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IMMUNOPHENOTYPING IN THE DIAGNOSIS AND CLASSIFICATION OF ACUTE LEUKEMIA: NATIONAL ONCOLOGY CENTER, ADEN

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ABSTRACT

Background: Acute leukemia comprises a heterogenous group of malignancies with variable clinical, morphologic, immunophenotypic and, molecular features. Flow cytometry is a crucial tool in the diagnosed and subtype hematological malignancy, especially acute leukemia, determining prognosis and monitoring response to therapy. By detecting various antigens presenting in various parts of cell, it is possible to know cell lineage and immaturity of the cell or group of cells. **Objective*:*** To evaluate immunophenotypic patterns of acute leukemia patient by multiparameter flowcytometry that help in the diagnosis and proper classification of acute leukemias. **Materials and Methods:** A descriptive study of acute leukemia cases was conducted at National Oncology Center Aden in Al -Sadaka Teaching Hospital over one year (January 2015 to June 2016). A total of 55 cases of acute leukemia diagnosed by multi parameter flow cytometry performed on peripheral blood and/ or fresh bone marrow aspirates. **Results:** 55 cases of acute leukemias were retrieved; 29(52.7%) of them were acute lymphoblastic leukemia (ALL), were B cell type (n=27) more than T cell type (n=2), and the remainder 26(47.3%), were proved by flowcytometry to be acute myeloblastic leukemia subtypes and one was acute myeloblastic leukemia (AML) with mixed phenotype (biphenotypic). Progenitors markers (CD34, HLA-DR, CD117 and TdT) were expressed more in acute myeloblastic leukemia more than in acute lymphoblastic leukemia blast cells, except TdT which was expressed by 13.8% of acute lymphoblastic leukemia (ALL) patients but not in acute myeloblastic leukemia patients. The B-lineage markers that expressed with higher percentage among ALL patients included CD19, CD10 and CD79a. Followed by CD20 and CD22. Only 2 patients with ALL expressed CD7 and cytoplasmic CD3 at the same times. Among the T-lineage markers, CD7 was aberrantly expressed in 26.9% and CD19 among B-lineage markers expressed 30.8% of AML patients. **Conclusion:** In our study the markers that expressed with higher percentage among ALL patients included CD19, CD10 and CD79a. The myeloid markers that were expressed markedly in AML patients included CD13, CD33 and cytoplasmic MPO.

KEYWORDS: *Acute leukemia; Immunophenotyping flowcytometry.*

INTRODUCTION

Acute leukemias are a heterogeneous group of malignancies with varying clinical, morphologic, immunologic, and molecular characteristics.[1]

Acute leukaemias are characterized by a defect in maturation, leading to an discrepancy between proliferation and maturation; since cells of the leukaemic clone continue to proliferate without maturing to end cells and dying there is continued expansion of the leukaemic clone and immature cells dominate.

The clinical manifestations of the leukemias are, directly or indirectly, due to the proliferation of leukemic cells and their infiltration into normal tissues. Augmented cell proliferation has metabolic consequences and infiltrating cells also disturb tissue function. Anemia, neutropenia and thrombocytopenia are important consequences of infiltration of the bone marrow, which in turn can lead to infection and hemorrhage.[2,3–7]

The French-American-British (FAB) classification of acute leukemia was first published in 1976 and was subsequently expanded, modified and clarified, (FAB) group established criteria of acute leukemia based on morphologic characteristics of the malignant clones. It defines three subtypes of acute lymphoblastic leukemia (ALL): L1, L2 and L3 and 8 subtypes of acute myeloblastic leukemia (AML).[8-12] The 2008 WHO classification of acute leukaemias is part of a broader classification of tumors of haemopoietic and lymphoid tissues.[13] It builds on the work of the French - American–British (FAB) group and on earlier WHO classifications published in 1999 and 2001. The principle

of WHO classification based on the phenotype (morphological and immunological) and on the underlying genetic abnormalities that determine disease characteristics.[14]

Refinement in classification of acute leukemias is accomplished by immunophenotyping. Differences in expression of surface membrane antigens or cytoplasmic components are used to identify and classify lymph proliferative disorders by cell of origin and stage of differentiation. This technique improves both accuracy and reproducibility of acute leukemia classification. It is considered particularly useful for identifying acute myeloid leukemia (AML) with lymphoid marker expression and, conversely, for ALL with myeloid marker expressions.[11,12]

In Yemen, there are little studies concern of acute leukemia, the last study in Aden by Iman Harize(2014) about clinical and hematological evaluation of acute leukemia in Aden hospitals, While in Sana'a Al-Ghazaly et al (2014) study concern about a ten year descriptive study of adult leukemia at Al-Jomhori teaching hospital in Sana'a Yemen the study involved sex, age, types of leukemia ,and seasonal distribution of leukemia in north, central and south areas.[16,17] Gamal Abdul-Hamid and Afif Nabhi (2010) studied the clinicoepidemiological features of adult leukemias in Aden, Yemen, concerning the distribution of leukemias according to type of malignancy, sex and age. Clinical manifestations and hematological parameters of leukemias.[18,19]

Recently in 2013 the flowcytomertry immunophenotyping introduced as diagnostic tool in Yemen in National Oncology Center, Sana'a(NOC), but till now no clear publish studies on immunophenotyping. In Saudi Arabia Al –Faleh et al (2015) whose study clinical features and outcome of acute myeloid leukemia, a single institution experience conducted at King Abdul-Aziz Medical City (KAMC) in Riyadh.[20] In Egypt Al-Mansoura study (2012) which study flowcytometric immunophenotypic profile of acute leukemia.

Cytogenetic and molecular studies have a crucial role in classification, prognosis and outcome of acute leukemic patients.

In our country like in several developing countries, where the cytogenetic and molecular studies are not available except that related to Philadelphia chromosome were introduced in 2013 in Sana'a.

The purpose of this study is to introduce the immunophenotyping analysis as one of the routing tests in diagnosis of acute leukemia for accurately determining the lineage of the malignant clone of leukemic blasts besides the light microscopic diagnosis of acute leukemia.

RESULT

The studied patients were 55 acute leukemias, 29(52.7%) of them were acute lymphoblastic leukemias and the remainder 26(47.3%) acute myeloblastic leukemias. Statistically, there is no significant difference between the percentages of ALL versus AML (p>0.05).

Table 1: Demographic characteristics of the studied patients with acute leukemias.

Item	AML (n = 26)		ALL (n = 29)		Total (n = 55)		*p*-value
	№	%	№	%	№	%	
- Sex:							
Male	17	65.4	17	58.6	34	61.8	0.61
Female	9	34.6	12	41.4	21	38.2	
- Age group (years):							
1 – 15	3	11.5	11	37.9	14	25.5	0.169
16 – 30	8	30.8	8	27.6	16	29.1	
31 – 45	5	19.2	4	13.8	9	16.4	
46 – 60	6	23.1	5	17.2	11	20.0	
> 60	4	15.4	1	3.4	5	9.1	
Mean ± SD (min.-max.)	39.1 ± 20.4 (1 – 70)		26.5 ± 19.4 (3 – 65)		32.5 ± 20.7 (1 – 70)		0.022
p-value of > 0.05 is considered statistically insignificant							

The sex distribution of the studied patients with acute leukemias showed significantly higher percentage of acute leukemias among males more than in females (61.8% vs. 38.2%, p=0.013). However, this trend was observed equally in both types of leukemias.

The peak age for acute leukemia in this study was from 16 to 30 years (30.8%). Followed by those less than 16 years of age (25.5%) and those from 46 to 60 years (20.0%).

Higher percentage of ALL patients (37.9%) was seen in younger age (1-15 years) while higher percentage of AML patients (30.8%) was seen in the age group (16-30 years).

It was noticed that the percentage of acute leukemia decreases after the age of 60 years (9.1%). In this study, the mean age of the studied patients with acute leukemia was 32.5 years. It was significantly higher among AML patients than in ALL patients (39.1 vs. 26.5 years) (p<0.05).

Table 2: Bone marrow examination in the studied patients with acute leukemias.

Bone marrow	AML (n = 26)		ALL (n = 29)		Total (n = 55)		*p*-value
	№	%	№	%	№	%	
Cellularity:							
Normocellular	1	3.8	3	10.3	4	7.3	0.220
Hypercellular	23	88.5	26	89.7	49	89.1	
Hypocellular	2	7.7	0	0.0	2	3.6	
Myeloid erythroid ratio:							
Normal	1	3.85	15	51.7	16	29.1	0.001*
Increase	24	92.30	4	13.8	28	50.9	
Decrease	1	3.85	10	34.5	11	20.0	
Erythroid precursors:							
Normal	3	11.5	6	20.7	9	16.4	0.394
Increase	0	0.0	1	3.4	1	1.8	
Decrease	23	88.5	22	75.9	45	81.8	
Myeloid precursors:							
Normal	1	3.8	3	10.3	4	7.3	0.005*
Increase	10	38.5	1	3.4	11	20.0	
Decrease	15	57.7	25	86.2	40	72.7	
Lymphoid precursors:							
Normal	6	23.1	2	6.9	8	14.5	0.0001*
Increase	2	7.7	25	86.2	27	49.1	
Decrease	18	69.2	2	6.9	20	36.4	
Megakaryocytic assessment:							
Normal	4	15.4	4	13.8	8	14.5	0.867
Decrease	22	84.6	25	86.2	47	85.5	
Increased eosinophils	1	3.8	2	6.9	3	5.5	0.542
Increased basophils	2	7.7	2	6.9	4	7.3	0.652
Increased Plasma cells	0	0.0	1	3.4	1	1.8	0.339
Bone marrow blast (%) Mean ± SD	73.1 ± 21.6		79.8 ± 16.4		76.7 ± 19.1		0.201
*statistically significant							

The majority of the studied acute leukemias were having hypercellular marrow (89.1%). Only two cases with AML were having hypocellular marrow. There is no significant difference in the percentage of hypercellularity between AML and ALL patients.

The myeloid to erythroid ratio is significantly increased in AML patients (92.3%) and normal or decreased in ALL patients (86.2%).

Erythroid precursors are equally depressed in AML as well as ALL patients (88.5% and 75.9% respectively). Myeloid series are significantly increased in AML patients (38.5%) and decreased in ALL patients (86.2%). Lymphoid precursors are significantly normal or decreased in AML patients (92.3%) and increased in ALL patients (86.2%).

Megakaryocytes are equally depressed in AML and ALL patients (84.6% and 86.2% respectively). Bone marrow eosinophilia, basophilia and plasmacytosis were seen in few percentages of acute leukemias (5.5%, 7.3% and 1.8% respectively). These are not significantly associated with either type of acute leukemias (p>0.05).

The percentage of blasts in bone marrow of patients with AML was not significantly differing than those with ALL (73.1% vs. 79.8%).

Table 3: Presentation of the studied patients with acute leukemias.

Presentation	AML (n = 26)		ALL (n = 29)		Total (n = 55)		*p*-value
	№	%	№	%	№	%	
New acute leukemia	19	73.1	24	82.8	43	78.2	0.683
Relapsed acute leukemia	4	15.4	3	10.3	7	12.7	

Secondary acute leukemia	3	11.5	2	6.9	5	9.1	
p-value of >0.05 is statistically insignificant							

About 78.2% of the studied patients with acute leukemias were new presentation of acute leukemia; 12.7% were relapsed acute leukemia and 9.1% were acute leukemia secondary to other leukemia (chronic myeloid leukemia in blast crisis). The relapsed acute leukemia patients were 7 cases. Four of them were AML patients in the first relapse, 2 ALL patients in the first relapse and 1 ALL patient in second relapse.

The source of sample in flow cytometry was variable, 33 samples were taken from the bone marrow (11 AML and 22 ALL) and 22 samples from the peripheral blood (15 AML and 7 ALL).

Those diagnosed as ALL were screened more by bone marrow samples (75.9%) than those diagnosed as AML (42.3%).

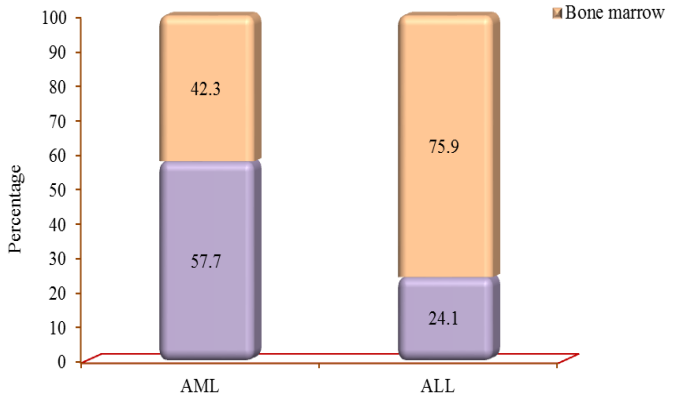

Fig. 1: The source flow cytometry sample in the studied patients with acute leukemias.

Table 4: Results of progenitors and lymphocytic cluster of differentiation in patients with acute leukemias.

Cluster of differentiation	Provisional diagnosis				Total (n = 55)	
	AML (n = 26)		ALL (n = 29)			
	№	%	№	%	№	%
- Markers of progenitors:						
CD34	22	84.6	16	55.2	38	69.1
HLA-DR	10	38.5	10	34.5	20	36.4
CD117	14	53.8	3	10.3	17	30.9
TdT	0	0.0	4	13.8	4	7.3
- B lineage markers:						
CD19	8	30.8	24	82.8	32	58.2
CD22	1	3.8	8	27.6	9	16.4
CD20	4	15.4	11	37.9	15	27.3
CD79a	3	11.5	16	55.2	19	34.5
CD10	3	11.5	22	75.9	25	45.5
cIg M	1	3.8	3	10.3	4	7.3
- T lineage markers:						
CD7	7	26.9	2	6.9	9	16.4
cCD3*	0	0.0	2	6.9	2	3.6
CD2	1	3.8	1	3.4	2	3.6
*c: Cytoplasmic						

This table showed that markers of progenitors were expressed more in AML more than in ALL blast cells, except TdT which was expressed by 13.8% of ALL patients but not in AML patients.

The B-lineage markers that expressed with higher percentage among ALL patients included CD19, CD10 and CD79a. Followed by CD20 and CD22.

Among the T-lineage markers, CD7 was aberrantly expressed in 26.9% of AML patients and CD2 in one AML patient. Only 2 patients with ALL expressed CD7 and cytoplasmic CD3 at the same times.

Table 5: Results of myelo-monocytic and aberrant cluster of differentiation in patients with acute leukemias.

Cluster of differentiation	Provisional diagnosis				Total (n = 55)	
	AML (n = 26)		ALL (n = 29)			
	№	%	№	%	№	%
- Myelo-monocytic markers:						
CD45	12	46.2	14	48.3	26	47.3
CD13	21	80.8	6	20.7	27	49.1
CD33	20	76.9	6	20.7	26	47.3
cMPO*	11	42.3	2	6.9	13	23.6
CD15	1	3.8	1	3.4	2	3.6
CD14	4	15.4	0	0.0	4	7.3
CD64	9	34.6	0	0.0	9	16.4
CD11c	1	3.8	0	0.0	1	1.8
- Aberrant expression:						
CD1a	0	0.0	1	3.4	1	1.8
CD4	4	15.4	3	10.3	7	12.7
CD8	1	3.8	2	6.9	3	5.5
CD5	1	3.8	1	3.4	2	3.6
*c: Cytoplasmic						

The hematopiotic marker (CD45) was expressed in nearly half of the studied patients with acute leukemias. The myeloid markers that were expressed markedly in AML patients included CD13, CD33 and cytoplasmic MPO. While the monocytic marker that expressed markedly in AML patients was CD64.

Aberrant expression included CD4 in 15.4% of AML patients and 10.3% of ALL patients.

Table 6: Common subtypes of leukemias in the studied patients.

Leukemia subtype	Provisional diagnosis				Total (n = 55)	
	AML (n = 26)		ALL (n = 29)			
	№	%	№	%	№	%
- Acute myeloblastic leukemias:						
AML-M1	1	3.8	–	–	1	1.8
AML-M2	6	23.1	–	–	6	10.9
AML-M3	1	3.8	–	–	1	1.8
AML-M4	2	7.7	–	–	2	3.6
AML-M5	3	11.5	–	–	3	5.5
AML (non APL)	9	34.6	3	10.3	12	21.8
- Acute lymphoblastic leukemias:						
B-ALL	3	11.5	24	82.8	27	49.1
T-ALL	–	–	2	6.9	2	3.6
- Mixed phenotype	1	3.8	–	–	1	1.8

Among the 26 patients provisionally diagnosed as AML, 22 were proved by flow cytometry to be AML subtypes and one was AML with mixed phenotype (biphenotypic). The remainder 3 patients were proved to be B-cell ALL.

The common subtype of AML was non-APL AML. Only one patients was diagnosed as APL.

For the 29 patients provisionally diagnosed as ALL, 26 were proved by flow cytometry to be ALL subtypes and the remainder 3 patients were proved to be AML (non-APL).

The totally diagnosed ALL patients by flow cytometry (n=29) were B cell type (n=27) more than T cell type (n=2).

Table 7: Treatment among the studied patients with acute leukemias.

Received treatment	AML (n = 26)		ALL (n = 29)		Total (n = 55)		*p*-value
	№	%	№	%	№	%	
Yes	20	76.9	27	93.1	47	85.5	0.094

No	6	23.1	2	6.9	8	14.5	
p-value of >0.05 is statistically insignificant							

Among the studied patients with AL, 85.5% of them received treatment and 14.5% not received treatment at the time of sampling for this study.

Table 8: Outcome of the studied patients with acute leukemias.

Outcome	AML (n = 26)		ALL (n = 29)		Total (n = 55)		*p*-value
	№	%	№	%	№	%	
Alive	7	26.9	11	37.9	18	32.7	0.094
Death	19	73.1	18	62.1	37	67.3	
p-value of >0.05 is statistically insignificant							

The outcome of the studied patients with AL showed higher percentage of death among them (67.3%). The percentage of death among AML was higher than in ALL (73.1% vs. 62.1%). difference was not statistically significant (p=0.405). There is no significant difference in the survival of patients with AML or ALL.

Median Survival

The median survival was 3.33 and 5.13 months in patients with AML and ALL respectively and the

Table 9: Kaplan-Meier for over all survive in acute leukemia patients all over the period of follow up.

Time (Month)	0	3	6	9	12	15	*p*-value
AML	26	13	7	4	2	0	
ALL	29	11	8	2	5	3	0.405*
Total	55	18	15	6	7	3	
* *Statistically insignificant*							

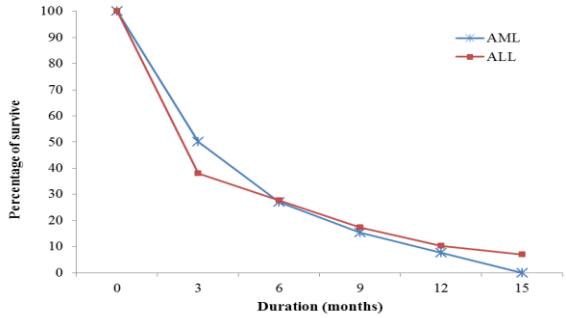

Fig. 2: Kaplan-Meier curves for over all survive in patients with acute leukemia all over the period of follow up.

When all the studied acute leukemias were taken together, they showed a median survival of 135 days with a standard error of 37.9 and 95% confidence interval of 60.6 – 209.4 days.

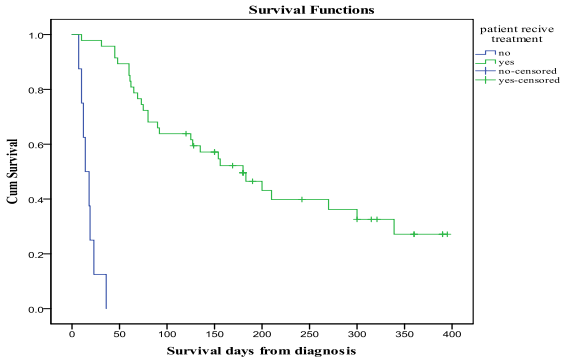

Fig. 3: Survival functions in patients with acute leukemia in relation to treatment.

Prognostic factors

In this table, it was evident that in patients with acute leukemia, the presence of age < 2 years or > 10 years, the presence of high LDH or hepatomegaly at presentation of patients were associated with worse overall survival (OS).

Other risk factors were not significantly associated with worse OR in the studied patients with acute leukemias.

Table 10: Cox regression survival analysis for overall survival in relation to different prognostic factors in patients with acute leukemia.

Prognostic factors	%	OR	95.0% CI	p-value
- Age (years):				
2 – 10	16.4	5.08	1.49 - 17.31	**0.009***
<2 or > 10	83.6			
- Sex:				
Female	38.2	0.712	0.28 – 1.77	0.446
Male	61.8			
- Hb (g/dl):				
≤ 10	90.9	2.36	0.25 - 22.62	0.46
> 10	9.1			
- WBC ($x10^6$/L):				
≤ 10	30.9	0.86	0.32 - 2.30	0.76
> 10	69.1			
- Platelets ($x10^6$/L):				
< 100	81.8	1.83	0.45 - 7.43	0.40
≥ 100	18.2			
- Albumin (g/dl):				
Normal	61.8	1.64	0.76 - 3.52	0.21
Low	38.2			
- LDH (U/L):				
Normal	27.3	3.59	1.16 - 11.06	**0.026***
High	72.7			
- Hepatomegaly	54.5	2.44	1.07 - 5.54	**0.033***
- Splenomegaly	50.9	1.12	0.49 – 2.53	0.79
- Lymphadenopathy	25.5	0.72	0.29 - 1.77	0.47
- CNS involvement	1.8	0.48	0.04 - 4.82	0.54

DISCUSSION

Leukemia ranked as a second common cancer for males and the fifth for females in southern Yemen in four governorate Aden, Lahje, Dhalea' and Abyen.[21]On the other hand, in Saudi Arabia leukemia is considered the third most common malignancy that affect males and the fifth in females for the year of 2009.National Cancer Institute USA, estimates the incidence of new leukemia cases were about 13.3 per 100,000 people in United States, based on cases and deaths (2009-2013).[22]

In this study, males were more affected with leukemia than females with significantly higher percentage (61.8% vs. 38.2%, *p*=0.013). These results are similar to other studies.[17,18,21,23]

The peak age for acute leukemia in this study was from 16 to 30 years (30.8%), followed by those less than 16 years of age (25.5%) and those from 46 to 60 years (20.0%). In contrast with Iman Harize (Aden), Mohammad Bashir (Peshawar) and Malaysia studies, the commonest age group of acute leukemia is the age group below 15 years (41.5%), followed by the age group 15-30 years (35.8%) and the age group 46-60 years (13.2%).[16,24,25]

More over, we figured that higher percentage of ALL patients (37.9%) was seen in younger age (1-15 years) while higher percentage of AML patients (30.8%) was seen in the age group (16-30years).This result was agreed with Osman et al. from Sudan who study acute myeloid leukemia in adult patients with frequent age group(17-40) years.[26.27] While Al- Ghazaly et. al who studied leukemia within ten years in Al-Jomhori teachings hospital in Sana'a city found that; AML was most common in the 40-59 years age group. In Swedish study, AML is a disease of the elder, with a median age of onset around ~70 years.[37] China study 54% of patients diagnosed of AML at 65 years or older and approximately a third diagnosed at ≥75 years of age.[38]

In this study, the mean age of the studied patients with acute leukemia was 32.5 years. It was significantly higher among AML patients than in ALL patients (39.1 vs. 26.5 years) (p<0.05). It was similar to a study from India by Subhash Chandra et.al, where the age in AML ranged from 3 years to 56 years with a mean age of 30

years and, in the ALL, the average age was 22years.[29] Harani et. al study reported that the mean age of acute myeloid leukemia patients in Karachi (2005) was 32 years.[31] Hassan et. al in his study on adult AML in the UAE and one recent Malaysian study by Meng et. al who reported that the median age was 39 years at diagnosis.[28,33], whereas in Al- Ghazaly et.al, the median age was 40 for AML and 18.5 for ALL.[17]

However for ALL, the disease is most common in the 14-19 years age group. In Abdul-Hamid and Nabhi study of Clinico-epidemiological features of leukemias in Aden Yemen, the age group most affected in patients with acute myeloid leukemia was 21-50 years and in acute lymphoblastic leukemia was 11-20 years.[18] While in Venkateswaran et. al from India study of acute leukemia, the average age of patients was 29-58 years.[23]

Sample in flow cytometry was variable of the source, 33 samples were taken from the bone marrow (11 AML and 22 ALL) and 22 samples from the peripheral blood (15 AML and 7 ALL). Those diagnosed as ALL were screened more by bone marrow samples (75.9%) than those diagnosed as AML (42.3%) .While in Indian study most of the patients (73.3%) diagnosed as acute leukemia from peripheral smear.[32]

In current period of medicine, when exact diagnosis is needed to manage the patient and also to explain prognosis, immunophenotyping is very useful for acute leukemia. It has diagnostic accuracy of almost 99 %. It can type acute leukemia into AML and ALL and ALL is further subclassified into B-ALL and T-ALL. There are four important methods of diagnosis in acute leukemia i.e. morphology, cytochemistry, cytogenetic and immunophenotyping.[39] Each one has got diagnostic and prognostic importance but obviously immunophenotyping is the best amongst these.

Markers of progenitors were expressed more in AML than in ALL blast cells, except TdT which was expressed by 13.8% of ALL patients but not in AML patients as mentioned in.

The CD34 expressed on many different cell types, especially on myeloblasts and very weakly on promyelocytes.[50] CD34 cells can be detected in cord blood, bone marrow and in the peripheral blood of normal subjects, where they constitute respectively about 1.5% and 0.1–0.01% of the elements.[51] The expression of CD34 has poor prognostic value, its absence was associated with a higher percentage of complete remissions.[52] In this study CD34 was positive in 84.6 % in AML patients and 55.2% in ALL patients. Similar result reported by Osman et al from Sudan wereCD34 reported in 78.7% in all cases of AML. While in Mansoura study from Eygypt CD34 found in 62.1% of non APL patient, and 76.3% of ALL patients, while in Indian study reported CD34 in 43.9% of ALL patients.[27,21,32]

Positivity of HLA-DR was in 38.5% of AML patients and 34.5% in ALL patients, with out significant difference between the types of leukemia. This result is parallel to that of an Indian study, while an Egyptian study revealed a high percentage of HLA-DR for both types of leukemias.[31,21] In AML M3 subtype in particular has its own unique immunophenotype which can be differentiated from other FAB subtypes of AML.[169] The combined use of HLA-DR and CD34 was much more helpful in distinguishing cases of non-APL AML from APL cases, than either of these antigens alone. HLADR and CD34 double negativity in APL was ranged to 80%.[21,48]

The expression of CD117 is normally expressed by bone marrow hematopoietic precursors, and can be detectable throughout the myeloid lineage until the promyelocyte maturation step and in the erythroid lineage until the pro-erythroblast stage.[50] The expression of CD117 in AML was 53.8% and 10.3 % in ALL patient. While in Sudanese study, the percentage of positive expression in AML patients was 83.8%. In Mansoura and Brian studies, the percentages of positive expression were 74.3% and 80% respectively,.[27,21,53] whereas, in an Indian and Indonesian studies, of ALL patients they were 2.4%,<5% respectively.[32,54]

In our study**,** TdT was positive and was expressed by 13.8% of ALL patients (table 3.8), while it was expressed by 97.4% in Mansoura study (2012) and Bachir et.al.[21,24]

The B-lineage markers that expressed with higher percentage among ALL patients included CD19 (82.8%), CD10 (75.9%) and CD79a (55.2%.) Followed by CD20 (37.9%) and CD22 (27.6%). Similar results were in Bhattacharyya et al. from India CD19 and cytoplasmic CD79a were the most commonly found to be positive in patients with B-ALL, while Subhash et al CD19 and common ALL antigen CD10 were most common antigen expression.[29, 32] In Mansoura study and Bachir et al all B-ALL cases express CD19.[21,24]

T-lineage-markers CD7 is the first T-associated antigen to appear during the maturation of T lymphocytes. CD7 and CD3 were common markers, Only 2 patients with ALL expressed CD7 and cytoplasmic CD3 at the same times. CD7 was not totally specific as it was demonstrated to cross react with AML cases.[55,56] Most cases express more than one T-lineage marker. Aberrant deletion of one or more pan T-cell antigens is common in this disease, however, and maybe a helpful diagnostic finding.[60] All the 2 cases of T-ALL show deletion of one or more of the T-cell antigens used (CD2, CD3, CD4, CD5, CD7and CD8). Vodinelich et al (1983), Kaleem et. al (1993) and Traweek et al(2003) showed that CD7 was the most often expressed T cell antigen.[79-81] While CD5 was the pan-T-cell antigen most often expressed by the T-cell cases in Venkateswaran et al.[23]

Cluster of differentiation 45(CD45) is a protein tyrosine phosphates that is present in all leukocytes with brightest expression on lymphocytes. In addition, it is of prognostic significance as its absence is associated with longer incident free survival in childhood B-cell ALL.[62,63] As well as CD45/side scatter (SSC) gating approach permits efficient discrimination between blasts and normal cells facilitating analysis of blasts present in low proportions. [64] The hematopoietic CD45 marker was expressed in nearly half of the studied patients with acute leukemia. CD45 was expressed in 97.2% of AML cases in Khalidi series.[65]

Myelo-monocytic-markers: Acute myeloid leukemia was defined immunologically by the expression of 2 or more of the following myeloid markers: myeloperoxidase (MPO), CD13, CD33, and CD117.[66] Myeloid markers that were expressed markedly in AML patients included CD13, CD33 and cytoplasmic MPO. Same result reported by zAl-faleh et al (2015) from Saudi Arabia.[20] while Mansoura study (2012),[21] from Egypt, and Kaleem et al (2003),[58] reported CD33 followed by CD13 were most common myeloid Antigens. Other study by Byrd et.al (2002),[61] showed that CD45, CD33, CD13 were the most commonly expressed Antigens.

Cluster of differentiation (CD13) is normally expressed on hematopoietic stem cells, on the mature and immature elements of the myeloid and monocytic lineages, as while as eosinophils and basophiles. Even though, frequently expressed, CD13 cannot be established in all cases of AML, its absence is related to a good prognosis.[214] In this study CD13 is positive in 80.8% of all AML cases. In Mansoura and Bradstock studies, CD13 was (77.9% and71% respectively) which is lower than those in in Brian and Ollivier studies (91%,95%).[21,68,53,66] Cluster of differentiation CD33 ,is a myeloid antigen and it appears during myeloid differentiation after CD13 at the hemopoietic precursor level . The intensity of expression of CD33 is high on monocytes, and dramatically decreases on basophils, neutrophils and eosinophils.[68] CD33 was positive in 76.1% of the AML cases of this study, compared to (91%), (87%) and (79%) in other studies.[53,66,68] The monocytic marker, expressed markedly in AML patients, was CD64 (34.6%) then CD14 (15.4%). Kaleem et al reported the same results, while Mansoura study reported that CD14was the most common monocytic antigen, followed by CD36.[58,21]

Aberrant expression: In numerous cases of acute leukemia, blasts of one lineage do not exhibit the markers of normal differentiation but expressed unusual markers in which myeloid associated antigens expressed in lymphoblasts and lymphoid associated antigen expressed in myeloblasts. This phenomenon is called aberrant phenotypes.[71,72]

From a prognostic point of view, aberrant antigen expression can adversely influence the clinical response, remission rate and overall survival in patients with acute leukemia.[72,79,80] In this study CD7 was aberrantly expressed in (26.9%) of AML patients in agreement with the results of Khurram et al(2010) and Jahedi et al (2014) while in difference to the results of El-Sissy et al. (2006) who reported that CD7 was expressed in a minority of his cases.[74,72,75] CD7 expression in AML correlates with a lower incidence of complete remission.[70] Other T lymphoid antigen expressions in AML patients in this study are: CD4 15.4%, CD2(3.8%), CD8 (3.8%) and CD5 (3.8%). Al-faleh et al (2015) reported that the most aberrant lymphoid antigens in AML patients were CD2,CD4 and CD7,[20] while Momani et al study (2016), CD4 was 4.5%.[76] Among B lymphoid antigenic expression, the commonest aberrant marker in AML patients were CD19 followed by CD 20. This results was similar to Momani et al from Jordon and Sarma et al (2015) from India.[76,73]

In our study the myeloid markers that were aberrantly expressed in ALL patients included CD13 and CD33 with 20.7% which lower than those of AL-Khayed et al (2015) and Momani et al, from Jordan (47% of CD33 and 37% of CD13), while in Seegmiller et al(2009) the common aberrant myeloid antigen was CD13, followed by CD33.[77,76,78]

Leukemia common subtypes

Among the 26 patients provisionally diagnosed as AML, 22 were proved by flow cytometry to be AML subtypes and one was AML with mixed phenotype (Biphenotypic). The remainder 3 patients were proved to be B-cell ALL.

The common subtype of AML was non-APL AML 34.6% while it was 3.8% in APL. In Mansoura study from Egypt non-APL was 77%.[21] These results have revealed a lower percentage of APL in our study, compared to those of the preceding studies that reported APL percentages ranging from 5 to 14% of all AML cases.[31,43-47]

Among AML cases which classify under FAB classification M2 was the commonest followed by M5. This results was agreement with most published data that indicated the predominance of M1-2 as the most common AML subtypes.[46,47,81,82] While in Harani et al.(2005) and Harakati et al (1998), who reported marked predominance of M4/5 subtypes varying between 42.2 and 73% of AML cases.[31,48]

For the 29 patients provisionally diagnosed as ALL, 26 were proved by flow cytometry to be ALL subtypes and the remainder 3 patients were proved to be AML.

The totally diagnosed ALL patients by flow cytometry were 29 patients, 27 patients were B cell type. These

results were in concordance with the majority available data.[55,84]

Common ALL (CD10 positive) in this study accounted for 75.9 % of B-ALL cases which is concomitant with Mansoura study, Gujral et al and Rego et al.[21,55,85]

In this study ALL-T cell type were 2 patients with (3.6%), while the proportion of TALL among known ALL Pakistani patients was 17.22%. and 25.5% in Mansoura study.[85,21]

In this study, the AL patients have shown a higher percentage of death (67.3%). The percentage of death among AML was higher than in ALL (73.1% vs. 62.1%) and the median survival was 3.33 and 5.13 months in patients with AML and ALL respectively and the difference was not statistically significant (p=0.405). There is no significant difference in the survival of patients with AML or ALL.

The studied acute leukemias, when taken together, showed a median survival of 135 days with a standard error of 37.9 and 95% confidence interval of 60.6 – 209.4 days, while in Harize study the median survival of patients with leukemia, was 234 days for AML and 407 days for ALL.[16] In Saudi Arabia AML was 343 days, in South Nigeria 180 days for AML and 240 days for ALL,[40] in Canada 381days for AML and in Iran 280 days for AML and 303 days for ALL.[48,87,88] Comparing our results with previous studies, the median survival was held back due to late presentation, incapability to maintain treatment, noncompliance with treatment regimen as a result of unawareness and most importantly poor or inadequate supportive management.

During the period of study, there were some factors that had an effect on our study such as lack of facilities, e.g. shortage of chemotherapy and blood banks services, lack of oncology centers(there is only one center treat leukemia cases in southern areas); as well as lack of transport, after the war, that make another obstacle that caused delay in diagnosisand, treatment. In relation to prognostic factors illustrated in results, it is found that the age of <2years or >10 years has an effect of five times of worse overall survivals. This prognostic factor is considered significant, the patients with acute leukemia; while, in previous literatures, adult or infant < one year had poor prognosis,[111] and the presence of high serum lactate dehydrogenises (LDH) or hepatomegaly during presentation of patients were associated with worse overall survival (OS). Serum LDH is almost certainly produced by the tumor cell. LDH level is moderately elevated in many cases of acute leukemia, irrespective of their cell type. Markedly, the elevated level of LDH is recorded in the majority of patients with AL and is suggestive of increased cell proliferation and turnover. LDH level has significant correlation with total tumor burden,[90,91] These observations are similar to those found in a study of Erickson and Morales, when they proved that estimation of serum LDH level has prognostic value.[92]

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