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Study of immunophenotypic markers and clinico-hematological findings in chronic lymphocytic leukemia

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Abstract

Background and objective: Chronic lymphocytic leukemia (CLL) is a clonal, mature B-cell neoplasm, its clinical features, cell morphology, and immunophenotyping are used to establish the diagnosis. Immunophenotyping by flow cytometry is the most accurate procedure for diagnosing CLL, there is no single marker exclusively expressed in CLL, however, a complex of immunophenotypic markers that incorporates several B-cell markers and assists in differentiating CLL from other mature B-cell neoplasms, the expression of these immunophenotypic markers is considered in the specific CLL scoring system (Moreue scoring system). this study aimed to assess the clinico – hematological features and immunophenotypic characteristics of CLL patients and to assess the role of immunophenotyping in the differential diagnosis of CLL.

Methods: an observational prospective and retrospective study was carried out at Nanakaly hospital for blood diseases and oncology in Erbil city, Iraq. That was conducted on 100 patients newly diagnosed with mature B cell neoplasm which included 68 cases of CLL and 32 cases with other mature B cell neoplasm (MBN), usinga convenience sampling method. The study period was from the1st of September 2021 to the end of April 2022. An interview questionnaire was used to collect the study data from the patients.

Results: The mean of age ± standard deviation of the CLL patients (58.85 ± 10.69) years and (66.2%) was male, The most frequent clinical presentations for CLL cases were fatigue (50%), lymphadenopathy (41.2%), and splenomegaly (41.2%), (50%) of CLL patients had anemia, thrombocytopenia was seen in (32.4%) and leukocytosis was seen (98.5%), all patients had lymphocytosis and (97%) had an absolute B lymphocyte count of more than 5 $x10^9$ /L. all the studied CLL patients expressed both CD45 and CD19 markers, the expression of the immunophenotypic markers was as following: CD5 (98.5%), CD23 (97.1%) and CD200 (97.1%), Half of the patients (50%) showed expression of monoclonal lambda light chain, sIgM (4.4%), CD20 (88.2%) and CD43 (54.4%) and CD38 (26.5%). The expression of the following marker had a significant role in the differentiation between CLL and other MBN: CD5, CD23, CD79b, FMC7, sIgM, CD200, and CD43.

Conclusion: Our results have shown that the clinical presentations and hematological profile of Iraqi CLL patients were not significantly different from that of previous local and global studies, the distinct immunophenotyping, as well as it has been found that immunophenotyping is a promising method for supporting the clinical and morphological characterization of CLL.

Keywords: Chronic lymphocytic leukemia; Lymphoproliferative neoplasms; Immunophenotyping; Flow cytometry.

Introduction

Chronic lymphocytic leukemia (CLL) Is the most common, slowly growing Mature B-cell neoplasm,[1,2] It characterized by Clonal growth and progressive accumulation of mature, dysfunctional

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monoclonal B-cells in the blood, bone marrow, lymph nodes, and spleen.[3,4] The disease's clinical course is extremely variable, ranging from asymptomatic to aggressive symptoms and progressive course of the disease.[5] The most common clinical findings are, lymphadenopathy, splenomegaly, hepatomegaly, Immune hemolytic anemia, and Evan's syndrome, also fever, night sweats, and weight loss may all occur. [6]CLL is a disease of elderly people, more common in males, it is the most frequent type of leukemia in the Western world and uncommon in Asia.[7]

The diagnosis of CLL, in most instances, can be verified by complete blood count demonstrating the sustained rise of monoclonal B-lymphocytes in peripheral blood more than 5 x 10^9/L for more than three months, The leukemic lymphocytes observed in the blood film are small, mature lymphocytes with a thin cytoplasmic border and a compact nucleus lacking apparent nucleoli and partly compacted chromatin also a characteristic finding is the presence of smudge cells (Gumprecht nuclear shadows).[8] as well as, immunophenotypic analysis by flow-cytometry regarded as a gold standard test in confirmation of CLL diagnosis and typically performed in all patients with lymphocytosis.[5] the leukemic cells of CLL have the characteristic immunophenotypic profile including positive expression of CD5, CD19 and CD23, and usually weak expression of CD20, CD22, CD79b, sIg (IgM, IgD) and negative expression of CD10 and FMC7,[9, 10] The population's clonality is demonstrated by monoclonal kappa and lambda light chains analysis ,the leukemic cells express either kappa or lambdalight chains.[11]

the immunophenotyping of CLL is considered in the specific scoring system for diagnosis of CLL and in the differentiation of CLL from other mature B-cell neoplasms.[8] Based on the most prevalent CLL marker profile, CD5+, CD23+, FMC7-, and weak expression of surface membrane immunoglobulin (sIg) and CD79b, they assigns a score of 1 or 0 to each of these five markers, scores for CLL range from 4 to 5 whereas score 3 is for atypical CLL and in other mature B-cell lymphoproliferative disorders the score range 0 to 2. [12]Although CLL is generally easy to diagnose based on its diagnostic criteria, Differentiation from other mature B -cell neoplasms or reactive diseases might be difficult in some cases, therefore, the blood smear and immunophenotyping should always be evaluated for the effective diagnostic process of CLL.

This study aimed to assess the immunophenotypic markers, clinical characteristics and hematological parameters of CLL patients and find out the importance of immunophenotypic markers in differentiation of CLL from other mature B cell neoplasms in Erbil, Iraq and compare them with previous regional and international studies.

Methods

This is observational, retrospective, and prospective study was conducted on 100 adults who have newly diagnosed with mature B cell neoplasms from January 2020 to May 2022 at Nanakaly hospital which is the main cancer hospital for adult and pediatric patients with benign and malignant hematology and oncology disorders in Erbil city. The data of the retrospective cases were obtained from the Laboratory's archives, whereas, the data of the prospective cases were directly collected from the patients, the diagnosis was made based on morphological and immunophenotypic results according to World Health Organization (WHO) classification (2016).

Clinical manifestations and physical examination (for lymphadenopathy, splenomegaly, hepatomegaly, and pallor were documented, and the hematological data recorded including hemoglobin level, white blood cell count, platelet count, absolute lymphocyte count, and percentage, Complete blood counts were undergone on a fully automatic hematology

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Analyzer (Medonic-USA), peripheral blood smears were freshly prepared and stained using Leishman's stain, the slides were examined under a microscope (Olympus-CX31-Japan) and the differential count was performed, then the results were reported. Immunophenotyping by flow cytometry was performed as a routine investigation for all cases of CLL and other mature B cell neoplasms. Eight colors flow cytometric analysis was performed using a FACSCanto II flow cytometer device (BD Biosciences, San Jose, California, USA), A sample of 3-5 ml peripheral blood was collected from each patient, and put in EDTA tubes, the antibody panel used in B lymphoproliferative disorders are: CD45 as a common white blood cell marker, Screening panel include: sIgM, kappa, lambda, CD19,CD20 and CD5

Secondary panel include:CD23, CD79b, CD10, CD200, CD43, FMC7, CD25, CD103, CD123, CD38, CD138. Then the samples were processed accordingly, the tubes were usually divided into two groups, one of them is only one tube for surface membrane immunoglobulin detection (kappa, lambda, CD19 and CD45) and they followed the wash–stain–lyse–wash method for this tube, The second group was composed of four to five tubes (according to the case), these tubes were used for detection of membrane antigens by Stain–Lyse–Wash method, Data were acquired

on a flow cytometer and translated into digital information on a computer as soon as possible. Analysis was performed on lymphocyte population gated according to forward and side scatter characteristics (FSC/SSC), A total of 20,000 cells were collected for evaluation, and the intensity of expression was evaluated as negative or positive with a cut-off of 20% (When at least 20% of gated lymphocytes expressed the studied antigen above the isotypic control, an antigen was regarded positive). and the intensity of expression (dim, moderate, bright) was evaluated. The absolute counts of B-cells in peripheral blood were determined using a dual platform method, B cell proportions in peripheral blood ((percentage of CD19+ cells/ lymphocytes) as measured by flow cytometry were combined with the absolute lymphocyte count obtained from CBC.

The statistical analyses were performed using the Statistical package for the social sciences (SPSS, version 25), data were presented as frequency, percentage mean ± SD, median, range (minimum-maximum value), The Chi-square test and the Student's t-test were used to evaluate the associations between proportion and to compare between data, the statistical significance level as set as *P* value ≤0.05

Results

A total of 100 mature B cell neoplasm patients have been evaluated in this study, 68 patients were diagnosed as CLL and 32 of them have other MBN, regarding CLL patients, 45 (66.2%) males and 23 (33.8%) females corresponding to a male – to – female ratio of (2.125/ 1). the mean age at presentation was (58.85 ± 10.69), the peak incidence was between the ages of 41 and 60 years (56.9%), the ages at diagnosis range between 35 years to 83 years with a median of 57.5 years, the most frequent clinical presentations for CLL cases were fatigue (50%), lymphadenopathy (41.2%) and splenomegaly (41.2%) (Table 1).

Based on hematologic parameters of CLL patients, the mean hemoglobin level was 12.4 ± 2.4 g/dL (range: 5.4-16.7g/dL), mean platelet count was 191.9 x 10^9/L ± 75.2 (range: 33 - 402 x 10^9/L), the mean WBC count was 63.4 x 10^9/L ± 76.8 (range: 8.1 - 556 x 10^9/L), the mean absolute lymphocyte count in peripheral blood was 53.6 x10^9/L ± 74.2 (range: 5.6-544.8 x 10^9/L), as well as the mean of absolute B lymphocyte count was 48.9 x 10^9/L ± 70.6 (range: 2.8– 506.6 x 10^9/L) and the mean value of lymphocyte percentage was 79.5% ± 11.2l (range: 47 % -98%) (Table 2).

Anemia was observed in (50%) of CLL patients, thrombocytopenia was seen in

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(32.4%) patients of and leukocytosis were seen in (98.5%) patients of WBC count of more than 11 x 10^9/L, and all CLL patients had lymphocytosis of an absolute lymphocyte count more than 3 x10^9/L, Furthermore, it was shown that the great majority of CLL patients(97%) had an absolute B lymphocyte count of more than 5 x10^9/L.

Table 1 Demographic and clinical characteristics of all patients (CLL and other MBN) (N:100).

Characteristics		**Patients group**	
		CLL (N:68)	**Other MBN (N:32)**
Gender N (%)	Male	45 (66.2)	23 (71.9%)
	Female	23 (33.8)	9 (28.1%)
	Ratio	(2.125/1)	
Age (year)	Mean ± SD	58.85 ± 10.69	57.81 ± 16.02
	Median	57.5	60
	Range	35 – 83	16-90
Clinical presentations N (%)	Fatigue	34 (50)	19 (59.4)
	Lymphadenopathy	28 (41.2)	14 (43.8)
	Splenomegaly	28 (41.2)	20 (62.5)
	Joint pain	21 (30.9)	8 (25)
	Abdominal pain	20 (29.4)	17 (53.1)
	Fever	18 (26.5)	4 (12.5)
	Asymptomatic	12 (17.6)	3 (9.4)

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Examination of peripheral blood smear showed mature lymphocytes with clumped chromatin and scantycytoplasm that were seen in all CLL patients and smudge cells were counted in almost all peripheral blood smear except two cases. Regarding the hematological parameters of other MBN patients are shown in table 2.

mean of absolute lymphocyte count was significantly high in CLL patients that presented with splenomegaly (41.2%), fatigue (50%) and abdominal pain (29.4%) (*P*-value 0.009, 0.036, 0.006 respectively), also the absolute lymphocyte count had a significant, strong, direct correlation with the WBC count in peripheral blood (r = 0.994, *P* <0.001), The comparison between clinical presentation and hematological parameter showed that both splenomegaly and fatigue had significant association with hemoglobin level, WBC count and platelet count (*P* = 0.038, 0.011, 0.008 respectively), (*P* = 0.039, 0.026, 0.08 respectively). lymphadenopathy had a significant association with Platelet count (*P* = 0.01), there was a significant relationship between abdominal pain and WBC count (*P* = 0.006), pallor and hepatomegaly had a significant association with hemoglobin level (*P* = 0.000) and (*P* = 0.05) respectively, epistaxis had a significant relation with hemoglobin level (*P* = 0.044) and platelet count (*P* = 0.029). shortness of breath (SOB) had a significant association with hemoglobin level (*P* = 0.001).

Table 2 The hematological parameters in CLL and other MBN patients.

Hematological parameter	CLL patients (N = 68)			Other MBN (N = 32)			*P* value	Normal range
	Mean ± SD	Median	range	Mean ± SD	Median	Range		
Hemoglobin in g/dl	12.4 ± 2.4	12.6	5.4 – 16.7	11.6 ± 1.56	12	6.8 – 14	0.095	12-15 /13-17*
WBC x 109/L	63.4 ± 76.8	42.5	8.1 – 556	55.3 ± 47.9	38	2 – 201	0.586	4 – 11
Platelet x 109/L	191.9 ± 75.2	195	33 – 402	161.4 ± 90.9	164.5	78 – 417	0.081	150 – 400
Lymphocyte count X109/L	53.6 ± 74.2	32.3	5.6 – 544.8	44.3 ± 89.8	26.1	104 – 156.8	0.506	1 – 3
Absolute B lymphocyte count	48.9± 70.6	25.3	2.8 – 506.6	36.5± 37.6	20.7	1.2 – 148.8	0.35	10-20% of lymphocyte count
Lymphocyte %	79.5 ± 11.2	80	47 – 98	78 ± 8.3	77	64 – 96	0.507	20 – 40

*male/female, SD= standard deviation

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Flow cytometry was used to analyze the immunophenotyping of all patients from peripheral blood, in relation to the CLL scoring system by assessing score value, patients who scored 4 or 5 were diagnosed as CLL, while who scored ≤ 3 were diagnosed as other MBN.

All CLL patients, in this series, expressed CD45 and CD19, whereas the great majority of them showed expression of CD5 (98.5%), CD23 (97.1%) and CD200 (97.1%). CD20 was expressed in (88.2%) and (54.4%) of patients who expressed CD43. Half of the patients (50%) showed expression of monoclonal lambda light chain, while only (11.8%) expressed kappa chain. CD38 was expressed in only (26.5%). (55.9%) of studied CLL patients showed expression of CD79b, and no one had FMC7 expression. (4.4%) expressed IgM (Table 3).

Table 3 The frequency of the immunophenotypic markers in CLL patients and other.

CD markers	CLL (N = 68) N (%)	Other MBN (N=32) N (%)	*P* value
CD45	68 (100)	32(100)	N/A
CD19	68 (100)	32(100)	N/A
CD5	67 (98.5)	21(65.6)	< 0.001
CD20	60 (88.2)	31 (96.9)	0.265
CD23	66 (97.1)	16 (50)	< 0.001
CD200	66 (97.1)	21(65.6)	< 0.001
CD43	37 (54.4)	10(31.3)	0.034
Kappa	8 (11.3)	12 (37.5)	0.006
Lambda	34 (50)	13 (40.6)	4
IgM	3 (4.4)	12 (37.5	< 0.001
CD79b	38 (55.9)	28 (87,5)	0.002
FMC7	0 (0)	17 (53.1)	< 0.001
CD38	18 (26.5)	4 (12.5)	0.130
CD25	23 (33.8)	12 (37.5)	0.823
CD103	3 (4.4)	4 (12.5)	0.206
CD123	1 (1.5)	4 (12.5)	0.035
CD138	6 (8.8)	5 (16.5)	0.322
Cyclin- D1	0 (0)	0 (0)	N/A
BCL2	0 (0)	0 (0)	N/A
CD10	0 (0)	0(0)	N/A
CD7	1 (1.5)	0(0)	1

*N/A= not applicable

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no patient expressed cyclin-D1, CD10, CD7 or BCL2. In our CLL patients, the most common expression patterns of CD45 (50%), CD5 (61.2%), CD19 (38.2%), CD23 (57.6%) were “dim to moderate” expression, while CD20 (65.7%), CD79b (71.1%), monoclonal kappa (75%) and lambda (61.8%) light chains, IgM (66.7%) were all had “dim” expression as most common pattern. CD200 was the only marker with “moderate” expression as the most frequent expression pattern (Table 4).

It was reported that CD5 marker was expressed more frequently in the CLL patients than in the other MBN patients (98.5% vs 65.6% with *P* <0.001), its predominant expression pattern was “dim-moderate” expression in both CLL and other MBN patients (*P* = 0.011). also, The CD23 antigen was discovered to be expressed in the majority of CLL patients compared to other MBN (97.1% vs 50% with *P* <0.001), its common expression pattern was “ dim-moderate” in CLL patients in comparison to other MBN patients that characterized mostly by “dim” expression (*P* <0.001), and similar finding was found for CD200 that expressed in most CLL cases compared with other MBN cases (97.1% vs 65.6% with *P* <0.001), the assessment of its expression pattern demonstrated that “moderate” expression was most frequent expression pattern for CLL patients and “dim” expression was the most frequent pattern for other MBN patients (*P* = 0.116). while regarding the expression of CD79b, we reported a relatively low frequency of expression in the CLL patients as compared to the other MBN patients (55.9% vs 87,5% with *P* = 0.002), its most frequent expression pattern was “dim” in both CLL and other MBN cases (*P* = 0.776). regarding FMC7 expression, it was not expressed in any CLL patients compared with other MBN patients (0 % vs 53.1% with *P* = 0.002), CD20 expression was approximately comparable in both CLL and other MBN patients (88.2%, 96.9% with *P* = 0.265), its most common expression pattern was “dim” expression in both CLL and other MBN patients (*P* = 0.009) (Table 4).

Regarding the relation between immunophenotypic markers and clinical presentation of CLL patients, there was a significant relation between CD20 expression with pallor (*P* = 0.02), and significant relation between CD43 expression and fever (*P* = 0.05).

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Table 4 The frequency of expression pattern of main immunophenotypic markers for CLL and other MBN patients.

Immunophenotypic markers	Expression pattern	CLL N (%)	Other MBN N (%)	*P* value
CD5	Dim	5 (7.5)	7 (33.3)	0.011
	Dim – moderate	41 (61.2)	9 (42.9)	
	Moderate	21 (31.3)	5 (23.8)	
	Moderate – bright	0 (0)	0 (0)	
	Bright	0 (0)	0 (0)	
CD 20	Dim	34 (65.7)	12 (38.7)	0.009
	Dim – moderate	21 (35)	8 (25.8)	
	Moderate	5 (8.3)	6 (19.4)	
	Moderate – bright	0 (0)	4 (12.9)	
	Bright	0 (0)	1 (3.2)	
CD23	Dim	6 (9.1)	11 (68.8)	< 0.001
	Dim – moderate	38 (57.6)	2 (12.5)	
	Moderate	21 (31.8)	3 (18.8)	
	Moderate – bright	1 (1.5)	0 (0)	
	Bright	0 (0)	0 (0)	
CD79b	Dim	27 (71.1)	14 (50)	0.092
	Dim – moderate	9 (23.7)	8 (28.6)	
	Moderate	2 (5.3)	6 (21.4)	
	Moderate – bright	0 (0)	0 (0)	
	Bright	0 (0)	0 (0)	
FMC7	Dim	0 (0)	5 (29.5)	N/A
	Dim – moderate	0 (0)	9 (52.9)	
	Moderate	0 (0)	3 (17.6)	
	Moderate – bright	0 (0)	0 (0)	
	Bright	0 (0)	0 (0)	
CD200	Dim	11 (16.7)	10(47.6)	0.116
	Dim – moderate	20 (30.3)	5 (23.8)	
	Moderate	35 (53)	6 (28.6)	
	Moderate – bright	0 (0)	0 (0)	
	Bright	0 (0)	0 (0)	
Kappa	Dim	6 (75)	8 (66.7)	0.776
	Dim – moderate	1 (12.5)	3 (25)	
	Moderate	1 (12.5)	1 (8.3)	
	Moderate – bright	0 (0)	0 (0)	
	Bright	0 (0)	0 (0)	
Lambda	Dim	21 (61.8)	4 (30.8)	0.041
	Dim – moderate	11 (32.4)	5 (38.5)	
	Moderate	2 (5.9)	4 (30.8)	
	Moderate – bright	0 (0)	0 (0)	
	Bright	0 (0)	0 (0)	
IgM	Dim	2 (66.7)	7 (58.3)	0.744
	Dim – moderate	1 (33.3)	3 (25)	
	Moderate	0 (0)	2 (16.7)	
	Moderate – bright	0 (0)	0 (0)	
	Bright	0 (0)	0 (0)	

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The result of the relation between immunophenotypic markers and hematological parameters revealed that only the negative FMC7 expression showed significant relation with WBC count (*P* = 0.031), absolute lymphocyte count (*P* = 0.035) and platelet count (*P* = 0.018). The final diagnosis of MBCN was significantly related to the Moreau score (*P* <0.001).

The great majority of all cases had score 4 (39%), followed by score 5 (29%), then (18%) had score 2, (11%) had score 3 and only (3%) had score 1 (Table 5).

Depending on the Moreau scoring system and specific immunophenotypic markers and other investigations, the final diagnosis of our studied patients confirmed as 68% of the patients as atypical CLL, 11% diagnosed as mantle cell lymphoma, 6% diagnosed as marginal zone lymphoma and 4% diagnosed as hairy cell leukemia (Table 6).

Table 5 The distribution of all studied patients (CLL and other MBN patients) according to the Moreau scoring system.

Final diagnosis	Score 5 N(%)	Score 4 N(%)	Score 3 N(%)	Score 2 N(%)	Score 1 N(%)
CLL (N=68)	29 (42.6)	39 (57.4)	0(0)	0(0)	0(0)
Other MBN (N=32)	0 (0)	0 (0)	11 (34.4)	18 (56.3)	3 (9.4)
P value	0.000	0.000	0.000	0.000	0.000

Table 6 Distribution of cases according to the final diagnosis.

Final diagnosis	N (%)
CLL	68 (68)
Mantle cell lymphoma	11 (11)
Atypical CLL	11 (11)
Marginal zone lymphoma	6 (6)
Hairy cell leukemia	4 (4)

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Discussion

In the present study, CLL was the most common type of mature B cell neoplasm (68%) and this was consistent with that documented in Baghdad[13] and Turkey[14] that confirmed CLL frequencies of 65.9%, 72.8% respectively The median age at diagnosis in the current study was 57.5 years with a peak incidence between 41 to 60 years which was close to that recorded previously in Erbil (65 years),[15] Baghdad (61 years)[13] and turkey (64.4 years).[14] In Western countries, on the other hand, the median age at diagnosis is more with a range (67 - 72 years), [16]Male dominance has been found in the current study (66.2%) with male to female ratio of (1.95/1) which was accordance with the results of Hasan *et al*,[15] Hellek *et al*[17]and Padaro *et al.*[18] In the present study the clinical symptoms of easy fatigability (50%), lymphadenopathy (41.2%), splenomegaly (41.2%), joint pain (30.9%), abdominal pain (29.4%), fever (26.5%) were the most presenting symptoms of CLL, and these symptoms were documented less frequently in the present study compared with previous study done in Erbil,[15] which recorded splenomegaly as most frequent clinical features (64.8%), lymphadenopathy(60%) and hepatomegaly (21.9%), a study done in Baghdad found that the most presenting features were lymphadenopathy (50%) followed by splenomegaly (35%), pallor (30%), hepatomegaly (22%) and fever (20%),[13] however, Previous studies have been well recorded the diversity of clinical presentations in CLL and their associations with ethnic background.[19, 20] According to the literature, 30–35% of CLL cases were diagnosed accidentally,[21] however the current study reported (17.6%) of patients were diagnosed accidentally, probably due to late-stage disease presentation, deficiencies in the referring system, and a loss of regular health checkup behavior in our population. in the present study, the most prominent findings in the peripheral blood of CLL patients at the time of diagnosis were leukocytosis which reported in (98.5%) and lymphocytosis was found in all patients (100%) (absolute lymphocyte count and high lymphocyte percentage), that was comparable with findings in: Erbil,[15] Egypt,[21] and France.[18] Anemia is a common and serious complication of chronic lymphocytic leukemia in the present study, the incidence of anemia was (50%) at the time of diagnosis which accordance with that reported in Iran (57%),[7] and Senegal (55%).[22] thrombocytopenia at presentation has always been proved to be a poor prognostic marker associated with stage IV disease. In the present study, Thrombocytopenia was documented in (32.4%) of CLL patients which were in agreement with the results of a study in Iran (31.4%), [7] and also with a study in Baghdad (25.2%).[13]

Immunophenotyping by Flow cytometry is an invaluable tool in diagnosisof mature B-cell neoplasms, including CLL,[5,8,23] Making precise diagnosis is crucial, Since the clinical development and response to therapy of CLL and other MBN vary. Moreau scoring system based on leukemic cells' expression of CD5, CD23, CD79b, FMC7, and surface membrane immunoglobulin (sIgM), has been applied in the diagnosis of CLL for about 25 years, as well as it helps distinguish CLL from other mature B cell neoplasms.[12] Even though CLL has a distinct immunophenotypic pattern when compared to other MBN, there are some cases have inconsistent immunophenotyping that made the differential diagnosis still a challenge.

In the current study, all CLL cases expressed CD45 and CD19, and the vast majority showed expression of CD5 (98.5%) and CD23 (97.1%) and less often an expression of CD79b (55.9%) and there was no case expressed FMC7(0%), These results are supported with the typical CLL pattern.[24] as these results were almost similar to that documented previously in Erbil with some differences in CD45 was

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expressed in (84.8%) and in the expression of FMC7(15%).[15] Also, the results of the current study was comparable to that documented in a study that has been done in Turkey regarding expression of CD19 (100%), CD5 (100%) and CD23 (96.7%), but there was difference in the expression of CD79b (38.8%) and FMC7 (42.1%),[25] the results of a study that has been done in Egypt showed almost identical results about the expression of CD19, CD5, CD23 whereas it showed more frequent expression for CD79b (73%) and FMC7 (25%).[26] The results of a study in Czech were consistent to the results of the current study about expression of CD19 (99.4%), CD5 (100%), CD79b (43.9%), but it reported different result about expression of CD23 (82.1%) and FMC7(2.9%),[27] Regarding the evaluation of sIg (IgM) expression in the present study revealed that the vast majority of CLL patients agreement with the results of study in Czech that reported the sIgM expressed in (10.6%),[27] The determination of the monoclonality of B lymphocyte based on the presence of monoclonal light chain restriction. In the present study, the monoclonality was determined in all CLL cases, monoclonal lambda light chain was more frequently expressed than monoclonal kappa light chain (50%), (11.3%) respectively, which were comparable to a study in Baghdad in terms of expression of monoclonal light chain type with less frequent rate, lambda (15%), kappa (7%),[13] CD20 was expressed in (88.2%) of CLL patients in the current study which was accordance to that reported previously in Erbil (75%),[15] as well as, in Turkey (93.4%),[25] the current study documented that CD200 was expressed in (97.1%) of the CLL patients that was accordance with the result of Falay *et al* study in Turkey in which CD200 expression was (96.7%),[28] as well as in a study from Czech was (98.3%),[27] In the present study, CD43 expressed in (54.4 %) of CLL patients that was less frequently expressed than the finding that documented by Falay *et al* in Turkey (98.3%),[28] The rate of CD38 expression among CLL patients in the present study was (26.5%), which was consistent with the result of Hasan *et al* (21%),[15] and Falay *et al* (24%),[28] In this series, CD25 expression was (33.8%) of CLL patients, it was comparable to the result of Starostka *et al* (39.3%).[29] It's easy to diagnose CLL with presence of its unique immunophenotypic pattern based on Moreue score, nevertheless, in some instances the diagnosis remains complicated because of presence of inconsistent immunophenotypic profile that making its differential diagnosis challenging,[30] regardless the developments in cytogenetics and molecular biology that have resulted in substantial improvements in the diagnosis of mature B cell neoplasms, immunophenotyping by flow-cytometry remains a gold standard method for accurate differential diagnosis.[31,32] the current study assessed the significance of the immunophenotypic markers in the differential diagnosis of CLL on the bases of their expression and expression pattern of them, CD45 was expressed in all studied patients (CLL and other MBN), it has been analyzed to recognize leukocyte subpopulation and serves as the basis for multiple gating methods,[23] CD19 was expressed in all studied patients (CLL and other MBN) establishing the B-lymphocyte lineage,[23] CD20 was highly expressed in all studied patients with less frequently expressed in CLL patients compared with other MBC patients, however the difference between them was non-significant, while regarding the expression pattern, the majority of CD20 positive CLL patients (65.7%) was expressed as Dim in of CLL compared with (38.7%) of other MBN patients, and the difference between them was significant. this result was consistent with that reported by Abbas *et al*[13] and Starostka *et al,*[29] CD5 was expressed in the vast majority of CLL patients which was significantly higher than that reported in other MBN patients, also the dim to moderate expression

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pattern was the most common in both group but significantly higher in CLL than other MBN, similar findings were reported by Marrero *et al* (95.3%),[33] the frequency of CD23 expression was significantly higher in CLL patients than other MBN patients and the pattern Dim- moderate was the predominant in CLL patients and significantly higher compared with other MBN, these results were comparable with that mentioned by Falay *et al*[25] and Starostka *et al*[29] in CLL cases, CD79b expression was significantly lower than that of other MBN cases (55.9% vs 87.5%), while CD79b Dim expression was the predominant pattern in CLL cases. Same findings were reported by Marrero *et al,*[33] The present study reported negative FMC7 expression in all CLL patients while it was expressed in (53.1%) of other MBN patients, these findings were accordance with the results that documented by Starostka *et al*[29] in which FMC7 was expressed in (2.9%) of CLL patients, the results of this study about sIgM demonstrating that only (4.4%) of CLL cases had positive sIgM expression whereas it was positively expressed in (37.7%) of other MBN cases, and the difference between these two groups was significant, likewise that reported by Köhnke *et al*,[34] the current study reported that CD200 was expressed in the vast majority of CLL (97.1%) and in (65.5%) of other MBN cases, this difference was significant, its most frequent expression pattern was moderate in CLL and Dim in other MBN cases in agreement with the results of Sawhney *et al.*[30] CLL patients had significantly higher CD43 expression frequency than that of other MBN patients, which was comparable with that reported in literature,[35] Based on these findings we regard the following markers to have significant role in the differential diagnosis of CLL: CD20, CD5, CD23, CD79b, FMC7, sIgM, CD200, CD43, and CD103.

Conclusion

our results have shown that the clinical presentations and hematological profile of Iraqi CLL patients were not significantly different from that of previous local and global studies, the distinct immunophenotyping, as well as it has been found that immunophenotyping is a promising method for supporting the clinical and morphological characterization of CLL, also Our study found that a certain immunophenotypic markers which differentiate CLL from other MBN. Applying the following marker combination: CD20, CD5, CD23, CD79b, FMC7, sIgM, CD200, CD43, and CD103. This study also found that none of the analyzed markers can be used singly to make definitive diagnosis of CLL using flow-cytometry.
Only the combination of these markers enables CLL to be differentiated from other MBN. Additionally, the CLL score value is essential in the differential diagnosis of CLL, since the majority of CLL patients at presentation had score of 4 or 5.

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Competing interests

The authors declare that they have no competing interests.

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