Original Article

Effectiveness of Chlorambucil as Front-line Therapy in Sudanese Patients with Chronic Lymphocytic Leukemia Attending Khartoum Oncology Hospital

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Abstract

Background: Chlorambucil was the standard of care therapy in chronic lymphocytic leukemia (CLL), it becomes restricted for a specific type of CLL population, due to the launching of more effective anticancer agents. However, in Sudan, chlorambucil remains the first-line therapy in CLL. Thus, the study aimed to determine the effectiveness of chlorambucil in Sudanese CLL patients who attended the Khartoum Oncology Hospital from January 2014 to October 2017. **Methods:** This was a descriptive retrospective hospital-based study in which files of patients who met the inclusion criteria were critically reviewed using a data collection sheet. The main response indicative parameters were lymphocytes count, lymph node enlargement, and organomegaly status. Effectiveness was assessed by measuring the overall response rate (ORR) as the primary endpoint and the progression-free survival (PFS) as the secondary endpoint. **Results:** A total of 64 patients were included, 62.5% of them were male. The majority of them (59.4%) were aged ≥65 years old. Clinically, 43.8% of these patients were at stage IV. Around 63.1% of the patients received high dosages of chlorambucil. The median PFS for chlorambucil was 18 months. The complete clinical and partial remission rates were 24.4% and 20%, respectively. The ORR was significantly higher with the higher dose of chlorambucil ($P = 0.019$). While in terms of PFS, there was an insignificant difference between high dose (15 months) and small dose (22 months) of chlorambucil. **Conclusion:** In CLL Sudanese patients, chlorambucil was shown to have low response rates. High doses of chlorambucil lead to induction of better ORR, but there was no additional benefit in PFS compared to those who received low doses of chlorambucil.

Keywords: Chlorambucil, chronic lymphocytic leukemia, Khartoum oncology hospital, overall response rate, progression-free survival

Introduction

Chronic lymphocytic leukemia (CLL) is "a neoplasm characterized by the relentless accumulation of CD5+ B lymphocytes in the peripheral blood, bone marrow, and secondary lymphoid organs (lymph nodes and spleen)."[1] In general, CLL is distinguished by a rise in the lymphocyte count in the peripheral blood of $\geq 5 \times 10^9$ L^{-1}.[1] Although there is substantial evidence of a genetic disposition in CLL, the etiology remains unclear.[2] CLL is considered the most frequent type of adult leukemia, and men are more likely than women to get it.[1] However, the incidence of CLL is affected by racial differences, non-Hispanic whites are the most likely to develop CLL, followed by blacks.[3] In addition, the pathogenesis of CLL is affected by genetic and familial predisposition factors.[1,4] Single nucleotide polymorphisms in almost 30 loci, including Interferon Regulatory Factor 4, Lymphoid Enhancer Binding Factor 1, B-cell lymphoma 2, and Telomerase Reverse Transcriptase, have been linked to familial CLL in genome-wide association studies.[1,3]

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Since the CLL is an incurable illness in most patients, CLL therapy aims to improve quality of life and prolong survival. As a result, for the vast majority of patients, survival is determined by the effectiveness and sequencing of therapy sequences administered during the course of the disease.[5,6] The first-line treatment of CLL should be determined by the patient's age, disease stage, comorbid conditions, performance status, and the agent's toxicity profile.[6] According to the National Comprehensive Cancer Network (NCCN), the preferred first-line treatment for all CLL patients is Bruton tyrosine kinase inhibitors and venetoclax. Whereas chlorambucil combined with Rituximab could be used as the second line in a frail patient with significant comorbidity or patients aged 65 years or older and younger patients with significant comorbidities.[6]

Chlorambucil is a bifunctional nitrogen mustard alkylating agent, forms covalent adducts with double-helical DNA, and suppresses cell growth. Interstrand cross-links are the most damaging of these adducts because they impede replication by causing DNA double-strand breaks. On the other hand, intrastrand cross-links caused by chlorambucil are effectively repaired by a specialized nucleotide excision repair enzyme.[7] In the past, chlorambucil was considered the best option for previously untreated CLL patients. The introduction of novel medicines, such as purine nucleoside analogs (PNAs) and, more importantly, their combination with PNAs (like fludarabine-cyclophosphamide-rituximab), narrowed the indications for CLL. Although chlorambucil utilization is less in monotherapy because of the availability of different treatment options, using chlorambucil in combination with anti-CD20 antibodies is still an option for fragile or unfit individuals.[8,9]

In Sudan, even introduction of new anticancer agents to treat CLL patients, chlorambucil is still commonly used as front-line therapy in CLL patients. Thus, this study was carried out to assess the effectiveness of chlorambucil as a front-line therapy for Sudanese CLL patients.

Methods

Study design and setting

The study was a descriptive retrospective hospital-based study. It was conducted at Khartoum Oncology Hospital, Khartoum, Sudan. The study population was CLL patients admitted to the hospital from January 2014 to October 2017.

Participants and sample size

Medical files of CCL patients admitted to Khartoum Oncology Hospital between January 2014 and October 2017 were reviewed. All patients diagnosed with CLL and treated with chlorambucil were included. Whereas all medical files with missing information were excluded from this study. At the initial screening stage, 568 cases were treated using chlorambucil. However, some of them have died, and files were not available at the time of data collection, some cases after complete the investigations and clinical assessments were diagnosed wrongly as CLL patients, and there is no follow-up in others, and some contained missing information. Therefore, the total number of included samples was 64 medical files [Figure 1].

All included CLL patients were CD5, CD19, CD20, and CD23 positive. Rai *et al*.'s classification was used to determine the clinical stage of disease at the time of treatment initiation,[10] accordingly patients in stage I, II, III, and IV disease with evidence of progression, and had no previous chemotherapy for CLL, and received a minimum of two cycles of chlorambucil chemotherapy were included in the study. Whereas patients who used oral corticosteroids chronically for a long period of time, or were diagnosed with autoimmune thrombocytopenia, had previous bone marrow transplants, or with active secondary malignancy were excluded from the study according to the CAM307 Protocol.[11,12]

Dosage for chlorambucil

The doses of chlorambucil administered by cycles every 28 days, either small dosages ranged from 0.05 to 0.1 mg/kg daily, or high doses ranged from 7 mg/m^2 day 1 to day 10, or 10 mg/m^2 day 1 to day 7, or 5. The total chlorambucil dose administered per 28-day cycle ranged from 60 mg (over 28 days) to 126 mg (over 7 days).

Outcome measures

The data were collected from the records using datasheet described case number, demographic information, prognostic factors, clinical staging at diagnosis, and baseline treatment indicative parameters (white blood cells, platelets, hemoglobin, lymphocytes, and lymph nodes or organomegaly occurrence) before starting treatments. The same treatment indicative parameters were also collected after the exact number of treatment cycles, date of starting treatment, date of progression, the type of regimen used, and whether complete remission (CR), partial response (PR), progression, or no response. In addition, all case reports were evaluated for the assessment of disease status and survival and reviewed for the monthly follow-up until the time of disease progression, administration of alternative therapy, or the date of data cutoff, whichever was first. The

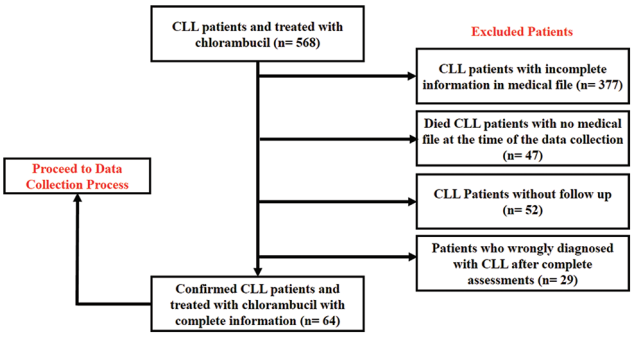

Figure 1: The flowchart of included chronic lymphocytic leukemia patients in the study. CLL: Chronic lymphocytic leukemia

cases that progressed had been followed for survival until the last date of the study.

The investigator determined the response criteria, and the cases had been classified to CR cases, PR cases, cases with progressive disease (PD), cases with stable disease (SD), relapse cases, or cases with refractory disease, based on the 2008 NCIWG criteria,[13] with the exception that CR was calculated without performing bone marrow test, i.e., normal blood counts and physical examination, and the case is stated as clinical CR (cCR).

The overall response rate (ORR) was calculated with the number of patients achieving a cCR and a PR, according to Meunier *et al.*[14] clinically, beneficial responses include CR and PR, while all others cases, including SD, PD, and death should be considered treatment failures. Overall survival was defined as the time from treatment initiation to the date of death or last follow-up. The progression-free survival (PFS) represented the time between initial therapy and relapse's first clinical or biological occurrence.[14]

Statistical analysis

The survival curves were estimated using the Kaplan–Meier method. The log-rank test was performed for survival curves comparison. The data were collected and filtered by Epi info 7.1 software (Centers for Disease Control; Bethesda, MD, USA), a program for epidemiologic statistics and graphs. Further analysis was done using Statistical Package for Social Sciences (SPSS) for Windows, Version 22.0 software (Armonk, NY, USA: IBM Corp). Descriptive statistics (frequency tables) were used, and the Chi-square test was performed to analyze the categorical variables. $P \leq 0.05$ were considered statistically significant.

Ethical approval statement

The ethical clearance (FPEC-23-2018) was obtained from the Ethical Committee of the Faculty of Pharmacy, University of Khartoum. Additional approval for checking the medical files was taken from the administration of Khartoum Oncology Hospital. Informed consent was not required for the study since the data considering for publication were retrospectively obtained and anonymized and encoded to sustain confidentiality throughout this observational study.

Results

The study involved 64 patients, 62.5% of whom were male and 37.5% were female. In addition, 59.4% were aged 65 years or older. Majority of the patients were illiterate (45.3%) or had a low-to-intermediate level of education (45.3%). Gezira and White Nile states were accounted for 28.2% of the cases [Table 1]. About 53.1% of patients had two comorbid diseases. The vast majority (43.8%) of individuals are at stage IV of the disease. Furthermore, 65.6% of the patients had a negative for both (CD38 and ZAP70) [Table 1].

Table 2 demonstrates the distribution of chlorambucil dose, prednisolone use, and clinical outcomes in Sudanese patients with CLL. Most (63.1%) of the patients got high dosages of chlorambucil, whereas 36.9% received small doses. However, 46.3% of patients had received prednisolone with a high dose of chlorambucil, whereas 17.4% received prednisolone in combination with a low dose of chlorambucil [Table 2].

Due to missing data, the response was determined only for 45 patients. Overall results of 2–6 treatment cycles in the 45 patients were cCR in 11 (24.4%), PR in 9 (20%), progression in 2 (4.4%), and no response in 23 (51.1%) [Table 2]. The calculated ORR was 44.4% in this study. High dose of chlorambucil significantly (P = 0.039) resulted in higher percentages of cCR, and PR compared to low dosage of chlorambucil [Table 2]. Prednisolone use was found to have no significant effect on the type of response, as no significant

Table 1: Distribution of sociodemographic, clinical characteristics of Sudanese patients with chronic lymphocytic leukemia (*n*=64)

Demographic and clinical data	*n* (%)
Gender	
Males	40 (62.5)
Females	24 (37.5)
Age (years)	
<65	26 (40.6)
≥65	38 (59.4)
Education level	
Illiterate	29 (45.3)
Low–intermediate education	29 (45.3)
High education	6 (9.4)
Residence (state)	
Gezira	9 (14.1)
White Nile	9 (14.1)
Khartoum	6 (9.4)
North Kordofan	6 (9.4)
South Kordofan	6 (9.4)
East Kordofan	5 (7.8)
North Sudan	5 (7.8)
Other states	18 (28)
Number of comorbid diseases	
≥3	9 (14.1)
2	34 (53.1)
1	21 (32.8)
CLL stage at diagnosis	
Stage I	9 (14.1)
Stage II	13 (20.3)
Stage III	14 (21.8)
Stage IV	28 (43.8)
Prognostic factors (CD38 and ZAP70) (*n*=32)	
CD38 positive	6 (18.8)
ZAP70 positive	4 (12.5)
Both (CD38 and ZAP70) positive	1 (3.1)
Both (CD38 and ZAP70) negative	21 (65.6)

CLL: Chronic lymphocytic leukemia, CD38: A cell surface protein in lymphocyte cells, ZAP70: An intracellular kinase expressed near the surface membrane of lymphocytes

difference was observed between low-dose chlorambucil alone and low-dose chlorambucil with prednisolone ($P = 0.069$), and high-dose chlorambucil alone with high-dose chlorambucil with prednisolone ($P = 0.498$). Furthermore, the median PFS from treatment initiation was 18 months [Figure 2a]. The median PFS for the high-dose arm was 15 months, while the low-dose chlorambucil was 22 months ($P = 0.233$) [Figure 2b]. Median PFS was 18 months in both cases where prednisolone was used or not with chlorambucil ($P = 0.631$) [Figure 2c].

Discussion

Political and economic instability and other more irritant health issues such as tropical and infectious illnesses are some of the causes contributing to Sudan's poor attention to cancer therapy. Khartoum Oncology Hospital is one of three specialized cancer centers in Sudan, delivering chemotherapy and radiation treatments to all 14 Sudanese states.[15,16] Further, the majority of patients that come to Khartoum Oncology Hospital are low-income, uneducated patients. From 2014 to 2017, 37,804 cancer patients visited Khartoum Oncology Hospital, with 561 CLL patients. CLL patients are more likely to become infected due to faulty immunological processes, and this risk increases with treatment due to therapy-related immunosuppression. In CLL patients, prophylactic intravenous immunoglobulin support for frequent hypogammaglobulinemia and immunization regimens against specific illnesses such as pneumococcus, influenza, hemophilia, tetanus, typhoid, and diphtheria, have been explored.[1] However, we have a lack of these prophylactic strategies in Sudan.

Patients in Sudan are mainly referred to Khartoum Oncology Hospital for treatment following a series of trials at basic and secondary care institutions. As a result, more than 40% of patients were diagnosed with Stage IV illness, which may add to the disease's difficulties in being controlled. Coming from afar was often a factor for insufficient follow-up during the treatment term.[15] Although the Khartoum Oncology Hospital is one of the three primary sources of cancer data in Sudan, inadequate follow-up documentation and the lack of death reports may contribute to erroneous results.[17,18]

Chlorambucil has been utilized as the standard of care treatment for all newly diagnosed patients in Khartoum Oncology Hospital, even though chlorambucil has been utilized in combination with anti-CD20 antibodies in NCCN recommendations for fragile or infirm patients.[8] Even when CR rates were clinically observed, no full remission in bone marrow was expected because the treatment aim for all patients was palliative care.[19] Further, no CR in terms of bone marrow was predicted; although the CR rates were measured clinically, bone marrow tests, lymph nodes, and organomegaly were not performed based on the hematologic readings.[14,20]

Cryptogenic abnormalities having therapeutic implications are del17p and/or TP53 mutations,[21,22] with which patients are considered to be at high risk of therapy resistance and early disease recurrence.[1] Only a few patients in this study had their

Table 2: Distribution of chlorambucil dosage, prednisolone usage, and clinical responses for Sudanese patients with chronic lymphocytic leukemia

Drug data	*n* (%)
Chlorambucil (*n*=64)	
High doses	41 (63.1)
Small doses	23 (36.9)
Prednisolone in patients with high doses of chlorambucil (*n*=41)	
Yes	19 (46.3)
No	22 (53.7)
Prednisolone in patients with low doses of chlorambucil (*n*=23)	
Yes	4 (17.4)
No	19 (82.6)
Overall clinical response (*n*=45)	
cCR	11 (24.5)
PR	9 (20)
No response	23 (51.1)
Progression	2 (4.4)

Chlorambucil dosage	Clinical response				*P*
	cCR	PR	No response	Progression	
High dose (*n*=28)	10	7	10	1	0.039
Low dose (*n*=17)	1	2	13	1	
Total (*n*=45)	11	9	23	2	

cCR: Clinical complete remission, PR: Partial response

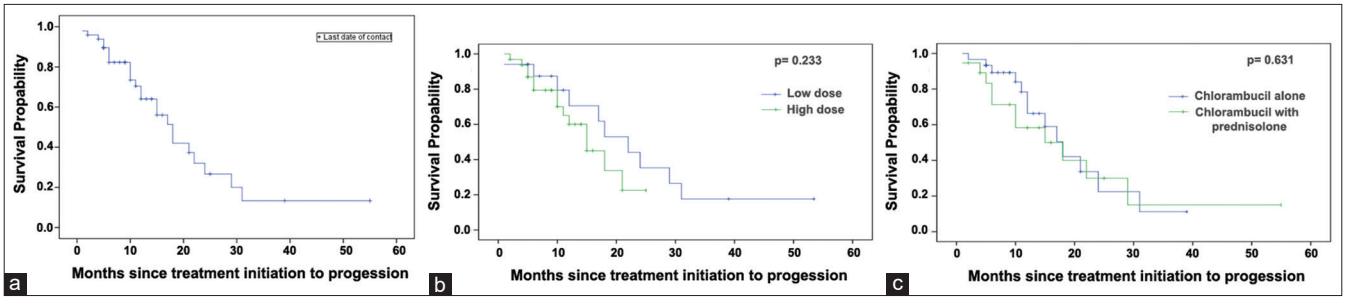

Figure 2: Survival estimate for Sudanese patients with chronic lymphocytic leukemia. (a) Overall progression-free survival. (b) Distribution of progression-free survival according to the dose of chlorambucil. (c) Distribution of progression-free survival according to the use of prednisolone or not with chlorambucil

cryptogenic abnormalities tested, and no patient was treated based on their relative cryptogenic abnormality.

The most common prognostic variables found in the investigated patients are CD38 and ZAP70. The expression of CD38 and ZAP70 predicts a bad prognosis and corresponds to some extent with IGHV mutational status but has little therapeutic influence and was hence not necessary.[1,20] All of these variables may contribute to a diminished response to chlorambucil, which is already determined to be modest yet relevant to the literature findings.

Chlorambucil is used in various dosages, either alone or in conjunction with prednisolone. The higher dose of chlorambucil resulted in a considerably higher ORR. Many clinical trials have suggested a link between chlorambucil dose or duration of treatment and CLL response;[23,24] in a meta-analysis, the ORR of patients treated with 10 mg/m^2/day for 7 days every 4 weeks for more than 6 months was 72%, compared to 31%–55% in patients treated with lower chlorambucil doses or shorter duration of chlorambucil therapy in other trials.[25]

The PFS was insignificantly higher with the small dose of chlorambucil by 7 months. This can be attributable to the fact that small dosages were often administered continuously, and data suggest that continuous dosing plans were more successful than intermittent schedules.[26] It should be noted that most patients were treated with lower doses of chlorambucil in the early years (2014 and 2015), and if there was advancement, the dose was increased to high-dose chlorambucil.[26] Most patients were started on high dosage chlorambucil in 2016 and 2017 due to a growing understanding of the response advantages of high doses, as previously reported in the literature.[26,27]

In terms of response and survival, prednisolone had no significant additive impact when used with chlorambucil. Many clinical trials revealed the beneficial effects of adding prednisolone with chlorambucil in treating CLL patients.[28,29] All prednisolone dosages employed in this trial were typical levels ranging from 30 mg/day or less, for up to <200 mg/day adds little if any value to chlorambucil treatment. Moreover, the use of prednisolone was limited in some patients due to hemolytic anemia and thrombocytopenia. Based on the literature, only high dosage corticosteroids had been shown to impact CLL therapy.[25,26]

The main limitations of this study were missing data and unavailable files; hence, there were a lot of medical files not included in this study. Second, it was conducted in a single hospital, so the results cannot be generalized to all oncology hospitals in Sudan. Despite these limitations, the findings of this study are novel as it is the first study about the effectiveness of chlorambucil in Sudanese CLL patients. Further multicenter studies with a larger population and well-documented cases are strongly recommended.

Conclusion

In this study, low response rates were achieved after using chlorambucil in treating CLL Sudanese patients. High chlorambucil dosages improved overall response, but there was no further advantage in PFS compared to those who received low doses of chlorambucil.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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