total leukocyte count >11000/cumm). As 32.63% of the patients presented with low to normal leukocyte count, it was inferred that thorough examination of peripheral blood smears was necessary in routine hematological examination to prevent missing out on early diagnosis of leukemia cases. Ansari et al did a similar study and found the following findings. The WBC count was highest in case of Acute Myeloid Leukemia without maturation with a mean of 97x109/L followed by the Acute Monocytic Leukemia and Acute Monocytic Leukemia subtypes (80-90 x109/L). The lowest WBC counts were observed in the Acute Promyelocytic Leukemia category as 10 x109/L.

Leucocytopenia (<11,000/ cu.mm) was found in 31 (32.63%) patients of the study population. Proportion of patients with leucocytopenia was found in higher proportion of patients of AML-M3 (50.0%), AML-M7 (50.0%) and AML-M1 (42.31%) as compared to patients with other morphological diagnosis.

Thrombocytopenia was seen in 91.58% (87/95) patients with platelet count <1.0lac/cumm. The platelet count ranged from

1100 cells/cu.mm to 4,10,000 cells/cu.mm. The mean value was 39643l/cu.mm ± 41903 cells/cu.mm (SD). The lowest value was seen in acute myeloid leukemia with maturation and AML M1 while the highest value was found in acute myeloid leukemia with maturation subtype. Acute promyelocytic leukemia had the lowest mean value while AML M7 had the highest mean value. AML M7 is also quoted in the literature to have higher platelet counts.(62)All the patients of acute myeloid leukemia with minimal differentiation, acute promyelocytic Leukemia, Acute Myelomonocytic Leukemia, and Acute Monocytic Leukemia showed thrombocytopenia. Though proportion of patients with platelet count >1 lac was higher in AML-M7 (50.0%) and AML-M6 (25.00%) as compared to patients with other morphological diagnosis but this difference was not found to be statistically significant.

PBS-blast count (<20%) was found in 27 (23.16%) patients. Thus, 23.16% cases were in aleukemic phase. Proportion of patients with PBS-blast count >20% was highest in acute myeloid leukemia with minimal differentiation and acute promyelocytic leukemia and significantly lower in Acute Monocytic Leukemia (25.0%) as compared to patients of other morphological diagnosis. The single patient diagnosed as AML-M5 had PBS-blast count <20% (ie was in aleukemic phase).AML M5, M4 and M7 had maximum number of aleukemic cases.

Comparison between haematological parameters in our study and Ihsan et al

Hb(mean) g/dl

Our Study Hb(mean) g/dl

Ihsan et al TLC(mean)
(cells/cumm)

Our Study TLC(mean)
(cells/cumm)

Ihsan et al Platelet (mean)(per cumm)

Our Study	Platelet (mean)(per cumm)					
Ihsan et al						
AML MO	6.8	8.4	68318	39700	26182	94000
AML M1	5.7	9.0	31242	52500	42150	83200
AML M2	6.14	7.8	45655	35000	44116	39800
AML M3	6.18	7.3	22975	25000	23450	53700
AML M4	4.73	8.1	46300	30100	35250	74900
AML M5	4.0	9.1	11600	62200	35000	63800
AML M6	6.23		14525		53625	
AML M7	6.35	6.9	15000	73500	64100	50800

Number of bone marrow blast cells were found to be significantly lower in patients diagnosed as AML M4 (38.00+17.26), AML M5 (58.00) and AML M6 (57.50+50.20) as compared to patients with other diagnosis. Highest blast count was seen in AML M2 followed by AML 3 and AML M1.The mean value of blast count was 71.18 ± 20.65.

Author	Auer rods seen
Neelam et al	40% casesof AML
Our study	54.7% cases of AML

We studied the details of nucleus in blast cells in the peripheral blood smears and bone marrow smears. The nucleus was rounded in majority of the blast cells in acute myeloid leukaemia while in acute monocytic leukaemia, the nucleus had an irregular shape due to indenta¬tions and lobulation. Nuclear chromatin was fine in blast cells of acute myeloid leukaemia. Similar findings were seen in the study by Ihsan M. Elhadi et al.(55)

Author	**Bone marrow Blasts**	**Subtype with highest blast count**	**Subtype with lowest blast count**
Advani et al	57.6% (34- 96)	Acute Myeloid Leukemia without maturation	M4
Our study	71.18(21 – 98)	M3	M4

In each case the slides were subsequently stained to assess their reactivity for myeloperoxidase (MPO) stain. It was seen that 64/

84 (76.19%) cases showed positive cytochemistry for MPO out of 84 cases diagnosed as AML M1, M2, M3 and M4 and excluding AML M5, M6 and M7 cases because they were predominantly negative correlating with the literature. 65.38% cases of AML M1 were positive for MPO. Maximum positivity was seen in AML M3 (100%) category, followed by AML M2(90.32%) while minimum in AML M5 and M7(0%). Statistically, the positivity of MPO in identifying the myeloid lineage was found to be significant. ($p<0.001$)

Amongst the undifferentiated leukemia cases as reported on morphological evaluation, 3 cases out of 6 showed positivity for MPO staining. All these six cases, when assessed for immunophenotyping diagnosis, showed expression of myeloid markers (CD13, CD33, CD117), thereby, confirming the myeloid lineage.

Concordance between Morphological and Immunophenotypic Diagnosis

Morphological Diagnosis	No. of cases	Immunophenotypic Diagnosis
AML	89	95
Undifferentiated	6	
	95	95

Agreement between Morphological against Immunophenotypic diagnosis for AML cases: 95/95 = 100%.

We compared our finding with other studies:

Year	Author	Total no. of cases studied	% MPO concordanceAML
2010	Mukda et al.(52)	114	89.6
2009	S Gujral et al(33)	964	75
2013	Belurkar et al.(44)	50	91.6
2014	Our study	95	69.47

With the results obtained, we reached to the conclusion that cytochemistry in our cases helped in establishing the lineage correctly in 76.19% cases. MPO is sensitive as well as specific for identifying precursors of myeloid lineage. Even though MPO enabled us to assign lineage in the 3 out of 6 cases of undifferentiated leukemia as categorized on morphology, the findings need to be confirmed with immunophenotyping. Nevertheless, the importance of MPO stain

cannot be subdued especially for centres where flowcytometry is yet to become a routine practice.

The diagnosis thus made on morphological and cytochemical grounds was further subjected to immunophenotypic analysis for confirmation.

When compared with previous studies, similar results were observed:

Year	Author	Total no. of cases studied	No. of AML cases with concordant diagnosis	Number of AML cases Percentage	
1998	Kheiri et al. (10)	93	37	33/37	89.1%
2004	Kresno et al. (48)	225	115	111/115	96.52%
2006	Qadir et al. (50)	646	525	522/525	99.43
2013	Belurkar et al(44)	50	12	12/12	100%
2015	Our study	95	95	95/95	100%

CD33 was the myeloid marker that was most commonly present in all the AML subtypes i.e.93.98% cases. CD13 was the next most common marker present in all the AML subtypes i.e.85.54% cases. In our study, the expression of marker CD13 was seen in 85.54% of cases (71/83). The expression of marker CD33 was seen in 93.98% of cases (78/83). Maximum positivity of CD33 was seen in M3,M4,M6 and M7 (100% cases) of AML followed by AML M2, M1 and M0. In a study by Ihsan et al, CD33 was found to have higher positivity among AML-M4 and AML-M5 with mean positivity of 75.9% and 76.6% respectively.

In our study, CD117 had maximum positivity seen in M6 and M7 (100% cases) of AML followed by AML M2, M1, M0, M4 and M3.Expression of marker MPO was seen in 78.69% of cases (48/61). Maximum positivity was seen in M3 (100%cases), followed by AML M2 and M1. Least positivity was seen in AML M7. Expression of marker CD34 was seen in 74.36% of cases (58/78). Maximum positivity was seen in M0 (100% cases) of AML followed by AML M2 and M1.Least positivity was seen in AML M7 and AML M3. In our study, the positivity of the stem cell marker CD34 was in the range of 0% to 99% among the various subtypes. Expression of marker HLADR was seen in 71.70% of cases (38/53). Maximum positivity was seen in M7,M1,M2 types of AML. Least positivity

was seen in AML M3. HLA-DR was in the range of 0% to 99.6%.

The positivity of the marker MPO was in the range of 0% to 99.3% among the various subtypes. 62.5% cases of AML M0 were positive for MPO on flowcytometry while on cytochemical staining by MPO, only 36.36% cases of AML M0 were positive. Hence, flowcytometry is a more sensitive method for MPO identification.

Acute Myeloid Leukemia With Minimal Differentiation, Acute Myeloid Leukemia without maturation and Acute Myeloid Leukemia with maturation cases were positive for CD34, CD13, CD33, CD117 and HLADR. Cases of acute promyelocytic leukemia showed strong MPO positivity. CD34 and HLA-DR expression was absent in most of the cases of promyelocytic leukemias. . This was supported in a study by Cesare Guglielmi et al in 199855 who had correlated the immunophenotype of adult and childhood acute promyelocytic leukaemia with morphology. They confirmed in APL an immunophenotype characterized by frequent expression of CD13, CD33 and CD9 and rare expression of HLA-DR, CD10, CD7 and CD11b. Similar findings were seen in the study by Elisabeth Paietta(38) and Bakke et al (76)

Although the diagnosis could not be supported by cytochemical staining, flowcytometry showed positivity for CD14 and CD11b along with other myeloid markers, thus confirming the finding of Acute Myelomonocytic Leukemia (Table 10). CD14 expression ranged between 11% to 87% and the highest was seen in the Acute Myelomonocytic Leukemia subtype.

Immunophenotyping could not be done on our only case of Acute Myelomonocytic Leukemia. Flowcytometry could not to be of any further use for our cases of AML6 and AML M7 as we did not have the specific markers for erythroid and megakaryocytic lineage.

Comparison of immune markers positivity in our study

	CD34	HLADR	CD13	CD33	CD117	MPO	CD7	CD19
AMLM0	100	80	81.82	90.91	70	62.50	27.27	40
AMLM1	81.82	90.91	79.17	91.67	86.96	88.89	31.82	36.36
AMLM2	83.33	82.35	85.19	92.59	92.59	94.12	8	34.78
AMLM3	20	20	90	100	44.44	100	11.11	12.50
AMLM4	75	75	100	100	50	66.67	0	50

AMLM5								
AMLM6	66.67	80	100	100	100	0	16.67	0
AMLM7	0	100	100	100	100	78.69	0	0
Mean(%) positivity		74.36	71.70	85.54	93.98	81.25	78.69	
18.18	31.43							

Comparison of immune markers positivity in study by Ihsan et al

	CD34	HLA	CD45	CD13	CD33	CD117	CD19	CD7
AML-M0	68.63%	47.14%	89.62%	56.81%	38.42%	61.50%	12.87%	31.89%
AML-M1	57.85%	48.80%	90.06%	51.12%	45.22%	55.81%	11.58%	19.94%
AML-M2	62.79%	51.99%	88.32%	49.85%	53.84%	56.68%	9.48%	33.37%
AML-M3	17.32%	13.13%	91.08%	58.94%	54.29%	22.49%	14.08%	22.26%
AML-M3V	17.32%	24.47%	96.87%	47.70%	67.50%	53.90%	1.52%	27.15%
AML-M4	41.64%	41.95%	93.86%	54.86%	65.98%	35.00%	2.88%	23.36%
AML-M5	23.38%	64.71%	94.88%	38.34%	76.64%	13.10%	0.84%	16.41%
AML-M7	32.35%	25.34%	91.92%	33.72%	56.12%	21.04%	7.72%	38.40%
Total	50.36%	42.87%	90.81%	51.73%	50.35%	49.44%	9.58%	27.37%

Similar study was done in Tata Memorial Hospital by Ansari et al in 2003 on 260 cases of AML, which showed the positivity of the stem cell marker CD34. CD34 was in the range of 18.5% to 66.7% among the various subtypes. The highest positivity was seen in the AMLM0 and AML M6. HLA-DR positivity was highest in case of AML M2 and was less in the AML M0 and M4 subtypes. CD34 and HLA-DR expression was absent in all cases of promyelocytic leukemias. CD33 was the myeloid marker that was most commonly present in all the AML subtypes. CD13 was the next most commonly expressed antigen showing 100% positivity in the AML M3, M4 and M6 categories and ranging from 80-90% in the other subtypes. CD14 and CD36 positivity was more commonly associated with the monocytic leukemias. CD14 expression ranged between 3.7% to 44.4% and the highest was seen in the AML M4. CD36 expression was highest in the AML M5 and less in AML M4 categories. In their study, expression of lymphoid antigens was seen in 15% cases of AML. CD7 was the most commonly expressed lymphoid antigen (11%) .

Author	CD13(%positivity)	CD33(%)	CD117(%)
S Gujral et al	92	88	73
Ihsan et al	51.5	49.8	
Our study	85.54	93.98	34

Author	HLA-DR	CD34	CD117	CD13			
S Gujral et(33) al(2009)		95	95	51	2	%	Acute Promyelocytic Leukemia cases Negative
Our study	80	80	55.56	10	% Acute Promyelocytic Leukemia cases Negative		

Also flowcytometry helped us to study the aberrant markers present in leukemia cases. Aberrant markers are the unusual immunophenotypes. These are described as cross lineage antigen expression, expression of markers that are asynchronous with the level of maturity and over or underexpression of antigens. In our study, 63 out of 95 cases (66.3%) showed expression of expected antigens only. The remaining 33.68% (32/95) showed presence of aberrant markers, most common being CD19 (31.43%) followed by CD7(18.18%) and CD2(9.09%). The diagnosis of mixed phenotypic leukemia was ruled out as the WHO criteria were not satisfied(Annexure 1).

Despite the presence of aberrant markers, myeloid origin of blasts is not doubtful because of additional confirmation using MPO (cytochemical and immunophenotyping MPO).

Year	Author	No. of AML cases	Cases showing aberrant markers
2010	Mukda et al. (52)	30	14 (46.67%)
2014	Mehdi Jaredi et al(79)	56	32 (57.1%)
2015	Our study	95	32 (33.68%)

Year	Author	No. of AML cases	CD19 +	CD7+	CD2+	
2010	Mukda et al. (52)	30	64.2%	35.71%	----	
2011	Ihsan et al(55)	106	13.6	45.1		
2007	Abdelhaleem et al(75)	59	22%	25.8%	15.5%	
2015	Our study	95	31.43%	18.18%	9.09%	

Year	Author	No. of AML cases	Cases showing aberrant markers							
CD	Acute Myeloid Leukemia Without Maturation		Acute Myeloid Leukemia With Maturation		Acute Promyelocytic Leukemia		Acute Myelomonocytic Leukemia		Acute Monocytic Leukemia	
2014	Mehdi Jaredi et al	56	32 (57.1%)		CD2	58.35	21.40	33.3	------	20
			CD7	72.7	28.5	-----	------	-----		
2015	Our study	95	32(33.68%)		CD2	12.5	4.76	25	-----	-----
			CD7	31.82	8.0	11.11	----	------		

Also, in a study by Ihsan et al, CD7 was mostly expressed in AML-M2 and AML-M3 (75%) and least in AML-M5, while CD19 was only expressed in cases of AML-M0 and AML-M7.

Cytogenetics was done in only 35 cases out of 95. Out of three translocations, t(15;17) was found to be the most common. It was detected in 20.59% (7/34) of cases. Infact, all the cases (100%) of AML M3 were positive for this translocation in which it was done. All the other subtypes of AML were negative for t(15;17) in the cases in which it was performed.

The translocation t(8;21) was found to be the next most common.It was detected in 11.76% (4/34) of cases. Infact, 4 out of 9 cases (44.44%) of AML M2 were positive for this translocation. All the other subtypes of AML were negative for t(8;21) in the cases in which it was performed. These findings were found to be statistically significant well correlated with the literature.(62)

The translocation t(16;16) was not detected in any of the AML subtypes, in the cases in which it was done.

The present study reemphasized the importance of morphological diagnosis of acute leukemia by scrupulous examination of peripheral blood and bone marrow aspirate smears even in the age of immunophenotypic diagnosis. In under-resourced hematology laboratories there is usually a missing step in the battery of required investigations. Moreover, when some of the advanced diagnostic instruments can be found then the problem

of chronic inadequate and irregular supply of kits and services would supervene. Therefore, the laboratory diagnosis would mostly depend on the more basic, but consistently available and well controlled, laboratory techniques that should at least include complete blood count (CBC) and peripheral blood morphology, after which a bone marrow study with aspirate will follow.(41)