

Evaluation Study of Different Techniques in Diagnosis of Acute Leukemia in Adults and Children Patients

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Abstract

Cytomorphology and cytochemistry are combined with immunophenotype by flow cytometry (FCM) to diagnosis and management the acute leukemia. The present study was designed to compare between the cytomorphology, cytochemistry and immunophenotype by flow cytometry at diagnosis and classification of acute leukemia to suggest a number of antibodies that can be adopted in the diagnosis by flow cytometry. The study included (160) cases were newly diagnosed (100 adults, 60 children). Morphological analysis was depended on peripheral blood smears examination by Leishman stain, cytochemical analysis with PAS and SBB stains and immunophenotype analysis used multiparameter. the results of this study shown that 60% of adult cases were AML, and 40% ALL. The mean age of adults was 48.45 years for AML cases and was 32.59 years for ALL adult cases. In children AML the mean age was 5.23 years and was 6.44 years in children ALL cases. **Our Conclusion** Also that the B.ALL was more commonly than T.ALL in both adults and children cases. Immunophenotypic detection by flow cytometry recorded (100%) successfully diagnosis in ALL cases, but it was successfully in (75%) of detected the subtypes of AML cases.

Keyword: Acute leukemia, AML, ALL, FCM, FAB classification; cytochemistry

Introduction

Acute leukemia is a complex disease, there are two main types of leukemia, myelogenous (AML) and lymphocytic (ALL), depended on the types of cell that are involved. On the other hand each type is subdivided into acute and chronic classes⁽¹⁾. That can affect each of adults and children at different ages⁽²⁾.

The diagnosis and sub-type classification of leukemia requires the simultaneous application of multiple techniques such as : Morphological assessment, Determination of cytochemical and immunophenotypic features and Evaluation of genetic abnormalities⁽³⁾.

The French-American-British (FAB) divided acute leukemia into various subtypes: M0 to M7 for myeloid

leukemia, and subdivided to three subtypes, L1, L2 and L3⁽⁴⁾.

Immunophenotyping by flow cytometry offers a rapid, precise and effective means for the diagnosis of acute leukemia. It provides valuable information of the leukaemic blasts by analysis of surface markers⁽⁵⁾.

Patients and Methods

This study included 160 cases were newly diagnosed with Acute Leukemia, 100 of them was adults ≥ 15 years (60 cases AML, and 40 ALL). While the other 60 cases were children ≤ 14 years (13 cases AML, and 47 ALL).

All cases were newly diagnosed. The samples collected from Hematology Unit of Baghdad Teaching Hospital in Medical City and Center Teaching Hospital of Pediatric. The collection period is from April to November 2019.

The cases were diagnosed according to FAB classification, based on 20% blast cells in peripheral

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blood (PB) film or bone marrow aspirate (BMA) as sign for diagnosis by cytomorphology by examination with Leishman stain, cytochemical with special stains Sudan Black B (SBB) and Periodic Acid-Schiff (PAS) and flow cytometry analysis was done by (BD FACSCanto II system 8 color). Using a panel of monoclonal antibodies

to classify acute leukemia which included CD13,CD33, CD117, CD34 and HLADR for AML, and each of CD3, CD5, CD7, CD10, CD19 and CD79a for T and B ALL respectively. Data were entered into Excel sheet and then transferred to SPSS and $P < 0.05$ was regarded as statistically significant.

Results

The age and gender of cases

In present study, in adults AML the highest case's number was with > 55 years with mean age 48.45 years. Also in adults ALL, the highest percentage was between 15-35 years with mean age 32.59 years. In children (age ≤ 14 years) the AML were 13 (21.67%) and mean age was 5.23 years and peak incident of AML children cases was < 5 years, whereas the ALL children were 47(78.33%) cases and mean age 6.44 years . the highest frequencies of children ALL cases were between (5-8 years).

The adult AML cases recorded 1.06:1 male to female ratio. Whereas the adult ALL cases the male to female ratio was 1.22:1. Children cases included male to female ratio 1.6:1 . while in children ALL cases the male to female ratio was 1.13:1 (figure 1) .

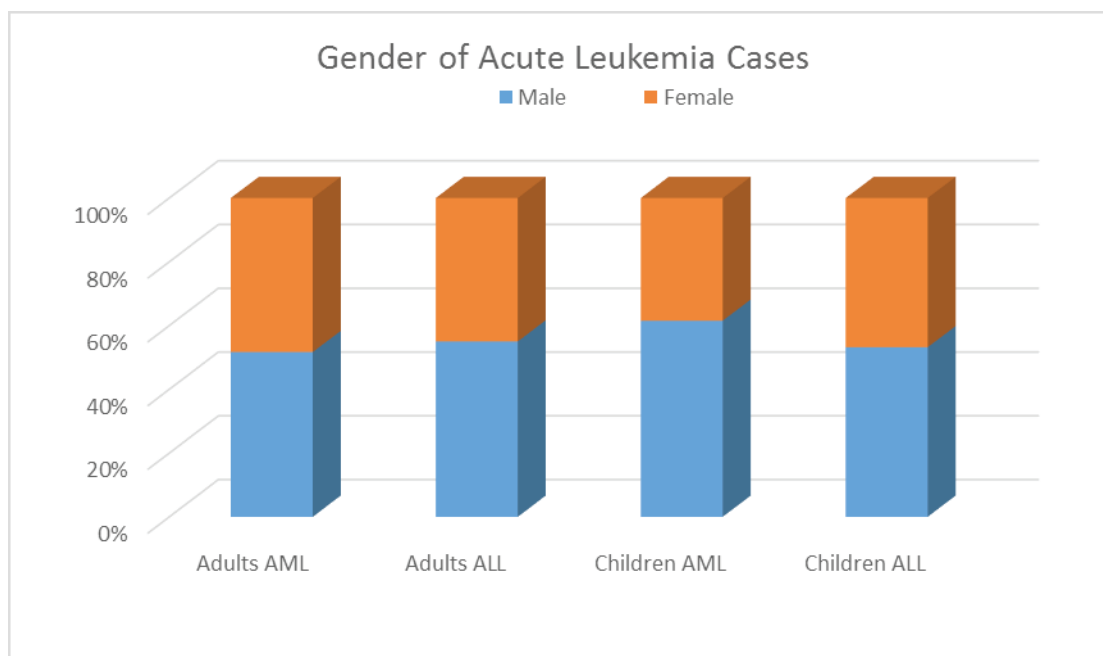


Figure 1: percentage of male and female of Acute Leukemia cases

Cytomorphological Diagnosis

In this study used peripheral blood smears examination the results were that adults AML 38(63%) cases were diagnosed with their subtypes according to FAB subclassification. While 18(30%) of cases diagnosed as just acute leukemia without subtype and recommended to immunophenotype and /or

cytochemistry analysis. Adults ALL 13(33%) cases were diagnosed without determination subtype, 10(25%) diagnosed as AML. And 17(42%) were diagnosis with their subtypes.

In children AML 6(46%) cases were diagnosed by cytomorphology with their subtypes, while 4(31%) cases diagnosis as acute leukemia without detect it's subtype.

In children ALL 11(23%) cases of them diagnosis as just acute leukemia, and 5(11%) given different diagnosis, 31(66%) cases which given diagnosis with subtype.

Cytochemical Diagnosis

The cytochemical detection was included SBB and PAS stains. SBB was positive in most cases of AML, While PAS stain in adults ALL cases was positively in 24(60%), **table 1**.

Table 1 the results of cytochemical diagnosis in acute leukemia cases:

Cytochemistry	AML		ALL	
	Adults	Children	Adults	Children
SBB	48(80%)	10(77%)	3(7.5%)	0%
PAS	3(5%)	1(7.7%)	24(60%)	40(85%)

Immunophenotype Diagnosis

this study included 73 cases newly AML diagnosed (60 adult , 13 children). Also 87 cases diagnosis as ALL (40 adults , 47 children).

Adults cases (83.3%), (85%) were positive to CD13 and CD117 respectively in same time all children cases were positive to both CD13 and CD117. CD33 was positive in (81%) in adults and was (84.6%) in children **table 2**. The subtypes of AML cases summarized in **table 3**.

In T.ALL all cases in both adults and children were positivity to CD5, follow CD7 which given percentage (92.8%) in adults and (88%) in children cases.

In adults B.ALL all cases given positive reaction for CD79a follow CD19 and HLADR. As well as the children cases recorded nearly the results that all children was positivity expression with CD79a and CD19 follow with CD10 and HLADR, **table 4**.

Table 2: The results of immunophenotype diagnosis with flowcytometry (FCM) of AML cases:

FCM markers	Adults	Children
CD13	50(83.3%)	100%
CD33	49(81.7%)	11(84.6%)
CD117	51(85%)	100%
CD34	46(76.7%)	10(77%)
HLADR	48(80%)	4(30%)

Table 3: the subtypes of AML cases.

Subtypes	Adults AML		Children AML	
	No.	%	No.	%
M0	10	16.67%	2	15.38%
M2	2	3.33%	0	0%
M3	7	11.67%	3	23.08%
M4	13	21.67%	4	30.77%
M5	9	15%	3	23.08%
M7	2	3.33%	0	0%
*Non M3	17	28.33%	1	7.69%
Total	60	100%	13	100%

*NonM3 mean that this cases may been all of the subtypes except M3 (ambiguous).

Table 4: The results of immunophenotype diagnosis with flowcytometry (FCM) of AML cases:

F.C.markers	Adults	Children
T.ALL		
CD3	11(78.5%)	12(70.5%)
CD5	14(100%)	17(100%)
CD7	13(92.8%)	15(88%)
CD34	8(57 %)	15(88%)
HLADR	8(57 %)	7(41%)
B.ALL		
CD10	18(72%)	25(86%)
CD19	19(76%)	29(100%)
CD79a	25(100%)	29(100%)
CD34	14(56%)	16(55%)
HLADR	19(76%)	25(86%)

Comparative assessment of acute leukemia diagnosis

In the table 6 which summarize the morphology, which is the cheapest method could used to a diagnosis within minutes to hours, while the immunophenotype give a result of diagnosis at one day and clinically helpful to characterize subtype of acute leukemia and it costly.

Table 5: comparative various diagnostic procedures :

	cytomorphology	cytochemistry	immunophenotype
AML			
Subtype detection required time The cost Type of sample Possibility of follow-up	60% Less than hour + P.B and/or B.M Weekly	79% Hours ++ B.M Not possible	75% one day +++++ P.B and/or B.M Not possible because highly cost
ALL			
Subtype detection required time The cost Type of sample Possibility of follow-up	55% Less than hour + P.B and/or B.M Weekly	73.5% Hours ++ B.M Not possible	100% one day +++++ P.B and/or B.M Not possible because highly cost

+ Cheapest; ++ cheap; +++ costly; ++++ most costly.

Number of cases successfully done by that method.

Discussion

The AML cases were more in adult cases that 60% of adult cases were diagnosed as AML, that demonstrated similar results to other studies (6-8). The acute myeloid leukemia is the commonest of acute leukemia in adults (7), and it is a third most common major cancer in Iraq (8).

The present results in children ALL cases were agreement with (9).

That the ALL is the mostly common of the cancer between the children and, the greatest common causes of death by cancer before twenty years of age (10).

One of the important prognostic factor in Acute Leukemia is the age (11). In present study shows that the mean age of adults AML cases were 48.45 years. This finding was comparable with other studies (12,13). But disagreed with (14,15). The peak incidence of AML adult cases in currently study was in age >55 years. But this study was higher than (16).

While agreement with (17). The mean age of ALL adults cases was 32.59 years. Which different with

(18,19). The mean age of children ALL cases in present study was agreement with (20). And the peak incidence were at the same line with (21).

The male to female ration results of adults AML cases in current study was different from (12), while in adults ALL cases were agreed with (22).

In children AML cases male to female ratio was 1.6:1. Which different from (23), while children ALL cases given nearly agreement with (21).

The cytochemistry found that the 80% of bone marrow aspirate (BMA) samples of adults AML and 77% of children AML were positive to SBB stain. On the other hand (BMA) samples in 60% adults ALL, 85% children ALL were positivity reaction to PAS stain. This positivity of cytochemical tests may be explained by the presence of specific enzymes or other substances in individual hematopoietic cells leading to development of color reaction. Morphologically, the presence or absence of myeloblasts has a central role in diagnosis of AML and suspicion of ALL respectively, as in FAB classification ALL is largely a diagnosis of exclusion.

In adults AML the markers CD 117 mostly positivity, followed CD13 and CD33. In children cases both CD117 and CD13 were positive in all cases (100%) followed by CD33. From this result that use of antibodies against for these antigens is possibly sufficient for an initial diagnosis of acute myeloid leukemia. Also the present results agreement with ^(24,25). Acute myeloid leukemia was defined immunologically by the expression of 2 or more of myeloid markers: CD13, CD33, and CD117 ⁽²⁵⁾.

The subtype M0 showed higher expression for CD34, CD117 and HLADR, while M4, M7 related with CD33 and CD117 that were much more helpful in distinguishing their subtypes in AML cases of adults. In this study results each of subtype M0 and M2 related with strong positivity more than other subtypes for CD13 and CD33 which may be diagnostic characteristic to this subtype of acute myeloid leukemia.

The current results found that T.ALL cases recorded percentage 100% positivity reaction to CD5 in both adults and children followed by CD7 in adults, while in children follow CD7 and CD34 there given same percentage value (88%).

The positivity CD79a in 100% of B.ALL adult cases. Whereas in children both CD79a and CD19 were 100%, agreement with ⁽²⁶⁾. From this result that use of antibodies against for these antigens is possibly sufficient for an initial diagnosis of acute lymphoid leukemia.

The B-ALL cases were mor than T-ALL for both adults and children cases, in results this study which similar to ⁽²⁷⁾.

Immunophenotypic detection by flow cytometry recorded (100%) successfully diagnosis in ALL cases, but it was successfully in (75%) of AML cases, the which recorded as AML (Non-M3). While other study in ⁽²⁸⁾ found that flow cytometry determined subtype in 94% of AML cases. That the large heterogeneity of myeloid-related lineages, no clinically relevant immunological classification of AML has been devised so far ⁽⁶⁾.

Depending on the current results, we conclude that the techniques used in diagnosis of acute leukemia in this study considered one complementary to other with ability using flow cytometric antibodies CD13 and CD117 as a cost effective diagnostic marker in acute

myeloid leukemia cases to support morphological findings. Also CD5 and CD7 were the most frequently expressed in T-ALL while CD79a and CD19 were most commonly associated with diagnosis of B-ALL. CD33 positivity reaction was strongly related with expression in AML-M2, M3, M4 and M7 subtype with adult cases, while it expression related with AML-M3 and M4 subtype in children AML cases.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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