RESEARCH ARTICLE

Editorial Process: Submission:05/18/2022 Acceptance:09/25/2022

Interleukin-10-1082A>G (rs1800896) Single Nucleotide Polymorphism is Not a Risk Factor of Chronic Lymphocytic Leukemia in Sudanese Population

Ameen Abdulaziz Basabaeen[1,2]*, Enaam Abdelrhman Abdelgader[3], Ebtihal Ahmed Babekir[1], Nour Mahmoud Abdelateif[1], Saadia Osman Abdelrahim[1], Omnia Mohamed Sharif[4], Osama Ali Altayeb[5], Eman Abbass Fadul[5], Ibrahim Khider Ibrahim[1]

Abstract

Objective: The present study was conducted to examine the association between the IL-10-1082A>G (rs 1800896) polymorphism and risk of Chronic Lymphocytic Leukemia and to assess the correlation between this polymorphism and clinicopathological characters. **Methods:** A case-control study was conducted in Khartoum state, Sudan, during the period from April 2017 to April 2018, involved 110 CLL patients and 80 healthy volunteers as a control group. Physical examination, complete blood count, and immunophenotype were performed in all patients to confirm the diagnosis. Clinical staging such as Rai and Binet were studied. CD38 and ZAP70 were performed by flow cytometry. Blood samples were collected from all participants; DNA was extracted by using ANALYTIKJENA Blood DNA Extraction Kit and analyzed IL-10-1082A>G polymorphism by using Allele Specific-Polymerase Chain Reaction. The statistical analysis was performed using statistical package for social sciences version 23.0 software. **Results:** Frequency of AA, AG, and GG genotypes was 32.7%, 55.5%, and 11.8% for the patient group and 31.25%, 51.25%, and 17.5% in the control group, respectively. The genotype of IL-10 (-1082A>G) did not associate with susceptibility of CLL in our population. The study showed that the G allele of the IL-10 gene (-1082A>G) is associated with the male sex. However, no significant association was found between -1082A>G genotype and clinicopathological characters. **Conclusion:** Our results do not support the involvement of the IL-10 −1082A>G promoter gene polymorphism in the increased CLL susceptibility. IL-10-1082G allele (IL-10-1082AG or IL-10-1082GG) was found more frequently in males. Furthermore, no association was observed between the IL-10-1082A>G polymorphism and clinical stages systems as well as established poor prognostic markers. Finally, within the group of patients with CLL, there was no difference in the age at diagnosis and hematological parameters according to genotype distributions.

Keywords: CLL- Interleukin-10- Single Nucleotide Polymorphism- IL-1082A>G

Asian Pac J Cancer Prev, **23 (9)**, 3229-3235

Introduction

Chronic lymphocytic leukemia is characterized by the progressive accumulation of monoclonal, small, mature-appearing $CD5^+$ B-cells in the peripheral blood, bone marrow, and secondary lymphoid tissue (Caligaris-Cappio and Hamblin, 1999). Genetic variation of some immunomodulatory cytokine may lead to different subtypes of lymphoma predisposition (Chatterjee et al., 2004).

Interleukin-10 is an anti-inflammatory cytokine important for proper immune system function (Iyer and Cheng, 2012). IL-10 which acts as a growth factor for normal activated human B and T lymphocytes stimulation and proliferation (El Far et al., 2004).

The gene encode IL-10 is located on chromosome 1(1q31-1q32) (Eskdale et al., 1997). Some evidence suggest of IL-10 gene that polymorphic variations in the promoter sequences of IL-10 gene may influence in the gene expression (Turner et al., 1997; Gibson et al., 2001). Moreover, SNPs in IL-10 gene promoter play a role in

[1]*Department of Hematology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan.* [2]*Ministry of Health & Population, Hadhramout, Yemen.* [3]*Department of Pathology, Faculty of Medicine, Al Neelain University, Khartoum, Sudan.* [4]*Department of Haematology, Faculty of Medical Laboratory Sciences, Sudan University of Science and Technology, Khartoum, Sudan.* [5]*Flow Cytometry Laboratory for Leukemia & Lymphoma Diagnosis, Khartoum, Sudan. *For Correspondence: ameenbasabaeen162@gmail.com*

pathogenesis of lymphoid disorders, and may increase a risk of NHL development, especially diffuse large B-cell lymphoma subtype (Dai et al., 2014).

Recently, a series of epidemiological studies have focused on the association between these SNPs of IL-10 (-3575T>A, -1082A>G,-819C>T and -592C>A) and the risk of CLL (Domingo-Domènech et al., 2007; Ennas et al., 2008; Lech-Maranda et al., 2008; Lech-Maranda et al., 2013; Ovsepyan et al., 2015).

IL-10-1082G allele were characterized by more advanced CLL (Rai stages III or IV and elevated LDH) (Lech-Maranda et al., 2013). Furthermore, this allele may be a marker which correlates with worse prognosis, fast progression of the disease and short TFS (Lech-Maranda et al., 2013). On contrast, Ovsepyan et al. shown single nucleotide replacement-1082G>A in IL-10 gene promoter region is probable genetic factor of CLL risk and of disease manifestation at advanced stages (Ovsepyan et al., 2015).

To the best of our knowledge, this is the first study with a large sample size conducted in Sudan to investigate the association between the IL-10-1082A>G polymorphism (rs1800896) and susceptibility of CLL and correlate with clinical presentation, hematological parameter, and some biological prognostic markers. The present study was conducted to examine the association between the IL-10-1082A>G polymorphism and the risk of CLL and to assess the correlation between IL-10-1082A>G polymorphism and clinical presentation, hematological parameter, and some biological prognostic markers among Sudanese patients with CLL.

Materials and Methods

Study Population

This study is a case-control study, conducted in Khartoum state, Sudan, in the period from April 2017 to April 2018, a total of 110 patients with CLL and 80 apparently healthy volunteers as a control group were recruited to participate in this study. Patients were obtained at Flow Cytometry Laboratory for Leukemia & Lymphoma Diagnosis, Khartoum; they were referred for Immunophenotype diagnosis.

All patients were diagnosed based on clinical history, physical examination and complete blood count. The peripheral blood is important to show morphological abnormalities and immunophenotypic criteria. All our patients have $\geq 5000\times10^{9}$ /L B lymphocyte, considered in our diagnosis according to International Workshop on Chronic Lymphocytic Leukemia (Hallek et al., 2008). The stage of the CLL was assessed by Rai and Binet classification (Rai et al., 1975; Binet et al., 1981). All patients were newly diagnosed without any previous treatment, whereas patients with other lymphoid neoplasms (both B and T-cell Lineage) were excluded.

Sample Collection

The peripheral blood was collected as samples from both groups included in the study (the patient group and control group). For the patient group, an amount of four milliliters (ml) of blood was collected from each patient in (EDTA) and divided equally into two tubes; one tube for complete blood count and immunophenotype test and the other tube for molecular analysis. For the control group, two (ml) of blood was collected from each healthy individual in (EDTA) for molecular analysis.

Determination of Blood Count

Two ml of peripheral blood were withdrawn from each patient; these samples were collected in EDTA tubes and preserved at room temperature (22-24^{o}C) then processed within 6-24h from the collection. Complete blood count was analyzed by using automated hematology analyzer (SYSMEX KX-21N, Japan). All results such total White Blood Cells, Absolute lymphocyte count, Hemoglobin level, Red Blood Cells and platelets were recorded.

Determination of Immunophenotyping and (CD38 & ZAP-70 expressions)

The diagnosis of CLL was confirmed in each patient by flow cytometry (EPICS XL Beckman Coulter Flow Cytometry, Miami, FL, USA), standard protocol of Beckman Coulter (COULTER, 2010) was used in fluorescent dye-labelled monoclonal antibody for CD45, CD3 CD5, CD10, CD19, CD20, CD22, CD23, FMC7, CD79b, kappa, and lambda light chain. A marker was considered positive at cutoff ≥30%. Cutoff point of 30% was selected as recommended by British Committee for Standards in Haematology guideline (Oscier et al., 2012). However, in order to confirm diagnostic CLL, a scoring system was applied depending on Moreau et al. 1997 (Moreau et al., 1997). ZAP-70 and CD38 were used as prognostic markers, with a cutoff point of 20% and 30%, respectively, as previously described (Basabaeen et al., 2019).

DNA extraction

DNA was extracted from all blood samples of patients and control groups by using ANALYTIKJENA Blood DNA Extraction Kit (Germany) (REF-845-KS-1020050), according to the manufacturer's instructions. The β-globin gene was used to assess the quality of DNA in all extracted samples, the primer sequences as previously described (Kerr et al., 2000). All specimens for β-globin gene were successful amplification. To evaluate the DNA quantification after DNA extraction, we measured DNA by using a NanoDrop spectrophotometer. Then DNA samples were routinely stored at -20^{o}C.

Genotyping of Interleukin-10-1082A>G (rs1800896)

Genotyping was carried out by using the allele specific polymerase chain reaction (AS-PCR), primers as described in our previous work (Sharif et al., 2019). Two separated PCR reaction mixtures of 20 µl were prepared for each sample. PCR was performed by using Maxime PCR Premix Kit (i-Taq), (iNtRON BIOTECHNOLOGY, South Korea), Cat. No. 25025), 4µl of genomic DNA, 0.5 µl of each primer, and 15 µl distilled water. PCR started at 94°C for 5 min, followed by denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 40 s, with a final extension at 72°C for 5 min. Thermocycling was using TECHNE Tc-412-UK PCR Thermal Cycler 96 well. The amplified products were run on 1.5%

agarose gel, and then stained with ethidium bromide for visualization under ultraviolet gel documentation system (Figure 1).

Statistical analysis

Data was analyzed using the statistical package for social sciences version 23.0 (Chicago, IL, USA). Numerical data were summarized as mean and standard deviation and n (%) of study participants, respectively. Logistic regression was used for calculation of odds ratio (OR) with confidence interval (CI) for risk estimation. The Hardy-Weinberg equilibrium was tested by goodness of fit X2 test to compare the observed genotypic frequencies in normal individual to the expected genotypic frequencies, and then calculated from the observed allelic frequencies. Chi-Square test was used for analyzing associations between categorical variables. One-Way ANOVA were used to compare the means of two groups. All P-values were two-sided, and < 0.05 was considered as the significance level.

Results

IL-10-1082A>G polymorphism in CLL patients group and control group

A total of 190 DNA samples were included in this study, namely, 110 CLL patients and 80 healthy controls. The CLL cases included 79 (71.8%) males and 31 (28.2%) females; the mean age was 62.97 ± 12.06 years. The controls included 57 (71.3%) males and 23 (28.7%) females; the mean age was62.9±11.88 years. There were no differences between the case and control groups on age, sex (P-value>0.05).

Out of 110 CLL cases included in the study, 36 (32.7%) showed the AA genotype of the IL-10-1082A>G polymorphism, whereas 61 (55.5%) displayed a heterozygous genotype and 13 (11.8%) were found to carry the GG genotype. Comparison of the total sample of patients with CLL and control group did not reveal any significant differences in genotype frequencies of polymorphic locus -1082A>G of IL-10 gene (Tables 1 and 2)

Moreover, CLL patients and healthy individuals presented with similar frequencies of -1082A alleles (60.45% and 56.87% for -1082A allele in patients and controls, respectively; (Table 1). IL-10-1082A>G polymorphism appeared not to be associated either with susceptibility to CLL. TheIL-10-1082A>G genotypes were then grouped into G+ alleles (combining the AG and GG genotypes into one group) and alleles encompassing the AA genotype appeared not to be associated either with susceptibility to CLL. The allele and genotype frequencies among patients with CLL and control subjects are summarized in (Table 1). The genotype distributions for the IL-10-1082A>G polymorphism in both 110 CLL patients and 80 control subjects were in Hardy–Weinberg equilibrium (HWE) and normally distributed with P-value > 0.05.

IL-10-1082A>G polymorphism and relation to some established prognostic markers

There was no difference in mean age at diagnosis for patients with the AA, AG, and GG genotypes (59.63, 63.98, and 67.46 years, respectively). However, among the patients with CLL, IL-10-1082G allele (IL-10-1082AG or IL-10-1082GG) was found more frequently in males. We also compared the distribution of IL-10-1082 genotypes in patients concerning Rai and Binet classification. However, no significant associations were observed. Also, did not observe any influence of the IL-10-1082 genotype distribution on CD38 expression and ZAP-70 expression as well as hematological parameters (Tables 2 and 3).

Discussion

CLL is characterized by a complicated etiology (Rogalinska and M Kilianska, 2010). It is suggested that CLL heterogeneity may be associated with the single nucleotide polymorphic variations within the genes governing leukocyte differentiation, life/death control, as well as cell-cell interactions (Żołnierczyk and Kiliańska, 2015). Many cytokines are known to be involved in the

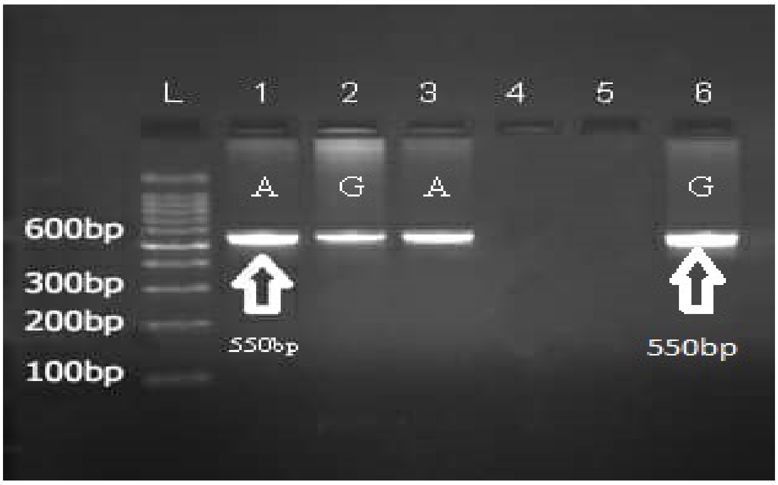

Figure 1. -1082A>G Genotyping Using AS-PCR. Lane L, 100 bp DNA molecular weight marker. Lanes 1 and 3, allele A is represented by the presence of a 550 bp PCR fragment. Lanes 2 and 6, allele G is represented by the presence of a 550 bp PCR fragment. Each two lanes represent one sample, lanes 1 and 2, heterozygous (AG); lanes 3 and 4, homozygous (AA); lanes 5 and 6, homozygous (GG).

Table 1. Frequency of the IL-10-1082A>G Genotype in Patients with Chronic Lymphocytic Leukemia and Control Group

Allele / Genotype	CLL group (N=110) (%)	Control group (N=80) (%)	OR (95% CI)	P- value
AA	36/110 (32.7)	25/80 (31.25)	1.07(0.58-1.99)	0.829
AG	61/110 (55.5)	41/80 (51.25)	1.18 (0.66-2.11)	0.566
GG	13/110 (11.8)	14/80 (17.5)	0.63 (0.28-1.43)	0.268
AG+GG	74/110 (67.3)	55/80 (68.75)	0.93(0.50-1.73)	0.829
A allele frequency	133 (60.45)	91 (56.87)	1.16 (0.77-1.75)	0.483
G allele frequency	87 (39.55)	69 (43.13)	0.86 (0.57-1.30)	
HWE: P *	0.093	0.688		

OR, odds ratio; 95% CI, 95% confidence interval; P*, significance probability for deviation from Hardy-Weinberg equilibrium (HWE), P value significant below 0.05.

pathogenesis of CLL (Allegra et al., 2020). IL-10 is a multifunctional cytokine; is key cytokine involved in the balance between cell-mediated and humoral immunity and lymphoid development (Moore et al., 2001; Mocellin et al., 2004). IL-10 stimulates the proliferation and differentiation of B and Th2 cells (Moore et al., 2001; Mocellin et al., 2004). There is, however, conflicting data regarding the effect of IL-10 on CLL cells, with some reports suggesting that IL-10 inhibits proliferation and induces apoptosis of malignant cells (Fluckiger et al., 1994), while others postulating that IL-10 protects against apoptotic cell death (Kitabayashi et al., 1995). The possible mechanism of involving IL-10 in the lymphoid development and disease pathogenesis are still debated (Lech-Maranda et al., 2012).

Several studies have investigated the association of IL-10-1082A>G gene polymorphism and CLL susceptibility, reporting conflicting results. In the present study, allelic frequencies and genotype distributions of IL-10-1082A>G SNP were similar in the CLL patients' group and matched controls group. It may suggest that this polymorphism has no impact on the susceptibility for the CLL occurrence. Our results are consistent with the authors who did not find any associations between IL-10-1082A>G polymorphisms and CLL risk occurrence. (Ennas et al., 2008; Lech-Maranda et al., 2013). In contrast, a contradictory result was obtained by Ovsepyan et al.,(2015) in Russia found Allele -1082A

Table 2. Associations between IL-10-1082A>G Genotype and CLL Prognostic Markers

Prognostic marker	Genotype			P- value
	AA	AG	GG	
No. of Cases	36	61	13	0.539[a]
HWE	40.2	52.6	17.2	
Mean Age	59.63y	63.98y	67.46y	0.082[b]
Sex (%)				
Male	23 (63.9)	43 (70.5)	13 (100%)	0.043[c]
Female	13 (36.1)	18 (29.5)	0 (0.0%)	
Binet Stage (%)				
A	12 (33.3)	21 (34.4)	0 (0.0%)	0.155[c]
B	12 (33.3)	17 (27.9)	6 (46.2)	
C	12 (33.3)	23 (37.7)	7 (53.8)	
Rai Stage (%)				
0	5 (13.9)	5 (8.2)	0 (0.0)	0.170[c]
I	9 (25.0)	11 (18.0)	3 (23.0)	
II	8 (22.2)	13 (21.3)	2 (15.4)	
III	6 (16.7)	26 (42.6)	4 (30.8)	
IV	8 (22.2)	6 (9.8)	4 (30.8)	
CD38 (%)				
<30%	22 (61.1)	37 (60.7)	10 (76.9)	0.529[c]
≥30%	14 (38.9)	24 (39.3)	3 (23.1)	
ZAP70 (%)				
<20%	27 (75.0)	40 (65.6)	7 (53.8)	0.346[c]
≥20%	9 (25.0)	21 (34.4)	6 (46.2)	

a, compared to the control group; b, ANOVA test; c, Chi Square test. (n=110), P value significant below 0.05.

Table 3. Associations between IL-10-1082A>G Genotype and Hematological Parameters

Parameter	Genotype						P. value
	AA		AG		GG		
	No.36		No.61		No.13		
	Mean	SD	Mean	SD	Mean	SD	
WBC×10^3/ul	81.92	70.82	90.89	78.29	132.36	65.92	0.112
Absolute- Lymphocyte×10^3/ul	73.06	68.3	79.37	72.24	121.04	63.3	0.1
Absolute B Lymphocyte×10^3/ul	67.16	67.73	72.13	68.04	111.51	63.58	0.116
RBC×10^6/ul	3.9	0.85	3.63	1	3.49	0.81	0.272
Lymphocyte %	85.58	8.9	83.9	8.1	89.15	10.05	0.129
Platelets×10^3/ul	193.41	98.42	195.94	112.75	146.23	76.63	0.29
Hemoglobin (g/dl)	11.57	1.98	11.06	2.69	10.4	2.51	0.316

ANOVA test; (n, 110). P value significant below 0.05

was significantly more incident in the CLL patients group in comparison with the control group, also found associations between allele -1082A and genotypes (-1082AA/-1082AG) and risk of CLL (Ovsepyan et al., 2015). The reasons for the discrepancies between different studies are not clear. This might be due to ethnicity.

Sharif et al. analyzed the association of IL-10-1082A>G polymorphism with Acute Myeloid Leukemia and concluded that the frequency of GA genotype was significantly higher in AML patients than in control subjects and they resulted that IL-10-1082A>G polymorphism was associated with AML in the Sudanese population (Sharif et al., 2019). Sharif et al., (2019) revealed that the allelic frequency of AA, GG, and GA genotypes was 53.3%, 36.7%, and 10% for the control group, respectively. The frequency IL-10-1082G>A genotype in the control group in this study displayed dissimilar frequencies to those in the control group reported by (Sharif et al., 2019). This may be due to different sample sizes, also may due to differences in ethnic populations from Sudan indicted significant intra-population differences in genotype distribution.

In the current study, genotype distribution revealed a higher frequency of heterozygous (AG) 55.5% than homozygous (AA, GG; 32.7, 11.8, respectively) in CLL patients. These frequencies agree with those previously reported in the literature (Guzowski et al., 2005; Domingo-Domènech et al., 2007; Ennas et al., 2008; Lech-Maranda et al., 2013; Ovsepyan et al., 2015).

In the past several years, some meta-analysis studies have been conducted on the association of IL-10 SNPs with NHL risk (Dai et al., 2014; Zhang et al., 2015; Li and Li, 2016). When stratified by NHL subtypes, all these meta-analysis studies revealed that IL-10 −1082A>G polymorphism was not associated with increased CLL risk (Dai et al., 2014; Zhang et al., 2015; Li and Li, 2016).

Regarding the association between this SNP and established prognostic markers in the present study, comparison of age at diagnosis between patients with different genotype IL-10 -1082A>G did not reveal any significant difference. Also previous studies have been unable to detect any significant difference in mean age among patients with different genotypes (Domingo-Domènech et al., 2007; Lech-Maranda et al., 2008; Lech-Maranda et al., 2013).

Nevertheless, data in this study suggested the CLL patients were carriers of G allele in IL-10−1082A>G promoter gene polymorphism is associated with the male sex, which can be partially explained by the fact that CLL is more prevalent in males than in females. This prognostic association has not been reported in previous studies (Domingo-Domènech et al., 2007; Ennas et al., 2008; Lech-Maranda et al., 2008; Lech-Maranda et al., 2013; Ovsepyan et al., 2015). But this supports that male to have carriers G allele than women (Lio et al., 2002). Thus, it is intriguing that the possession of −1082G genotype, suggested being associated with IL-10 high production.

We were unable to show any significant association between this polymorphism and clinical-stage systems (Rai and Binet) on CLL patients. A similar result was obtained by (Lech-Maranda et al., 2008) found no significant effect of this polymorphism on the Rai stage system. Moreover, Domingo-Domenech et al. explained IL-10-1082A>G was not associated with established prognostic factors such as the Rai stage system (Domingo-Domènech et al., 2007). While another study conducted by Lech-Maranda et al found the presence of IL-10-1082G allele (IL-10-1082AG or GG) was associated with the Rai stage III or IV (Lech-Maranda et al., 2013). In contrast, Ovsepyan et al. explained -1082AA genotype is significantly more incidents in patients with more advanced Binet stages (B+C) than patients with early-stage (Ovsepyan et al., 2015). This discrepancy may be the result of racial differences. Besides, in this study, about 90% of patients displayed advanced Rai stages and 70% were at Binet stage B or C. As expected, reverse patterns with highest patient percentages at stage (Rai 0 and Binet A) and lowest percentages at advanced stages (III, IV, and C) were reported in developed countries.

Our study demonstrated no associations between IL-10-1082A>G polymorphism and CD38 expression or ZAP-70 expression. The results of the current study are consistent with those of Lech-Maranda et al. and suggest that no association was found between IL-10: −1082A>G genotype or haplotype distribution and clinical characteristics of CLL patients at diagnosis, including CD38 expression (Lech-Maranda et al., 2008). More recently Lech-Maranda et al., (2013) have also been unable to detect any association between IL-10-1082A>G and prognostic factors except the presence of IL-10-1082G allele (IL-10-1082AG or GG) was associated with the elevated serum levels of lactate dehydrogenase and advanced Rai stage system.

In conclusion, our results do not support the involvement of the IL-10−1082A>G promoter gene polymorphism in the increased CLL susceptibility. Also, in our group of CLL patients no significant differences were found in the distribution of IL-10−1082A>G alleles and genotypes as compared to controls. IL-10-1082G allele (IL-10-1082AG or IL-10-1082GG) was found more frequently in males. Furthermore, no association was observed between the IL-10 −1082A>G SNP and clinical stages systems as well as established poor prognostic markers. Finally, within the group of patients with CLL there was no difference in the age at diagnosis and hematological parameters according to genotype distributions.

Author Contribution Statement

AAB & EAA & IKI conceived the study design, participated in data collection, performed the statistical analysis, interpreted the results, and revised the manuscript. EAB, NMA, & SOA participated in the statistical analysis and drafted the manuscript. OAA and EAF participated in the data collection, carried out the laboratory work, and prepared the results. AAB& OMS were performed the molecular analysis. All authors read and approved the final manuscript.

Acknowledgments

We would like to thank the staff of the Hematology Department at Al Neelain University for facilities and supporting and we are grateful to the staff of Flow Cytometry Laboratory for Leukemia & Lymphoma for their collaboration. Finally special thanks to the patients for being cooperative, despite their pains.

Availability of Data and Materials

The individual data are available in the archives of the Flow Cytometry for Leukemia & Lymphoma Diagnosis, Khartoum, Sudan and can be obtained from the corresponding author on request.

Consent for Publication

Not applicable.

Ethics Approval and Consent to Participate

Ethical clearance was obtained from the Institutional Review Board at Al Neelain University. The principal investigator obtained written informed consent from all participants prior to their inclusion in the study.

Funding

The research did not receive any fund or financial support.

Conflict of interest

The authors declare that they have no conflict interests.

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