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Evaluation of related flow cytometry parameters with FAB subtype of acute myeloid leukemia associated with histological assessment

Rasha Hamid Ayub[1*], Ayad Hameed Ibraheem[2]

1- Department of Biology, College of Education, University of Samarra (rashahamid@uosamarra.edu.iq)

2- College of Veterinary Medicine, University of Tikrit

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Abstract

The acute leukemia is a group of malignant disorders of the haemopoietic cells, characteristically related with increases of number of leucocytes in the blood. The present study is aimed to evaluation the expression of immunophenic antibodies which used on blast cells to diagnosis and classification acute leukemia by flow cytometry to define their relationship with age, gender and French-American–British (FAB) subtypes, and assessment complete blood count (CBC) including white blood cells WBCs, hemoglobin HGB, and platelets PLTs, addition to define histological changes of bone marrow biopsy (BMB) of acute leukemia patients. This study included 73 patients were newly diagnosed with acute myeloid leukemia (AML), 60 of them was adults (range 15 –77 years), and the other 13 cases were children (range 1–14 years). The patients divided into three groups: 15-35, 36-55, and 56-77 years, while children patients divided into: 0-4, 5-8 and 9-14 years. The results shown that fever and paleness were the most common clinical feature among acute myeloid leukemia patients (adults and children).

Introduction

Leukemia is malignancy disorder of the hematopoietic stem cells that can affect adults and children, [1,2]. That characterized by an abnormal proliferation of immature neoplastic leukocytes blasts in the bone marrow and peripheral blood [1]. Leukemia was first noted by German pathologist Rudolf Virchow and John Bennett in 1845 [3]. Leukemia is grouped by the type of cell that predominates in the peripheral blood and the bone marrow is defined according to cell lineage as (either myeloid or lymphoid) and grouped by the rate of cell growth (either acute or chronic). When blasts or other immature cells predominate, the leukemia is classified as acute, the predominance of more mature cell types being associated with chronic leukemia [4, 5].

In 1976 the French American British (FAB) group developed a system of nomenclature that separated the acute myeloid from acute lymphoid leukemias. FAB divided acute myeloid leukemia into various subtypes: M0 to M7 as shows in table 1 [5], based on using

morphological cell type from which the leukemia developed, and the level of maturity of the cells. And then using routine staining to appearance of leukemia cells under the microscope [5].

Table (1): The French American Britain (FAB) classification of AML [4, 5].

FAB subtype	Name
M0	Undifferentiated acute myeloblastic
M1	Acute myeloblastic leukemia with minimal maturation
M2	Acute myeloblastic leukemia with maturation
M3	Acute promyelocytic leukemia
M4	Acute myelomonocytic leukemia
M5	Acute monocytic leukemia
M6	Acute erythroid leukemia
M7	Acute megakaryoblast leukemia

Study Design and Sample Collection

A total of 73 subjects, 60 adults cases(31 male, 29 female) and 13 children cases(8 male, 5 female) with AML attending Heamatology Unit of Baghdad Teaching Hospital in Medical City and Center Teaching Hospital of Pediatric for diagnosis and treatment during the period from April to November 2019, along with control (healthy) were included in this study.

The cases were diagnosed according to FAB classification, based on 20% blast cells in peripheral 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 myeloid leukemia which included CD13,CD33, CD117, CD34 and HLADR. Bone marrow biopsies paraffin embedded samples were collected from the patients archives under pledges and administrative procedures.

The specimen was fixed in 10% formalin, for 24 hour prior to decalcifying, dehydrating and embedded in paraffin wax by the usual histological procedures. The blocks were serially sectioned at 5 μm thickness by a manual microtome (AS Anglia scientific, Germany) so as to make histological slides ready for staining. The sections stained with hematoxylin and eosin (H&E). Data were entered into Excel sheet and then transferred to SPSS and $P < 0.05$ was regarded as statistically significant.

Results

In this study adult cases (age rang 15-77 years) were 60 cases (31 male, 29 female) with a mean age of (48.45 years). The highest patient's number was with > 55 years while, in the children (age rang 1–14 years) AML cases were 13 (8 male, 5 female) the mean age was (5.23) years. The peak incident children patients was <5 years. The male to female ratio was 1.06:1 in adult cases whereas male to female ratio in children cases 1.6:1.

Subtype Non-M3 in adults and M4 in children were the most commonly FAB subtype for AML cases. Table 2 summarized FAB subtype of studies cases.

Table 2: Distribution subtype of AML cases according to the FAB classification

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)

Clinical features

Fever and paleness were the most common clinical feature among acute myeloid leukemia of both adults and children. Bleeding or purple spot on the skin caused by internal bleeding was found in 9 cases of adult AML patients (five cases were females while two cases of them were males), as well as in children cases that 23% of them were complaining bone pain. Hepatosplenomegaly was mostly occurred in children cases were recorded (23%) while in adults recorded (11%) . Organ enlargement like liver and spleen was one of distinguish characterized acute leukemia patients Table 3.

Table 3: clinical features and organ enlargement in AML cases.

Clinical features	AML	
	Adults %	**Children%**
Fever	41(68.3%)	9(69.2%)
Bleeding	9(15%)	0(0%)
Paleness	27(45%)	7(53.8%)
Hepatomegaly	3(5%)	2(15.3%)
Splenomegaly	4(6.6%)	0(0%)
Hepatosplenomegaly	11(18%)	3(23%)
Lymphadenopathy	10(16.5%)	2(15.3%)
Bone Pain	6(10%)	3(23%)

Hematological laboratory parameters

Abnormalities of complete blood count (CBC) analysis including leucopenia or leukocytosis, and/or neutropenia, anemia, thrombocytopenia and/or presence of immature (blast) cells in peripheral blood film of patients. this abnormalities of CBC and presence of blast cells in peripheral blood film in patients are summarized in table 4.

Table 4: abnormalities CBC and blast percentage of acute leukemia patients:

Clinical features		AML	
		Adults	Children
WBCs	< 4	9(15%)	0
	(4-11 10^9 /L)	11(18.3%)	0
	> 11	40(66.7%)	13(100%)
HGB	< 12	58(96.7%)	12(92.3%)
	(12 -17 g/dL)	2(3.3%)	1(7.7%)
	> 17	0	0
PlTs	<130	50(83.3%)	12(92.3%)
	(130–400 10^9 /L)	10(16.7%)	1(7.7%)
	>400	0	0
P.B. Blast%	<20%	9(15%)	4(30.8%)
	≥ 20%	51(85%)	9(69.2%)

Relationship Age with Immunophenotype markers

In adults cases, according to age groups the results in both CD117 and HALDR give significant differences (P<0.05) with lowest mean value in patient's group (36-55) years. Table 5 shows significant of all adult groups.

Table 5: Expression of immunophenotype markers with age groups of adults AML cases.

age / AML Markers	15 - 35 No.15	36 – 55 No.20	56 – 77 No.25	P-Value
	Mean	Mean	Mean	
CD13	67.10 a	64.38 a	62.56 a	0.943
CD33	75.93 a	78.77 a	76.33 a	0.964
CD117	74.36 a	51.00 b	72.06 a	* 0.054
CD34	57.57 a	42.85 a	45.40 a	0.565
HALDR	62.43 a	43.10 b	68.22 a	* 0.049

(*) significant differences (P<0.05)

In children cases all CD13, CD117 and HALDR given significant differences (P<0.05), and group (9 – 14 years) were highest mean in all cytometric markers, table 6.

Table 6: Expression of immunopheno type markers with age groups of children AML patients:

age / AML Markers	0 – 4	5 – 8	9 – 14	P-Value
	Mean	Mean	Mean	
CD13	73.20 a	56.00 b	81.76 a	*0.026
CD33	84.3 a	73.3 a	95.0 a	0.532
CD117	81.2 a	50.5 b	87.0 a	*0.039
CD34	78.83 a	78.50 a	90.00 a	0.697
HLADR	25.50 b	45.00 b	61.00 a	*0.023

Relationship Gender with Immunophenotypic markers

AML cases in this study were not given significant differences between gender and immunophenotype as shown in table 7.

Table 7: Expression immunophenotypic markers according to gender of adults AML cases.

Gender / AML Markers	**Male**	**Female**	**P-Value**
	Mean	Mean	
CD13	59.7	69.5	0.364
CD33	72.0	82.0	0.723
CD117	70.1	63.1	0.465
CD34	46.7	50.2	0.763
HALDR	63.9	54.2	0.378

Children cases not recorded significant differences**,** as shown in table 8.

Table 8: Expression immunophenotypic markers according to gender of children AML cases.

Gender / AML Markers	**Male**	**Female**	**P-Value**
	Mean	Mean	
CD13	73.4	64.2	0.512
CD33	85.4	79.3	0.723
CD117	71.0	71.0	1.000
CD34	84.8	75.3	0.440
HALDR	56.5	49.0	0.730

Relationship immunophenotype with FAB subtype

In adults CD13 was strongly expression in M2 subtype with all cases, while positivity it is lower with M7 and Non-M3 cases. M7 subtype showed positivity expression with all adults AML cases. Stem cells marker CD34 strongly expression associated with M0, whereas it lower positivity recorded in M2, M7 and Non-M3. HLADR marker was negative expression with M3

and M7, while highest positivity expression was with M0 cases. Table 9. There was preferential expression of CD33 among M2, M3, M4 and subtype M7.

Table 9: Results of immunophenotyping percentage of positive cases for each FAB subtype in adults

Sub-Types	CD13	CD33	CD117	CD34	HLADR
M0	60%	80%	90%	100%	90%
M2	100%	100%	50%	50%	50%
M3	86%	100%	85%	85%	0%
M4	69%	100%	92%	92%	80%
M5	88%	84%	63%	77%	77%
M7	50%	100%	100%	50%	0%
Non M3	58%	76%	70%	58%	76%

Children cases including subtypes: M0, M3, M4, M5 and NonM3 (which not given specific diagnosis). Immunophenic antibody CD13 was positivity expression for all subtypes of all cases, each subtype M0 and Non-M3 was negative expression associated of CD33. In subtypes M0 the cases given negative expression to HLADR, while all cases of Non-M3 showed significant positive for HLADR. CD117 and CD34 recorded strongly positive expression for cases associated with M0 and Non-M3. Table 10.

Table 10: Results of immunophenotyping percentage of positive cases for each FAB subtype in children

AML Markers	M0	M3	Non M3	M4	M5
CD13	100%	100%	100%	100%	100%
CD33	0	100%	0	100%	66%
CD117	100%	66%	100%	75%	33%
CD34	100%	33%	100%	50%	66%
HLADR	0	33%	100%	50%	66%

Assessment of Bone marrow Biopsy

This study showed increased cellularity of biopsy in 94% of adult cases as shown in figure 1 A, while all children were hypercellularity at diagnosis. Most cases showed diffuse infiltration by leukemic cells. That cellular including several dystrophic megakaryocytes as shown in figure 1B which showed increased an abnormal clusters of megakaryoblast to elderly case. Also the diffuse infiltration maybe with primitive cells replacing other hemopoietic elements as showed with most adult cases, as well as fibrosis figure 1C and architectural disturbances of bone marrow as fatty marrow or increases fatty vacuoles were appeared with adults cases of AML as shown figure 1C, 1D respectively.

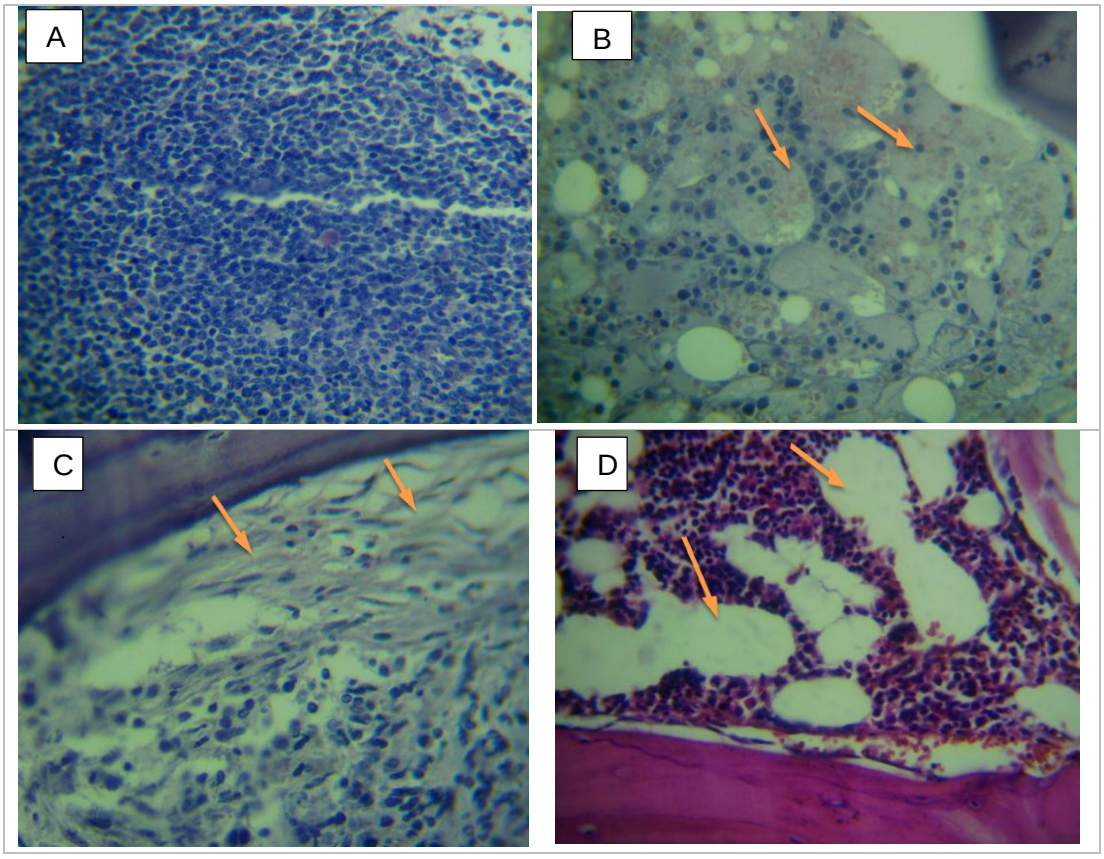

Fig. 1: (A) hypercellular marrow with myeloblast (male with AML in 71years at newly diagnosis), (B) biopsy section showing increased and abnormally clustered megakaryocytes(arrow) for (AML male 68 years), (C) section appears fibrosis (arrow)(female AML in 70 years) (D) hypercellular with fatty vacuoles (arrow) (AML.M3 of female in 29 years old) (H&E 40X).

Discussion

In current study, the AML was more in adult patients and that agreement with [6-8] . The mean age of adults AML cases were 48.45 years. This finding was comparable with other Iraqi studies [9, 10]. The peak incidence of AML adult patients in currently study was in age >55 years. But result of this study was higher than [11]. with male to female ratio was 1.06:1. This results showed nearly equal between male and female patients, that different with Iraqi study [9] which found that female patients were more than male patients.

In children AML cases 8(61.5%) males and 5(38.46%) females with the male to female ratio was 1.6:1. This result disagreement with [12]. the Non-M3 28.33% was the higher subtype followed by M4 21.67% and M0 16.67% for adult AML cases. This result difference from other Iraqi study by [13-15]. Children patient in this study showed that AML.M4 (30.77%) was most common followed by M3 and M5 (23.08% for both of them) of studied cases. This result different from Sudanese study [16] , current result agreement with study [12]. This might be attributed to differences in sample sizes or due to environmental factors.
Fever and paleness was the most common clinical feature among acute myeloid leukemia cases of both adults and children. That result was agreement with [17]. Fever and paleness was may be result from bacterial, fungal or viral infection or may be from leukemic burden of cells on tissues or organs by leukemic process itself.

In this study, 16.5% of adults and 15.3% of children AML were showed lymphnode enlargement, in addition to hepatomegaly and hepatosplenomegaly were occurred in both adults and children these results were consistent with a study [18]. Many of Iraqi and international studies showed that the more than half of adult patients had hepatomegaly, splenomegaly or lymph nod enlargement at diagnosis that could be appreciated with physical examination also that the degree of organomegaly was more noticeable in children than in adults. Bone pain appeared in the acute myeloid leukemia cases is nearly similar to other Iraqi studies [19, 9]. Bone pain is a direct consequence of bone marrow expansion. Children often present with a limp or the inability to walk due to the pain caused by the leukemic infiltration of the periosteum (bone covering) or due to the actual bone itself causing osteoporosis or bone erosion [20].

Leukocytosis (WBC count >11X 109/L) was most commonly in all acute myeloid leukemia cases. Hemoglobin concentration (HGB) in present study were less than normal value which refer to anemia, and Thrombocytopenia most commonly in both adults and children cases. Current study don't found any case with thrombocytosis. These findings similar with other studies [8,21, 22]. There caused by infiltration of bone marrow with leukemic cells which resulting failure the normal hematopiessis. In adults and children CD117 recorded significant differences ($p<0.05$) were related with age and the highest mean was at the group 35-55 years and group (5-8) years. As well as in the same age group for children (5-8) years, CD13 showed significant differences ($p<0.05$), the age groups (0-4 and 5-8) each of them given significant differences. without any significant differences between surface markers and gender in acute myeloid leukemia. 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.

Bone Marrow cellularity change with age, so reference to the age of the patient very important. In healthy pediatric samples. Bone marrow biopsy (BMB) use to supply correct blasts count. The highest blast count result should be used if bone marrow biopsy different from bone marrow aspirate (BMA). Bone marrow biopsy allows an assessment of marrow cellularity, dominant cell line, presence of fibrosis and other stromal changes, dysmegakaryopoiesis (which is more easily detected in histology preparations than in smears) and finally, architectural disorganization. In normal marrow, granulopoietic precursors are mainly found in the paratrabecular region, while erythroid and megakaryocytes are more or less confined to the central marrow cavities [23]. The clonal proliferation of hematopoietic stem cell is thought to produce growth factors and an abnormal cytokine release that mediates a bone marrow reaction that leads to fibrosis of the bone marrow [24].

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تقييم معاييرالتدفق الخلوي وعلاقتها بالتصنيف الفرعي لمرضى سرطان الدم النخاعي الحاد المرتبطة بالتقييم النسيجي

رشا حامد أيوب 1*، أياد حميد إبراهيم 2

1- قسم علوم الحياة، كلية التربية ، جامعة سامراء (rashahamid@uosamarra.edu.iq)

2- كلية الطب البيطري، جامعة تكريت

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الكلمات المفتاحية:

سرطان الدم النخاعي الحاد، التدفق الخلوي، التصنيف الفرعي، صورة الدم الكاملة،النخاع العظمي

الخلاصة:

اللوكيميا الحادة (ابيضاض الدم الخبيث) أحد امراض الدم الناتج عن خلل واضطرابات في عملية انتاج خلايا الدم بسبب فقدان السيطرة على انقسام وتكاثر وتمايز خلايا الدم. صممت الدراسة لتقييم المعايير الدموية والمضادات المناعية المستخدمة في التدفق الخلوي لمعرفة مدى علاقة الاستجابة لهذه المضادات مع العمر، الجنس والتصنيف الفرعي لسرطان الدم النخاعي مع تقييم التغييرات النسيجية لنخاع العظم لدى المرضى. تضمنت الدراسة 73 مريض (60 بالغ) قسمت الى ثلاثة مجاميع عمرية (15- 35، 36-55 و 56-77 سنة) وتضمنت الدراسة 13 طفل تم تقسيمهم أيضا الى ثلاثة مجاميع عمرية (1-4، 5-8 و 9-14 سنة). جمعت العينات من مركز امراض الدم - مستشفى بغداد التعليمي في مدينة الطب و من مستشفى الطفل التعليمي المركزي، للفترة من شهر نيسان الى شهر تشرين الثاني للعام 2019 . أظهرت النتائج أن الحمى والشحوب كانت السمة السريرية الأكثر شيوعًا بين مرضى ابيضاض الدم النخاعي الحاد (البالغين والأطفال).