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BONE MARROW MORPHOLOGY AND CYTOCHEMICAL STAINING IN DIAGNOSIS AND CLASSIFICATION OF ACUTE LEUKEMIA

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ABSTRACTS

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 numbers 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%). Cytochemical analysis of leukemia, when coupled with morphology confirm the diagnosis in (95%) of AML cases and (80%) of ALL cases. **Conclusions**: The most common type of acute leukemia observed in our study was acute lymphoblast leukemia (ALL) (58.5%), followed by AML 37.7% and undifferentiated leukemia in 3.8% . The AML subtype most common one was myeloblastic leukemia with maturation (M2) (40%).

KEYWORDS: Acute leukemia; FAB classification; Aden hospitals.

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]

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]

In 2001, a World Health Organization (WHO) expert group proposed an updated system for the classification of leukaemia and lymphoma and in 2008 a further updating of the WHO classification incorporated new knowledge and gave a greater importance to molecular genetics features.[8] 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 acute leukemia either AML or ALL, some studies the pattern of adult leukemias in Yemen, concerning age, sex, area of residence whether rural or urban and the type of leukemia were collected and analyzed.[9,10]

Cytochemical stains are extremely useful in the diagnosis and classification of acute leukemias. 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]

Despite the widespread use of immunophenotyping in the diagnosis of hematopoietic neoplasms, cytochemical studies are still of diagnostic importance.[11] This is particularly true of the acute leukemias, although a large panel of cytochemical tests is probably not necessary in most cases.[6,12] In rare patients with inconclusive flow cytometry results, cytochemical stains may provide information which can confirm a diagnosis.[12] 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.[13]

In limited resource centers, 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

This is descriptive study conducted at two Aden governmental hospitals from January 2011 to September 2012. The total numbers 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.

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). Bone marrow aspiration either from supra sternal or posterior iliac crest, with enough slides for Leishman stain and two cytochemistry stains, myeloperoxidase (MPO) for myeloid leukemia and Periodic acid Schiff (PAS) for lymphoid leukemia.

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

RESULTS

Fifty three patients were studied (37 males and 16 females), 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 $\times 10^9$/l. Similarly the platelet count was ranged from 6.0 to 250.0$\times 10^9$/l. Cytochemical analysis of leukemia, when coupled with morphology confirm the diagnosis in (95%) of AML cases and (80%) of ALL cases.

Table 1: Distribution of adults and pediatricpatients 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 1; 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%).

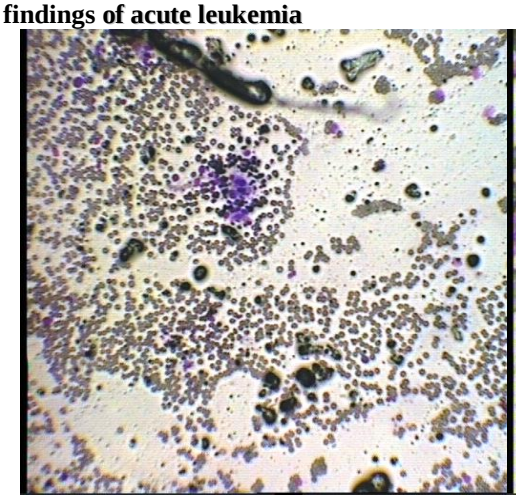
Table 2: FAB classification subtypes among patients with acute leukemia.

Acute leukemia subtypes	Subtype	No.	%
Acute myeloid leukemia	**M1**	1	5
	M2	8	40
	M3	2	10
	M4	5	25
	M5	4	20

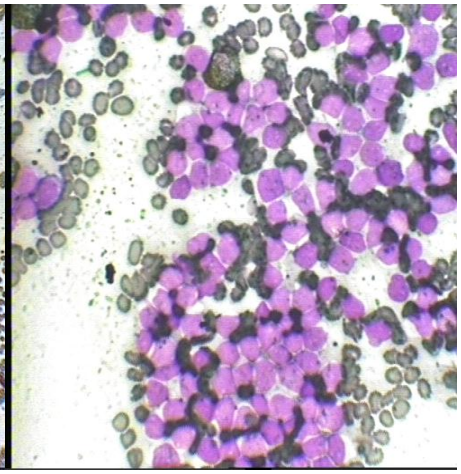
	Total	20	100
Acute lymphoblastic leukemia	**L1**	16	51.6
	L2	14	45.2
	L3	1	3.2
	Total	31	100
Undifferentiated Leukemia		2	3.8
Total		53	100
Note: Percentage calculated in relation to the total population of patient (53)			

Table 2 shows that the most common subtype of acutemyeloidleukemia wasM2(40%)followed by M4 (25%) and M5 (20%).Inacute lymphoblastic leukemia L1was (51.6%) followed by ALL-L2 which was (45.2%).

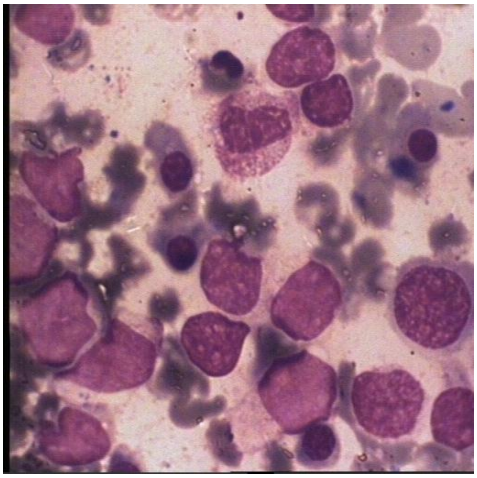
Bone marrow findings of acute leukemia



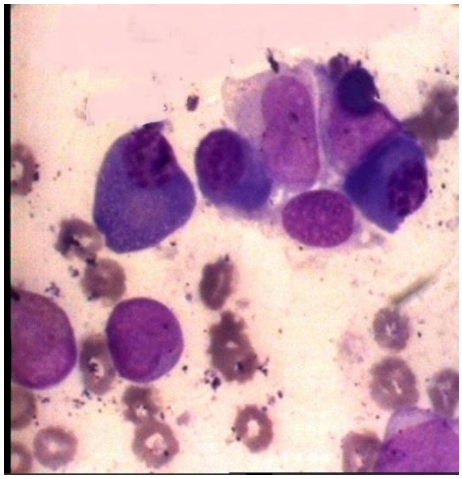
Slide 1: BM smear of M5 with hypocellular and peripheral WBC count 400/µl. (MGG^{x}10, Leishman stain).



Slide 2: BM smear of ALL with hypercellular BM (MGG^{x}40, Leishman stain).



Slide 3: BM smear of AML-M5, showing increase of erythroid precursore. (MGG^{x}100, Leishman stain).



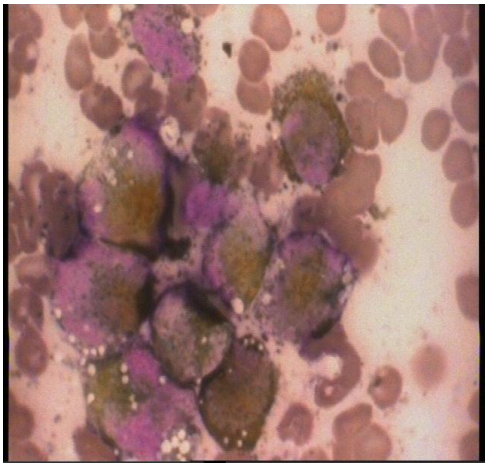
Slide 4: BM smear of M4, with increase of plasma cells. (MGG^{x}100, Leishman stain)

Table 3: Score for Mean percentage of MPO positive blast cells in acute myeloid leukemia subtypes, No.=20.

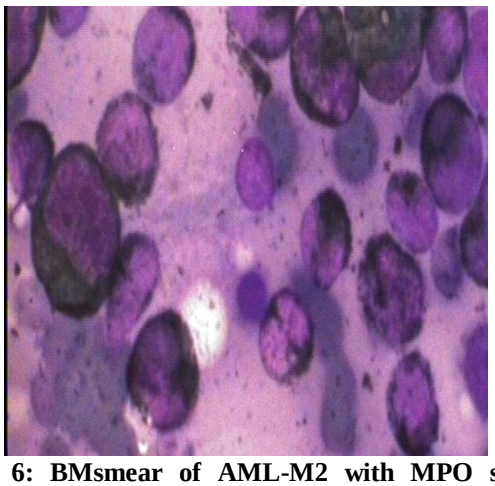
Score for blast cells	**M1**	**M2**	**M3**	**M4**	**M5**	**P-value**
	NO= 1	**NO= 8**	**NO= 2**	**NO= 5**	**NO= 4**	
Score 0 Mean ± SD	31.9	37.4 ± 35.7	000	59.2± 19.1	90.9 ± 2.3	0.022
Score 1	6.4	9.5 ± 9.9	7.2 ± 7.0	16.5± 10.9	5.2 ± 3.6	0.508

Mean ± SD						
Score 2 Mean ± SD	46.3	20.5 ±10.8	24.2± 21.5	14.9 ± 10.2	2.8 ± 1.5	0.038
Score 3 Mean ± SD	14.4	32.7 ± 35.7	68.7± 28.5	9.4 ± 8.6	0.2 ± 0.2	0.081
Score MPO positive cells as follows: 0: negative reaction. 1: Faint localized reaction. 2: Strong reaction localized to cytoplasm. 3: Heavy overall reaction partially obscuring the nucleus. *% taken from the total column * One Way ANOVA test p<0.05						

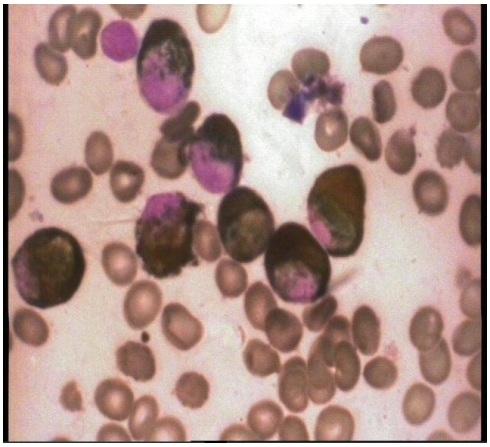
The mean percentage of MPO positive blast cells in AML subtypes scoring 0, presented more in M5 with (90.9%) followed by M4 with (59.2%); there were no negative blast cells in M3 (p = 0.022). In score 1, there is no difference between the AML subtypes with (p = 0.508). In score 3 positive blast cells of AMLpatients could be seen more more in M3 with (68.7%) followed by M2 with (32.7%) and p = 0.081.



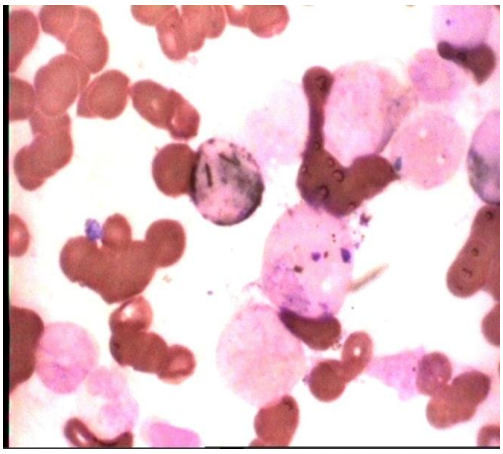
Slide 5: BM of AML-M2 (relapse) with MPO stain: shows strong positive MPO stain with 98% of blasts cells. (MGG^{x}100, counter stain Giemsa).



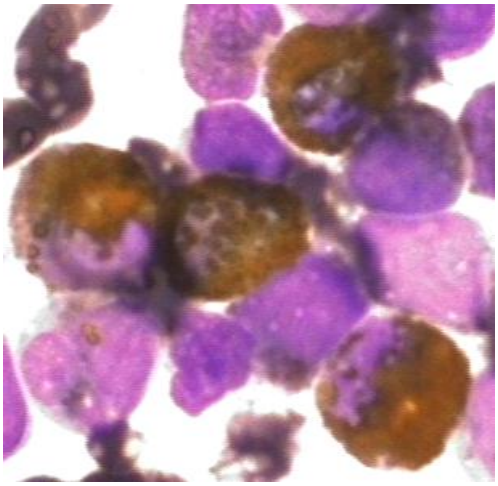
Slide 6: BMsmear of AML-M2 with MPO stain: Marrow aspirate smear shows positive MPO stain with 81% of blast cells. (MGG^{x}100, counter stain Giemsa).



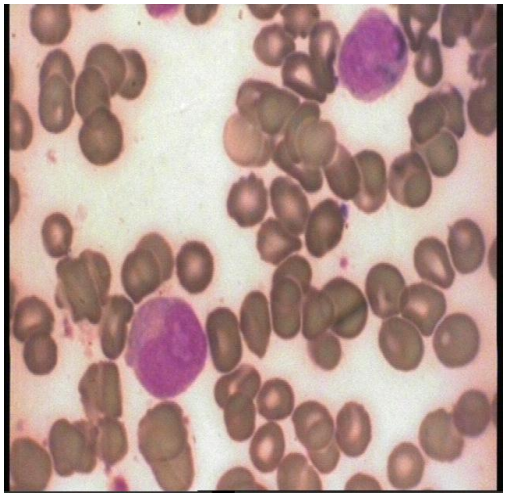
Slide 7: BM smear of AML-M3 with MPO stain: shows positive MPO stain with 100% of blast cells. (MGG^{x}100, counter stain Giemsa).



Slide 8: BM smear of AML-M4 with MPO stain: myeloblast cells showing blast cell with Auer rods.(MGG^{x}100, counter stain Giemsa).



Slide 9: BM smear of AML-M4: shows positive MPOstain 22% of blasts cells and note that mature eosinophilic cells show large golden granules. (MGG^{x}100, counter stain Giemsa).



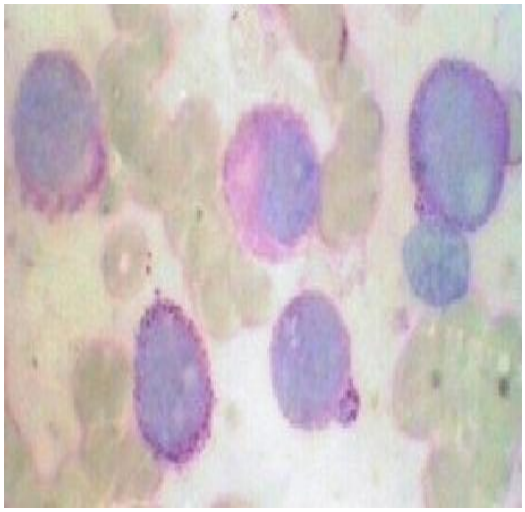
Slide 10: BM smear of AML-M5 with MPO stain;Two monoblast cells one was negative and the other was positive with localized reaction to cytoplasm. (MGG^{x}100, counter stain Giemsa).

Table 4: Mean percentage of positive blast cells of PAS stain in ALL subtypes, No = 24

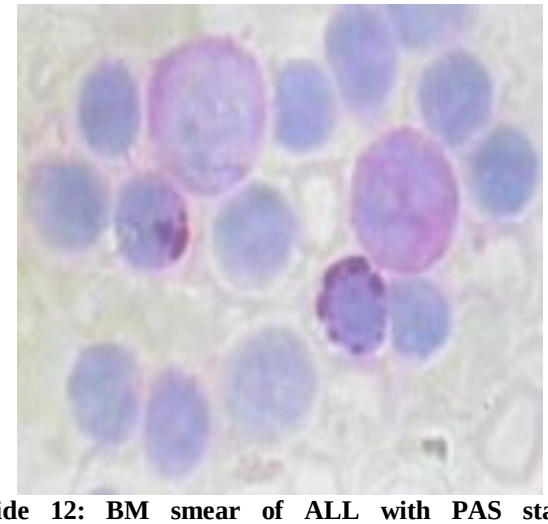
ALL subtypes	Meanpercentage of positive blast PAS stain with range	NO	P-value
L1	24.9 (5-76)	13	0.689
L2	20.7 (5-91)	11	

Note: excluded one case (ALL-L3) because there was only one case and exclude negative PAS cases of ALL
% taken from the total column
* T- test, p<0.05

In table 4 the mean of positive blast cells of PAS stain presented more in ALL-L1patients(with 24.9% of blast cells) and in ALL-L2 was (20.7%) with (P= 0.689).



Slide 11: BM smear of AML-M4 with PAS stain: blast cells with diffuse granulare PAS positive. (MGG^{x}100, counter stain Harris hematoxylin).



Slide 12: BM smear of ALL with PAS stain: Lymphoblast cells with PAS block positive cells, note that diffuse PAS positive pattern of tow granulocyte cells. (MGG^{x}100, counter stain Harris hematoxylin).

Table 5: The results of the cytochemical staining in patients with AML and ALL, No.= 51.

Stains	AML		ALL	
	No	%	No	%
MPO				
Positive	19	95	0	0
negative	1	5	31	100
Total	20	100	31	100
PAS				
Positive	3	15	25	80.6
negative	17	85	6	19.4
Total	20	100	31	100
MPO positive ≥3% of myeloblast cells % taken from the total column Note: Percentage calculated in relation to the total population of patient (51)				

Table 5: shows that the MPO positive cases were (95%) of AML patients, while in the PAS stain, positive cases of ALL patients were (80.6%) and those with negative PAS stainscases were(19.4%).

Table 6: Sensitivity and specificity testes of cytochemistry stains in patients with AML and ALL No.= 51.

Stains	Sensitivity test %	Specificity test %
MPO in AML	95	100
PAS in ALL	80.6	85
Note: were 2 cases excluded as undifferentiated		

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. While 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.

Table 7: Concordance of cytochemistry stains on morphology, No.= 53.

Stains	AML		ALL		UD	
	No	%	No	%	No	%
Morphology						
Initial morphology diagnosis	21	100	30	100	2	100
Concordance of cytochemistry stains on morphology						
Concordance of cytochemistry on morphology	18	85.7	23	76.7	0	0
Discordance of cytochemistry on morphology	2	9.5	1	3.3	0	0
Negative cytochemistry	1	4.8	6	20.0	2	100
MPO positive ≥3% of myeloblast cells % taken from the total column Note: Percentage calculated in relation to the total population of patient (53)						

Table 7: shows that cytochemistry stains concordance on morphology in (85.7%) of AML cases, and in (76.7%) of ALL cases.

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.[14] 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.[15]

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.[6,7] WHO classification of AL is not practiced widely at national levels because of financial constraints (included immunophenotyping and cytogenetic studies).[7] So that FAB is still favorite and popular among hematologists in limited resource centers.

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] FAB M2 with (40%) was the commonest AML type, it is similar to other studies, 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 the common type.[17]

Acute lymphoblastic leukemia is the most common malignant disease affecting children accounting for approximately 30% of childhood cancers.[18] FAB classification showed a higher percentage of FAB L1 subtype in children and FAB L2 subtype in older children and adults.[19] 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 similar other previous studies.[17,20]

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.[21] The diagnosis of acute leukemia requires blasts that comprise 30% or more of bone marrow cells, (The WHO classification proposes 20% blasts).

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.[22]

In the present study, decreased erythroid precursors were present in most patients of AML and ALL (90% and 96.8% respectively).[7,23] 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.[24] 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.[24] Auer rods were present in 20% of AML cases, while in other studies it is about 40%.[24] 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.[24] Large cell size was seen in 12.9% ALL cases, Lilleyman J S, et al, study was seen at (7%).[25]

In the present study, in the FAB M1 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 . While in FAB M2 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.[26] MPO reactions in FAB M2 cases are variable; some cases 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.[7,24] 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.[27] In this study, there was one case (12.5%) FAB M2, and with characteristic perinuclear hof, indented nuclei and strongly positive MPO.

In acute hypergranular promyelocytic leukaemia type (M3), the predominant cells were promyelocyte cells (57%),the cytoplasm of which contains coarse with brightly staining granules 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.[28] In hypogranular variant type, which had few granules with few Auer rods. No dysplastic changes were found in the three lineages of all 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 white cell count (WBC) were noted in hypergranular leukemia, while higher WBC were seen in variant type.[29] MPO was strongly positive (100%) of blast cells in both hypergranular and variant types, this result was similar to other studies.[29]

In this study, 5 cases were diagnosed as FAB M4 and one case (20%) as FAB M4 with eosinophilia. The criterion for morphological diagnosis of FAB M4, are recognition of agranulocytic 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.[30] A minimum of 5% of bone marrow eosinophils has been suggested as a criterion for the recognition of eosinophilic differentiation.[7,30] 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.[30,31]

The FAB criteria for the recognition of monocytic differentiation is the presence of fluoride-sensitive naphthol AS acetate esterase (NASA) or NASDA activity,[24,32] 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).[23]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.[32] 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.[7]

AML/TLD is characterized as a subtype of de novo AMLthat shows morphological dysplasia of mature hematopoieticcells on a background of leukemic blast cells.[11]

The frequency of trilineage dysplasia in AML was reported as 11.6% in Goasguen and colleagues and 12% in Tamura and associates.[33] In two lineages, accounting for the higher percentage (38%) of cases in that category in the Daniel A, et al study.[34] In the present study, two lineages dysplasia were found in 25% of AML, and three lineages dysplasia in 10% of AML 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.[35] The present study showed L1 (16 cases) mainly small cells withhigh nuclocytoplasmic (N/C) ratio, poorly visible nucleoli and regular nuclear outline.

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.[7] In this study, one case had few coarse azurophilic granules or unusual Chédiak-like inclusions, which showed 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.[36] 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,[7,23] 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.[7] The PAS stain often shows characteristic block positivity in 75% of ALL cases.[6,37] 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) , and other cases (96%) showed mix of single and granular block. Snower and associates reported PAS stain positivity in 52% ALL.[38] 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, [7]In this study, there was three cases (15%) of AML with block positivity; two cases myelomonocytic (FAB M4) and one monocyticleukemias. This was similar to a study of Snower and associates, where there was (14%) of AML cases had PAS-positive blocks.[38]

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 . On the other hand, when used, there must be a constant awareness of their lack of specificity.[38]

AML cases could be assigned correct lineage based on morphology and cytochemical staining (PAS, MPO),[13] 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.[12] 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.[12]

In this study, The sensitivity and specificity of the MPO stain alone for myeloblastic 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).[13]

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.[38]

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