Childhood Acute Lymphoblastic Leukaemia: Arabian Perspective

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The immunophenotypic pattern of acute lymphoblastic leukemia (ALL) varies with race and geographical location. The pattern in Eastern Saudi Arabia was not reported before.

Objective: This study was performed to compare the pattern of childhood ALL immunophenotype in Eastern Saudi Arabia with those previously reported in other parts of the world.

Design, settings, & subjects: Analysis of 32 consecutive cases of childhood ALL seen in Eastern Saudi Arabia (1991-1996) was performed. Immunophenotype was analyzed by flowcytometry of blood and bone marrow samples.

Results: In the 32 analysed cases, common ALL was the commonest phenotype (68.7%). Pre-B and B-ALL accounted for 9.3% each. Early pre-B ALL and T-ALL were the least common each accounting for 6.2%. Mixed lineage leukemia occurred in 3% of cases.

Interpretation: These findings suggest lower prevalence of T-ALL and early Pre-B ALL in Arabs. The most prevalent phenotype is common ALL as in the rest of the world.

Conclusion: In this study we found that the immunophenotypic pattern of childhood ALL is different in Arabs which may influence the outcome of the disease in this population.

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Geographical and racial variation in the prevalence and immunophenotypic pattern of ALL is a subject of much interest. Experience in USA with multiracial population indicates that acute leukaemia in childhood is more common in white caucasians than in children of African ancestry; this racial difference in the annual incidence (42 per million/year vs 25 per million/year) is also reflected in male:female ratio; this being 1.4:1 in whites and 1:1 in African Americans. It was also noticed that ALL of T-cell origin appears to be more common in children of African ancestry in the USA, whereas CDIO+ ALL of B-cell lineage is less frequent as compared with white caucasians. Similarly it has been reported that precursor-B cell ALL is less frequent, whereas, mature B-ALL and T-ALL are more common in Palestinian Arabs[1]. Immunophenotypic studies in childhood regarded ALL as universal tool for predicting prognosis, treatment outcome and relapse. Furthermore certain immunotype show close association with overt or cryptic cytogenetic abnormalities and thus constitute stronger predictor of prognosis and drug resistance. Thus t(1;19) carries bad prognosis in pre-B-ALL, but not in early pre-B-ALL. Similarly early pre-B-ALL and T-ALL are resistant to L-asparaginase whereas common ALL and pre-B-ALL are responsive to this agent[2,3].

The population of the Eastern Province of Saudi Arabia is rather homogenous and in-bred and has the highest gene frequency in the country for sickle cell anemia. Although precise incidence of childhood acute leukaemia in Eastern Saudi Arabia is not known, in an epidemiological study based on 95 new cases (all ages) seen during 1987-1988, a crude incidence rate of 5.2 and 3.6 per 100,000 per year has been reported in Saudi males and females respectively. Acute leukaemia represented two-third of these cases of which 58% were of lymphoid origin[4]. Immunophenotypic studies of such cases in this region has not been documented so far, but in a previous report diagnosed on the basis of morphology and cytochemistry, LI accounted for two third of ALL cases in childhood[5]. The present report is part of a continuing study of leukaemia in the Eastern Province of Saudi Arabia and here we attempt to present for the first time the immunophenotypic pattern of childhood ALL in this rather distinct population.

METHODS

Thirty two children (age <15 years) with ALL were investigated for their immunophenotypic pattern from August 1991 to April 1996. Blood and bone marrow samples were received in EDTA.

Reagents: Monoclonal antibodies required for immuno-phenotyping of acute leukaemia were purchased from Becton-Dickinson Immuno-cytometry system (San Jose, CA, USA). Single and two colour flow cytometric analysis was carried out using FITC-labelled antibodies against CD3, CD7,

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CD15, CD19, CD20, CD25, CD34 and kappa light chain and PE-labelled antibodies against CD4, CD5, CD8, CD13, CD14, CD22, CD23, CD33, CD16/CD56 and lambda light chains. Where possible Tdt, slg and cytoplasmic μ chain were demonstrated by fluorescence microscopy using FITC-labelled antibodies. Whole blood staining and sample preparation was carried out using manufacturer's protocol. For manual staining (Tdt, cytoplasmic μ)and for the demonstration of surface immunoglobulin, cells were separated on Ficoll-Hypaque density gradient.

Flow Cytometry: Stained leukaemia cells were analyzed with Becton-Dickinson flow cytometer (FACScan) equipped with argon-laser emitting 488 nm coherent light at 15 μ power. Negative control (ie. Isotype-control) consisted of FITC- and PE-labelled antibody to key hole limpet haemocyanin. Automatic (or manual) gating was performed to include more than 90% of blast cell population. This fraction (gate) was defined by analysis of forward and side scatter and the pattern of fluorescence using monoclonal antibody to CD45 and CD14 (leucogate) to exclude non-leucocytes and monocytes. Each measurement contained at least 3-5 thousand events. Single and dual parameters scattergrams were examined using on-board software. Statistical analysis was carried out using "Simulset" and where possible "lysis" software package provided by the manufacturer. Results in excess of 20% positivity of blast cells for a given antigenic marker by flow cytometry and 10% when assessed by immunocytochemistry were regarded as positive. Since peripheral blood leukocytes do not normally show CDIO or CD34 positivity, the arbitary cut off point used for these markers was 10%; even normal bone marrow cells do ordinarily show positivity in excess of 10% for these two markers.

Morphological and Cytochemical Characteristics: The blast cells of acute myeloid leukaemia and of ALL were classified according to FAB criteria. Cytochemistry included staining for myeloperoxidase, esterases, lipids (Sudan black), glycogen (PAS) and acid phosphatase.

Immunophenotypic Sub-grouping of ALL: ALL was divided into B-lineage and T-lineage leukaemias according to present thinking regarding cell-surface and cytoplasmic antigen expression[2,6-9]; although some of the currently used terminologies are at times confusing[10].

B-lineage leukaemia was categorized as: (a) Early Pre-B-ALL (also described by some as pre-pre-B-ALL, Null cell ALL, Pro-B ALL) based on CD34, DR and CD19 positivity and CDIO negativity, (b) Common-ALL based on CD10 positivity in addition to the presence of other markers eg. CD19, DR and varying percentage of CD34, (c) Pre-B-ALL if cytoplasmic m chain was demonstrable and (d) B-ALL, if surface immunoglobulin (slg) was detectable. Transitional B-ALL[2,8] characterized by presence of m chain only on the cell surface was not studied and is therefore included in category (c). T-lineage ALL consisted of (a) Early/intermediate T-ALL (CD7+, CD3-, DR- (in children), CD10±, CD34±) and late (mature) T-ALL (CD3+, CD7+, CD34-, CDIO-). CDI expression was not studied to separate early from intermediate T-ALL. Biphenotypic Acute Leukaemia was recognized on the basis of co-expression of CD33 (or CD13/CD15/CD16) and CD19 as detected either by two-colour fluorescence or when single colour flourescence was used, the sum total of these two markers were > 120%.

RESULT

Of the 32 children with ALL, 30 (93.8%) were B-lineage ALL and 2 (6.2%) were T-lineage ALL. Their ages varied from < 1 year to 15 years with overall M:F ratio of 1.8:1. All cases of early pre-B-ALL (two patients), B-ALL (3 patients) and T-lineage ALL (2 patients) were males. Only 2 cases of childhood T-lineage ALL were seen; their ages were 9 months and 10 years respectively. Both had early T-ALL.

The most frequent sub-group was CD10+ common-ALL (68.7%), this was followed by pre-B (9.3%,) and B-ALL (9.3%). Early pre-B (null cell) ALL represented 6.2% of cases (Table 1).

Table 1. Childhood acute lymphoblastic leukaemia in Eastern Saudi Arabia

Immunophenotypic Classification	No.	%	Age Range Yrs	Median	M:F ratio
Early pre-B ALL	2	6.2	5-13	9	2:0
Common All	22	68.7	1-15	4	1.4:1
Pre-B ALL	3	9.3	11/2-5	2	1:2
B-ALL	3	9.3	1-15	3	3:0
T-ALL	2	6.2	<1-10	5	4:0
TOTAL	32	100	<1-15	5	1.8:1

In 3% cases of childhood ALL co-expression of myeloid marker eg. CD33, CD15 or CD16 was observed (ie. biphenotypic ALL). Most patients with ALL had L_1 morphology (72.5%) followed by L_2 (24%) and L_3 (3.5%).

DISCUSSION

Identification of leukaemia on the basis of morphology and cytochemistry complimented by immunophenotyping, cytogenetic and molecular biological techniques has not only provided a rational basis for its classification, but has also helped to develop prognostic subgroups with varying drug sensitivities. Furthermore, it has allowed comparative studies in various races and geographical boundaries providing insight into the epidemiology of leukaemias. Interest in immunotyping of acute lymphoblastic leukaemia was generated in 1973 by the identification of T-cell ALL by sheep red cell resetting technique[11], this was soon followed by the description of B-lineage ALL[12] in particular the common ALL[13], the cytoplasmic μ chain positive pre-B ALL and the early pre-B ALL[14], which was at first designated null cell ALL[15]. Transition B-ALL with surface expression of only m chain is the latest addition to this list. A number of clinical and biological features of leukaemia influence the chances of cure, the chief being the age, leucocyte count and haemoglobin level at diagnosis and the sex of the patient. This has led to the concept of "risk group", giving intensive treatment only to those who are likely to encounter treatment failure and at the same time avoiding highly toxic drugs in those who are likely to be cured[2,10]. Inspite of reservations

expressed by some authorities that the immunophenotyping may not be of independent significance in prognosis after allowing for age, sex, and leukocyte count[16], there are others who believe that leukaemia phenotyping has prognostic connotation, either directly as a surrogate indicator of other prognostic markers such as p-glycoprotein expression, drug resistance and pea-agglutinin binding characteristics of leukaemic cells[3,17,18]. Trials based on immunophenotype-specific therapy will settle this question[8]; there is already indication that slg positive B-ALL previously regarded incurable responds favourably if given short term intensive therapy commonly used for non-Hodgkin's lymphoma[16].

Racial and geographic variations in the immunophenotypic pattern of ALL are well established[1,2,19]. In our population 6.2% cases of childhood ALL were of early pre-B type; this frequency is much lower than reported frequency of 14% in the USA[3]. Similarly, 6.2% frequency of T-lineage ALL in our series is much lower than the reported frequency of 14% and 24% respectively in European and American studies[20]. Surprisingly Arabs in Gaza have higher prevalence of T-ALL than pre B-ALL[1]. In the present study, we have separated pre-B-ALL from common ALL on the basis of cytoplasmic μ chain; about 10% of cases belonged to this group[21]. This sub-group has not been previously identified in cases reported from this country. Expression of CD34 and CD10 on leukaemic lymphoblasts (which form important basis for classification) is closely linked to prognosis. Thus expression of CD34 on < 5% blast cells (P<0.02) or CD10 on <20% blast cells (P<0.001) was associated with poor prognosis in an univariate cox regression analysis of risk factors[22], high expression of both CD34 and CD10 on the other hand was of good prognostic significance[23]. In the USA, children of African ancestry[1] have low incidence of CD10+ ALL and high incidence of T-ALL no such pattern was seen in Saudi children. It is noteworthy that Arabs from Gaza as opposed to our Arab population also show high incidence of T-ALL[1], incidently occurrence of B-ALL is also higher in this group, and at least in the latter respect Saudis resemble Arabs from Gaza. It has been reported that 5-10% cases of childhood ALL (as compared with 10-20% of adult ALL) show lineage infidelity and express myeloid markers[24], in the present study 3% patients showed co-expression of CD19 and CD33. Although no correlation was found between FAB classification and immunophenotypic pattern LI morphology in 72% of our cases and L3 in 3.5% cases is in keeping with the data from the West[6].

The present report is part of a continuing collaborative study and an update will follow in due course. We are also studying the prognostic significance of the shedding of CD9 by leukaemic blast cells. Study of transitional B-ALL in Saudi population has been added to our protocol. We also plan to study the reported good prognostic significance of the lack of CD45 expression in childhood ALL and the association of myeloid marker in T-cell ALL with lower remission induction.

CONCLUSION

This study documents for the first time the immunophenotypic patterns of childhood ALL in Arabs from Eastern Saudi Arabia and provides evidence that the prevalence of some of the subtypes is lower than which has been published in the literature from the western world. These findings not only highlight the significance of race and geography in disease pattern and prevalence, but also provide rationale for possible immunophenotype - specific therapy of our leukemia cases.

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