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Clinical, Hematological and Immunophenotypic Profiles in Chronic Lymphocytic Leukemia in Kurdistan-Iraq

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Abstract

Background and objectives: Chronic lymphocytic leukemia is the accumulation of morphologically mature lymphocytes in blood and lymphatic tissues. We aimed to outline the clinico-hematological and immunophenotypic patterns of patients with chronic lymphocytic leukemia in Kurdistan-Iraq.

Methods: This is a retrospective cohort study on 154 patients referred to Sulaimani Public Health Lab and Nanakaly Hospital - Hawler from January 1st 2013 to December 31st 2017 and diagnosed as chronic lymphocytic leukemia. Demographic, clinical, and laboratory data were recorded. Clinical stages and immunological scoring were determined.

Results: Median patient age at diagnosis was 70 years with a range of 39-82 and a male: female ratio of 1.8:1. Thirty five patients (22.7%) were diagnosed incidentally, and 85 (55.2%) had B-symptoms at presentation. Lymphadenopathy was the most common presenting feature, seen in 84 (54.6%) patients ,while splenomegaly reported in 40 (26%)patients and hepatomegaly in 7(4.6) patients. Forty four (28.6%) patients had anemia at diagnosis, while 150(97.3%) patients presented with leukocytosis, and 21(13.6%) patients had thrombocytopenia. Leukemic B-cells have a specific immunophenotypic profile: CD19, CD20 ^{weak}, CD22 ^{weak}, CD5 ^{bright}, CD23^{+}, CD79b^{weak,} FMC7 $^{weak/-}$, CD200 and weak immunoglobulin light chain restriction. The applied scoring system revealed that 112 (72.7%) patients scored four and the rest scored five out of five. Moreover, sixty five (42.2%) patients presented with advanced Rai stage and 62(40.3%) patients presented with stage C Binet system.

Conclusions: Patients characteristics were not distinct from those reported elsewhere, though a higher proportion of our patients presented with an advanced clinical stage.

Keywords: Chronic lymphocytic leukemia; Staging; Flow cytometry.

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Introduction

Chronic lymphocytic leukemia (CLL) is a slowly progressive accumulation of morphologically and immunologically mature lymphocytes in the blood, bone marrow, lymph nodes, and spleen [1,2]. It is the most common type of leukemia in western countries with an annual incidence rate of about 5 new cases per 100000 populations, while it is much less common in other parts of the world like Japan and China [2, 3]. The median patient age at diagnosis is 72 years and the incidence increases with age reaching 30-50 per 100,000 in patients older than 80 years, with a male: female ratio of 1.3:1 to 2:1[2,4].The etiology of CLL is still unclear, but most likely genetics and environmental factors paly a considerable role in its occurrence [5,6].The clinical features at diagnosis are extremely diverse; approximately 25% of the patients are asymptomatic and have early stage (Rai 0 or I) disease [1,2]. When symptomatic, lymphadenopathy (LAP) is the most common presenting feature, though fatigue, splenomegaly, hepatomegaly, fever, night sweats, weight loss and major hemorrhage are common clinical features as well[7].Flow cytometric immunophenotyping of CLL is the most reliable methodology to confirm the presence of clonal B- cells. A composite panel of different B-cell markers is applied to help distinguish CLL from other mature B-cell neoplasms (MBCN)[8]. The profile expression panel of five B-cell markers is integrated into a specialized CLL scoring system (modified Matutes score) in order to distinguish typical CLL (score 4-5) from other MBCN (0-3)[8]. Two clinical staging systems are applied to assess the outcome of CLL patients and these staging systems are based on physical examination and results of routine blood count; the Rai and Binet staging systems [9,10], Table (1).

Table (1): the Rai and Binet staging systems

Stages	**Descriptions**
Rai staging system	
Stage 0	Lymphocytosis with no LAP, splenomegaly, hepatomegaly, anemia or thrombocytopenia
Stage I	Lymphocytosis with LAP but with no splenomegaly, hepatomegaly, anemia or thrombocytopenia

Stage II	Lymphocytosis, splenomegaly, and maybe hepatomegaly and LAP, but with no anemia or thrombocytopenia
Stage III	Lymphocytosis with anemia, and maybe splenomegaly, hepatomegaly and LAP, but with no thrombocytopenia
Stage IV	Lymphocytosis with thrombocytopenia, and maybe splenomegaly, hepatomegaly and LAP.
Binet staging system	
Stage A	<3 areas of lymphoid organs with no anemia or thrombocytopenia
Stage B	≥3 areas of lymphoid organs with no anemia or thrombocytopenia
Stage C	Anemia (<10 g/dl),and or thrombocytopenia(<100 $x10^9$/l) and any number of lymphoid organs may be enlarged

To the best of our knowledge, this is the first study performed in Sulaimani and the largest in Kurdistan and whole Iraq to investigate the clinico-hematological and immunological parameters of CLL patients and to assess the staging classification according to the previous staging systems.

Patients and methods

This work is a retrospective cohort study conducted on 154 patients who were newly diagnosed with CLL at Sulaimani Public Health Laboratory (94 patients), and Nanakaly Hospital in Hawler (60 patients), Kurdistan Regional Government (KRG), Iraq, from January 1st 2013 to December 31st 2017. The patients were diagnosed according to the clinical history, physical examination and immunophenotypic criteria (using flow cytometry). Staging was performed using both Rai and Binet classifications and CLL scoring systems was applied to characterize the cases of typical CLL[8].In all cases, the laboratory diagnostic work–up comprised a combination of cytomorphology and immunophenotyping using flow cytometry [BD FACS Calibur flow cytometer and FACS Canto II (BD Biosciences, San Jose, CA, USA)] performed on peripheral blood and/or bone marrow aspiration. The panel of monoclonal antibodies was as follows: CD5, CD23, CD19, CD20, CD22, CD79b, FMC7, CD38, CD200, Kappa and Lambda immunoglobulin (Ig)light chains. The modified Matutes score was calculated as described previously.

Table (2): Chronic lymphocytic leukemia scoring system[8.]

Markers	Points	
	0	1
CD5	Negative	Positive
CD23	Negative	Positive
FMC7	Positive	Negative or weak
SIg	Medium/high	Weak
CD79b	Medium/high	Negative or weak

Data documented and analyzed using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 25.Descriptive statistics presented as median, mean (± standard deviation) and frequencies as percentages. The work was approved by the ethics committee of Kurdistan Board of Medical Specialties (KBMS).

Results

The distribution of 154 CLL cases among different age categories is shown in figue(1). The median patients age at diagnosis was 70 years old (range 39-82 years) and the majority of cases were aggregated in≥ 60 years age categories. Younger patients (<39 years) and patients over 80 years patients formed a minor population. Moreover, 99 patients (64.3%) were males and 55(35.7%) were females (male: female ratio of 1.8:1) and male predominance was reported at all age groups, Table(3).

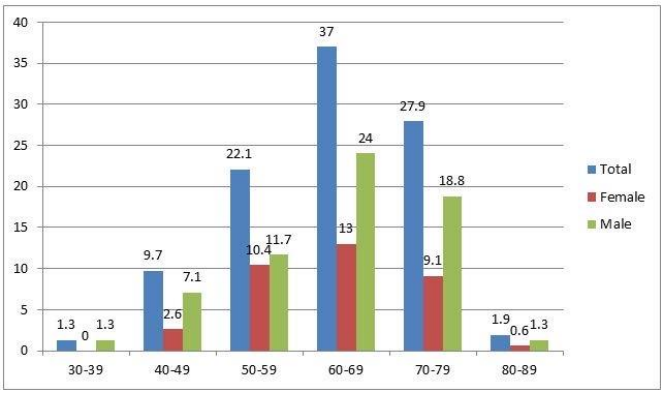

Figure (1): The distribution of age and gender among CLL patients.

Blood film examination revealed the predominance of small mature looking lymphoid cells with scanty cytoplasm , round nucleus and clumped chromatin in 140 (90.9%) of cases. Other 8(5.2%) cases showed the presence of small-medium sized lymphoid cells with a relatively clumped chromatin and small nucleoli (prolymphocytes) , forming less than 11% of total lymphocytes count .In addition, a heterogeneous population of small mature and larger lymphoid cells, with more dispersed chromatic and more abundant cytoplasm were reported in the rest 6 (3.9%) cases. At presentation ,35 patients (22.7%) were diagnosed incidentally during routine examination, and85 (55.2%) patients had B-symptoms .Lymphadenopathy (LAP) was the most common presenting feature, seen in 84 (54.6%) patients ,while splenomegaly was seen in 40 (26%) patients and hepatomegaly in 7 (4.6) patients Table(3).

Table (3): The demographic, clinical and hemogram parameters of the patient.

Variables	**Range**
Age (in years)	39-82
Males	99(64.35)
Females	55(35.7%)
Incidental diagnosis (%)	35 (22.7)
B symptoms (%)	85 (55.2)
LAP (%)	84 (54.6)
Splenomegaly (%)	40 (26)
Hepatosplenomegaly (%)	7 (4.6)
Hb (g/dl) mean11.7 ± 2.2	(4.8 - 16.7)
WBC ($x10^9$/L) mean 73.2 ± 88.7	(7 – 504)
Platelet ($x10^9$/L)mean178.5 ± 78.4	(30 – 431)

Immunophenotyping using flow cytometry analysis had shown that all cases expressed the B –cell markers; CD19, CD20 ^{weak} in addition to CD5 ^{bright} and CD23 (98.7%), while CD22 ^{weak}, CD79b ^{weak} were less frequently reported, in 55.2% and 64.9% respectively. FMC7^{weak} was the least expressed marker among the panel of B-cells markers used to diagnose CLL cases (7.8%). Clonality on the other hand was reported in 146 (94.8%) cases, with Kappa light chain restriction detected in 90(61.6%) of CLL cases and Lambda light chain reported in 56(38.4%) cases .CD38 was expressed by leukemic B-cells in 49(31.8%) cases (Table 4). Two CLL cases lacked the expression of CD23; therefore, cyclin D1 was performed on lymph node biopsy to

exclude mantle cell lymphoma, which was shown to be negative. Chronic lymphocytic leukemia scoring system, based on using a panel of five membrane markers (Table2) revealed that 112 (72.7%) patients, scored four out of five points, and the rest (27.3%) scored five.

Table (4): The results (%) of cases positive for different of CD markers among the 154 CLL patients.

CD markers		Positive (%)	Negative (%)
CD19		154 (100)	—
CD20		154 (100)	—
CD22		85 (55.2)	—
CD5		154 (100)	—
CD23		152 (98.7)	2 (1.3)
CD79b		100 (64.9)	54 (35.1)
FMC7		12 (7.8)	142 (92.2)
CD38		49 (31.8)	105 (68.2)
Ig light chain	Kappa	90 (61.6)	56(38.4)
	Lambda	56 (38.4)	90(61.6)
CD200		*104 (100)	

*Performed in 104 cases.

Sixty five (42.2%) patients presented with advanced Rai stages (III and IV) and 62(40.3%) patients presented with stage C Binet staging system.

Table (5): Rai and Binet clinical staging of the patients.

Staging		Frequency (%)
Rai system	Stage 0	34 (22.1)
	Stage I	28 (18.2)
	Stage II	27 (17.5)
	Stage III	44 (28.6)
	Stage IV	21 (13.6)
Binet system	Stage A	83 (53.9)
	Stage B	9 (5.8)
	Stage C	62 (40.3)

Discussion

Chronic lymphocytic leukemia is a neoplasm composed of small mature B-cells which co-express CD5 and CD23. While it's the most common leukemia in Western countries, it's the least frequent in developing countries including Iraq[11]. The

median age of CLL patients in this study (70 years) is higher than a figure reported by an earlier local study from Baghdad/Iraq by Nallawi et al[12] (60 years) and two other Iraqi figures by Hasan (65years)[4] and Naji (60years)[1], while it's comparable with previously reported figures worldwide[14-16]. Furthermore, this work had confirmed male predominance among CLL patients in various age categories in accordance with previous studies[4, 13,17]. Reports from developed countries had revealed that the majority of CLL cases are diagnosed on the basis of routine blood investigations in asymptomatic subjects[18].In contrast, only 22.7 % of our patients were diagnosed incidentally, while just over half had B-symptoms at presentation (55.2%). This finding was further highlighted by the advanced stages our patients tend to present with (Rai stage III / IV 42.2 %) and (40.3%) Binet stage C in agreement with previous studies from developing countries which showed the largest proportion of their studied CLL patients had presented with advanced clinical stage, and the figures ranged from 32%-63.3% for Rai stage III and IV)and (58-63.2% for Binet stage C)[4,12,13,16] and in contrast to developed countries reported CLL cases[17,18]. The latter might be attributed to a variation in the biology of the disease, lack of regular medical checkup and defects in referring system[2, 21]. Generally in this study, the clinical and hematological features in our patients were similar to those reported from previous studies[2, 4, 15,16].In addition to the clinic-hematological parameters, this study had highlighted the immunophenotype of 154 patients with de novo CLL and found that CD19,CD5,CD20 and CD23 (98.7 %) were expressed in all the patients, while CD 22 , FMC7 and CD 79b were less consistently expressed on leukemic B-cells and our results are in line with previous studies which documented that the following immunophenotypic profile is characteristic ofCLL:CD19,CD5^{bright},CD23,CD20^{weak},CD22 ^{weak}, CD79b^{weak}, FMC7 $^{weak/-}$ and weak immunoglobulin light chain restriction characterize B-cells in CLL[20,22]. Furthermore, some previous studies had suggested that CD200 can be a novel marker in the differentiation between MBCN and CLL[23,24]. Furthermore, a new scoring system had been suggested by T Kőhnke et al[25] which introduces CD200 instead of s-Ig (CLL flow score) and proved to diagnose CLL with significantly higher specificity. Expression of CD38 (> 30%), ZAP70 (> 20%)or CD49d is associated with adverse prognosis. In this study CD38 was a part of

the panel applied for CLL diagnosis and was less frequently expressed (31.8%); just similar to what was reported by earlier studies, ranging from 21%-32%[4, 26,27].Considering surface light restriction, 94.8% of cases showed Ig-light chain restriction with a higher frequency of Kappa (61.6%) over Lambda (38.4%), and Matutes et *al*[8] Ivancevic et *al*[9] and had shown a comparable results regarding Ig light chain expression (92% and 95% respectively). Moreover, an absent Ig- light chain at presentation in a minority of CLL cases at presentation is considered as an aberrant feature of leukemic B- cells in CLL[8, 9].

Conclusions

Apart from advanced stages at presentation, this study has shown that the clinico–hematological and immunophenotypic characteristics of our patients are comparable to previous regional and international data. Further studies are required to determine the specificity of CD200 in differentiating CLL from other MBCN. In addition, it's prudent to predict the survival by correlating both CD markers and genetic studies.

Conflicts of interest

The authors report no conflict of interest.

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