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Article

Immunophenotypic and Morphological Spectrum of Leukemia in Diyala Governorate: CD Markers as Key Tools in Diagnosis and Classification

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ABSTRACT

leukemia is classified into two main types acute and chronic, the acute leukemia is further subdivided into, acute lymphocytic leukemia, which accounts for about three-quarters of acute leukemia, and acute myeloid leukemia accounting for most of the rest of the acute leukemia cases, and the chronic leukemia is further divided into chronic lymphocytic leukemia and chronic myeloid leukemia. The diagnosis and classification of leukemia rely on the simultaneous application of multiple techniques. Cytomorphology and histomorphology are combined with cytochemistry and multiparameter flow cytometry to assign the diagnostic sample to the correct entity. Every type of leukemia has a unique set of CD markers, which now serve as both the base for diagnosis and the classification of hematological malignancies. Immunophenotyping improves both the accuracy and reproducibility of acute leukemia classification. Acute Leukemia more common than Chronic Leukemia. - acute myeloid leukemia is more common than chronic lymphocytic leukemia. Chronic lymphocytic leukemia is more common than chronic myeloid leukemia. Combination of both morphology and Immunophenotyping are important for diagnosis of leukemia.

Keywords Leukemia, CD markers, diagnosis of leukemia.

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1. INTRODUCTION

Leukemia, is a malignant disease that results from abnormal blood cell synthesis in the bone marrow and blood-forming organs which can be classified based on the degree of progression, the etiology is poorly expressed, and the majority of authors believe it to be complex. The risk factors include chromosomal abnormalities (Down syndrome), ionizing radiation exposure, viral infections (Epstein-Barr), chemical substances (benzene), and families with a history of or members who have had leukemia [1]. leukemia is classified into two main types acute and chronic , the acute leukemia is further subdivided into ,acute lymphocytic leukemia (ALL), which accounts for about three-quarters of acute leukemia, and acute myeloid leukemia (AML) accounting for most of the rest of the acute leukemia cases [2].and the chronic leukemia is further divided into chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML). AML occurs at all ages from childhood, while ALL occurs at all ages, from birth to adulthood of age, but the incidence peaks from (2-6) years [3,4]. The diagnosis and classification of leukemia rely on the simultaneous application of multiple techniques. Cytomorphology and histomorphology are combined with cytochemistry and multiparameter flow cytometry to assign the diagnostic sample to the correct entity [6]. Based on the lineage of the blast cells under the influence of cytokines, or colony stimulating factors, the acute leukemia is characterized as acute myeloid leukemia (AML) or acute lymphoid leukemia (ALL). Distinct subsets of surface molecules are expressed by precursor cells from different lineages or blast cells in acute leukemia, many of which are now known as cluster of differentiation (CD) antigens. Flow cytometry is used to analyze the expression of CD antigens on leukocyte

(6). Every type of leukemia has a unique set of CD markers, which now serve as both the base for diagnosis and the classification of hematological malignancies. Immunophenotyping improves both the accuracy and reproducibility of acute leukemia classification [7]. The aims of this study were to determine the incidence of different types of leukemia and assess the incidence of abnormal expression of CD markers in cases of leukemia in the Iraq-Diyala Governorate.

2. METHOD

Across sectional study carried out during the period of one year from February 2021 to February 2022 a total of (40) patients attended Al-Shams Medical Laboratory in Iraq-Diayla Governorate referred from different physicians as a suspected case of hematological malignancy.

All patient included in this study were subjected to panel of tests including the following:

Complete blood count.

Blood film Examination which done by consultant hematologist.

After diagnosis by blood film, patients referred to Baghdad Medical City- Hematology Center-Flow cytometry Department for doing flow cytometry.

Flow cytometry is to confirm diagnosis of leukemia which is initially done by blood film and for identification subtypes of leukemia.

Complete blood count (CBC) using Auto-analyzer Sysmex, with examination of Leishman-stained peripheral blood (PB) smears for differential leucocytes count and blast cells percentage. Immunophenotyping of blast cells in peripheral blood samples was done using BD FACS Caliber Flow Cytometer.

Analysis of data. The overall clinical data of leukemia was analyzed to identify the gender ratio, average age at the first diagnosis. Dichotomous variables were expressed as percentages.

3. RESULTS AND DISCUSSION (10 PT)

In current study for leukemia classification, we used a combination of French- American –British (FAB) Classification looking for morphology of blast and World Health Organization classification (WHO) looking for immunological markers of blast cells.

Using data collecting from result of peripheral blood count, blood film examination and flow cytometry result, the leukemic cases were classified to acute and chronic, and acute is further classify to acute lymphoblastic leukemia and acute myeloid leukemia

3.1. Acute myeloid leukemia

The results presented in this chapter were based on analysis of 40 newly diagnosed patients with leukemia, 23 males (57.5%) and 17 female (42.5 %) with age range from 2 -71 year for male and from 3 months-71 years for female, from total number of leukemia, 23 case (57.5%) were acute leukemia and other 17 case (42.5%) were chronic leukemia as show in (Table 3.1) below.

while the chronic leukemic is classify to chronic lymphocytic and chronic myeloid leukemia so we arranged patient in four group as following as show in (table 1) below:

Table 1. General Characters of patients N = 40

Age (year)	Range (3months-71yeras) Average (38.02)		
	Men (2-71yeras) Average (37.04)		
	Women (3months-70yeras) Average (39.43)		
Sex	Men	N= 23	(57.5 %)
	Women	N= 17	(42.5 %)
Types of Leukemia			
Acute Myeloid Leukemia	Average age (2-60 years)	N= 12	(30%)
	Men	N= 7	(58.3%)
	Women	N= 5	(41.7%)
Acute Lymphoid Leukemia	Average age (3months-19 years)	N= 11	(27.5%)
	Men	N= 6	(54.5%)
	Women	N= 5	(45.5%)
Chronic Lymphoid Leukemia	Average age (50- 71years)	N= 11	(27.5%)
	Men	N= 5	(45.5%)
	Women	N= 6	(54.5%)
Chronic Myeloid Leukemia	Average age (33- 57years)	N= 6	(15%)
	Men	N= 3	(50%)
	Women	N= 3	(50%)

3.1.1. Acute myeloid leukemia

Group I: Acute myeloid leukemia (AML) consists of 12 patient (30%). with age range 2-60 years and distributed as following as showing in (Table 2) below to:

AML-M0 One patient (8.3%).
AML-M2 Two patient (16.7%).
AML-M3 Two patient (16.7%).
AML-M4 Three patient (25%).
AML-M5 Three patient (25%).

Table 2. Acute Myeloid Leukemia (AML) total number 12 patients

Types	Numbers	percentages
AML-M0	1	(8.3%)
AML-M2	2	(16.7%)
AML-M3	2	(16.7%)
AML-M4	3	(25%)
AML-M5	3	(25%)

AML-M7	1	(8.3%)

3.1. 2. Group of Acute lymphoblastic leukemia

Group II: Acute lymphoblastic leukemia (ALL) consists of 11 patient (27.5%), with age range 3 months-19 years. and distributed as following as showing in (table 3) below:

Eight patient (72.2 %) have L1 Subtype (B-Cell ALL).

Three patient (27.3 %) have L2 Subtype (T-Cell ALL).

Group III: chronic lymphocytic leukemia (CLL) consists of 11 patient (27.5%). With age range 50–71-year-old.

Group IV: Chronic myeloid leukemia (CML) consists of 6 patient (15 %). With age range 33-57 years. Table (3)

Table 3. Acute Lymphoid Leukemia (ALL) total number 11 patients

Types	Numbers	percentages
B-cells ALL	8	(72.7%)
T-cells ALL	3	(27.3%)

3.2. CD Markers Panels

Using large panels of different monoclonal antibody, use of different component against the cluster of differentiation (CD) of surface antigen, the acute leukemia in both type and chronic lymphocytic leukemia shows the following result express in (table 4,5)

Table 4. Acute leukemia

Variable	AML N= 12	B-ALL N= 8	T-ALL N= 3
HLADR	8 (66.6%)	---	---
CD19	---	8(100%)	---
CD13	11(91.6)	3(37.5%)	---
CD33	11(91.6)	---	1(33.3%)
CD14	2(16.6)	---	---
CD5	---	---	3 (100%)
CD7	4(33.3)	---	3(100%)
CD3	---	---	3(100%)
TdT	---	5(62.5%)	---

Table 5. Chronic Lymphocytic leukemia CLL

Variable	CLL N= 11
LAIR1	7(63.6%)
CD45	11(100%)
CD5	11(100%)
CD11c	5(45.4%)
CD19	11(100%)
CD20	11(100%)
CD23	11(100%)
CD31	9(81.8%)
CD38	4(40%)
CD43	11(100%)
CD79b	5(45.4%)
CD81	11(100%)
CD200	11(100%)
SmIgκ	2(18.1%)
SmIgλ	3(27.2%)

4. Discussion

Acute leukemia are a group of malignancy with clinical, morphological immunological and molecular pattern and display pattern of surface expression (CD Antigen). Accurate diagnosis and classification of leukemia are the basis of appropriate management of patient and for accurate diagnosis we need application of multiple techniques. Precursor cells from different blast cells in leukemia express different subdivision of surface molecules many of which are now defined as cluster of differentiation (CD) antigen, every leukemic type has a specific set of CD markers, what constitutes nowadays current classification of hematopoietic malignancy and now used widely in classification [7,8]. The expression of CD markers on leukocytes can be done by special techniques known as flowcytometry. Immunophenotyping detection in combination with morphology improve both accuracy and reproducibility of leukemic diagnosis. Flowcytometry is a technique used to analyze multiple phenotypic and functional parameters simultaneously within a signal cell or a group of cells. In current study firstly we examine the morphology of leukemic cells under microscope through examination of blood film and then we applying the Immunophenotyping to reach the definite diagnosis of newly cases of leukemia and according to this patients with leukemia included in study were classified into four group, group I : AML consist of 12 patient group II: ALL consist of 11 patient, 8 of them are B.cells ALL and 3 of them are T.cells ALL, , Group III: CLL consist of 11 patient , Group IV: CML consist of 6 patient, The age range of all cases at time of diagnosis were from 3 months to 71 years and this was in consistent with results of previous two study [9] as the age of patient at the time of diagnosis were ranged 1-78 years and 1.5-82 years respectively. Regarding AML, Current study show that, The majority of AML (90%) of cases included in this study were in adults with age range between 17-60 years old age while only (10 %) of AML cases including in this study were among children with age range between 2-12 years age, also on applying FAB classification on AML cases including in the study, AML M4-M5 cases was the most common subtype of AML , and this result are agree with result of previous study [10]. and disagree with other previous study (11 from 7) in which the AML-M2 was the most common subtype [11]. Regarding ALL, this study shows that, majority of ALL (80%) of cases included in this study were in Children with age range between 3 months-10 years old age while only (20 %) of ALL cases including in this study were among adult with age range between 15-19 years age. low-cytometry result of ALL show that, Eight patients with precursor B-cells ALL and Three patients with precursor T-cells ALL, [12]. In addition Immunophenotyping of blast cells using flow cytometry show that the common type was precursor B-Cells type(CD 10 +) which is most usual in children [13-15]. Also, current study result show that, patient affected by CLL. Were old age with range 50–71-year-old,and The age range of patient diagnosis to have CML in this study was range between 33-57years and this agree with fact

that this type of leukemia usual occur commonly in middle and old age population with equal sex incidence [16,17].

5. CONCLUSION

leukemias within the examined population. Among acute subtypes, Acute Myeloid Leukemia represents the most frequently encountered form, followed by Acute Lymphoblastic Leukemia (ALL. In contrast, within chronic leukemias, Chronic Lymphocytic Leukemia is more common than Chronic Myeloid Leukemia. The results further emphasize that accurate diagnosis requires the integration of both morphological evaluation and immunophenotyping, particularly through the use of CD marker profiling, which remains essential for proper classification, subtyping, and treatment planning.

Recommendations

1. **Expand the study to a larger cohort** across multiple healthcare centers to better estimate the true incidence and distribution of leukemia at the governorate and national levels.
2. **Standardize diagnostic protocols** by combining morphology, flow cytometry, and immunophenotyping to ensure diagnostic accuracy and improve subtype distinction.
3. **Enhance laboratory capacity** with advanced immunophenotyping panels to support early detection and reduce diagnostic uncertainty.
4. **Encourage multidisciplinary collaboration** between hematologists, pathologists, and laboratory specialists to improve patient management and clinical outcomes.

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