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CLINICAL AND HEMATOLOGICAL EVALUATION OF ACUTE LEUKEMIA IN ADEN HOSPITALS, YEMEN

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ABSTRACT

Background: Acute leukemia (AL) is serious heterogeneous neoplastic haemopoietic disease. Therefore, parameters are needed to classify this disease into subtypes. The aim of present study is to determine the frequency of various subtypes of acute leukemia using French-American-British (FAB) criteria in our population, and to study the clinical and hematological presentations of acute leukemia. **Materials and Methods:** This is descriptive study conducted at two Aden governmental hospitals from January 2011 to September 2012. The total number of subjects were 53 patients that included both adults and children. History and physical examination, complete blood count, bone marrow aspiration and cytochemistry stains were done for all patients. **Results:** Fifty three patients were studied (males= 37, females= 16), with male to female ratio 2.3:1. The age ranged between 18 months to 76 years with a mean age of 22 years. Acute Lymphoblast leukemia (ALL) was 58.5%, acute myeloid leukemia (AML) 37.7% and undifferentiated leukemia 3.8%. The predominant subtype of ALL was L1 (51.6%) followed by L2 (45.2%). In AML; M2 (40%) was the predominant subtype followed by M4 (25%) and M5 (20%). All patients were anemic; their hemoglobin was 3.5 -12 g /dl. The WBC count was quite variable ranged from 0.40 to 300.0 ×109/l. Similarly the platelet count was ranged from 6.0 to 250.0×109/l. Cytochemical analysis of leukemia, when coupled with morphology confirm the diagnosis in (95%) of AML cases and (80%) of ALL cases. In AML, patient's death is more significant than ALL (P=0.055), and most of the deaths occurred in older patients with no statistical significance (P=0.08). In ALL, the age and sex were found to be significant prognostic factors. Other prognostic factors included WBC count, percentage of cytochemical positive blast cells with no significant in both type of acute leukemia. **Conclusions:** The most common type of acute leukemia observed in our study was acute lymphoblast leukemia (ALL) (58.5%), and in the AML subtype the most common one was myeloblastic leukemia with maturation (M2) (40%).

KEYWORDS: Acute leukemia; FAB classification; Aden hospitals.

INTRODUCTION

Leukaemia is a disease resulting from the neoplastic proliferation of haemopoietic or lymphoid cells.[1] Acute leukemia's are characterized by sudden uncontrolled growth of malignantly transformed hematopoietic progenitor cells. These cells accumulate within bone marrow leads to suppression of the growth and differentiation of normal blood cells.[1,2]

Symptoms results from varying degree of anemia, neutropenia, and thrombocytopenia and from tissues infiltration, the underlying pathophysiology consists of a maturational arrest of bone marrow cells in the earliest stages of development. The mechanism of this arrest is under study, but in many cases, it involves the activation of abnormal genes through chromosomal translocations and other genetic abnormalities.[2-5]

The acute leukemias (AL) are divided into 2 categories, depending upon their cell of origin. Leukemia evolving from the myeloid/granulocyte cell line is called acute myelogenous leukemia (AML). Lymphocytic precursors give rise to acute lymphocytic leukemia (ALL).[3,6]

The modern classification of acute leukemia dates back to 1976, when international group of investigators from France, America and Britain developed a uniform classification system designated as French-American-British (FAB) classification, which was subsequently modified in 1985.[7-9] The major advantage of the FAB classification system is its ease of use, so its proposal was adopted internationally. In 2001, a World Health Organization (WHO) expert group proposed an updated system for the classification of leukaemia and lymphoma incorporating clinical features, haematological and histological features, immunophenotyping and the results

of cytogenetic and, to a lesser extent, molecular genetic analysis.[10]

In 2008 a further updating of the WHO classification incorporated new knowledge and gave a greater importance to molecular genetics features.[11] As the diagnosis and classification of leukaemia comes to rely increasingly on sophisticated and expensive investigations, that are not practical for many developing countries, some suggestions as to how leukaemia might be diagnosed in under-resourced laboratories.

In Yemen, there is lack of studies concern of AL either AML or ALL, in pediatric there was study of Mohammad Hammod (1998) about acute leukemia that concern in clinical and hematological presenting features of AL in AL-Wahda teaching hospital.[12] Jameel AL-Ghazaly (2005) studded the pattern of adult leukemias at Al-Jomhori hospital (Sana'a), concerning age, sex, area of residence whether rural or urban and the type of leukemia were collected and analyzed.[13] Gamal Abdul-Hamid (2012) studied the pattern of hematological malignancies at Al-Gamhouria Teaching Hospital in Aden, this study involved sex, age, and the frequencies for the main hematological disorders and present the different subtypes of the Hodgkin's and non-Hodgkin's lymphomas.[14]

In Iraq study (2003) Adel Hessen were found M3 is commonest subtype of AML, many subtypes of AML defined by FAB criteria had characteristic clinical and haematological distinctive features at medical presentation.[15] Mohammad Bashir study (Peshawar), the clinical presentation of acute leukemias was so variable and atypical that in most of the cases the diagnosis is delayed for a quite long time, due to which the prognosis becomes poor.[16]

Cytochemical stains are extremely useful in the diagnosis and classification of acute leukemias.[7] They allow correct identification of myeloid and lymphoid acute leukemias, as well as providing the basis for subclassification of the acute myeloid leukemias by the French-American-British criteria and the World Health Organization classification (WHO).[7,8,9]

Despite the widespread use of immunophenotyping in the diagnosis of hematopoietic neoplasms, cytochemical studies are still of diagnostic importance (Scott, 1993).[17] This is particularly true of the acute leukemias, although a large panel of cytochemical tests is probably not necessary in most cases.[6,18] In rare patients with inconclusive flow cytometry results, cytochemical stains may provide information which can confirm a diagnosis (Mhawech et al., 2001).[18] Myeloperoxidase or Sudan black B cytochemical stains remains the hallmark of a diagnosis of acute myeloid leukemia (AML) in most cases. Some cases, such as minimally differentiated AML and monoblastic leukemias, are myeloperoxidase-negative.[9]

In Yemen like in many developing countries, where the genetic and immunophentyping are not available, the use of cytochemical stains is still have a value in the diagnosis of leukemia. The purpose of this study is to introduce in laboratory practice cytochemical stains in order to be a routine test in identifying the subtypes of acute leukemia as well as to identify common clinical and hematological presenting features of acute leukemia, and to determine the frequency of acute leukemia subtypes according to (FAB) classification using morphologic and cytochemical stains.

MATERIAL AND METHODS

Prospective descriptive study conducted at Al-Gamhouria Teaching Hospital and Al -Wahda Teaching Hospital in the period at 5th January 2011 to 30th September 2012. Fifty six patients with acute leukemia, with thirty nine newly diagnosed acute leukemia that were not readily classified into conventional (FAB) diagnostic categories, six secondary acute leukemias, eight relapse acute leukemias and three excluded cases, they were taken up for study from the Al-Gamhouria Teaching Hospital and Al - Wahda Teaching Hospital.

Data was collected from patients with newly, secondary acute leukemia, and relapse acute leukemia by presence of blast cells in peripheral blood. For patient under the age of 12 years old, one of the parents was interviewed. The interview included a detailed relevant history for personal data, chief complains, medical history which included past history of anemia, infection and family history of cancer. Physical examination of liver, spleen, lymph nodes, and gum hypertrophy was done. The collected data reported in a previously prepared questionnaire for the purpose of this study.

Blood counts obtained from venous blood, 5 ml was collected from each patient in EDTA tube and sent for complete blood cell (CBC) count by an automated blood counter (Sysmex KX-21N). The EDTA blood samples were also tested for ESR and blood cell morphology.

Bone marrow aspiration either from supra sternal or posterior iliac crist, with enough slides for Leishman stain and two cytochemistry stains, one for myeloid leukemia myeloperoxidase (MPO) and other for lymphoid leukemia Periodic acid Schiff (PAS) (see annex 2) the non specific esterase's stains that uses for monocytoid leukemia were not available.

Statistical analysis: The employed technique of data collection which is an open – closed questionnaire covering all necessary variable needed to accomplished the study.

The data collection in the questionnaire have been entered the SPSS program and analysis in order to find out the frequency, percentage, and mean values with standard deviations, Chi-Squared test for qualitative variables, t student test for the difference of tow means,

and Kruskal Wallis test for the difference of three or more means for quantitive variables, with the 95% confidence limits. P- Value of ≤0.05 was considered as statistically significant.

RESULT

The study population consisted of 53 patients with acute leukemia being studied during period from 5th January 2011 to 30th September 2012 in two Aden hospitals (Al-Gamhouria Teaching Hospital and Al - Wahda Teaching Hospital).

Table 1: Distribution of patients with acute leukemia according to demographical characteristics, No. =53.

Characteristics	No.	%
Gender		
Male	37	69.8
Female	16	30.2
Age Groups (Years)		
≤ 15	22	41.5
16 – 30	19	35.8
31 – 45	2	3.8
46 – 60	7	13.2
≥ 61	3	5.7
Mean ± SD (range)	22±19.1 (1.5- 76)	
Residency		
Lahj	19	35.8
Aden	13	24.5
Abyan	8	15.1
Shabwa	6	11.3
Hadramout	2	3.8
Aldalah	3	5.7
Other	2	3.8
Note: percentage calculated in relation to the total population of patient (53)		

Table1 shows that acute leukemia was more prevalent in males than females (male: female ratio 2.3:1). Patients at age ≤ 15 were most common with (41.5%) followed by the age group 16- 30 (35.8%). Regarding residency, patients from Lahj governorate constituted the highest percentile (35.8%) followed by Aden governorate (24.5%), Abyan (15.1%), Shabwoa (11.3%) and other governorates (13.3%).

Table 2: Distribution of adults and pediatric patients in acute leukemia, No.=53.

Type of acute leukemia	Adults >15 years old		Pediatric ≤15 years old		p- value
	No	%	No	%	
AML	17	54.5	3	14	0.002
ALL	12	38.5	19	86	
Undifferentiated Leukemia	2	6.5	0	0	
Total	31	100	22	100	
% taken from the total column *Chi-square test p<0.05.					

Table 2 shows that (54.5%) of adult patients had AML, while (38.5%) had ALL and (6.5%) undifferentiated leukemia. In children, the ALL was the commonest type of leukemia (86%) and AML was (14%).

Table 3: Distribution of patients with acute leukemia according to pattern of occurrence.

Pattern of occurrence	No.	%
New (primary)	39	73.6
Relapse	8	15.1
Secondary AL:		
Hematological diseases	5	9.4
Therapy-related AML	1	1.9
Total	6	11.3
Note: Percentage calculated in relation to the total population of patients (53)		

According to pattern of occurrence of acute leukemia, the new cases were 73.6%, relapse cases 15.1% and secondary cases 11.3%.

Table 4: FAB classification subtypes among patients with acute leukemia, NO. =53.

Acute leukemia subtypes	subtype	No.	%
Acute myeloid leukemia	M1	1	5
	M2	8	40
	M3	2	10
	M4	5	25
	M5	4	20
	Total	20	100
Acute lymphoblastic leukemia	L1	16	51.6
	L2	14	45.2
	L3	1	3.2
	Total	31	100
Undifferentiated Leukemia		2	3.8
Total		53	100
Note: Percentage calculated in relation to the total populatiof patient (53)			

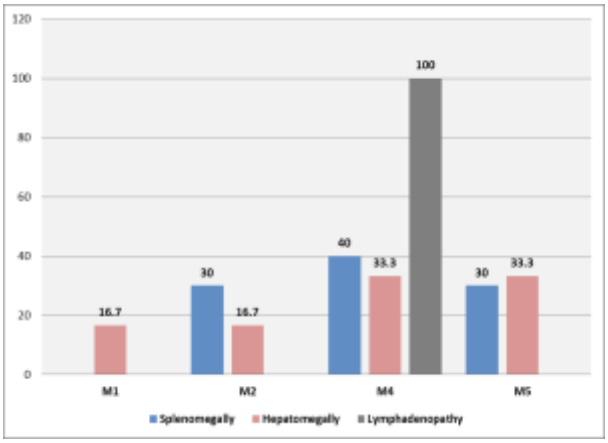
Table 4 shows that the most common subtype of acute myeloid leukemia was M2 (40%) followed by M4 (25%) and M5 (20%). In acute lymphoblastic leukemia L1was (51.6%) followed by ALL-L2 which was (45.2%).

Table 5: Frequency of symptoms and signs in patients with AML and ALL No. =51.

Clinical features	AML No=20		ALL No=31		Total No=51		p- value *
	Yes (%)	No(%)	Yes (%)	No (%)	no.	%	
Symptoms							
Fatigue	17 (85)	3 (15)	25(80.6)	6(19.4)	42	82.4	0.982*
Fever	17 (85)	3 (15)	23(74.2)	8(25.8)	40	78.4	0.493*
Loss of appetite	12 (60)	8 (40)	18(58.1)	13(41.9)	30	58.8	0. 891
Sweating	8 (40)	12 (60)	9(29)	22(71)	17	33.3	0.417
Bleeding per gum	7 (35)	13 (56)	3(9.7)	28(90.3)	10	19.6	0.036*
Bone pain	12 (60)	8 (40)	20(64.5)	11(35.5)	32	62.8	0.745
Weight loss	10 (50)	10 (50)	18(58.1)	13(41.9)	28	54.9	0.572
Recurrent infection	9 (45)	11 (55)	10(32.3)	21(67.7)	19	37.3	0.358
Bleeding	10 (50)	10 (50)	8(25.8)	23(74.2)	18	35.3	0.078
Signs							
Paler	13(65)	8 (40)	19(61.3)	12(38.7)	32	62.7	0.789
Bruising	6(30)	14(70)	7(22.6)	24(77.4)	13	25.5	0.553
Gum hypertrophy	2(10)	18(90)	0	31(100)	2	3.9	0.197*
Splenomegaly	10(50)	10(50)	23(74.2)	8(25.8)	33	64.7	0.169
Hepatomegally	6(30)	14(70)	16(51.6)	15(48.4)	22	43.1	0.523
Lymphadenopathy	3(15)	17(85)	20(64.5)	11(35.5)	23	45.1	0.005*
Note: - Were 2 cases excluded as undifferentiated Percentage were calculate by total of each column Chi-square test $p<0.05$, *Fisher exact test.							

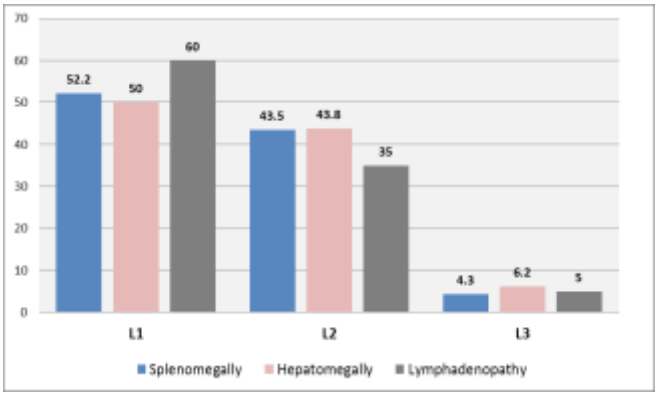
In table 5, the most common symptoms of both types of acute leukemia were fatigue, (85%) for AML and (80.6%) for ALL followed by fever (85% for AML and 74.2% for ALL). There was no statistical significant difference between symptoms and types of acute leukemia except bleeding per gum where (p=0.036).

Gum hypertrophy presented in 10% of AML patients. Splenomegaly, hepatomegally and Lymphadenopathy present more in patients with ALL (74.2%, 51.6% and 64.5% respectively). Lymphadenopathy shows significant statistical difference with types of acute leukemia (p= 0.005).



Graph 1: Distribution of organomegaly in AML subtypes.

Organomegally was more common in M4 than other subtypes of AML, splenomegaly was (40%), hepatomegaly (33.3%) and lymphadenopathy (100%). Followed by M5, splenomegaly (30%) and hepatomegaly (33.3%), and then M2 where splenomegaly (30%) and hepatomegaly (16.7%).



Graph 2: Distribution of organomegaly in ALL subtypes.

In ALL- L1 splenomegaly was (52.2%) and lymphadenopathy (60%), whereas in ALL-L2 splenomegaly was (43.5%) and lymphadenopathy (35%).

Table 6: Mean duration of symptoms prior to presentation (days) among patients with newly diagnosed acute leukemia types No. =36.

Type of acute leukemia	Mean	p-value
AML (No.13)	36	0.509
ALL (No. 23)	48	
Note: - were 2 cases excluded as undifferentiated, and cases with extreme value T-test, p<0.05.		

The mean duration of symptoms (days) in patients who newly diagnosed, was 48 days in ALL and 36 days in AML. There was no significant statistical difference between duration of symptoms and types of acute leukemia.

Table 7: Peripheral blood findings in patients with AML and ALL.

Blood investigation	AML No. = 20		ALL No.= 31		P-value
	Mean	Range	Mean	Range	
Hemoglobin (g/d)	7.13	4.3-10	7.51	3.5-12	0.695
Red blood cells count ($\times 10^{12}$ /l)	2.60	1.6-5.37	2.98	1.3-6.6	0.383
Total white blood cell count ($\times 10^{9}$/l)	65.022	0.400-300.0	44.703	1.600-233.00	0.032

Platelet count (×10 ^{9}/l)	73.65	13.0-239.0	84.42	6.0-250.00	0.427
MCV	90.22	71.7-105	83.3	68-101	0.476
MCH	28.13	20.7-35.5	26.4	20.1-32.9	0.982
MCHC	30.94	26.1-37.9	30.7	15-35.4	0.779
Erythrocyte sedimentation rate (mm/hr)	70.95	5-135	77.5	5-142	0.068
Mean of peripheral blasts	58.7	7-99	78.1	2-99	0.021
Note: - were 2 cases excluded as undifferentiated * T-test, p<0.05.					

The total WBC count was more elevated in AML patients with 65.0 ×10^{9}/L, while in ALL patients it was 44.70 ×10^{9}/L. There was significant statistical difference between WBC count and types of leukemia (p= 0.032).

The mean erythrocyte sedimentation rate (ESR) was more elevated more in ALL than AML patients with 77 mm/h r in ALL and 70 mm/hr in AML patients (p= 0.068). The mean of the peripheral blast was 78.1 in ALL patients and 58.7 in AML patients. There was a significant statistical difference between the mean of peripheral blast and types of leukemia (p= 0.021).

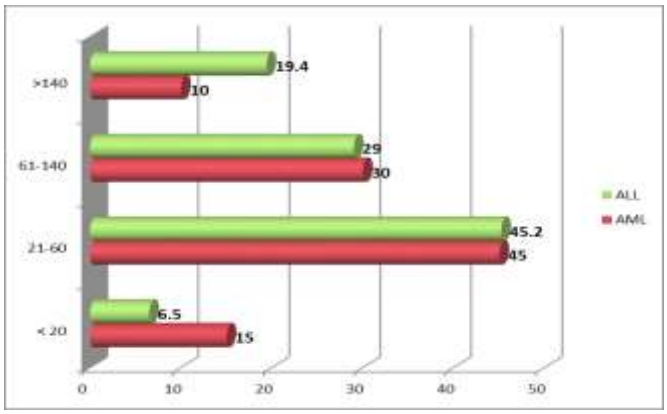
Table 8: Distribution of peripheral blood count in patients with AML and ALL according to severity, No =51.

	AML No=20	%	**ALL No=31**	%	**P-value**
Degree of anemia g/d					
Severe anemia < 7	10	50	13	41.9	0.821
Moderate anemia 7-9	6	30	10	32.3	
Mild anemia 10-12	4	20	7	22.6	
Non anemic >12	0	0.0	1	3.2	
Total	20	100	31	100	
Distribution of platelet counts×10 ^{9}/l					
< 20	3	15	2	6.5	0.662
20 – 60	9	45	14	45.2	
61 -140	6	30	9	29.0	
>140	2	10	6	19.4	
Total	20	100	31	100	
Distribution of WBC counts×10 ^{9}/l					
<4.0	3	15	5	16.1	0.835
4.0 – 10	5	25	7	22.6	
11 – 20	2	10	5	16.1	
21 – 50	3	15	4	12.9	
51 – 100	2	10	6	19.4	
> 100	5	25	4	12.8	
Total	20	100	31	100	
Distribution of Absolute neutrophils counts×10 ^{9}/l					
< 0.5	4	20	9	29	0.389
0.5 – 2	5	25	11	35.5	
> 2	11	55	11	35.5	
Total	20	100	31	100	
Note: were 2 cases excluded as undifferentiated % taken from the total column * Chi-square test p<0.05					

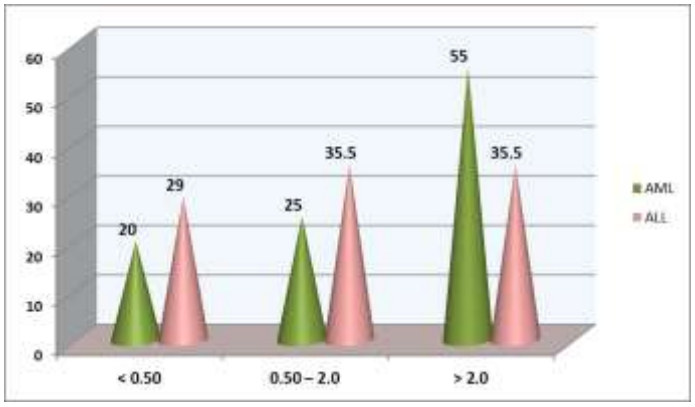
The most common degree of anemia in both types of acute leukemia, was severe anemia, constituting (50%) in AML and (41.9%) in ALL patients. The platelet counts less than 60×10 ^{9}/l was present in (60%) of AML patients and in (51.7%) of ALL patients. A normal platelet count of >140×10 ^{9}/l was present in 19.4 % of ALL patients and in (10%) of AML patients.

Patients with WBC count of ≤10 ×10 ^{9}/L were found in (40%) of AML and in (38.7%) of ALL patients, a WBC count >10×10 ^{9}/L present in (60%) of AML and in (61.2%) of ALL. Severe neutropenia (<0.5X10^{9}/L) was present in (20%) of AML patients and in (29%) of ALL patients.

There was no significant statistical difference between (degree of anemia, platelet counts, WBC counts and ANC) and types of acute leukemia.



Graph 3: Distribution of platelet counts in patients with AML and ALL.



Graph 4: Distribution of Absolute neutrophils count in patients with AML and ALL.

Table 9: Morphological abnormalities of blood film in patients with AML and ALL No.=51.

Blood film finding	AML *no=20*		ALL *no=31*		Total		p-value
	No.	%	No.	%	No.	%	
Red blood cells:							
Target cell	6	30	9	29	15	29.4	0.914
Polychromatia cell	14	70	19	61.3	33	64.7	
Normoblast	11	55	12	38.7	23	45.1	
Tear drop cell	4	20	7	22.6	11	21.6	
White blood cells:							
Pseudopelger-Huet anomaly	8	40	7	22.6	15	29.4	0.183
Blast cell	20	100	31	100	51	100	1.000
Platelets:							
Few platelets per field	15	75	24	77.4	39	76.5	0.842
Note: - were 2 cases excluded as undifferentiated % taken from the total column * Chi-square test p<0.05.							

Table 9 shows that there were a least difference in the morphological abnormalities of red blood cells findings, between ALL and AML patients and P value were (0.914). There was no significant statistical difference between abnormalities in blood smears in patients with acute leukemia and types of leukemia.

DISCUSSION

Leukemias are the second most common malignancy seen in both adult sexes and the first most common malignancy in adult male patients in southeastern Yemen.[19] This is in contrast to the data based on NCI SEER Program (National Cancer institute and Surveillance, Epidemiology and End Results) (1975-2003) which reported adult leukemias to be the tenth

form of malignancy affecting approximately 12.8 persons per 100,000 in the United States annually.[20]

In this study, males were more affected with leukemia than females with M: F ratio of 2.3:1. These results are similar to other studies.[12-16,19-38]

The commonest age group of AL 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%). This result was similar to Mohammad Bashir (Peshawar) and Malaysia studies.[16,19] Hassan et al (Pakistan) reported 62 cases of AML, where all morphological subtypes occurred in patients aging between 25 and 29 years,[20] while in other studies it had two peaks in the extremities of life.[21-31]

Varying degrees of the distribution of types of leukemia in adults and children have been noticed. In children, the ALL is the commonest type of leukemia (19/22 cases with 86%) and AML (3/22 cases with 14%) which was similar to other studies.[12,16,19,23,24] In children, the mean age of ALL was 6.6 years, this was in an agreement with Middle East Childhood Cancer Alliance (MECCA) where the mean age of ALL was 6.1 years.[39] In Muhammad Idris (Pakistan) study, the median age being 4.5 years, in South- Nigeria the presenting mean age was 4.4 years.[24,27] For adult AML, the mean age was 41.6 years which is in an agreement with the pattern of other local studies, (AL-Ghazaly, Sana'a was 39.1 years and Abdul-Hamid's study in Aden was 35 years),[13,14] In other studies such as Aga Khan (Pakistan), it was 32year, Saudi Arabia was 31.4 years, while in Japan the study reported 45 years and so did other studies.[15,16,18,161,162]

This result seems to be lower than the expected mean age reported in western countries where AML peaks in incidence after the 6th life decade.[28,29]

In geographic distribution, most cases were from Lahj governarate (35.8%), followed by Aden and Abyan. In study of Bawazir, Abdul-Hamid, et al (1998) on cancer in the south eastern governorates of Yemen, more than 50% of the patients were from Aden, followed by patients from Lahej, Abyan, Hadhramout and Shabwa.[40]

For most patients with acute leukemia, the cause of the disease is unknown. More than 90% of secondary leukemias are of myeloid origin and have a particularly poor outcome.[154] In the presented study 6 cases were secondary AL, whereas 83.3% of these cases were AML and 16.7% were undifferentiated leukemia.

FAB system provide structured criteria for the diagnosis of various subtypes of AL and is based mainly on morphological and cytochemical features; for some of the categories, immunophenotyping is necessary.[6,9] WHO classification of AL is not practiced widely at national levels because of financial constraints (included immunophenotyping and cytogenetic studies).[9] So that FAB is still favorite and popular among hematologists in many developing countries and including Yemen.

From the 53 cases diagnosed as acute leukemia, there was preponderance of acute lymphoblastic leukemia 31 cases (58.5%) of acute leukemia cases, acute myeloid leukemia 20 cases (37.7%) of AL cases and undifferentiated leukemia constituted 2 cases (3.8%) of AL cases. These results are similar to other studies that include both adult and pediatric acute leukemia patients.[16,19,24,35] FAB M2 with (40%) was the commonest AML type, it is similar to other studies,[23,31] followed by (25%) of FAB M4, Nakase et al showed FAB M4 as common subtype in the Australian population compared to the Japanese population, where FAB M2 is common.[25] However, two studies one from Iraqi reported predominance of FAB M3, and the other from Saudi Arabia with predominance of FAB M4.[15,22]

Acute lymphoblastic leukemia is the most common malignant disease affecting children accounting for approximately 30% of childhood cancers. FAB classification showed a higher percentage of FAB L1 subtype in children and FAB L2 subtype in older children and adults. FAB L1 subtype with 51.6% cases was the commonest type of acute lymphoid leukemia, FAB L2 constituted 45.2% of cases and FAB L3 3.2% of cases, this is the same as in other studies.[23,27,32]

Patients with AL present most frequently with signs of the uncontrolled growth of leukemic cells in bone marrow, lymphoid structures, and other sites of extramedullary spread (*e.g.*, CNS, lymph nodes, liver, spleen, and kidney).[33,41] The most common present symptoms in this study were fatigue, fever, pallor, purpura, and bone pain, as in lectures.[34] Fatigue was (82.4%) and fever was (78.4%) respectively, fatigue in these patients was the result of anemia, while fever may be attributable to infections. Other studies have shown similar pattern of presentation; in Mohammad Hammod study, fever 94% and fatigue 72%; in Saudi Arabi, fever 34.6%; while in Peshawar study, fever 90% and fatigue 84%; and in South Nigeria, fatigue 98% and fever 94%.[12,16,21,23] Weight loss (54.9%) was due to hypermetabolism or impaired metabolism, where the rate of catabolism is higher than that of anabolism,[41] these results similar to other studies.[12,6,21,23,29, 34]

Bleeding problems ranged from mild such as (petechiae, bruising, and mucosal bleeding to severe such as CNS hemorrhage). Bleeding was present in 50% of AML and in 25.8% of ALL. Bleeding per gum was present in 35% of AML and in 9.7% of ALL, while bruising had showed no difference between AML and ALL (30 % and 22.6%). In Mohammad Hammond's study bleeding present in 50% of AL, while in a Saudi study bruising represents 30.7% of AML. In South Nigeria bleeding in AML was 50% and in 20.6% of ALL; and in an Iraqi study, bleeding was 41% of AML.[12,15,22,24]

In the present study, splenomegaly was present in 50% of AML patients and hepatomegaly in 30%. In Saudi Arabia study, splenomegaly was 30.7% and 13% was hepatomegaly of AML patients.[162] In South Nigeria splenomegaly was 20% and hepatomegaly was10% and so in other studies.[16,24,30,32]

In the present study, 74% of ALL cases had splenomegaly, 51.6 % had hepatomegaly and 64.5% had lymphadenopathy. In Peshawar's study splenomegaly was 75%, hepatomegaly was 67.8% and lymphadenopathy was 64.3% of ALL,[16] while in South Nigeria splenomegaly was 32% and 20.5% was hepatomegaly.[24]

Gingival enlargement was found in 10% of AML cases of present study, in Iraqi study was found in 15.8% and in Pakistani study in 27% of AML cases.[15,16]

The average duration of symptoms prior to presentation was about 36 days in AML and 48 days in ALL with an average of few days to weeks. This result is similar to that in an Iraqi study and more than that the South Nigerian study.[15,24] Late presentation is common in many developing countries in contrast to early presentation seen in the developed countries. The reasons for late presentation are ignorance and poverty and the low quality of health services. Late presentation has become a serious impediment in the management of patients with hematological malignancies as well as other malignancies.[176] Patients who present late tend to have unfavorable outcome with treatment. It is noticed that in the ALL children have low average duration of symptoms prior to presentation (30 days) than adults ALL (60 days) and P value is (0.002), this may be because there is more care to children complaints than adults.

Haematological parameters on presentation showed that patients with acute leukemia had low mean hemoglobin level, low platelet count and low to very high white blood cell count.

Anemia was present in most cases; it ranged from mild to very severe degree. Mean hemoglobin level of AML was (7.13g/dL), it was same as other studies.[15,16,21,32]

In the present study, moderate to severe anemia (Hb <9.0 g/dl) was observed in (80 %) of AML and in (74.2%) of ALL patients, which is similar to Peshawar's study that reported (80%) of AL had Hb concentration of <9.0 g/dl.[16] In an Iraqi study (84%) of AML patients had Hb concentration of <10.0 g/dl, in Iranian study (73.7%) of AML and (67.8%) of ALL patients had Hb concentration of ≤10.0 g/dl, and in a Malawi study, more than 90% of AL had Hb concentration of <9.0 g/dl.[15,173,177]

The majority of the patients (82.3%) presented with thrombocytopenia. Marked thrombocytopenia (<60 $X10^9$/L) was found in (60% of AML and 51.7 of ALL). These results are within the range of reported studies such as Mohammad Hammod, Aden (56%) of ALL patients presented with marked thrombocytopenia, and so do the other studies.[12,15,16,37]

The mean white blood cell count was $65\pm90\times10^9$/L at presentation for AML, this corresponds with Aga Khan 63.4×10^9/L and in South Nigeria, which was 62×10^9/L for AML,[21,24] in contrast to a western study where mean WBC in a Canadian study was 11.1×10^9/L for AML.[31]

About (60%) of patients with AML had WBC count more than normal >10 $X10^9$/L; this is similar to an Iraqi study (53.8%), an Iranian study (46.6 %) and Peshawar's study which showed that 78.6 % had WBC count more than normal.[15,16,37] In this study (61.7%) of ALL patients had WBC count >10 $X10^9$/L while in Mohammad Hammod's study it was (68.6%) of ALL cases, and in Peshawar's study it was (78.6%), and in an Iranian study (35.2 %).[12,16,37] While in western study only 20% of ALL presented with high WBC count.[38]

Patients with higher WBC count have bad prognosis due to hyperviscosity, vascular infarction of CNS and lungs, and more complications of bone-marrow failure.[41] Therefore, it may be one of the factors for poor prognosis in our setup, as compared to Western countries.

Absolute severe neutropenia (<$0.5X10^9$/L) was observed in (20 %) AML and in (29%) ALL. Our results were lower than those presented in Iraqi and Philippini studies (49.2% and 64% respectively).[15,42]

The erythrocyte sedimentation rate (ESR) was elevated significantly in both types of AL but were more in ALL because of the long duration of disease than AML. ESR is often used as an indicator of active disease, although this test is considered a nonspecific test.[43]

In a blood smear, red cell morphology was mildly abnormal, with exaggerated variation in cell size and occasional poikilocytes. Nucleated red cells were present in both types of AL but were more in AML. Less often, extreme abnormalities of red cell size, shape, and hemoglobin content were present, these findings were similar to the lectures.

Despite of the recent advances in field of molecular hematology and flow cytometry, bone marrow examination remains the cornerstone in the diagnosis of acute leukemia. The diagnosis of acute leukemia requires blasts that comprise 30% or more of bone marrow cells, (The WHO classification proposes to change this to 20% blasts).[10] For differentiating ALL and AML, a bone marrow aspirate is necessary.

Bone marrow examination at presentation revealed depression of normal three cell lines in most acute leukemia patients.

In present study, bone marrow findings in patients with acute leukemia showed hypercellular bone in most patients with AML and ALL. Hypoplastic AML findings in bone marrow were seen in one case (5%) AML, in Al-Kali et al study (USA) on large numbers of patients it was (9%) of AML patients.[44]

In the present study, decreased erythroid precursors were present in most patients of AML and ALL (90% and 96.8% respectively), as in lectures.[9] While increased of erythroid precursors seen in 5% of AML cases, but <50% of total nucleated bone marrow cells.

The nucleus was round in (54.8%) ALL and in (30%) AML, in Neelma's search it is in most cases of ALL and AML.[45] However, the nucleus had indentated in 20% of AML, while in Neelma search it was 10%. Nuclear chromatin was fine in blast cells of acute myeloid leukaemia, and coarser in acute lymphoblastic leukaemia. However, in (15%) AML cases the chromatin was coarse, while in Neelma search it was (32%) of AML,[45] (on cytochemistry they proved to be myelo-blasts). Auer rods were present in 20% of AML cases, while in other studies it is about 40%.[21,45] Cytoplasmic budding was present in 45 % of AML cases, while in Neelma search 35 % of AML. In acute lymphoblastic leukaemia group, there were (12.9%) cases with vacuoles in the cytoplasm in Ramanowsky stained preparations, this was similar to Neelma's study.[45] Large cell size was seen in 12.9% ALL cases, Lilleyman J S, et al, study was seen at (7%).[46]

In the present study, in the FAB M1 (one case) the blasts are often predominantly type I, which was morphologically similar to lymphoblasts, no Auer rods were seen in this case; myeloperoxidase (MPO) stain of BM was more than (50%) positive of blast cells (Slide:9,31). While in FAB M2 (8 cases) the blasts are predominantly type II blasts, Auer rods were present in (37.5%) of FAB M2, these results were similar to Roland Mertelsmann, et al.[47] MPO reactions in FAB M2 cases are variable; some case with low MPO reactions <20% and other cases > 90% of blast cells; the PAS reaction is negative with a weak diffuse reaction, which is an agreement with other studies.[9,45,48] Dysplastic features, such as hypersegmented neutrophil were found in (12.5%) of M2, and Hyposegmented neutrophils (Pseudo-Pelger Huet) abnormalities in (50%).

In Steven A. et al, study on 13 cases of therapy-related AML (t-AML), found that in 8 of 13 cases, the blasts were large with indented nuclei, a perinuclear hof, and salmon-colored cytoplasmic granules was FAB M2.[49] In this study, there was one case (12.5%) FAB M2 was post therapy (radiotherapy), and with characteristic perinuclear hof, indented nuclei and strongly positive MPO.

In the FAB M3 (2 cases); one was hypergranular promyelocytic and other was variant leukaemia. In acute hypergranular promyelocytic leukaemia type, the predominant cells were promyelocyte cells (57%), the cytoplasm of which contains coarse with brightly staining granules (Slide-13), but no Auer rods were seen. In Matsuo T, et al, study they were noted in fewer than 50% of acute promyelocytic leukemia cases had Auer rods.[50] The other case of M3 was the variant type, which had few granules with few Auer rods. No dysplastic changes were found in the three lineages of both cases. The WBC were found low in hypergranular (1.5×10^9) while elevated in variant (22×10^9), this finding was similar to other studies; where a low a white cell count (WBC) were noted in hypergranular leukemia, while higher WBC were seen in variant type.[51] MPO was strongly positive (100%) of blast cells in both hypergranular and variant types, this result was similar to other studies.

In this study, 5 cases were diagnosed as FAB M4 and one case (20%) as FAB M4 with esinophilia. The criterion for morphological diagnosis of FAB M4, are recognition of a granulocytic component must be at least 20% of non-erythroid cells, and marrow monocytic component (monoblasts to monocytes) ≥20% of non-erythroid. Alessandro Pulsoni (Italy) reported (22%) typical eosinophilia was observed in 400 cases of M4-AML. A minimum of 5% of bone marrow eosinophils has been suggested as a criterion for the recognition of eosinophilic differentiation.[9] In this study, one case of FAB M4 had esinophilia (14%) of total marrow cells, (there was both neutrophilic and eosinophilic differentiation). Abnormal eosinophils are detectable, it shows irregular staining granules and there is some mature eosinophils showing vacuolation and nuclear hypolobulation these finding was similar to other studies.[52]

The FAB criteria for the recognition of monocytic differentiation is the presence of fluoride-sensitive naphthol AS acetate esterase (NASA) or NASDA activity,[45,53] but in our condition, when these stains are not available, we depend on morphology in three cases and the available immunophenotyping for two cases.

FAB M5 is subdivided into M5a, poorly differentiated (>80% monocytic cells including monoblasts), and M5b, well differentiated (80% monocytic, predominantly promonocytes and monocytes). In this study monocytic leukaemias (4 cases), there was one patient (25%) with M5a and 3 patients (75%) with M5b. The dysplastic features were observed in 2 cases (50%), this result was similar to other studies in which dysplastic features were common in M5.[53,54] MPO staining in monoblasts was scattered and had a lower score than other AML subtypes, in M5a it was negative, promonocyte in FAB-M5b were more frequently MPO or SBB positive, as in lectures.[9]

AML/TLD is characterized as a subtype of de novo AML that shows morphological dysplasia of mature

hematopoietic cells on a background of leukemic blast cells.[17]

The frequency of trilineage dysplasia in AML was reported as 11.6% in Goasguen and colleagues and 12% in Tamura and associates.[55,56] In two lineages, accounting for the higher percentage (38%) of cases in that category in the Daniel A, et al study.[57] In the present study, two lineages dysplasia were found in 25% of AML, and three lineages dysplasia in 10% of AML cases (Slides:21-30). In the present study, 5 cases (71.4%) were new cases (de novo), and 2 cases were secondary cases.

The French, American, and British (FAB) system classification of ALL is based only on the way that leukemia cells are seen under the microscope after routine staining, are recognized 3 groups: L1, L2, and L3. In L1 ALL morphology, it is small cells, up to twice the diameter of a red cell. The present study showed L1 (16 cases) mainly small cells with high nuclocytoplasmic (N/C) ratio, poorly visible nucleoli and regular nuclear outline, as seen in (Slide: 17).

In FAB L2 (14 cases), the blasts were larger and, there was more heterogeneous population, L2 may be indistinguishable from the M1 variant of myeloid leukaemia, and the differentiation was made primarily by myeloperoxidase (MPO) staining.[9] In this study, one case had few coarse azurophilic granules or unusual Chédiak-like inclusions (Slide: 19), which negative MPO and positive PAS. This finding is similar to Sharma S, etal, study, that described a case of ALL with intracytoplasmic inclusions, these inclusions stained negative for MPO, Sudan Black E (SBB) and ANAE and positive for PAS, and so other study by Brunning RD, et al.[58,59] The L3 cells are medium sized to large and are characterized by basophilic and moderately abundant cytoplasm with prominent cytoplasmic vacuolation in the bone marrow, but is not necessarily in the peripheral blood,[9] In this study only one case with characteristic FAB L3.

Lymphoblasts showed negative reactions for MPO and the great majority of neutrophils of ALL were strong MPO positive, which was similar to lectures.[9] The PAS stain often shows characteristic block positivity in 75% of ALL cases.[6,60] In the present study, PAS stain was positive in 80.7% of ALL cases, and 4% of these cases showed only a single block positivity (FAB L1) (Slide:39), and other cases (96%) showed mix of single and granular block. Snower and associates reported PAS stain positivity in 52% ALL.[61]

In B-lineage ALL, the PAS stain often shows a characteristic block positivity. This is seen also, perhaps less often, in T-lineage ALL. In ALL, the blocks and coarse granules of positively staining material are present in PAS-negative cytoplasm. Whereas in the case of the block positivity that is seen much less often in cases of AML (mainly in monoblasts and erythroblasts), the PAS-positive blocks are in cells with a background diffuse or finely granular positivity,[9] In this study, there was three cases (15%) of AML with block positivity; two case myelomonocytic (FAB M4) and one monocytic leukemias. This was similar to a study of Snower and associates, where there was (14%) of AML cases had PAS-positive blocks.[61]

So, although PAS staining can be useful in the diagnosis of ALL, it is important to recognize that PAS block positivity alone is not a sufficient basis for this diagnosis. Because of their lack of specificity, cytochemical stains should be regarded as redundant in the diagnosis of ALL unless immunophenotyping is unavailable (as in our case). On the other hand, when used, there must be a constant awareness of their lack of specificity.[61,62]

There were studies that found correlation between PAS positivity and blast vacuolation. In Lilleyman's study, found relationship between the presence of vacuoles, PAS positivity, a relatively low WBC and the presence of the common ALL antigens (CD10) P<0.0001. When cases had both vacuoles and PAS positivity the chance of CD10 being positive was 98%.[63] In the present study, there were found associated with the presence of cytoplasmic vacuoles and PAS positivity, low mean presenting WBC and low mean age than cases with cytoplasmic vacuoles (P= 0.078) (Slide: 43,44).

The relapse marrows (2 AML and 6 ALL) were also reviewed in the acute leukemia to see if there were any changes in morphological diagnosis. In most relapses, the cell type was found to have remained the same as at the time of initial diagnosis. In a one case (50%) AML (Slide; 12, 33), there was a change; the blasts were large, vaculated, and appeared less differentiated compared to the initial marrow. In this case a definitive diagnosis was difficult without referring to initial marrow aspirates. Roland Mertelsmann, et al, reported in his study, that most relapse marrows were with no changes in morphological diagnosis and in a few cases, where there was a change, the cells appeared less differentiated, i.e., M2 became Ml, and rarely M5b became M5a and the blasts were large and appeared primitive with more basophilic cytoplasm.[47] For ALL relapses (2 L1 and 4 L2), and at initial diagnosis (4 L1 and 2 L2), where there were 2 cases (33%), FAB L1 changed to FAB L2. The commonest features in this study, the cells size tended to become larger and the prominence of nucleoli. In a study of (Lilleyman, et al 1995), a similar finding, six (5%) were classified as FAB type L2 at diagnosis, compared with 18 (13%) at relapse.[64] At another study, 33 patients (23 children and 10 adults), nineteen patients changed their FAB type from L1 to L2, and the event was as frequent in children as adults.[65]

Cases of acute leukemia showing morphological and cytochemical features of both myeloid and lymphoid differentiation or not being classified were categorized as

acute leukemia, cytochemically unclassified. In this study, cytochemical stains led to a change of diagnosis in 3 cases (5.7%) of total cases (2 cases from AML to ALL, and one case from ALL to AML), and helped to confirm the diagnosis in 95% of AML cases and in 80.5% of ALL cases. In a study of Roland Mertelsmann, et al, study cytochemical stains were essential in establishing the diagnosis in 9%, leading to a change of diagnosis by one observer team in 14%, and helping to confirm the diagnosis in 32% of cases.[47] Sushma Belurkar, et al reported a total of 22/33 (66.7%) ALL cases and 11/12 (91.6%) AML cases could be assigned correct lineage based on morphology and cytochemical staining (PAS, MPO),[19] and when study the correlation between morphologic and flowcytometry diagnosis showed complete partial concordance in 86% of the cases. Cytochemical stains used in the study by Mhaweek et al, included Sudan-black, specific esterase (alfa-naphthyl ASD chloroacetate esterase), non-specific esterase (alfa-naphthyl butyrate esterase), Periodic acid-Schiff, and acid phosphatase.[18] Definite diagnoses were made for all 10 of their AML cases, whereas diagnoses were possible in only 79.4% patients with ALL when only morphology and cytochemical staining was used. In the rest of the cases, cytochemistry did not aid in diagnosis and hence they opted for flowcytometry to render a definitive diagnosis.[18]

Although SBB may be more sensitive in some leukaemic blasts that are myeloperoxidase negative and may show weak SBB staining, myeloperoxidase is a highly specific cytochemical marker for myeloblasts.[66] Rare cases of SBB positive ALL have been reported.[67,68] In this study, The sensitivity and specificity of the MPO stain alone for myloblastic leukemia was 95% (19 true positives of 20) and 100% (no false positives), respectively. In Sushma Belurkar, et al study the sensitivity of the MPO stain alone for myloblastic leukemia was 91.6% (11 true positives of 12).[19]

In this study, the sensitivity and specificity of the PAS stain alone for lymphoblastic leukemia was 80.6% (25 true positives of 31) and 85% (three false positives), respectively. Snower and associates reported that the sensitivity and specificity of the PAS stain alone for lymphoblastic leukemia was 52% (15 true positives of 29) and 81% (four false positives), respectively.[61]

In our study, the median survival of patients with leukaemia, was 234 days for AML and for ALL 407 days. It is more than South Nigeria (AML 180 days and ALL 240 days),[24] in Saudi Arabia (AML 343 days), in Canada (AML 381 days) and in Iran (AML 280 days and for ALL 303 days).[22,31,37] The lower median survival noted in our study was a result of multiple factors which included late presentation, inability to maintain treatment, non-compliance with treatment regimen as a result of ignorance and most importantly poor or inadequate supportive management.

There are several factors that determine the possible prognostic outcome of patients with AML (age, WBC count, cytogenetic abnormalities, time to achieve complete remission, immunophenotype, dysplastic feature and MPO percentage).

In this study, patients below 50 years old had more survivals than those above 50 years with (P= 0.030), which is similar to other studies.[18,29,37,69] In the WBC count study, there were two groups (WBC count $<50\times10^9$/L and $>50\times10^9$/L), at 6 months survival we had equal result for both groups (P= 1.000) and the result change at 1year survival was (P= 0.491*)* where the survival was more in the group with WBC less than 50×10^9/L. This result difference from other studies where the WBC count had a significant effect on prognosis, and was considered as an independent adverse prognostic factor for achieving complete remission and was associated with shorter over survival.[31,37,69] This because that we have a limited number of cases (12 cases) and because many patients, with very high WBC count died before starting chemotherapy.

Although, there were 7 cases that had MLD, only 2 cases (28.6%) received chemotherapy, and the other cases died before therapy start. The present study suggests that the MLD cases had less survivals than cases without MLD (P= 0.028), this result is corresponds with other studies. Goasguen, et al found that the presence of dysgranulopoiesis was associated with a significant decrease in the ability to achieve a complete remission, while Tamura, et al, found the presence of trilineage dysplasia to be associated with lower disease-free survival, and Gahn et al, found that dysplastic changes of granulocytes and megakaryocytes were associated with a worse event-free survival in a series of 102 patients with AML.[55,56,60] Other studies demonstrate that the category of dysplasia cannot be accepted as a general and independent criterion for the classification of AML or for the definition of prognostic subgroups.

It has previously been suggested that the percentage of MPO positive blast cells have an impact on clinical outcomes of AML.[71,72] MPO expressions at diagnosis in AML patients could be one of the indicators that help identifying patients who benefit from transplantation.[71] The ECOG study shows that patients with more than 50% MPO-positive blasts had a significantly better CR rate in the AML M1 subtype (P 0.003).[72] A similar Japan Adult Leukemia Study Group (JALSG) reported a significant importance of the percentage more than 50% of MPO positive blast cells for survivals in a large population.[211] However, Suĭć M, et al, in his study of FAB M2 AML, reported opposite results that a high proportion of MPO positive blasts before treatment may have constituted a significantly unfavorable prognostic factor.[73] So far, the clinical prognostic value of MPO expression in AML has been controversial.

In our study, MPO expressions (Patients were divided into two groups using the percentage of MPO-positive blast, high ≥50% and low <50%), at 6 month survival, we didn't find difference between the two groups of AML (P= 1.000), and at 12 month this value was changed to (P = 0.273) whereas cases with more MPO expressions in blast cells had better outcome. In this study, no prognostic significance of the percentage of MPO positive blast cells in AML; it may be due to limited number of cases.

In acute lymphoblastic leukemia, the age of patients with ALL (adult and pediatric groups) significantly correlates with the clinical outcome, whereas better outcome in pediatric group with P-value (0.011) in one year survival, and in 18 month survival P-value (0.054). This result recognized the more favorable outcomes for pediatric group over adult group and is in a concordance with other studies, where long-term survival achieved in over 80% of pediatric and cure rates for adults with ALL remain relatively low, with only 40% of patients cured.[74,75] In an Iranian study the survival more in patients less than 15years old P value 0.001.[37]

Along with age, the initial peripheral blood leukocyte count is one of the first identified prognostic factors in childhood ALL. Since 1996, based upon guidelines developed by the Cancer Therapy Evaluation Program (CTEP) of the National Cancer Institute (NCI), a leukocyte count of 50×10^9/L has typically been used as the cut-off to classify patients as either high risk or low/standard risk.[62] In this study, at 18 months survival, there were (37.5%) of cases with WBC $>50\times10^9$/L was alive, while (61.1%) of the cases with WBC count $<50\times10^9$/L was alive. The significant difference between the two groups could not be observed as the duration of follow up was only 18 months. In Japanian study a significant result was reported at 100×10^9/L but not at 50×10^9/L.[76]Alison M and Howard J recognize that children with WBC above 50×10^9/L being at higher risk, in an Iranian study initial higher WBC count was significantly related to death with p=0.001.[37,77]

Male children with ALL continue to show poorer prognosis. The Surveillance Epidemiology and End Results (SEER) is an example of a large database that allows for the assessment of the effect of sex on leukemia reported a continuous poorer survival boys. In this study at 18 months survival there was a prognostic significance in sex differentiation with P=0.021, all females who received chemotherapy (100%) were alive and only 42.9% of males were alive. In Japan female with more survival p=0.0025, and so other studies.[76,77]

The correlation of Periodic acid Schiff (PAS) reaction with the prognosis, have yielded a conflicting and confusing literature. Some studies suggest that strong PAS-positivity indicates a good prognosis,[78,79] but others claim that it has no such significance (Lanham GR, et al).[80] In the first studies, appears that the PAS reaction can identify longer survivors among patients with ALL; but only, in the absence of features, it is strongly associated with a poor prognosis.

In this study, (Patients were divided into three groups using the percentage of PAS positive lymphoblast, <1 %, 1-10 and >10%). In comparing groups, at six months survival and one year no significant difference between groups (P= 0.682) and (P= 0.836) respectively. At eighteen month survival, all cases of ALL who less than 1% PAS positive lymphoblast were dead (100%), and equal results with other groups (P= 0.117). So, we did not found any relation between PAS reaction and prognostic survivors, this may be due to the short period of survival follow up. This results were corresponds with Lanham GR, et al study and difference with other studies of J S Lilleyman, V. Mills and J S Lilleyman, J A Britton.[78-80]

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