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Original Research Article

Patterns of microRNA and CD Expression Associated with Chronic Lymphocytic Leukemia in a Set of Sudanese Patients

Ekhlas A. Abuelfadol[1], Ahmed Abdalla Agabeldour[2], Inaam Eloshary Mohammed Hayaty [3], Mahadi Musa Mohammed Abdallah[4], Hassan Yousif Adam Regal[5], Khalil Ali Ibraheim[6], Ahmed Abdallah[7], Rogeia Mahmoud Niyle[8], Salah Eldinn Eltahir Gumaa[9], Hassan Mohamed Musa Salih[10], Hussain Gadelkarim Ahmed[11, 12*]

[1]Departement of Hematology, Faculty of Medical Laboratory Sciences, University of Kordofan, El-Obeid, Sudan
[2]Department of Pathology, Faculty of Medicine, University of Kordofan, El-Obeid, Sudan
[3]El-Obeid Obstetrics and Gynecology Teaching Hospital, El-Obeid, Sudan
[4]Ministry of Health, El-Obeid Obstetrics and Gynecology teaching Hospital, El-Obeid, Sudan
[5]Department of Parasitology, Faulty of Medical Laboratory Sciences, University of Kordofan, El-Obeid, Sudan
[6]Department of Surgery, Faculty of Medicine, University of Kordofan, Doctor's Khalil Clinic, El-Obeid Teaching Hospital-Ophthalmology Department, El-Obeid, Sudan
[7]Departemnt of Obstetrics and Gynecology, University of Kordofan, El-Obeid Obstetrics and Gynecology Teaching Hospital, Alfayhaa Obs & Gyn Clinic, El-Obeid, Sudan
[8]Department of Medicine, University of Kordofan El-Obeid Teaching Hospital, Dermatology Department, El-Obeid International Hospital, Tebyan Clinic, El-Obeid, Sudan
[9]Department of Radiology, El-Obeid Teaching Hospital, El-Obeid International Hospital, Department of Radiology, DR Salah Clinic, El-Obeid, Sudan
[10]Sudan Sickle Cell Anemia Center, El-Obeid, Sudan
[11]Prof Medical Research Consultancy Center- MRCC, El-Obeid, Sudan
[12]Department of Histopathology and Cytology, FMLS, University of Khartoum, Sudan

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***Corresponding author:** Hussain Gadelkarim Ahmed
Prof Medical Research Consultancy Center- MRCC, El-Obeid, Sudan

Abstract

Background*:** CLL is an adult leukemia presented with a clonal accumulation of lymphocytes. Immunophenotypic changes can be effective in predicting clinical course, the survival of patients, and determining first-line treatment. ***Methodology: The current research used lymphoma data obtained from El-Obeid Oncology Center. Included were details pertaining to lymphoma patients who received their diagnoses between January 2018 and January 2020. One hundred patients, including sixty-one with CLL and forty-nine without, made up the full coverage sample. Immunohistochemistry and molecular analyses were conducted on a subset of individuals. Additionally, several patients underwent flow cytometry analysis for some CD markers as well as microRNA prediction. ***Results*:** CD19 was shown to be positively expressed in all CLL patients studied (100%), while 88% of non-CLL patients also showed the same expression. Ninety-three percent of the CLL patients and nine percent of the non-CLL patients examined showed CD5-positive expressions. Only 16.7% of CLL patients and 66.7% of non-CLL patients tested positive for CD22. Among the CLL patients studied, 92.3% showed CD23 positive expressions, while no non-CLL patients did. Every single patient with CLL and every single patient without CLL tested negative for CD38. We observed the presence of FMC70 in 16.7% of the analyzed CLL samples and in two out of the three non-CLL cases. We evaluated the predictive significance of microRNA in a cohort of 12 patients, finding that around 83% showed positive expression. ***Conclusion*:** CLL is more prevalent in Sudan than the reported global epidemiology and more common among elder men than non-CLL variants. According to the molecular prognostic markers, CLL has bad prognostic indicators.

Keywords: Chronic lymphocytic leukemia, CD markers, microRNA, Sudan.

INTRODUCTION

CLL is a commonly occurring variant of leukemia. Chronic CLL is the most prevalent kind of leukemia in Western countries, representing 25% of all occurrences [1]. It commonly manifests in older individuals and exhibits a highly unpredictable clinical progression. Different genetic changes cause leukemia by messing up the control of cell death and division in a

group of B-cells that are all the same [2]. Malignant B cells, which display specific attributes associated with mature, healthy B lymphocytes, including the presence of operational B-cell receptors, distinguish CLL. The interaction between CLL cells and the microenvironment in secondary lymphatic organs causes BCR signaling to cause CLL cells to multiply [3].

The assessment of cytogenetic abnormalities and surface biomarkers in neoplastic CLL cells has the potential to have a major impact on CLL prognosis. Furthermore, analyzing changes in CD marker expressions throughout. People with CLL can have serious health problems like organomegaly, immunodeficiency, and advanced disease stages because CD markers on lymphocytes and other cells can change. The interaction of CD markers with the CLL microenvironment mediates this influence. Because CD markers are important in the development of CLL, finding changes in CD marker expression in cells affected by CLL may help in figuring out how well these people will do [4].

Small noncoding ribonucleic acids, known as microRNAs, play a crucial role in regulating various essential physiological and biological processes, including cell division, proliferation, and apoptosis. Much of the miRNAs work as either tumor suppressors or oncogenes. This means that any genetic or epigenetic problem that changes their structure and/or function could cause tumors to form and grow. Multiple investigations on miRNAs have demonstrated their crucial role in the prognosis of various malignancies, such as CLL. Furthermore, their epigenetic modifications, such as methylation, might serve as indicators of disease advancement and the effectiveness of treatment [5]. The reference is Ali et al., 2024. The researchers observed a notable increase in miR-29a expression levels among patients with CLL. Furthermore, as previously described [6], they observed a strong correlation between elevated miR-29a expression and unfavorable prognostic indicators, such as high levels of CD38 and ZAP70.

Despite the prevalence of lymphoma in Sudan, recent publications on the disease have been scarce. This is why this study set out to fill that gap by examining the microRNA and CD expression patterns associated with CLL in a group of Sudanese patients.

MATERIALS AND METHODS

The current research used lymphoma data obtained from El-Obeid Oncology Center. Included were details pertaining to lymphoma patients who received their diagnoses between January 2018 and January 2020. One hundred patients, including sixty-one with CLL and forty-nine without, made up the full coverage sample. According to conventional histopathology, the patient has lymphoma. To validate the previous diagnosis and classify the lymphomas into CLL and non-CLL lymphoma types, the histopathological diagnosis of the tissue samples was reevaluated. We conducted additional tests, such as immunohistochemistry and molecular analyses, on a subset of individuals. Additionally, several patients underwent flow cytometry analysis for some CD markers as well as for microRNA prediction.

Data analysis: The obtained information sets were entered into computer software, Statistical Package for Social Sciences (SPSS version 16; SPSS Inc., Chicago, IL). The chi-square test was used, and $P < 0.05$ was considered significant.

Ethical consent: The protocol of this study was established in accordance with the 2013 Declaration of Helsinki, and this study was approved by the ethics committee of the Prof. Medical Research Consultancy Center (MRCC). The approval number was MRCC.

RESULTS

The present study comprised a cohort of 100 individuals diagnosed with lymphoma, ranging in age from 15 to 80 years, with an average age of 60 years. The sample consisted of 100 patients, with 57 being male and 43 being female. Approximately 14% of the patients were under the age of 40, despite the majority (56%) being over 60. The gender distribution exhibited a reasonably comparable pattern across several age groups, as indicated in Table 1 and Figure 1. 77% of the patients are urban inhabitants. According to the data presented in Table 1 and Figure 1, the majority of patients were identified as housewives, followed by self-employed individuals and farmers, accounting for 31%, 28%, and 13%, respectively.

Table 1: Distribution of the study subjects by demographic characteristics

Variable	Males	Females	Total
Age			
≤ 40 years	7	7	14
41-50	4	4	8
51-60	12	10	22
61-70	15	13	28
≥ 71	19	9	28
Total	57	43	100

Variable	Males	Females	Total
Residence			
Urban	43	34	77
Rural	14	9	23
Total	57	43	100
Occupation			
Self-employed	27	1	28
Farmer	12	1	13
Employer	3	2	5
Retired	7	1	8
Housewife	0	31	31
Student	2	1	3
Other	1	0	1
Total	52	37	89

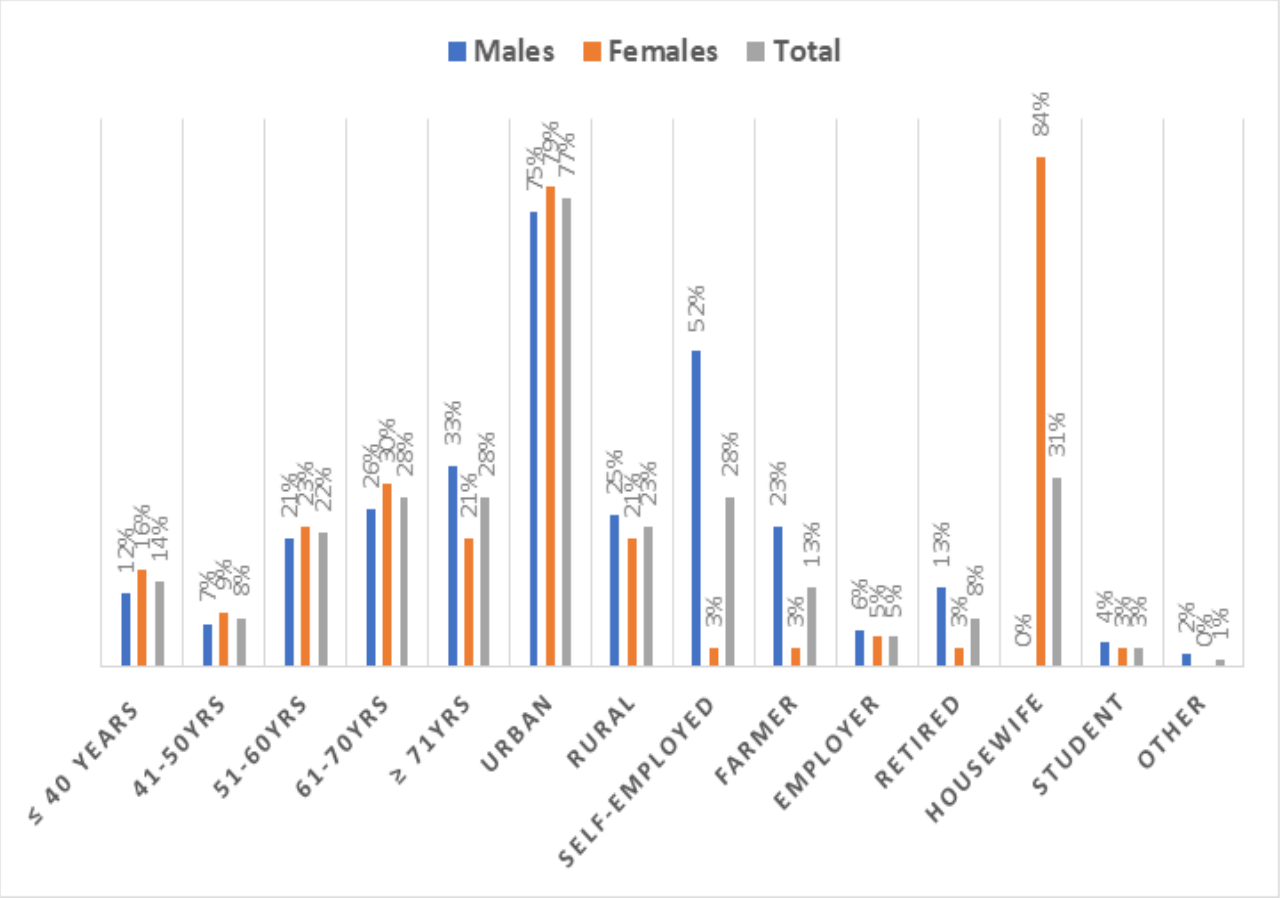

Figure 1: Description of the patients by demographic characteristics

Table 2 and Figure 2 presented the distribution of lymphoma types and demographic features. Approximately 65% of males and 56% of females were affected by CLL. Although lymphoma was more prevalent among older individuals, chronic lymphocytic leukemia (CLL) was more commonly observed in those aged over 70 years, accounting for 22 out of 61 cases (36%). On the other hand, non-CLL was on the rise among individuals under 40 years old, including 10 out of 39 cases (26%). The study found that there is a significant association between the risk of CLL in older individuals and the relative risk (RR) and the 95% confidence interval (95%CI). The RR (95% CI) is 1.7 (1.1017 to 2.638), with a p-value of 0.0166 and a z-score of 2.395. The risk of non-CLL in younger individuals is 1.1172 (95% CI = 0.6597 to 1.8921), with a p-value of 0.6801 and a z-score of 0.412.

The majority of participants in this study were people residing in metropolitan areas. The majority of patients with CLL were housewives, followed by self-employed individuals and farmers, accounting for 18 out of 52 (35%), 17 out of 52 (33%), and 9 out of 52 (17%) patients, respectively, as shown in Table 2 and Figure 2.

Table 2: Distribution of the lymphoma type and demographic characteristics

Variable	CLL	Non-CLL	Total
Gender			
Males	37	20	57
Females	24	29	43
	61	49	100
Age			
≤ 40 years	4	10	14
41-50	3	5	8
51-60	14	8	22
61-70	18	9	27
≥ 71	22	7	29
Total	61	39	100
Residence			
Urban	48	29	77
Rural	13	10	23
Total	61	39	100
Occupation			
Self-employed	17	11	28
Farmer	9	4	13
Employer	3	2	5
Retired	4	4	8
Housewife	18	13	31
Student	0	3	3
Other	1	0	1
Total	52	37	89

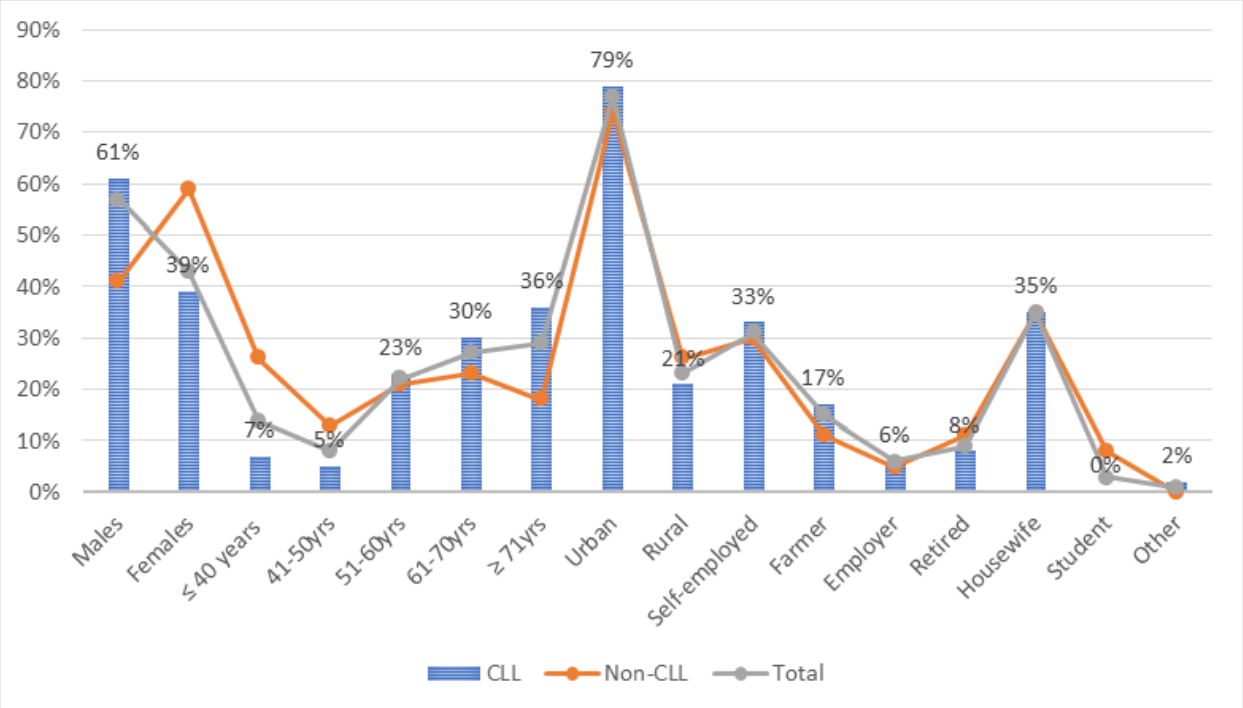

Figure 2: Lymphoma type and demographic characteristics

The distribution of CD molecular markers for CLL and non-CLL lymphoma types is shown in Table 3 and Figure 3. CD19 was shown to be positively expressed in all CLL patients studied (100%), while 88% of non-CLL patients also showed the same expression. Half of the CLL patients tested positive for CD20, but 62.5% of the non-CLL patients did as well. Fifteen percent of the CLL patients and twelve percent of the non-CLL patients tested positive for CD3. Ninety-three percent of the CLL patients and nine percent of the non-CLL patients examined showed CD5-positive expressions. Only 16.7% of CLL patients and 66.7% of

non-CLL patients tested positive for CD22. Among the CLL patients studied, 92.3% showed CD23 positive expressions, while no non-CLL patients did. Every single patient with CLL and every single patient without CLL tested negative for CD38.

Only one case out of the CLL patients evaluated had CD79a positive expressions. Among the CLL patients analyzed, 16.7% showed CD79b positive expressions, but 66.7% of the non-CLL individuals did as well. Among both the CLL and non-CLL patients evaluated, 50% showed CD30 positive expression.

Table 3: Distribution of CLL and non-CLL lymphoma types and CD molecular markers

Variable	CLL	Non-CLL	Total
CD19			
Positive	5	7	12
Negative	0	1	1
Total	5	8	13
CD20			
Positive	7	8	15
Negative	7	2	9
Total	14	10	24
CD3			
Positive	2	1	3
Negative	11	10	21
Total	13	11	24
CD5			
Positive	13	2	15
Negative	1	4	5
Total	14	6	20
CD22			
Positive	2	2	4
Negative	10	1	11
Total	12	3	15
CD23			
Positive	12	0	12
Negative	1	3	4
Total	13	3	16
CD38			
Positive	0	0	0
Negative	5	2	7
Total	5	2	7
CD79a			
Positive	1	0	1
Negative	0	0	0
Total	1	0	1
CD79b			
Positive	2	2	4
Negative	10	1	11
Total	12	3	15
CD30			
Positive	2	1	3
Negative	0	1	1
Total	2	2	4

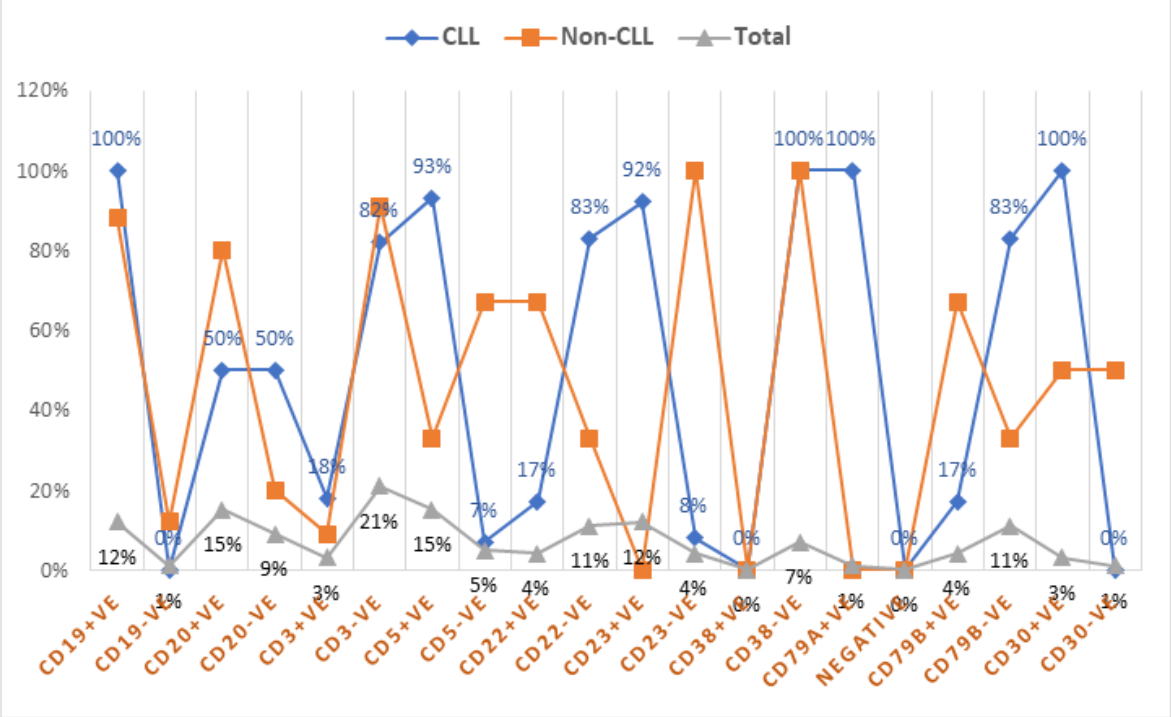

Figure 3: CLL and non-CLL lymphoma types and CD molecular markers

The data presented in Table 4 and Figure 4 exclusively revealed the presence of ZAP70 in the examined CLL samples. We observed the presence of FMC70 in 16.7% of the analyzed CLL samples and in two out of the three tested non-CLL cases. We observed a prevalence of 20% for strong positive expressions of IgHLambda, while the remaining 80% showed mild positive expressions. About 80% of non-CLL cases showed positive expression of the BCR/ABL fusion gene, while CLL cases showed no such expression. In CLL, we observed a chromosomal translocation, but one instance showed a negative deletion of 17p13. We evaluated the predictive significance of microRNA in a cohort of 12 patients, finding that around 83% exhibited positive expression.

Table 4: Distribution of genetic change in CLL lymphoma and non CLL verities

Variable	CLL	Non-CLL	Total
ZAP70			
Positive	1	0	1
Negative	2	0	2
Total	3	0	3
FMC7			
Positive	2	2	4
Negative	10	1	11
Total	12	3	15
IgHLambda			
Strong (+)	2	2	4
Weak (+)	8	0	8
Total	10	2	12
BCR/ABL fusion gene			
Positive	0	4	4
Negative	1	0	1
Total	1	4	5
Translocation			
t(15;17) negative	0	1	1
t(9;22) positive	0	1	1
Deletion	0		
17p13 negative	1	0	1

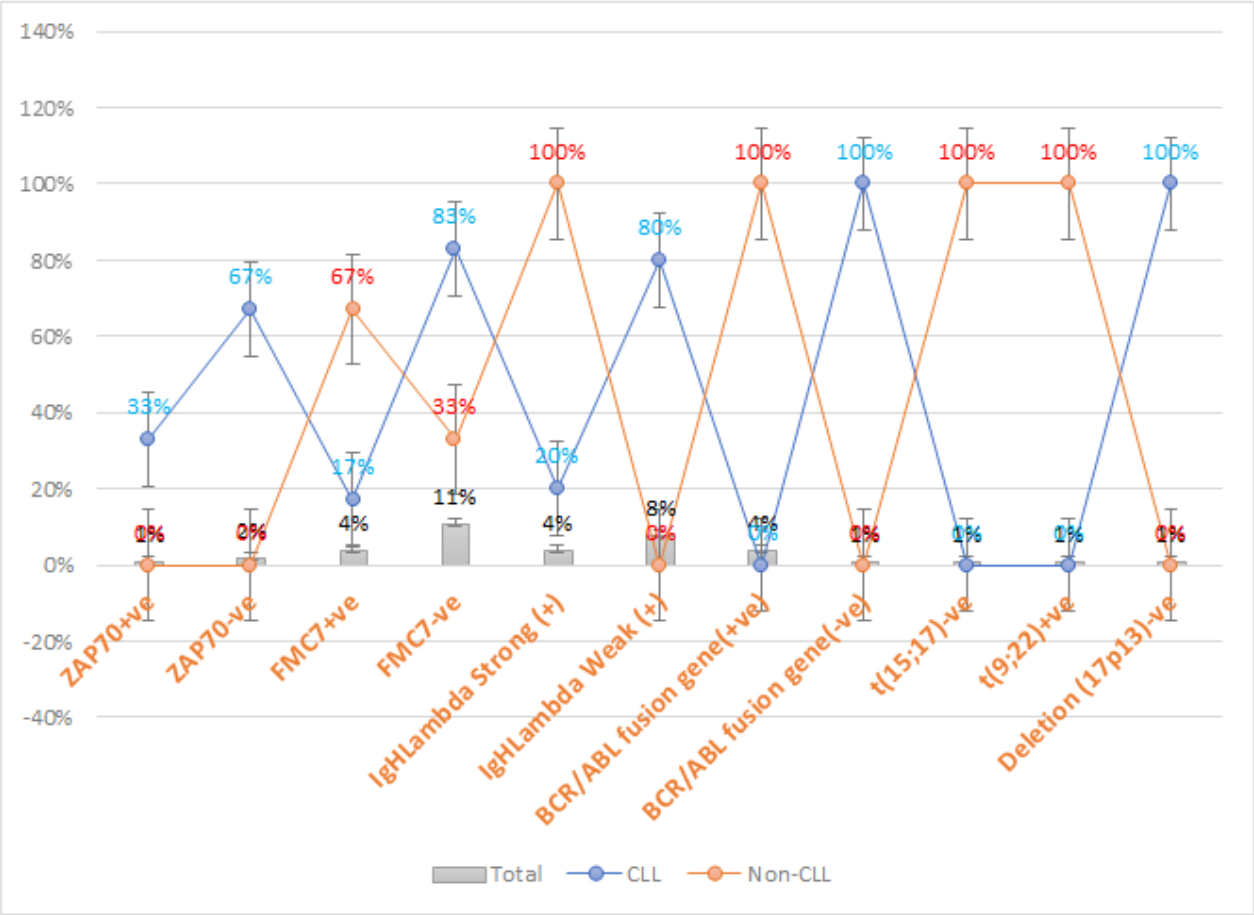

Figure 4: Genetic changes in CLL lymphoma and non CLL verities

DISCUSSION

In Sudan, there is a significant lack of available data pertaining to various types of cancer, including lymphomas, with a special emphasis on CLL. The current study aims to address the current state of lymphoma in a country where extended conflict has severely damaged the healthcare system.

The results of the current study indicate that CLL is the prevailing form of lymphoma in Sudan. CLL constitutes around 25–30% of the total leukemia cases in Western countries [7]. Our study's elevated prevalence may be due to the relatively small sample size.

Males exhibited a higher susceptibility to CLL compared to females in this study. Despite the fact that an individual's sex is associated with one of the highest known risks for developing leukemia and lymphomas, there is a lack of emphasis on these hazards. Both chronic lymphocytic leukemia and lymphocytic lymphoma exhibit an atypical pattern, previously unrecorded, characterized by a significant male overrepresentation that is peculiar to the age ranges of the 40s and 60s [8].

Reports have already linked increased radiation exposure to the rising incidence of CLL among urban inhabitants [9].

In the present study, we noted that CLL is significantly more common among the elderly population compared to other leukemias. A comprehensive evaluation of the epidemiological characteristics of CLL is warranted, especially in the current context of global population aging. A high incidence has been achieved in males and older people [10]. CLL incidence increases with age, reaching 37.9/100,000 in patients over 85 years [11]. The median age of patients at the time of diagnosis of chronic lymphocytic leukemia (CLL) is 71 years, and the incidence of this hematologic malignancy increases steadily with age [12]. In studies from Europe and the USA, only 5%–11% of patients with CLL are younger than 50–55 years at diagnosis [13].

In the current study, although the number of tested patients for the different CD markers was small, most of the results were in line with those reported in the literature. CD19-positive expression was noted in all tested cases of CLL. CD20, CD5, CD22, and CD23 were the most frequently expressed. CLL requires the presence of ≥5000 clonal B-lymphocytes/μL, the coexistence of CD19, CD20, CD5, and CD23, the restriction of light chain immunoglobulin, and the lack of expression of the antigens CD22 and CD79b [14].

In the past few years, new parameters have been considered for prognostication. One such marker that has been the subject of speculation and has been found useful by some studies is zeta-associated protein 70 (ZAP-70) and CD38 [15]. The higher expression of these miRNAs was correlated with negative ZAP70 and CD38 expression [16]. The concept is used to predict the presence of miRNAs [17]. The emerging role of microRNAs (miRNAs) in CLL pathophysiology and their possible application in therapy are worth noting. MiRNAs are one of the most important regulatory molecules for gene expression. They can be both oncogenes and tumor suppressor genes in CLL, and the loss of control of certain miRNAs has been linked to prognosis, progression, and drug resistance. In this review, we describe the role of the miRNAs that primarily impact the disease and how these miRNAs could be used as therapeutic tools. Certainly, the use of miRNAs in clinical practice is still limited in CLL. Despite several studies suggesting their efficacy on the disease, either alone or in combination with other drugs, many issues remain unresolved, particularly regarding their biological and safety profile [16]. The differential expression of several important microRNAs with pro- or anti-CLL functions in B lymphocytes in different geographical populations is likely to contribute to the difference in CLL incidence between different ethnic groups [18].

Although the present study brings to light undeclared paused data from Sudan, it has some limitations, including the small groups tested for molecular markers.

CONCLUSION

CLL is more prevalent in Sudan than the reported global epidemiology and more common among elder men than non-CLL variants. According to the molecular prognostic markers, CLL has bad prognostic indicators.

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