CLINICAL & BASIC RESEARCH

Clinical Aspects, Immunophenotypic Analysis and Survival Rate of Chronic Lymphocytic Leukaemia Patients in Erbil City, Iraq

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الجوانب السريرية (الإكلينيكية)، تحليل النمط المناعي الظاهري ومعدل البقاء على قيد الحياة لمرضى سرطان الدم الليمفاوي المزمن في مدينة أربيل، العراق

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ABSTRACT: *Objectives:* Chronic lymphocytic leukaemia (CLL) is characterised by an accumulation of clonal B cells in the blood, bone marrow and lymphatic tissue. This study aimed to evaluate the clinical and immunophenotypic characteristics and survival rate of CLL patients. *Methods:* This retrospective study was conducted at the Nanakaly Hospital for Blood Diseases & Oncology in Erbil, Iraq, between January 2011 and December 2017. A total of 105 CLL patients were assessed to determine clinical presentation and staging, immunophenotype and survival rate. *Results:* The median age of the patients was 65 years and 63.8% were male. The main clinical presentations were splenomegaly (64.8%), pallor (61.9%) and lymphadenopathy (60%). More than half of the patients presented at an advanced clinical stage according to the Rai and Binet staging systems (59.1% and 55.2%, respectively). All CLL cases expressed both cluster of differentiation (CD)19 and CD5, 67.6% had monoclonal kappa light chains and 21% expressed CD38. The five-year overall survival (OS) rate was 61.3%. The mean duration of five-year survival was 41.3 months (95% confidence interval: 36.4–46.3 months). There were no correlations between survival and sociodemographic, clinical or laboratory characteristics. *Conclusion:* In comparison to the existing Western literature, Iraqi CLL patients more frequently presented with hepatosplenomegaly and at a more advanced clinical stage. In addition, the five-year OS rate was much lower.

Keywords: Lymphoproliferative Disorders; Chronic Lymphocytic Leukemia; Immunophenotyping; Survival Rates; Iraq.

الملخص: **الهدف:** يتميز سرطان الدم الليمفاوي المزمن بتراكم خلايا ب النسيلة في الدم ونخاع العظام والنسيج الليمفاوي. هدفت هذه الدراسة الي تقييم الخصائص السريرية و النمط المناعي الظاهري ومعدل البقاء على قيد الحياة للمرضى المصابين بسرطان الدم الليمفاوي المزمن. **الطريقة:** أجريت هذه الدراسة الأسترجاعية في مستشفى ناناكالي لأمراض الدم والأورام في أربيل، العراق، بين يناير 2011 وديسمبر 2017. تم تقييم ما مجموعه 105 مصابا بمرض سرطان الدم الليمفاوي المزمن لتحديد العرض السريري و مرحلة المرض و النمط المناعي الظاهري ومعدل البقاء على قيد الحياة. **النتائج:** كان متوسط عمر المرضى 65 سنة بينهم 63.8% من الذكور. وكانت العلامات السريرية الرئيسية عبارة عن تضخم الطحال (64.8%)، شحوب (61.9%) واعتلال العقد اللمفاوية (60%). كان أكثر من نصف المرضى في مرحلة سريرية متقدمة وفقا لجداول راي وبينيت (59.1% و 55.2% على التوالي). أظهر تحليل النمط المناعي الظاهري لجميع المرضى كلا من مجموعة التمايز 19 و 5، بينما أظهر 67.6% من المرضى سلاسل كابا الخفيفة وحيدة النسيلة و 21% منهم كان لديهم مجموعة التمايز 38. وكان المعدل العام للبقاء على قيد الحياة لمدة خمس سنوات هو 61.3%. وكان متوسط مدة البقاء على قيد الحياة لمدة خمس سنوات 41.3 شهرا (فاصل الثقة 95%: 36.4–46.3 أشهر). لم تكن هناك ارتباط بين معدل البقاء على قيد الحياة والصفات الاجتماعية والديموغرافية والسريرية والمخبرية للمرضى. **الخلاصة:** بالمقارنة مع المطبوعات الغربية الحالية، فإن مرضى سرطان الدم الليمفاوي المزمن العراقيين لديهم بنسبة اكثر من تضخم الطحال والكبد ويتم تشخيصهم في مرحلة سريرية أكثر تقدمًا. بالإضافة إلى ذلك، كان معدل البقاء على قيد الحياة لمدة خمس سنوات فيهم أقل بكثير.

الكلمات المفتاحية: الاضطرابات التكاثرية الليمفاوية؛ سرطان الدم الليمفاوي المزمن؛ النمط المناعي الظاهري؛ معدلات البقاء على قيد الحياة؛ العراق.

Advances in Knowledge

- This study provides the clinical and immunophenotypic findings and the survival rate of chronic lymphocytic leukaemia (CLL) patients in Erbil City, Iraq.
- The current sample of CLL patients more frequently presented with hepatosplenomegaly, although less frequently with lymphadenopathy, compared to populations reported from Western countries. In addition, the Iraqi patients tended to present at a more advanced clinical stage and had lower survival rates than Western patients.

Application to Patient Care

- The current results highlight the importance of implementing more advanced work-up tools for Iraqi CLL patients, such as a thorough cytogenetic evaluation.

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CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) IS the most common type of leukaemia among adults in Western countries; however, it is relatively rare in Asia.[1,2] It is characterised by the build-up of lymphocytes in the blood, bone marrow and lymphatic tissue. The median age at diagnosis ranges from 67–72 years and the condition is more common in males.[1,2] The clinical course of CLL is highly variable, ranging from no symptoms to the rapid development of features of high-risk disease.[3] Lymph node swelling is the most common presenting feature of CLL, although fever, night sweats and weight loss are sometimes seen. The most common physical findings are lymphadenopathy, splenomegaly and, less frequently, hepatomegaly.[3]

A diagnosis of CLL can be established via a complete blood count (CBC) showing the progressive accumulation of clonal B cells (>5,000 B lymphocytes/mL) over a period of at least three months and an immunophenotypic study demonstrating clonal lymphocytes expressing B cell markers and cluster of differentiation (CD)5.[4] Both the modified Rai and Binet clinical staging systems are widely used to classify CLL patients into different prognostic groups according to the extent of lymph node involvement, enlargement of the liver and/or spleen and blood findings (i.e. anaemia and thrombocytopaenia).[5,6]

The median survival rate for patients with CLL ranges from 18 months to more than 10 years, depending on prognostic factors like age, gender, disease stage, performance status, lymphocyte doubling time, absolute lymphocyte count (ALC) and β-2 microglobulin serum levels.[7] Furthermore, the expression of certain biological markers such as CD38 or CD49d, deletion of chromosome 17p13 and the mutation status of the *immunoglobulin heavy-chain variable region*, *tumour protein-53* (*TP53*), *Notch homolog 1*, *splicing factor-3B subunit-1* (*SF3B1*), *baculoviral inhibitor of apoptosis protein-1 repeat-containing protein-3* (*BIRC3*), *myeloid differentiation primary response 88* and *zeta chain T cell receptor-associated protein kinase-70* (*ZAP70*) genes also have a prognostic impact.[8] The current study aimed to assess and compare the clinical characteristics, immunophenotypic findings and survival rate of CLL patients in Erbil, Iraq, with previously reported international and regional data.

Methods

This retrospective study was carried out between January 2011 and December 2017 at the Nanakaly Hospital for Blood Diseases & Oncology, a regional referral hospital for adult and paediatric patients with benign and malignant haematology and solid oncology disorders in Erbil, Iraq. All patients attending the hospital during the study period and diagnosed with CLL as confirmed by CBC and immunophenotyping were invited to participate in the study.

The main clinical presentation and characteristics of each patient was documented, including the presence of lymphadenopathy, pallor, hepatomegaly and splenomegaly. In addition, the patients underwent a CBC and immunophenotyping study by flow cytometry using a FACSCanto II flow cytometer device (BD Biosciences, San Jose, California, USA) and/or immunohistochemistry. The laboratory analysis included a reticulocyte count, erythrocyte sedimentation rate, direct antiglobulin test (DAT), bone marrow examination, serology tests (i.e. to determine lactate dehydrogenase [LDH] levels) and liver and renal function tests. In indicated cases, patients also underwent various imaging studies, including ultrasonography of the abdomen, a chest X-ray and computed tomography. In each case, clinical stage was determined

Table 1: Baseline characteristics of chronic lymphocytic leukaemia patients in Erbil, Iraq (N = 105)

Characteristic	n (%)
Age in years	
<50	10 (9.5)
50–59	21 (20)
60–69	40 (38.1)
≥70	34 (32.4)
Gender	
Male	67 (63.8)
Female	38 (36.2)
Clinical presentation*	
Splenomegaly†	68 (64.8)
Pallor	65 (61.9)
Peripheral lymphadenopathy	63 (60)
Hepatomegaly	23 (21.9)
Rai clinical stage	
0	14 (13.3)
I	9 (8.6)
II	20 (19)
III	19 (18.1)
IV	43 (41)
Binet clinical stage	
A	29 (27.6)
B	18 (17.1)
C	58 (55.2)

*Percentages do not add up to 100% as some patients may have had more than one clinical presentation. †For patients with splenomegaly, the mean longitudinal axis of the spleen was 14.7 ± 3.4 cm.

Table 2: Laboratory results of chronic lymphocytic leukaemia patients in Erbil, Iraq (N = 105)

Investigation	Mean ± SD	Median	Range	Normal range
Haemoglobin in g/dL	10.6 ± 2.3	11	5–15.4	13.5–17.5/11.5–15.5*
Whole blood count × 10⁹/L	94.8 ± 106.6	65	8.9–491	4–11
Absolute lymphocyte count × 10⁹/L	87 ± 104.5	58.5	6–462.5	1–4.8
Absolute neutrophil count × 10⁹/L	6.7 ± 5.7	6	0.2–54	1.8–7.7
Platelet count × 10⁹/L	140 ± 74.2	115	30–382	150–400
Reticulocytes, %	2.1 ± 2.0	1.7	0.1–10	0.5–1.5
Erythrocyte sedimentation rate in mm/hour	44.3 ± 33.3	37	2–145	0–10/3–15*
Lactate dehydrogenase in U/L	400.1 ± 159.9	378	108–905	100–250
Aspartate aminotransferase in U/L	24 ± 10.9	22	8.9–99	8–48
Alanine transaminase in U/L	18.1 ± 9.9	17	4–50.3	7–55
Blood urea in mg/dL	44.2 ± 21.3	41	16.3–160	15–40
Serum creatinine in mg/dL	1 ± 0.4	1	0.3–3	0.7–1.3

*SD = standard deviation. *Males/females.*
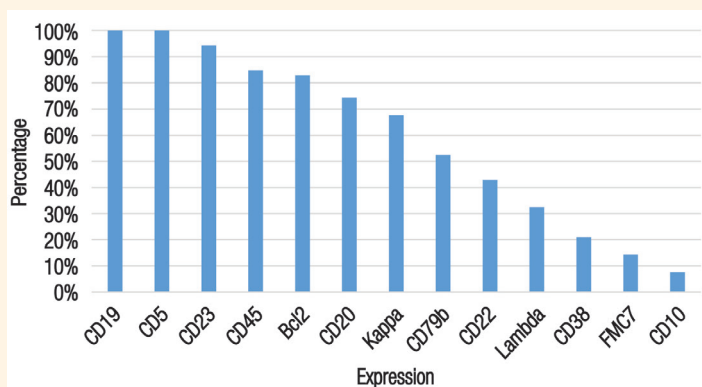
Figure 1: Immunophenotypic findings of chronic lymphocytic leukaemia patients in Erbil, Iraq (N = 105).
CD = cluster of differentiation; Bcl2 = B cell lymphoma 2; FMC7 = Flinder Medical Centre-7.

Table 3: Treatment provided to chronic lymphocytic leukaemia patients in Erbil, Iraq (N = 105)

Treatment*	n (%)
FCR	24 (22.9)
BR	19 (18.1)
FC	2 (1.9)
Chlorambucil	9 (8.6)
Chlorambucil plus prednisolone	10 (9.5)
Rituximab and chlorambucil	10 (9.5)
COP	4 (3.8)
Rituximab and COP	5 (4.8)
Rituximab and CHOP	7 (6.7)
Rituximab	2 (1.9)
No treatment	17 (16.2)

*FCR = fludarabine, cyclophosphamide and rituximab; BR = bendamustine plus rituximab; FC = fludarabine plus cyclophosphamide; COP = cyclophosphamide, oncovin and prednisolone; CHOP =cyclophosphamide, hydroxydaunomycin, oncovin and prednisolone. *Percentages do not add up to 100% as four patients were treated with more than one protocol.*

according to both the Rai and Binet staging systems.[5,6] Survival rates were calculated, including the overall survival (OS) rate over the study period and the five-year OS rate.

Data were analysed using the Statistical Package for the Social Sciences (SPSS), Version 22.0 (IBM Corp., Armonk, New York, USA). A Chi-squared test was used to compare associations between proportions. Kaplan-Meier survival curves were plotted and the log-rank Mantel-Cox test was used to compare mean survival times. A *P* value of ≤0.050 was considered statistically significant.

This study was approved by the Scientific and Ethical Committees of the College of Medicine, Hawler Medical University, Erbil.

Results

A total of 143 CLL patients attended the hospital during the study period. However, 38 patients were excluded due to a lack of adequate data or follow-up. As such,

Table 4: Correlation between survival rate and selected characteristics among chronic lymphocytic leukaemia patients in Erbil, Iraq (N = 105)

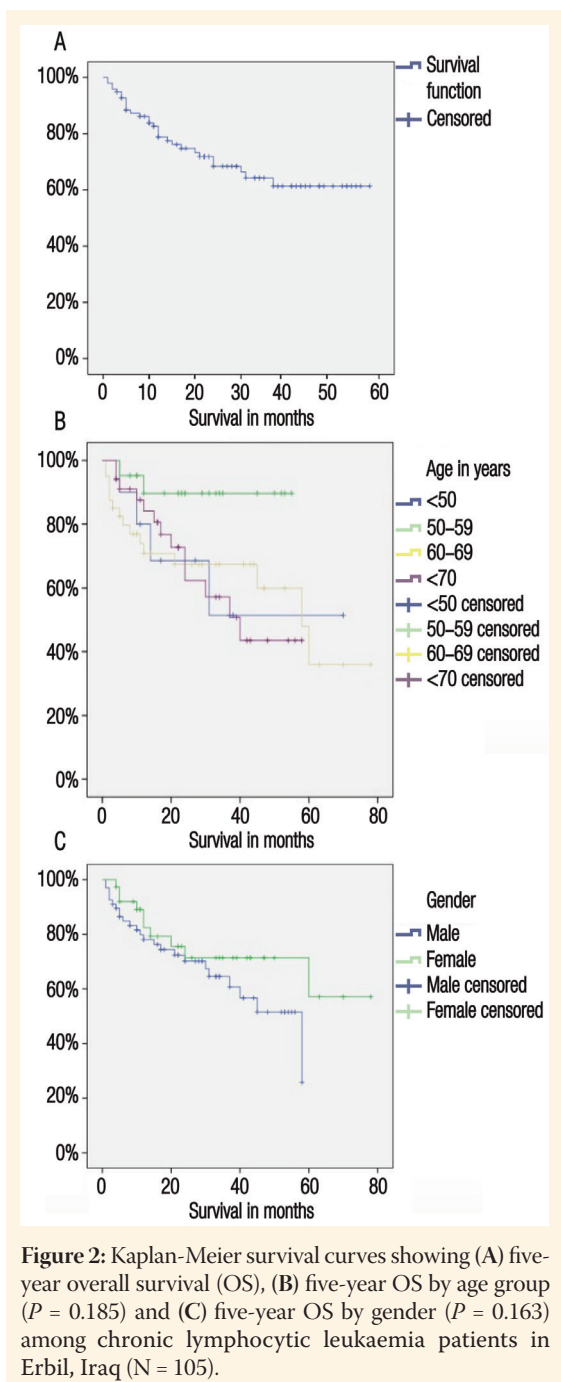| Characteristic	n (%)		*P* value
	Survived (n = 71)	Died (n = 34)	
Age in years			
<50	6 (60)	4 (40)	
50–59	19 (90.5)	2 (9.5)	0.098
60–69	25 (62.5)	15 (37.5)	
≥70	21 (61.8)	13 (38.2)	
Gender			
Male	43 (64.2)	24 (35.8)	0.163
Female	28 (73.7)	10 (26.3)	
Lymphadenopathy			
Yes	41 (65.1)	22 (34.9)	0.496
No	30 (71.4)	12 (28.6)	
Splenomegaly			
Yes	46 (67.6)	22 (32.4)	0.993
No	25 (67.6)	12 (32.4)	
Hepatomegaly			
Yes	14 (60.9)	9 (39.1)	0.434
No	57 (69.5)	25 (30.5)	
Rai clinical stage			
0	12 (85.7)	2 (14.3)	
I	4 (44.4)	5 (55.6)	
II	15 (75)	5 (25)	0.190
III	14 (73.7)	5 (26.3)	
IV	26 (60.5)	17 (39.5)	
Binet clinical stage			
A	21 (72.4)	8 (27.6)	
B	13 (72.2)	5 (27.8)	0.648
C	37 (63.8)	21 (36.2)	
Hb level in g/dL			
≤11	54 (65.9)	28 (34.1)	0.465
>11	17 (73.9)	6 (26.1)	
WBC × 10⁹/L			
<65	37 (69.8)	16 (30.2)	0.628
≥65	34 (65.4)	18 (34.6)	
Platelet count × 10⁹/L			
<150	44 (69.8)	19 (30.2)	0.551
≥150	27 (64.3)	15 (35.7)	
LDH in U/L			
<250	17 (81)	4 (19)	0.144
≥250	54 (64.3)	30 (35.7)	

Hb = haemoglobin; WBC = white blood cells; LDH = lactate dehydrogenase.

105 patients were included in the final analysis. Of these, 67 (63.8%) were male and 38 (36.2%) were female. The mean age at presentation was 63.3 ± 10.7 years. Most patients (70.5%) were ≥60 years, with only 9.5% <50 years. The age at diagnosis ranged from 28–81 years old, with a median of 65 years. The main clinical presentations were splenomegaly (64.8%) pallor (61.9%) and lymphadenopathy (60%). Hepatomegaly was observed in 21.9% of patients. Modified Rai staging showed a high risk (stage III or IV) of CLL in 59.1%, an intermediate risk (stage I or II) in 27.6% and a low risk (stage 0) in 13.3% of patients. In terms of Binet staging, 55.2% of patients were stage C, 17.1% were stage B and 27.6% were stage A [Table 1]. Most patients (72.4%) were from Erbil and other cities in the Kurdistan region of Iraq, while the rest were from other northern and western Iraqi cities.

The mean haemoglobin level was 10.6 ± 2.3 g/dL (range: 5–15.4 g/dL). Mean platelet and white blood cell counts were 140 ± 74.2 × 10⁹/L (range: 30–382 × 10⁹/L) and 94.8 ± 106.6 × 10⁹/L (range: 8.9–491 × 10⁹/L), respectively. The mean ALC was 87 ± 104.5 × 10⁹/L (range: 6–462.5 × 10⁹/L) [Table 2]. Overall, LDH levels were elevated in 82.8% of patients and 11.4% had autoimmune haemolytic anaemia (AIHA) with positive DAT results. Anaemia was observed in 83 (79.1%) patients, comprising 83.6% and 71.1% of male and female patients, respectively. A total of 63 (60%) and 28 (26.7%) patients presented with thrombocytopaenia and incidental lymphocytosis, respectively. None of the patients had Richter's syndrome.

All patients underwent immunophenotyping analysis via flow cytometry and/or immunohistochemistry from either peripheral blood (79.1%) or bone marrow (21%) samples. All cases expressed both CD19 and CD5, while the vast majority expressed CD23 (94.3%), CD45 (84.8%) and B cell lymphoma 2 (82.9%). About two-thirds of the patients (67.6%) expressed monoclonal kappa light chains, while just under one-third (32.4%) expressed lambda chains. Only 21% of cases expressed CD38 [Figure 1]. In terms of treatment, most patients received either fludarabine, cyclophosphamide and rituximab (22.9%) or bendamustine plus rituximab (18.1%). The rest underwent various other chemotherapy protocols (46.7%) or did not receive any treatment at all (16.2%) [Table 3].

The mean follow-up period was 27.7 months. A total of 34 (32.4%) patients died during the study period, of which 24 (70.6%) were male and 10 (29.4%) were female, resulting in an OS rate of 67.6%. Although survival was greater among those aged 50–59 years (90.5% versus 60–62.5% in other age groups) and among females (73.7% versus 64.2% in males), these differences were not statistically significant (*P* = 0.098 and 0.163,

Figure 2: Kaplan-Meier survival curves showing **(A)** five-year overall survival (OS), **(B)** five-year OS by age group (P = 0.185) and **(C)** five-year OS by gender (P = 0.163) among chronic lymphocytic leukaemia patients in Erbil, Iraq (N = 105).

respectively). Other clinical and laboratory characteristics were also not significantly correlated with survival (P >0.050 each) [Table 4]. The five-year OS rate was 61.3%. The mean duration of five-year survival was 60 months (95% confidence interval: 36.4–46.3 months); however, the median survival could not be estimated and could have occurred beyond the study period. The mean duration of survival was longer among females and those aged 50–59 years old; however, these differences were not statistically significant (P = 0.185 and 0.163, respectively) [Figure 2].

Discussion

In the current study, the median age at diagnosis of CLL patients in Erbil, Iraq, was similar to that reported in neighbouring countries such as Iran and Turkey (65 years versus 60.73 and 64 years, respectively).[2,9] In contrast, the median age at diagnosis in Western countries is much higher, ranging from 67–72 years old.[1,10,11] However, the frequency of male CLL patients in the current study was identical to that reported in Italy (64%).[12] In terms of clinical presentation, spleno-megaly (64.8% versus 54%) and hepatomegaly (21.9% versus 14%) were reported more frequently in the current study compared to Western patients, while lymphadenopathy was less frequent (60% versus 87%).[3] In Turkey, rates of splenomegaly, hepatomegaly and lymphadenopathy differed at 55.4%, 54.6% and 79.2%, respectively.[9] In Iran, Payandeh *et al.* also reported varying rates of organomegaly (34%) and lymphaden-opathy (38.7%).[2] The diversity of clinical presentations in CLL and correlations with ethnicity have been well documented in previous research.[13]

The incidence of anaemia and AIHA in the current study (78.1% and 11%, respectively) was comp-arable to that reported in previous research in which AIHA was documented in up to 11% of late-stage CLL patients, with positive DAT results seen in up to 15% of patients.[14] In the present study, 60% of patients had thrombocytopaenia; this finding is understandable given that most patients presented at an advanced clinical stage, such as Rai stage III or IV (59.1%) or Binet stage C (55.2%). According to data from other developing countries such as Iran, Turkey and India, patients in this region are often first seen at more advanced stages (38.5%, 33.3% and 41%, respectively).[2,15,16] However, in developed countries, only 10–20% of patients present at an advanced stage.[17] These findings may indicate that routine CBC analysis is less frequently performed in developing countries or that hospitals in this region lack proper evaluation and management protocols or experience delays in the referral of patients with incidental lymphocytosis. It is also possible that there is a difference in the biology of the disease (e.g. in terms of such patients having a less mutated subtype of CLL).[4]

In the present study, most cases expressed CD19, CD5 and CD23, with CD79b less frequently expressed; these findings are consistent with the characteristic profile of CLL disease.[18] For those patients not expressing CD23—considered atypical CLL cases—other markers could help to differentiate CLL from mantle cell lymph-oma, such as the negative expression of Flinder Medical

Centre-7.[19] Overall, CD38 expression at any level is regarded as a poor prognostic indicator; it was expressed in 21% of cases in the present study, which is comparable to the findings of Hojjat Farsangi *et al.* (27.6%).[20] In the current study, 67.6% of patients had monoclonal kappa light chains, with 32.4% expressing lambda chains. Hojjat Fersangi *et al.* reported rates of expression of kappa and lambda chains to be 71.3% and 28.7%, respectively, among Iranian CLL patients.[20] Stamatopoulos *et al.* also reported similar findings in Greece.[21]

The OS (67.6%) in the current study was comparable to data reported from Iran (64%), although the five-year OS rate (61.3%) was higher than results from Turkey (36.5%) and India (51%).[2,22,23] However, data from Germany and the USA indicate much higher five-year relative survival rates (80.2% and 82.4%, respectively).[24] The mean duration of five-year survival in the current study was 41.3 months, which is similar to data reported from Iran (38.5 months).[2] This may be related to variations in a number of patient-related characteristics, such as clinical stage at diagnosis. The reason why the five-year OS rate in the present study was lower than the overall OS is likely because patients whom were more recently diagnosed (i.e. in 2015–2017) had not yet received their five-year follow-up at the time of the analysis.

Early diagnosis plays a major role in improving survival in CLL patients.[25] Other possible causes for low five-year OS rates are the lack of availability of equipment to conduct thorough cytogenetic evaluations for factors known to have a prognostic impact—such as deletions of chromosomes 11q, 13q, 17p and trisomy 12 as well as *TP53*, *NOTCH1*, *SF3B1* and *BIRC3* gene mutations.[8] In addition, insufficient access to adequate or novel chemotherapy agents like obinutuzumab, ibrutinib, idelalisib and venetoclax as a frontline treatment or for relapsed/refractory cases may negatively affect survival.[26]

No significant correlations were noted between survival rates and selected sociodemographic, clinical or laboratory characteristics in the present study. These findings partially agree with those previously reported by Shvidel *et al.*; however, they contradict de Faria *et al*'s study.[27,28] The difference between these findings is probably due to variations in sample size, the lack of genetic assessment conducted in the current study and the well-known impact of ethnicity.[13] In addition, data regarding CD49d and *ZAP70* expression, which have a major prognostic impact on CLL patients, were not available.[8] Further research is therefore recommended to evaluate these factors.

Conclusion

The median age, male-to-female ratio and clinical and immunophenotypic characteristics of Iraqi CLL patients in the current study did not differ greatly from previous regional and international data; however, patients more frequently presented at an advanced stage and had a lower five-year OS rate compared to Western populations. In addition, Iraqi patients more frequently presented with organomegaly, including hepatomegaly, although less frequently with lymphadenopathy.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FUNDING

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