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RESEARCH ARTICLE

CORRELATION OF CLINICO-HAEMATOLOGICAL AND IMMUNOPHENOTYPIC PROFILE OF PATIENTS OF ACUTE LEUKEMIA

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ABSTRACT

Objective: To study the cytomorphological features of acute leukemia patients and relating them to their immunophenotypic features.

Results: Out of 50 cases of acute leukemia, 32(64%) cases were diagnosed as AML, 18 (36%) cases as ALL. Clinical features like fever, generalised weakness, bleeding tendencies, pain abdomen and loss of appetite were observed. On clinical examination pallor, bony tenderness, organomegaly and lymphadenopathy were seen. In peripheral blood examination: anemia, leucocytosis, and thrombocytopenia were observed. Cyto-morphologically, 48.2% ALL and 51.8% AML cases were diagnosed, classified according to FAB as ALL L1 (72.2%) and ALL L2 (27.8%). In AML cases, AML M2 (77.5%) was followed by AML M4 (12.4%). B-lymphoid lineage CD markers included CD19, CD79a and CD34 in (100%) followed by CD10 (86.4%), CD79a (63.6%), HLA-DR (85.61%), Tdt and CD10 (76.9%), CD20 in (46.5%) of cases. In T-ALL all 5 (100%) cases expressed cyCD3 and CD5. In AML cases markers were CD13 in (93.7%) followed by CD34 (81.2%), MPO and CD33 (78.1%) each, CD117 in (71.8%), HLA-DR in (75.8%). CD 64 was a sensitive monocytoid marker being positive in 100% of cases of AML-M4. Two of 50 cases of acute leukemia got lineage correction on FCA. 1(2%) case of ALL L2 and 1(2%) case of AML M2 diagnosed on morphology were reassigned lineage to AML M2 and T-ALL respectively.

Conclusion: The study concluded a concordance between cytomorphology and immunophenotyping. However a detailed evaluation from peripheral blood film to cytochemistry and morphology on bone marrow and immunophenotyping is necessary for diagnosing acute leukemia.

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INTRODUCTION

Leukemias are a group of disorder arising from the dysregulated clonal expansion of immature lymphoid or myeloid progenitor cells that are blocked at a particular stage of differentiation. The diagnosis of acute leukemia traditionally has been relied on the evaluation of peripheral blood counts, bone marrow morphology and cytochemistry staining. Diagnosis uniformity has been enhanced with introduction and later modification of the French-American-British (FAB) classification system, which is based upon morphological and cytochemical characteristic and provides as a standardization of criteria for the subclassification of myeloid and lymphoid leukemias. However, it is still insufficient for the classification of leukemias as morphologic characteristic may overlap and cytochemistry may be negative or equivocal. In most cases of poorly differentiated leukemias, the lineage cannot be definitely diagnosed by these methods. Problems with the FAB classification include inter-observer variability and poor correlation with survival (Naeem, 2014). Assignment of lineage is critical in the diagnostic evaluation of acute leukemia, as treatment for acute myeloid leukemia and acute lymphoblastic leukemia differs. Myeloid and lymphoid lineage may be distinguished based on cellular morphology, cytochemical staining, and expression of lineage-specific antigens (Ghosh, 2002). Refinement in classification of acute leukemias is accomplished by immunophenotyping. Differences in expression of surface membrane antigens or cytoplasmic components are used to identify and classify lymphoproliferative disorders by cell of origin and stage of differentiation (Schwonzen, 2007).

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MATERIAL AND METHODS

The cross-sectional study was conducted on 50 patients diagnosed as acute leukemia who reported to medicine OPD, Haematology Clinic or admitted to medicine wards of Pt. B.D. Sharma Post Graduate Institute of Medical Science Rohtak, Department of Medicine of age more than 15years of age, diagnosed by complete haemogram and bone marrow examination showing blasts >20% (Qadir, 2006). Routine investigations along with complete haemogram, bone marrow aspiration for morphology and cytochemistry and immunophenotyping.

OBSERVATIONS AND RESULTS

According to analysis based on cytomorphology and immunophenotyping the acute leukemia cases were classified as ALL and AML. In our study AML comprised of 32 (64%) and ALL 18 (36%) patients. In ALL cases B-ALL 13(72.2%) contributed more than T-ALL 5(38.4%). Males 10 (41.7%) predominated over females 8 (30.8%) in ALL patients whereas in AML patients number of females i.e.18 (69.2%) exceeded number of males 14 (58.3%), however females predominated over males. Majority of acute leukemia were seen in fourth decade followed by sixth decade for AML. The youngest patient was 15 years old and the oldest patient was 68 years old. It was observed that the maximum numbers of ALL cases were in the fourth to fifth decade forming 22.2% of total ALL cases. Fever in 10(70.6%) cases was the most common complaint followed by generalised weakness in 8(61.5%) cases of B-ALL, whereas fever and generalised weakness both were equally seen in 3(60%) of cases of T-ALL. Fever was observed in 24(75%) cases followed by generalised weakness in 18 (56.2%) cases and bleeding tendencies in 3 (9.3%) cases of AML. Other less common presenting features were pain abdomen in 2(15.3%) cases of B-ALL and 7(21.8%) of AML, weight loss in 1(7.6%)of B-ALL and 1(3.1%) of AML and loss of appetite in 1(7.6%) cases of B-ALL and in 5 (15.6%) cases of AML (Figure1).

respectively. Lymphadenopathy was observed in 7(53.8%) cases of B-ALL, 2 (40%) cases of T-ALL and 10(31.2%) cases of AML. CNS involvement was seen in 2 (15.3%) of B-ALL and 1 (20%) of T-ALL. Gum hypertrophy was found in 1 (3.1%) of AML case (Table 1).

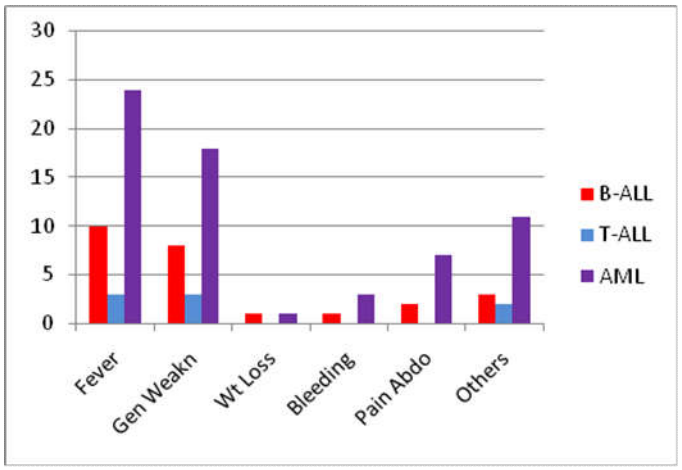

Figure 1. Clinical features of B-ALL, T-ALL, AML

Table 1. Clinical examination of B-ALL, T-ALL, AML

	B-ALL(n=13)	T-ALL(n=5)	AML
Pallor	13 (100%)	5 (100%)	29(90.6%)
Bleeding	7 (53.8%)	3 (60%)	13(40.6%)
Organomegaly	10 (76.9%)	3 (60%)	10(31.2%)
Lymphadenopathy	7 (53.8%)	2 (40%)	10(31.2%)
Gum hypertrophy	0 (0%)	0(0%)	1(3.1%)
CNS involvement	2 (15.3%)	1 (20%)	0(0%)
Bony tenderness	9 (69.2%)	4 (80%)	25(78.1%)

PBF showed highest and lowest TLC observed was $280x10^9$/L and $4x10^9$/L respectively and platelet counts ranged from 20-$130x10^9$/L in ALL. The blasts percentage on PBF ranged from 40%-95% for ALL and 30-90% for AML. TLC varied from highest of $180x10^9$/L to lowest of $2x10^9$/L with platelet count as low as $20x10^9$/L in patients of AML (Table 2).

Table 2. Haematological findings of B-ALL, T-ALL, AML

	B-ALL(n=22)		**T-ALL(n=6)**		**AML(n=32)**	
	Mean	Range	Mean	Range	Mean	Range
Hb levels(gm/dl)	6.53	4.5-10	8.1	4.5-12.2	7.23	3.5-11.8
TLCx109/L	64815.38	4600-280000	62000	40000-10000	38934.37	2000-180000
Platelet countx109/L	46153.84	20-130000	24000	20000-40000	65781.25	20000-200000
Blast % on PBF	76%	40%-95%	73%	50%-85%	66%	30%-90%

Table 3. Comparison of Cases of AML and ALL on Cyto-morphology with other studies

	AML					ALL		
	M0	M1	M2	M3	M4	L1	L2	L3
Kosasih *et al*[7]	1.3%	16.6%	29.1%	9.3%	18.5%	57.4%	42.6%	0%
Belukar *et al*[8]	------	11.3%	33.9%	3.7%	30.1%	------	------	------
Silicean *et al*[9]	------	2%	14%	-----	4%	26%	12%	4%
Present study	3.1%	3.1%	77.5%	3.1%	12.4%	72.2%	27.8%	------

Examination showed pallor 13(100%) in B-ALL, 5(100%) in T-ALL and 29(90.6%) in AML was the most frequently observed clinical sign. Organomegaly was found in 10 (76.9%) cases of B-ALL cases, 3(60%) of cases of T-ALL and 10(31.2%) of AML, bony tenderness in 9 (69.2%) cases of B-ALL, 4 (80%) cases of T-ALL and 25(78.1%) of cases of AML. Bleeding manifestations were found in 7 (53.8%), 3 (60%) and 13(40.6%) cases of B-ALL, T-ALL and AML

In ALL, it was observed that ALL L1 13 (72.2%) cases were more common than ALL L2 5 (27.8%).In AML, M2 was more common i.e. 25(77.5%) cases followed by M4/M5 4 (12.4%) and M0, M1, M3 each constituted 1 (3.1%) cases. No case of ALL L3, AML M6 and M7 was detected (Table 3). In ALL CD45 Antigen showed dim expression in 16(88.8%) cases and heterogenous expression in 2(11.1%) cases of acute leukemia.

In B-ALL, the most common CD markers expressed were CD34, CD19 and CD79a in 13 (100%) cases each, HLA-DR in 11 (85.61%) cases each, Tdt and CD10 in 10 (76.9%) , CD20 in 6 (46.5%) cases. In T-ALL all 5 (100%) cases expressed cyCD3 and CD5.Others markers positive included CD34, CD7, CD45 and sCD3. CD 19, CD10, CD34, CD79a and HLA-DR were significant statistically ($p<0.05$) in cases of B-ALL. CD3, cyCD3, CD5, CD4 and CD7 were significant statistically ($p<0.05$) in patients of T-ALL A Dim CD45 expression was observed in all the AML cases. CD13 was the most common antigen expressed i.e. in 30(93.7%) cases followed by CD34 in 26(81.2%) cases, MPO and CD33 in 25(78.1%) each cases, CD117 in 23(71.8%) cases, HLA-DR in 22 (75.8%) cases. CD45 was seen in 24(100%) of cases followed by CD34 and CD13 in 22 (91.7%) of cases each, HLA-DR and MPO in 19 (79.2%) of cases, CD33 and CD117 in 17 (70.8%) of AML-M2. On correlation between cyto-morphological and immunophenotypic profile, 17 (94.4%) of cases and 31 (96.9%) of cases were categorised as ALL and AML respectively both on cytomorphology and immunophenotyping where as 1 (3.1%) ALL case on cytomorphology was diagnosed as AML by FCA and one case of AML turned out to be ALL on immunophenotyping. Total of 48 (95.5%) of acute leukemia cases were concordant on both morphology and immunophenotyping while 4.5% cases were discordant. Cronbach's Alpha value is 0.955 which shows a high concordance between the cytomorphologic and immunophenotypic profile (Figure 2).

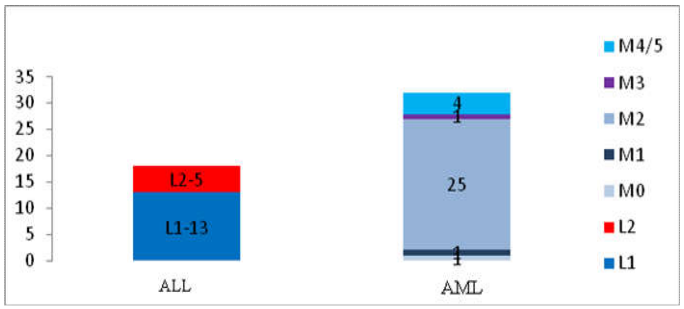

Figure 2. Cytomorphological profile of acute leukemia based on FAB classification

DISCUSSION

According to analysis based on cytomorphology and immunophenotyping the acute leukemia cases were classified as ALL and AML. In our study AML comprised of 32 (64%) and ALL 28 (36%) patients, findings being consistent with studies by Scherrer *et al* and Salem *et al*. (Salem, 2012).

Clinical features: Fever in 10(70.6%) cases and generalised weakness in 8(61.5%) cases were the most common symptoms found in both B-ALL cases while fever and generalised weakness both were equally seen in 3(60%) of cases of T-ALL. Bhattacharya *et al*. (2014) and Naeem *et al*. (2014) also reported fever as the commonest presenting feature followed by bleeding manifestations. Fever in 24 (75%) cases was the most common chief complaint followed by generalised weakness in 18(56.2%) cases and bleeding tendencies in 3(9.3%) cases of acute myeloid leukemia. In a study by Ghosh *et al* (Ghosh, 2003) Generalised body weakness was present in most of the patients.

Clinical Examination: Pallor 13(100%) in B-ALL and 5(100%) in T-ALL was the most frequently observed clinical sign followed by organomegaly was observed in 10(76.9%) cases of B-ALL cases and 3(60%) of cases of T-ALL. Lymphadenopathy in 7(53.8%) cases of B-ALL and 2(40%) cases of T-ALL and CNS involvement seen in 2(15.3%) of B-ALL and 1(20%) of T-ALL. In a similar study Bhattacharya *et al*[6] reported pallor as a common finding followed by organomegaly and lymphadenopathy with CNS involvement in 1.7% of cases which was consistent with our study. In a study by Naeem[1] lymphadenopathy was found in 63% of patients of B-ALL. Pallor 29(90.6%) was the most frequently observed clinical sign followed by bony tenderness in 25(50%) cases of AML. Organomegaly was found in 15(46.9%) of cases, bleeding manifestations seen in 10(31.2%) of cases and lymphadenopathy was observed in 10 (31.2%) cases. Ghosh *et al*[2] observed that pallor was found in 82%, lymphadenopathy in 36.2%, organomegaly in 26.2% of cases of AML which is consistent with our study.

Acute Lymphoblastic Leukemia: Based on FCA, out of 18 cases, 72.2% and 27.8% cases of ALL were diagnosed as B-ALL and T-ALL respectively. In B-ALL, the most common CD markers expressed were CD34, CD19 and CD79a in 13(100%) cases each, HLA-DR in 11(85.61%) cases each, Tdt and CD10 in 10(76.9%) , CD20 in 6(46.5%) cases. CD19, CD10, CD34, CD79a and HLA-DR were significant statistically ($p<0.05$) and helped in establishment of lymphoid lineage of B-cell type. Bhattacharyya *et al*[6] and Belurkar *et al*[8] reported CD19 as most sensitive marker along with CD10, CD79a, CD20 and Tdt in B lymphoid lineage assignment. Augilera *et al.* (2001) reported CD79a the most often expressed antigen. On the other hand Mukda *et al.* (2011) mentioned both CD19 and CD79a as the most often expressed B-ALL antigens. In T-ALL all 5(100%) cases expressed cyCD3 and CD5. Others markers positive included CD34, CD7, CD45 and Tdt. CD5 and cyCD3 were expressed in all T-ALL cases i.e. 5(100%), CD7 and CD34 in 3(60%) of cases, CD3 in 2(40%) and Tdt in 1(20%) of cases.CD5, cyCD3, CD7, CD3 were significant statistically ($p<0.05$). Bhattacharyya *et al.* (2014) and Belurkar *et al*. (2013) reported that cyCD3 was the most frequent expressed antigen along with CD3 and CD7. Augilera *et al*. (2001) concluded that cyCD3 was the best marker for T-ALL cases. Tiensiwankul *et al.* (1999) observed CD5 expression by all the cases of T-ALL along with CD7.

Acute Myeloid Leukemia: Thirty two cases of AML were diagnosed and categorised further. CD13, CD34, MPO and CD33 were the most common myeloid marker expressed in 30(93.7%), 26(81.2%), and 25(78.1%) AML cases of CD34 and MPO respectively, followed by CD117 in 23(71.8%) and HLA-DR in 22 (75.8%) of cases. Zheng *et al.* (2008) also concluded the above findings. On immunophenotyping our study reveal a predominance of AML M2 i.e. 24 (75%) cases followed by AML M4/M5 in 4 (12.5%) cases. Ansari *et al.* (2003) stated that the commonest AML subtype in their study was AML-M2. CD45 was seen in 24(100%) of cases followed by CD34 and CD13 in 22 (91.7%) of cases each, HLA-DR and MPO in 19 (79.2%) of cases, CD33 and CD117 in 17 (70.8%) of AML-M2.Silicean *et al.* (2013) showed CD34 expression in highest frequency, followed by CD 117, CD33, CD 13, HLA-DR and MPO. CD13, CD33, CD117, CD64 and MPO were expressed in all the 4 cases of AML M4/M5 and was comparable with Venketsvaran *et al*. (2012) Dunphy *et al.* (2007) reported 4 out of 9 cases of AML M4 which were based on dim CD64 positivity and concluded that CD64 is a more sensitive and specific marker for distinguishing AMLs with a

monocytic component. AML M3 is an unusual and distinctive disease clinically, morphologically, immunophenotypically and genotypically. Only one case (3.1%) of AML M3 was diagnosed. FCA showed positivity for CD13, CD33, CD117, CD64, MPO and was concomitant with other studies by Selicean *et al*. (2013) and Kaleem *et al* (Kaleem, 2003). It was found that 2 cases diagnosed as ALL L2 and AML M2 on morphology expressed different lineage on FCA. Hence a lineage correction was done. The ALL L2 on morphology, was diagnosed as AML-M2 with CD13, CD33, MPo, HLA-DRand CD 117 being positive myeloid markers. Another case of AML M2 on morphology was assigned lymphoid lineage of T cell type i.e. CD5, CD7, Tdt, cyCD3 on immunophenotyping. It was reviewed again morphologically and reported as T-ALL. Qadir *et al.* (2006) also reported lineage correction in 2% cases. Therefore, immunophenotyping with a limited panel must be routinely performed for the correct diagnosis of acute leukemia.

Conclusion

To conclude, our study proved to be helpful in assigning correct lineage to leukemia cells and support the use of particular panel of CD markers as a better diagnostic tool after preliminary investigations. Although considered superior, flowcytometric analysis must always be performed in conjunction with cytomorphology.

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