Morphological Pattern of Childhood Lymphoblastic Leukaemia at Shaikh Zayed Hospital, Lahore (1994-1999)

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This study was conducted at Haematology Department of Shaikh Zayed Hospital, Lahore. During the period of six years (1994-1999), 76 cases of acute lymboblastic leukaemia (ALL) were diagnosed in children upto 15 years of age (age range 0.6-15 years). These cases included 53 males (69.7%) and 23 females (30.3%) with a male/female ratio of 2.3:1. Amongst the ALL cases L1 was the most frequent subtype accounting for 48 patients (63.2%), followed by L2, 26 patients (34.2%) and L3, 2 patients (2.6%). ALL cases showed a distinct peak in the children upto 5 years of age and a decline thereafter in older age groups. ALL-L1 mainly contributed for this early age peak. L1 showed a clear predominance in younger age groups whereas L2 cases showed a progressively increasing relative frequency in older age groups.

Key words: Acute lymphoblastic leukaemia l(ALL), French American British (FAB) Sodium Flouride (NaF)

Acute lymphoblastic leukaemia (ALL) is the most common form of leukaemia in children reported to account for approximately 80% of the total leukaemias[1,2]. Acute non-lymphoblastic leukaemia (ANLL) or acute myeloid leukaemia (AML) representing approximately 17% with chronic myeloid leukaemia (CML) contributing for the remainder[1].

In most countries the total leukaemia rate is highest under 5 years of age with a subsequent decline in incidence thereafter. ALL mainly accounts for this early age peak[3-6].

ALL is primarily a disease of young children; in contrast to AML which mainly occurs in adults and infants younger than 1 year[6]. ALL/AML ratio in children is usually reported around 4:1[1,2,5,7]. ALL affects males more often than females in all age groups[2,3].

ALL is a malignant disease of lymphopoietic system that is manifested by slow but uncontrolled growth of abnormal, poorly differentiated lymphoid cells. These abnormal lymphoid cells can be found in the bone marrow, spleen and lymph nodes. Normal bone marrow elements usually are replaced or displaced by abnormal cells[8].

The French American British (FAB) classification recognizes three morphological subtypes: L1, L2 and L3[9]. The L1 subtype is characterized by small, uniform lymphoblast population and is the most common subtype in children. The L2 subtype is characterized by large, pleomorphic lymphoblast population and is more common in adults. The L3 subtype represents a large, homogeneous lymphoblast cell population that resembles Burkitt lymphoma; this form typically occurs in children and young adults[9,10].

In the present study we conducted analysis of 76 consecutive cases of childhood ALL diagnosed by the Haematology Department of Shaikh Zayed Hospital, Lahore over a period of 6 years (1994-1999) to observe the FAB classification and its sex and age related pattern.

Patients and methods

This study was conducted at the Department of Haematology, Shaikh Zayed Hospital, Lahore. The study period extended from January 1, 1994 to December 31, 1999 (six years). Amongst 116 consecutive cases of childhood leukaemia, 76 cases of ALL were diagnosed during this period. Children of both sexes aged 15 years or younger were included in this study.

The diagnosis of ALL was made on examination of pre-treatment blood films and bone marrow aspirates performed in all cases. All the bone marrow and peripheral blood smears stained with routine giemsa stain were re-examined to confirm the original diagnosis. Special stains like Sudan Black B, myeloperoxidase, naphthol AS-D chloracetate esterase, alpha naphthyl acetate esterase, acid phosphatase and periodic acid schiff (PAS) had been used in various combinations to differentiate and diagnose the different types of acute leukaemia. All the smears stained with different cytochemical stains were re-evaluated.

Cytochemical stains specific for myeloid lineage like Sudan Black B, myeloperoxidase and naphthol AS-D chloracetate esterase were negative in all the ALL cases. PAS and acid phosphatase showed a variable pattern. Alpha naphthyl acetate esterase (NaF sensitive) was also negative in all these cases.

Characteristic cytomorphology of the blast cells and absence of positivity with cytochemical stains specific for myeloid and monocytic lineage confirmed their lymphoid origin. All the cases confirmed as ALL were subtyped according to the criteria laid down by French-American-British (FAB) cooperative group[9]. The morphological features used for subtyping ALL included; 1) nuclear cytoplasmic (N/C) ratio; 2) presence, prominence and frequency of nucleoli; 3) outline of nuclear membrane, and 4) cell size. The scoring system for L1 and L2 proposed by Bennett et al[11] was used to differentiate between the two subtypes. The system is out lined in Table 1. According to

which score ranged from the minimum score of -4 to maximum +2. Score 0 to +2 was assigned L1 subtype whereas -1 to -4 was labelled as L2 subtype.

Patients were subdivided into 4 age groups. First group included neonates and infants upto 1 year of age. Second group represented more than 1 year and upto 5 years (>1-5 years), third group more than 5 years and upto 10 years (>5-10 years) and the fourth group more than 10 years and upto 15 years of age (>10-15 years). Age in months was converted to decimal fraction of the year.

Cases which were unclassifiable, or suspected to represent mixed lineage origin on the morphological grounds were not included.

All the patients confirmed as ALL were further analysed to observe the pattern of age and sex and its relevance to FAB classification and relative incidence of its various subtypes.

Results

A total of 76 consecutive cases of childhood ALL were diagnosed over a period of 6 years (1994-1999). ALL constituted 65.5% of the total 116 cases of childhood leukaemia diagnosed during the same duration. Rest of the childhood leukaemia cases comprised of AML, 32 cases (27.6%), CML, 6 cases (5.2%) and acute bilineal leukaemia, 2 cases (1.7%) (Table 2). ALL / AML ratio was 2.37:1.

Fifty three (69.7%) of the ALL cases were males and 23 (30.3%) were females (M/F ratio 2.3:1) (Table 3). M/F ratio for L1 and L2 patients was 2.4:1 and 2.25:1, respectively (Table 4).

Median age at the time of diagnosis for ALL cases was 6 years (range 0.6-15 years). For ALL-L1 and L2 subtypes median age was 5 years (range 1.5-12 years) and 9.5 years (range 0.6-15 years), respectively (Table 4). Out of the two cases of ALL-L3 subtype one was a female child of 3 ½ year and the other a 4 ½ year old male (Table 4).

Amongst the ALL patients 48 presented with L1 subtype (63.2%), followed by 26 patients with L2 subtype (34.2%) and 2 patients with L3 subtype (2.6%) (Table 5). L1/L2 ratio was 1.8:1 (Table 6). L1/L2 ratio in different age groups excluding infants upto 1 year of age showed a progressively increasing relative frequency of L2 subtype in advancing age groups (Table 6).

ALL showed a peak with 33 cases (43.4%) in the age group of >1-5 years (Table 7; Fig. 1). This peak was mainly contributed by L1 subtype which also showed peak incidence with 26 cases (54.2%) in the same age group (Table 7; Fig. 2). Amongst ALL-L2 subtype maximum number of 11 cases (42.3%) were observed in age group of >5-10 years (Table 7; Fig. 2).

Table 1: Scoring system for L1 and L2.

Criteria*	Score~
High N/C ratio ≥ 75% of cells	+
Low N/C ratio ≥ 25% of cells	-
Nucleoli: 0 to 1 (small) ≥ 75% of cells	+
Nucleoli: 1 or more (prominent) ≥ 25% of cells	-
Irregular nuclear membrane ≥ 25% of cells	-
Large cells ≥ 50% of cells	-

*The following are not scored: (1) intermediate or indeterminate criteria; (2) regular nuclear membrane in ≥ 75% of cells, and (3) < 50% large cells, regardless of cell size heterogeneity.
~Positive (+) or negative (-)
Possible score: -4 to +2
Bennett et al. (1981)

Table 2: Childhood leukaemia.

Type	n	%age
Acute lymphoblastic leukaemia (ALL)	76	65.5
Acute myeloid leukaemia (AML)	32	27.6
Acute bilineal leukaemia	2	1.7
Chronic myeloid leukaemia (CML)	6	5.2
Total	116	100.00

Table 3: ALL: Sex distribution (n=76).

Sex	n	%age
Male	53	69.7
Female	23	30.3
Total	76	100.00
Male : Female ratio	2.3:1	

Table 4: ALL: presenting age and sex distribution in ALL and its subtypes

Subtype	No. of cases			M/F Ratio	Age (Years)		
	Male	Female	Total		Range	Median	Mean
L1	34	14	48	2.4:1	1.5-12	5.0	5.7
L2	18	8	26	2.25:1	0.6-15	9.5	8.9
L3	1	1	2	1:1	3.5-4.5	-	4
ALL (L1+L2+L3)	53	23	76	2.3:1	0.6-15	6.0	6.8

Table 5:ALL: FAB subtype distribution (n=76).

Type	No. of cases	Percent
L1	48	63.2
L2	26	34.2
L3	2	2.6
Total	76	100.00

Table 6: ALL: Comparative frequency of L1 and L2 in different age groups.

Age group (Years)	L1 (n=48)	L2 (n=26)	L1/L2 ratio
≤ 1	-	1	0:1
> 1-5	26	5	5.2:1
> 5-10	16	11	1.5:1
> 10-15	6	9	1:1.5
Total	48	26	1.8:1

Table 7: Age related distribution of ALL and its subtypes (n=76).

Age Group (Years)	Acute lymphpblastic leukaemia (ALL)						ALL (L1+L2+L3)	
	L1(n=48)		L2(n=26)		L3(n=2)			
	No.	%	No.	%	No.	%	No.	%
≤ 1	-	-	1	3.9	-	-	1	1.3
> 1-5	26	54.2	5	19.2	2	100	33	43.4
> 5-10	16	33.3	11	42.3	-	-	27	35.5
> 10-15	6	12.5	9	34.6	-	-	15	19.8
Total	48	100	26	100	2	100	76	100

Table 8:Incidence of ALL subtypes in different childhood series.

Authors (Years)	No. of cases	L1 (%)	L2 (%)	L3 (%)
Keleti et al. (1978)[18]	229	63	36	1
Coccia et al. (1979)[19]	324	78	22	-
Hann et al (1979)[20] Palmer et al. (1980)[22]	209	73	24	3
Viana et al (1981)[21]	223	71	25	4
Bennett et al. (1981)[11]	61	80	17	3
Zafar MN (1985)[23] (5% cases not classified)	80	55	40	-
Hassan et al. (1992)[15]	37	60	35	5
Lilleyman et al. (1992)[17]	2135	89	11	Not included
Iftikhar and Kazi (1993)[14]	209	79.9	19.6	0.5
Present study (2000)	76	63.2	34.2	2.6

Discussion

Acute lymphoblastic leukaemia (ALL) is primarily a disease of young children. In our study ALL constituted 65.5% of the total childhood leukaemia cases. ALL therefore remained the leading type of leukaemia occurring in childhood as has been observed in almost all the studies on childhood leukaemias worldwide[3,7,12]. The relative frequency of 65.5% for ALL observed in our study is significantly lower than the figure of 80% or higher reported for childhood ALL in the western population[1,2]. On contrary, AML showed a higher frequency among our children. This pattern is reflected by an overall ALL/AML ratio of 2.37:1 observed in this study in contrast to the ALL/AML ratio of 4:1 or higher, reported for children in United States[1,2,5] and Malaysia[7]. ALL/AML ratio reported in different Pakistani studies ranges from 2.14:1 to 4.7:1[13,16,25].

Males dominated females with an overall M/F rate ratio of 2.3. Male predominance was virtually observed in all age groups and both L1 and L2 subtypes. Male excess has been reported in most of the studies on childhood ALL at home and abroad[3,13,15,17]. There is however some variation observed in M/F rate ratio with most in 1.1 to 1.5 range. A typical male excess of 20-40% is usually reported in total leukaemia and ALL[2, 3]. Much larger male excess has therefore been noted in our study. In a large Pakistani study Iftikhar and Kazi[14] have reported an even higher male excess with M/F rate ratio of 4.1.

Amongst ALL cases L1 was the most frequent subtype (63.2%), followed by L2 (34.2%) and L3 (2.6%). While comparing the relative frequency of different subtypes of ALL in different childhood series worldwide it is evident that the frequency of L2 subtype was significantly higher than most of the western studies except Keleti et al.[11,17-22] (Table 8). In Pakistan Iftikhar and Kazi[14] from Lahore reported a much lower frequency of 19.6% for L2 in children. This is however to be noted that this study included children only upto 12 years of age. In other studies Hassan et al.[15] from Rawalpindi and Zafar[23] from Karachi have reported an even higher frequency of L2 in children under 15 years of age (Table 8).

In most populations the patterns of ALL are similar to those of total leukaemia with an early age peak and decline thereafter[3]. This early age peak in the incidence of childhood ALL, usually in children upto 5 years of age has been reported in several international studies[2,3,5,6,12]. A similar pattern of ALL with a peak in children upto 5 years was observed in the present study with a subsequent progressive decline in the older age groups (Fig. 1). This early age peak was mainly contributed by L1 subtype which also showed a similar decline afterwards with the advancing age.

A peak of ALL incidence before the age of 10 years and subsequent decline with increasing age until 30 years has been reported from China[24].

ALL-L1 and L2 subtypes showed a contrasting pattern of distribution in different age groups. Whereas L1 showed a peak incidence in age group of >1-5 years with subsequent decline in older age groups, L2 showed a progressively increasing relative frequency in advancing age groups (Table 6; Fig. 2). The L1/L2 ratio which was 5.2:1 in age group of >1-5 years was therefore reversed in favour of L2 (1:1.5) in age group of >10-15 years (Table 6). It was also noted that the median age of L1 and L2 subtypes was 5 years and 9.5 years respectively, at the time of diagnosis. The distribution by age in the two main morphological subtypes, L1 and L2 showed a clear predominance of L1 in the younger age groups and the frequency of L2 cases increased gradually in older children

as they approached adolescence. A similar pattern of L1 and L2 frequency has been reported by Bennett et al.[11].

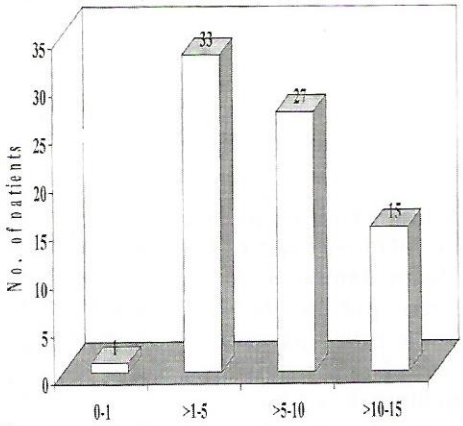

Fig. 1. ALL: Distribution in different age groups (n=76)

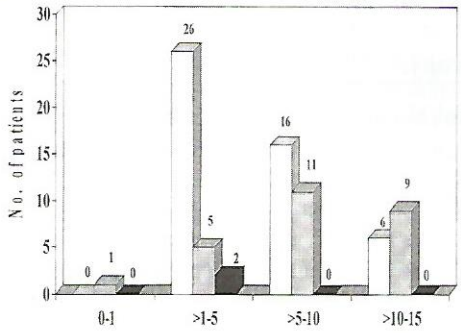

Fig.2. ALL: Distribution of FAB subtypes in different age groups.

Conclusions

- Acute lymphoblastic leukaemia (ALL) was found to be the most common form of leukaemia diagnosed in children upto 15 years of age. The relative frequency of ALL was however noted to be significantly lower when compared with the developed nations.
- Much larger male excess than the typical 20-40% increase in ALL was observed in our children.
- Although L1 was the most common subtype of ALL, L2 showed a significantly higher frequency rate than what has been reported in most of the western studies. This partly accounts for the relatively poor prognosis of childhood ALL in our population.
- The distribution by age in the two main subtypes L1 and L2 showed a clear predominance of L1 in the younger age groups and the frequency of L2 cases increased gradually with age.
- As it has been observed in the developed countries, a distinct peak in the childhood ALL cases was also documented in this study. ALL-L1 mainly contributed for this early age peak.

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