Immunophenotypic Study of Leukaemia Cases

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ABSTRACT

This study presents an immunophenotypic study of 739 cases of various types of leukaemia admitted to the Riyadh medical Complex, Riyadh, Saudi Arabia from January 1988 to December 2003. Paterns of observation for morphologically acute leukaemias were observed and classified into sub-types based on French-American-British (FAB) classification.

(Keywords: clinical research, Leukaemia, Leukemia, cancer, morphological differences)

INTRODUCTION

The significance of immunophenotypic studies for leukaemia classification, and for prediction of prognosis, response to therapy and relapse is well established. In the present study a total of 739 cases of various types of leukaemia admitted in Riyadh Medical Complex (Central Hospital), Riyadh (Saudi Arabia) were studied in Medical Laboratory from January 1988 to December 2003. The incidence of leukaemia was observed to be 0.15% of the total hospital population during this period.

The AML (Acute Myeloid Leukaemia) group was the most frequent (34.78%), followed by the ALL (Acute Lymphoblastic Leukaemia) group (24.36%), CML (Chronic Myeloid Leukaemia) (20.43%), CLL (Chronic Lymphocytic Leukaemia) (19.76%), LSCL (Lymphosarcoma cell Leukaemia) (0,40%), and HCL (Hairy Cell Leukaemia) (0.27%). Morphologically acute leukaemias were further classified into subtypes on the basis of FAB classification (Bennett et al, 1976; Bennett et al, 1985; Behrens and Kidd, 1987).

In acute leukaemias, in the group of ALL, the following pattern was observed; Common-ALL > T-ALL > B-ALL > Null-ALL > Pre B-ALL. Among FAB subtypes of ALL, in the L1 type, the following pattern was observed; Common–ALL > T-ALL > Null-ALL > Pre B-ALL. In the L2 type, the following pattern was observed; Common-ALL > T-ALL > Null-ALL and Pre B-ALL and in the L3 type only B-ALL was seen.

In the AML group, all the cases were positive for myeloid markers. In addition, glycophorin, and platelet antigens were positive for AML-M6 and AML-M7, respectively. In childhood ALL, prognosis in common (ALL and Pre B-ALL is best), while in adult ALL, Common–ALL and Null-ALL have a poor prognosis. In chronic leukaemias (CLL and LSCL), the most common immunologic marker was B-type. In HCL, only the B-type was seen.

MATERIALS AND METHODS

Advances in flow cytometry technology and the availability of commercially produced monoclonal antibodies have opened new horizons for the diagnosis and classification of acute leukaemia. The procedure provides a supportive analysis to the routine FAB classification scheme of acute leukaemias involving morphological and cytochemical characteristics. Besides the assignment of lineage, the results of this investigation also help in characterization of differentiation stage of acute leukaemia. This in turn, helps in treatment selection, optimization, and monitoring of leukaemia therapy.

Specimen Collection – Bone marrow aspiration samples were received in the laboratory anticoagulated in either EDTA or sodium heparin tubes (Vacutainer, Becton-Dickinson, Rutherford, NJ, USA). 3.0 cc of peripheral blood, containing at least 20% blast cells, was collected in an EDTA tube, aseptically, by venipuncture.

Reagents – Monoclonal antibodies were purchased from Becton-Dickinson Immunocytometry Systems, Inc. (BDIS, San Jose, CA; USA). The panel selected was based on the recommendations of the British Committee for Standards in Haematology (1994).

Methods – Methods of flow cytometry and immuno-fluorescence tests were followed as described by Khan, 2000; British Committee for Standards in Haematology, 1994; Griffin et al, 1983; Khalil et al, 1995; Neame et al, 1986; and Todd-Henry, 1996. Prior to flow cytometric analysis, the morphologic characteristics (FAB subtypes) of the blast population were determined by light microscopy, using special cytochemical stains. The method was followed as described by Khan, loc cit; Bennett et al, 1982; Khan et al, 1991; Spence et al, 1988; Todd-Henry, loc cit.

OBSERVATIONS

Immunophenotypes of acute leukaemias – In the group of ALL, out of 180 cases (24.36%), the most common immunophenotype was Common-ALL – 129 cases (17.46%), followed by T-ALL – 21 cases (2.84%), B-ALL – 11 cases (1.49%), Null (Early pre-B) ALL – 10 cases (1.35%), and Pre-B- ALL 9 cases (1.22%). Among AML, all of the 257 cases (34.78%) were positive for myeloid markers (Table 1 and Figure 1).

Table 1: Immunophenotypic Classifications of Acute Leukaemias.

Immunophenotypes	Cases	%
ALL	180	24.36
Common-ALL	129	17.46
Null (Early pre-B)-ALL	10	1.35
Pre-B-ALL	9	1.22
B-ALL	11	1.49
T-ALL	21	2.84
AML	257	34.78

Among ALL – FAB subtypes, in ALL-L1, Common – ALL was the most common (2.03%), followed by T-ALL (1.35%), Null-ALL (0.41%), and Pre-B-ALL (0.27%). In ALL-L2 – Common –ALL was the most common (15.43%), followed by T-ALL (1.49%), and Null-ALL & Pre-B-ALL (0.95% each). In ALL-L3, only B-ALL (1.49%) seen (Table 2).

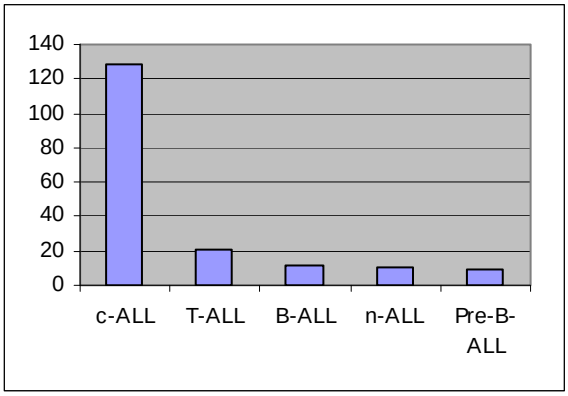

Figure 1: Immunophenotypic Classifications of Acute Leukaemias.

Blasts of AML were positive for CD13 and CD33, with special antibodies required for AML-M6 (glycophorin +ve) and AML-M7 (platelet antigens +ve). For blasts of ALL, precursor of B-ALL was positive for CD19 and cCD22, with Common– ALL which was CD10 +ve, null type which was CD10-ve, and pre B-ALL which was cIg+ve and was either CD10+ve or CD10-ve. B-ALL was SmIg+ve. T-ALL was CD7+ve and cCD3+ve.

Blasts of AML were TdT-ve, Precursor B-ALL were TdT+ve, whereas B-ALL was TdT-ve. T-ALL was TdT+ve, where as chronic (mature) T-cell proliferations were TdT-ve (Table 2).

Immunophenotypes of chronic leukaemias – In 146 cases (19.76%) of CLL, the most common immunologic marker was B-type – 142 cases (19.22%); 4 cases (0.54%) belonged to T-type. In LSCL, out of 3 cases (0.40%) studied, 2 cases (0.27%) were of B-type and one case (0.13%) was of T-type. Two cases (0,27%) of HCL studied belonged to immunologic marker B-type. B-type CLL, LSCL and HCL were positive for CD19, CD20, CD21, CD22 and CD24. T-type CLL and LSCL were positive for CD3 and CD4 (Table 3 and Figure 2).

DISCUSSIONS AND CONCLUSIONS

Immunological marker study (immuno-phenotyping) is used to distinguish AML from ALL and is particularly useful in sub-classifying ALL. It also carries prognostic significance.

Table 2: Immunophenotypes of FAB Subtypes of AML and ALL.

	AML 257 (34.78%)								ALL 180 (24.36%)		
	M0	M1	M2	M3	M4	M5	M6	M7	L1	L2	L3
	3 (0.41%)	15 (2.03%)	68 (9.20%)	64 (8.66%)	72 (9.74%)	20 (2.71%)	14 (1.89%)	1 (0.14%)	30 (4.06%)	139 (18.81%)	11 (1.49%)
Myeloid Markers											
(CD13+, CD33+, TdT-)	+	+	+	+	+	+	+	+	-	-	-
Glycophorin+	-	-	-	-	-	-	+	-	-	-	-
Platelet antigens+	-	-	-	-	-	-	-	+	-	-	-
Lympoid Markers											
Precursor B-ALL (TdT+, CD19+, cCD 22+)											
i) Common ALL (CD10+)	-	-	-	-	-	-	-	-	+ 15 (2.03%)	+ 144 (15.43%)	-
ii) Null-ALL (CD10-)	-	-	-	-	-	-	-	-	+ 3 (0.41%)	+ 7 (0.95%)	-
iii) Pre-B-ALL (CD10+ or -, cIg+)	-	-	-	-	-	-	-	-	+ 2 (0.27%)	+ 7 (0.95%)	-
B-ALL											
(TdT-, sIg+)	-	-	-	-	-	-	-	-	-	-	+ 11 (1.49%)
T-ALL											
(CD7+, cCD3+, TdT+)	-	-	-	-	-	-	-	-	+ 10 (1.35%)	+ 11 (1.49%)	-

+ = Positive; - = Negative; Parentheses = Showing number of cases and percentage; c = Cytoplasmic; s = Surface; Ig = Immunoglobulin; CD = Cluster Designation Antigens; TdT = Terminal deoxynucleotidyl Transferase.

Table 3: Immunophenotypes of Chronic Leukaemias.

			Markers			
			B (CD 19, CD 20, CD 21, CD 22, CD 24)		T (CD 3, CD 4)	
Types	Cases	%	Cases	%	Cases	%
CLL	146	19.76	142	19.22	4	0.54
LSCL	3	0.40	2	0.27	1	0.13
HCL	2	0.27	2	0.27	-	-
CML	151	20.43	-	-	-	-

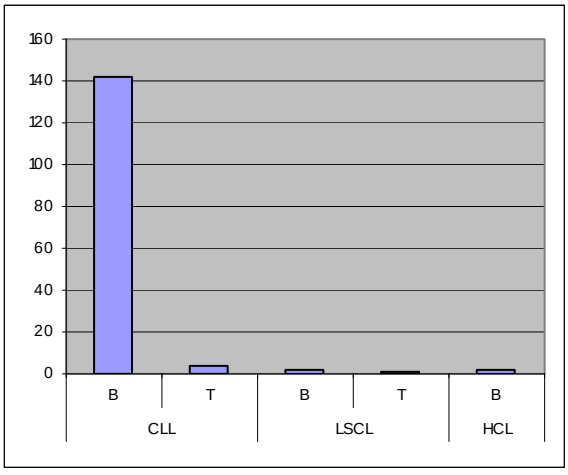

Figure 2: Order of Frequency of Immunophenotypes of Chronic Leukaemias of Lymphoid Origin.

In childhood ALL, patients with Common-ALL and pre-B-ALL do best; while those with Null-ALL (without Common ALL antigen {CALLA}) and T-cell ALL do less well; patients with rare B-cell ALL tend to do very poorly. In adult ALL, patients with Null or Common immunologic subtypes have a poor prognosis and patients with rare B-cell ALL do very poorly. These observation are similar to those reported by Besa et al, 1992; Hoffbrand & Pettit, 1993; Hoffbrand & Pettit, 2006; Haferlach et al, 1997.

In the present study, in the entire group of acute leukaemia, ALL was 24.36%. In this percentage, the most common immunophenotype was Common-ALL (17.46%), followed by T-ALL (2.84%), B-ALL (1.49%), Null-ALL (1.35%), and pre-B-ALL (1.22%). Among AML, all of the 257 cases (34.78%) were positive for myeloid markers. There was diagnostic role of glycophorin for M6 and platelet antigen for M7. These observations are similar to those reported by Roberts, et al (1992).

In the ALL group separately, the relative subtype frequency was as follows: the most common type was Common ALL (71.7%), followed by T-ALL (11.7%), B-ALL (6.1%), early pre-B(Null)ALL (5.5%), and pre-B-ALL (5.0%). The relative frequencies of various subsets of ALL in the present study were compared with other workers in Saudi Arabia as well as with the western population in Table 4.. It is obvious from the above table that in the present study Common ALL was the most common phenotype (71.7%) in Central Saudi Arabia. Roberts et al (1990) and Khalil et al (1994), both from Central Saudi Arabia, also reported Common ALL as the most common phenotype (57.0% & 86.5% respectively). In Eastern Saudi Arabia, Al-Sheikh et al (1999) also found Common ALL as the most common phenotype (65.2%). In the Western population, Common ALL was the most common type : 63.0% (Ludwig et al, 1994 in Germany); 52.5% (Rivera & Crist, 1995 in USA); 49.0% (Kaspers et al, 1996 in Holland).

In the present study, incidence of early pre-B (Null) type was low (5,5%) in comparison to 22.1% found by Roberts et al, loc cit (Central Saudi Arabia). However, it was close to other reports: 4.5% by Khalil et al, loc cit (Central Saudi Arabia) and 6.5% by Al-Sheikh et al, loc cit (Eastern Saudi Arabia). It was low in comparison to the reports from Western population: 14.0% (Rivera & Crist, loc cit {USA}); 14.0% (Kaspers et al, loc cit {Holland}). However, it was close to the report of 5.0% by Ludwig et al, loc cit (Germany). Incidence of pre-B-ALL (5.0%) in the present study, was close to 6.5% found by Al-Sheikh et al, loc cit (Eastern Saudi Arabia). These results were also low in comparison to the reports of Western population: 16.0% (Germany) Ludwig et al, loc cit; 22.5% (USA) Rivera & Crist, loc cit; and 21.0% (Holland) Kaspers et al, loc cit.

In the present study, incidence of B-ALL (6.1%) which carried poor prognosis was high in comparison to the Western population: 3.0% (Germany) Ludwig et al, loc cit: 0.5% (USA) Rivera & Crist, loc cit; and 2.0% (Holland) Kaspers et al, loc cit. In Saudi Arabia (Central), it was high in comparison to 3.0% found by Khalil et al, loc cit. However, it was low in comparison to 8.6% found by Roberts et al, loc cit (Central Saudi Arabia) and 9.7% found by Al-Sheikh et al, loc cit (Eastern Saudi Arabia).

Incidence of T-ALL (11.7%), in the present study, was low in comparison to the Western population: 13.0% (Germany) Ludwig et al, loc cit; 21.0% (USA) Rivera & Crist, loc cit; and 14.0% (Holland) Kaspers et al, loc cit. In Saudi Arabia, it was low in comparison to 12.3% (Central Saudi Arabia) Roberts et al, loc cit and 13.0% (Eastern Saudi Arabia) Al-Sheikh et al, loc cit. However, it was high in comparison to 6.0% (Central Saudi Arabia) Khalil et al, loc cit. The Arab population in Central & Eastern Saudi Arabia also differed from Arabs in Gaza, where T-ALL appeared to be more frequent (Greaves et al, 1993).

In the present study, regarding correlation between FAB classification and immuno-phenotypic pattern, in the group of ALL, out of 180 cases (24.36%), Common ALL was the most common in ALL-L2 type – 114 cases (15.43%), followed by T-ALL – 11 cases (1.49%), Null-ALL – 7 cases (0.95%), and pre-B-ALL – 7 cases (0.95%). B-ALL was only seen in ALL-L3 – 11 cases (1.49%). In ALL-L1, Common ALL was the most common – 15 cases (2.03%), followed by T-ALL – 10 cases (1.35%), Null-ALL – 3 cases (0.41%), and pre-B-ALL – 2 cases ((0.27%). No such type of correlation could be found in the study of other workers in Saudi Arabia.

Regarding immunophenotypes of chronic leukaemias, in the present study, in 146 cases (19.76%) of CLL, the most common immunologic marker was B-type – 142 cases (19.22%); while 4 cases (0.54%) belonged to T-type. In LSCL, out of 3 cases studied, 2 cases (0.27%) were of B-type and one case (0.13%) was of T-type. Two cases (0.27%) of HCL studied, belonged to immunologic marker B-type. This is similar to the observation by Henry, 1989; Besa et al, loc cit; and Mazza, 1995.

Table 4: Immunophenotypic Pattern of ALL in the present Study (Central Saudi Arabia) – A Comparison Between Different Studies.

Study	Immunophenotypes				
	Early pre-B(Null) ALL	Common ALL	Pre-B-ALL	B-ALL	T-ALL
Present Study Central Saudi Arabia	5.5%	71.7%	5.0%	6.1%	11.7%
Roberts et al (1990) Central Saudi Arabia	22.1%	57.0%*		8.6%	12.3%
Khalil et al (1994) Central Saudi Arabia	4.5%	86.5%*		3.0%	6.0%
Ludwig et al (1994) Germany	5.0%	63.0%	16.0%	3.0%	13.0%
Rivera & Crist (1995) USA	14.0%	52.5%	22.5%	0.5%	21.0%
Kaspers et al (1996) Holland	14.0%	49.0%	21.0%	2.0%	14.0%
Al-Sheikh et al (1999) Eastern Saudi Arabia	6.5%	65.2%	6.5%	9.7%	13.0%

** Common ALL and Pre-B ALL were reported together.*

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