Original article

Significance Aberrant Of CD133 Expression and Their Clinical Impact in Acute Lymphoblastic Leukemia among Sudanese Patients in Khartoum State

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is a malignant disease that arises from several genetic mutations in a single B- or T-lymphoid precursor, resulting in altered blast cell survival, proliferation, and maturation. **Objectives:** This current study was aimed to determine the significance of aberrant CD133 and 45 expressions in Sudanese Patients with Acute lymphoblastic Leukemia, and their clinical significance in Khartoum state. **Materials and Methods:** One hundred Sudanese participants were enrolled in this study as follows: 88 Patients diagnosed with ALL as a case and 12 healthy controls donors were randomly selected. 2.5-5 mL of EDTA blood/bone marrow for flowcytometry from each patient and controls subject. **Results:** Eighty-eight newly diagnosed Sudanese patients with acute lymphocytic leukemia (ALL) were involved in this study, their age average is (15.7) and their stander deviation (SD) is 17.4. The frequency of aberrant markers concerning control groups was significantly associated with all patients in CD 45 with a P value (0.0001), while there was no difference between cases and control in the frequency of CD 133 antigen, there was no significant difference between age groups in the expression of different aberrant antigens. The study was significantly more expressed CD133 in males than females. Also no significant difference in hematological parameters between patients with or without expression of Aberrant CD 45 and 133 markers, while we found significantly high TWBCs in T. cell leukemia. **Conclusion:** Aberrant myeloid antigens CD45 was significantly associated with childhood and adult ALL and may be considered as important prognostic factors, while CD 133 were not associated with ALL.

Keywords: *Acute lymphoblastic Leukemia, CD45, CD133, flow cytometer-Sudan*

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease that arises from several genetic mutations in a single B or T-lymphoid precursor, resulting in altered blast cell survival, proliferation, and maturation. Eventually, this leads to the lethal accumulation of leukemic cells. The risk of developing ALL is more common in children younger than 5 years of age of 50. Overall, about 4 out of every 10 cases of ALL are in adults [2].

ALL is not common cancer, accounting for less than half of 1% of all cancers in the United States. The average person's lifetime risk of getting ALL is about 1 in 1000. The risk is slightly higher in males than in females and higher in whites than in African Americans [3]. Children may do better than adults because of differences like childhood and adult ALL, variances in treatment (children's bodies can often tolerate aggressive treatment better than adults), or some combination of these [4,5].

The incidence of ALL is assessed at 1.6 per 100000 population, in 2016 alone, an estimated 6590 new cases were diagnosed, with over 1400 deaths due to ALL [6]. The incidence of ALL follows a bimodal distribution, with the first highest occurring in childhood and the second-highest occurring around the age of 50 [7]. While dose intensification strategies have led to significant progress in outcomes for pediatric patients, the prognosis for the

elderly remains very poor. Even with a high rate of response to induction chemotherapy, only 30-40% of adult patients with ALL will achieve long-term remission [8].

CD 45 Antigen also known as protein tyrosine phosphatase, receptor type C, also known as PTPRC is an enzyme in humans that is encoded by the PTPRC gene. PTPRC, which was originally called leukocyte common antigen (LCA) [9,10]. CD45 is a pan-leukocyte protein with tyrosine phosphatase activity involved in the regulation of signal transduction in hematopoiesis. CD45 does not colocalize with lipid rafts on murine and human non-transformed hematopoietic cells, but CD45 positioning within lipid rafts is modified during their oncogenic transformation to acute myeloid leukemia. CD45 colocalizes with lipid rafts on AML cells, which contributes to elevated GM-CSF signal intensity involved in the proliferation of leukemic cells [11]. Many alternatively spliced transcripts variants of this gene, which encode distinct isoforms, have been reported [9]. CD133 Antigen also known as prominin-1, is a glycoprotein in humans that is encoded by the PROM1 gene [9,10]. It is a member of pentaspan transmembrane glycoproteins, which specifically localize to cellular protrusions. While the precise function of CD133 remains unknown, it has been proposed that it acts as an organizer of cell membrane topology [12]. Today, CD133 is the most commonly used marker for the isolation of cancer stem cells (CSC). Its population derives from different tumors, mainly from various gliomas and carcinomas [13].

Materials and Methods

The current study was a case-control study done in Khartoum State in the period from 2019 to 2021. One hundred individuals were included in our study. Out of the One hundred, 88 patients were diagnosed with Acute lymphoblastic leukemia. Patients who had other forms of dysplastic disorders and tumors were excluded. 2.5-5 ml of blood for flow cytometer was drawn in EDTA. The laboratory investigations included: A Complete blood count by using an automated hematology analyzer Sysmex kx21 and an Immunophenotyping by flow cytometry.

Immunophenotyping: All samples and healthy controls were analyzed using a flow cytometer (FC500 Beckman Coulter, Miami - USA), following the instructions of immune phenotyping kits offered by the IMMUNOSTEP Company.

Principle of flow cytometer: The underlying principle of flow cytometry is related to light scattering and fluorescence emission. Which occurs as light from the excitation source (commonly a laser beam) strikes the moving particles. The data obtained could give valuable information about biochemical, biophysical, and molecular aspects of particles. Light scattering is directly related to structural and morphological properties of the cell, while fluorescence emission derived from a fluorescence probe is proportional to the amount of fluorescent probe bound to the cell or cellular component.

Data were analyzed by using the Chi-square test and Fisher exact test. The numerical variables were presented in mean and stander deviation (SD). Two-sided P-values of < 0.05 were considered significant. All the statistics were performed using the SPSS software version 20 and Graph Pad Prism software version 5.
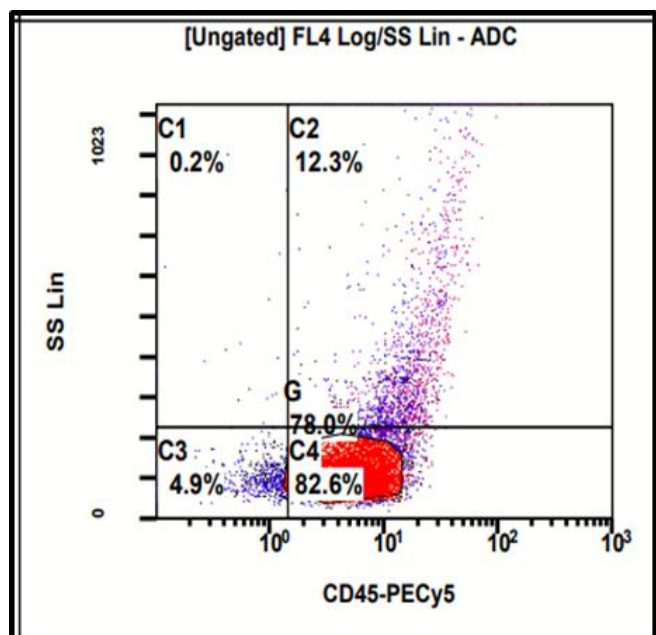
Results

Eighty-eight ALL patients were involved in this study, the mean and SD age of patients were 15.7 and 17.4 years old, while the mean and SD of the control was 15.9 and 11.8.

The detection of aberrant CD markers was done by using flow cytometer techniques and the results were obtained in histograms representing the concentration of each aberrant antigens. Shown below is an example of a patient's results and control results summarized in figure (1)
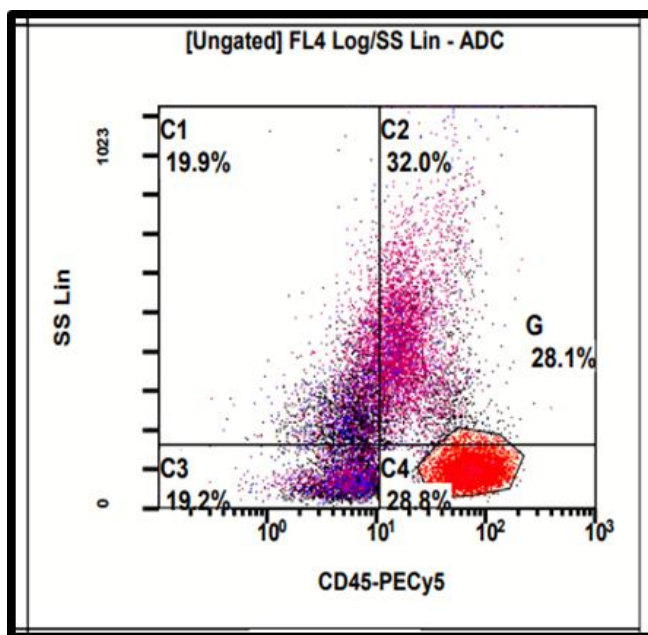
A: Control sample with negative result in CD 45

B: ALL sample with positive results for CD45

Eighty-eight participants were stratified by detection of aberrant CD markers (myeloid antigens), Frequency of each marker as illustrated in figure (2) which showed around 94.3% of the patients was CD45 positive and 30.7% were positive for CD 133.

The distribution of the aberrant markers concerning control groups was significantly associated with All patients in CD 45 with a P value (0.0001) (as shown in figure (3), while there was no difference between cases and control in the frequency of CD 133 antigen which statically not significant with P. value (0.17).
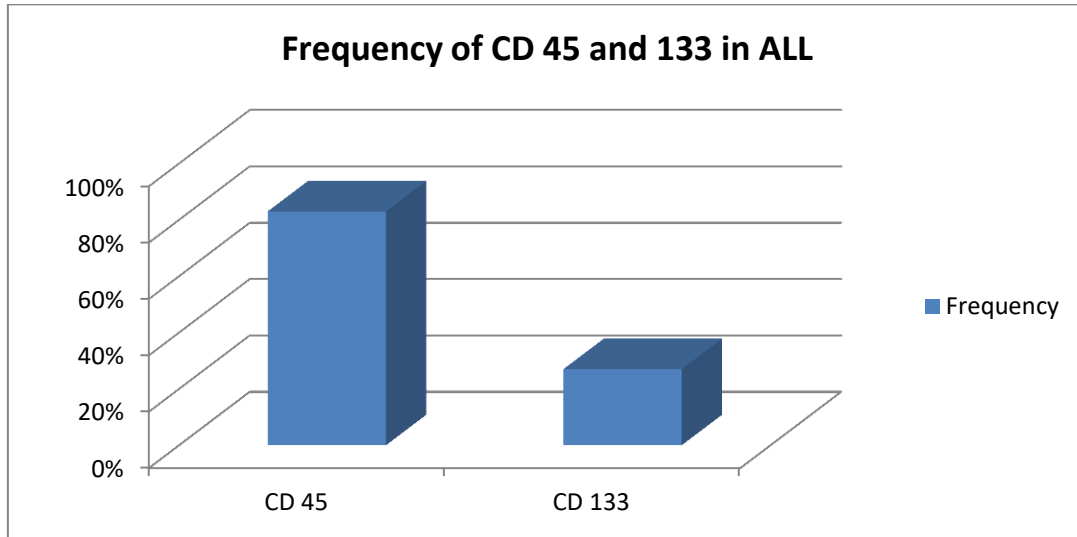
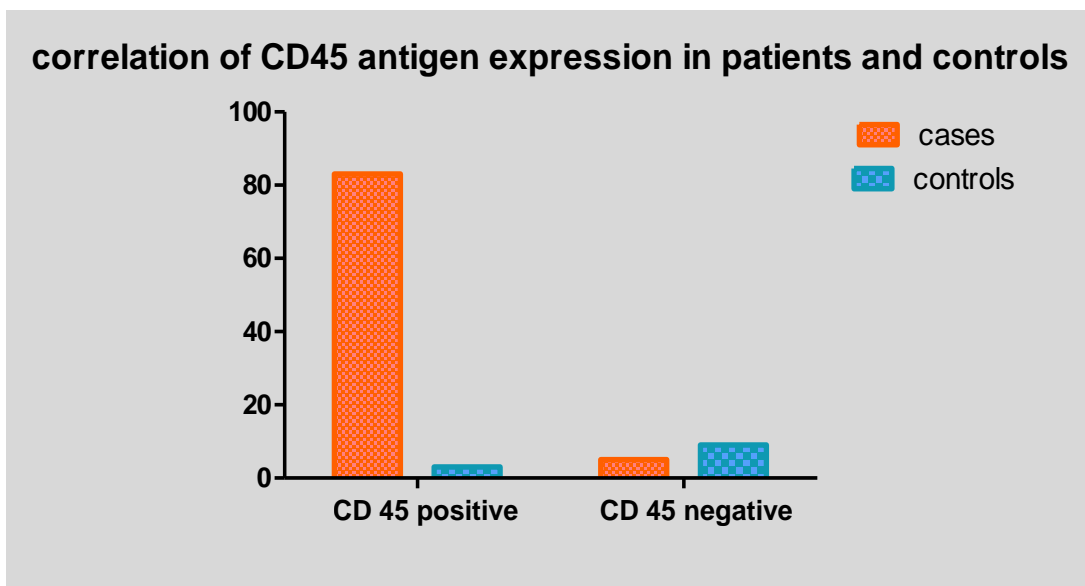**Figure 2: Frequency of CD45 and CD 133 makers in ALL patients**

Figure 3: Correlation of CD 45 expression in case and control with a P value (0.0001)

The general age distribution groups were shown in figure (4) were the most common group were between age 0-20 that represent 79 % of the whole study group, while the age distribution according to myeloid expression (CD45 and CD133) was shown in figure (5) with no significant between age groups in the expression of different aberrant antigens. The distribution of gender was more frequent male than female in cases and control group (figure 6)
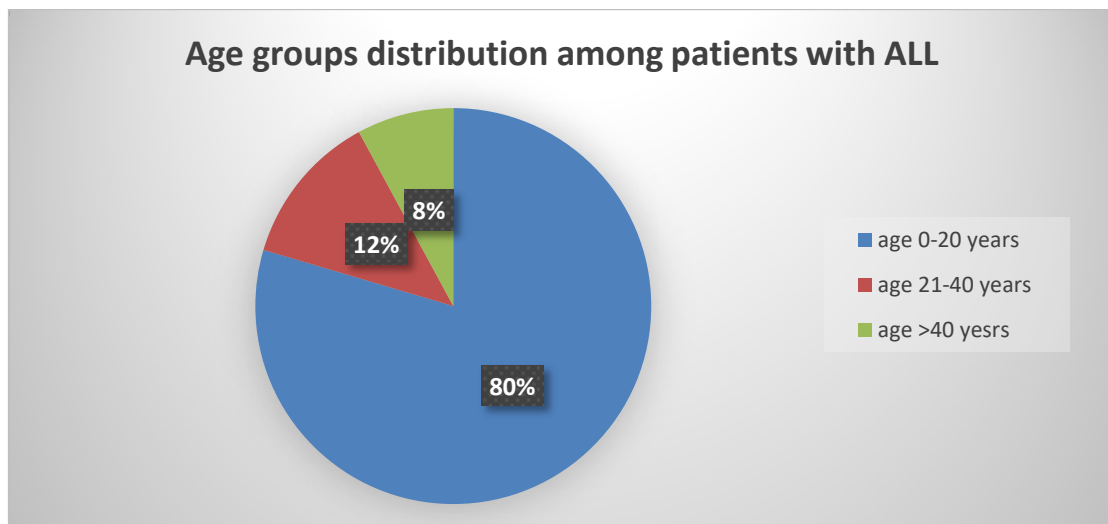

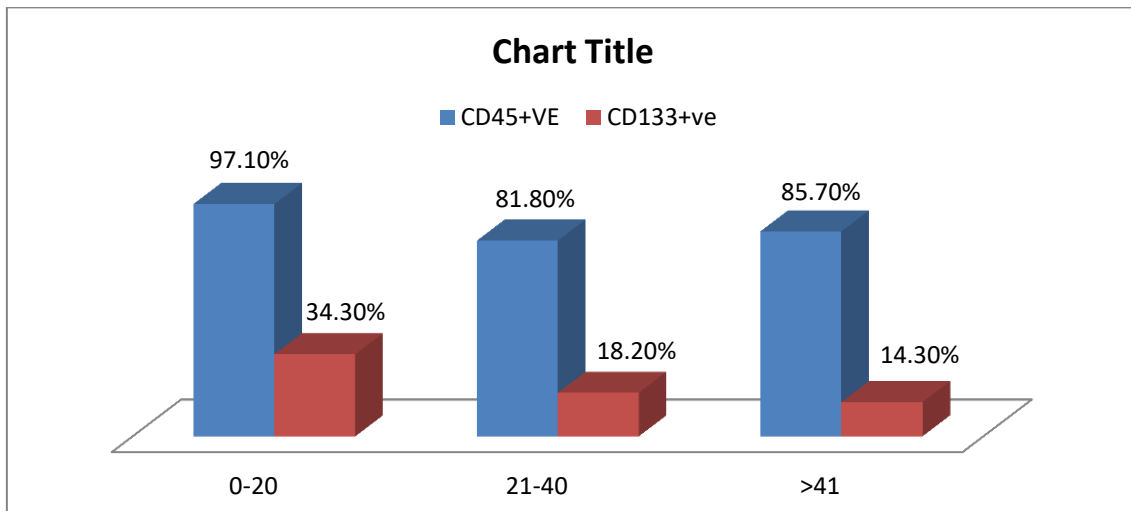**Figure 4: Age groups distribution among patients with ALL**

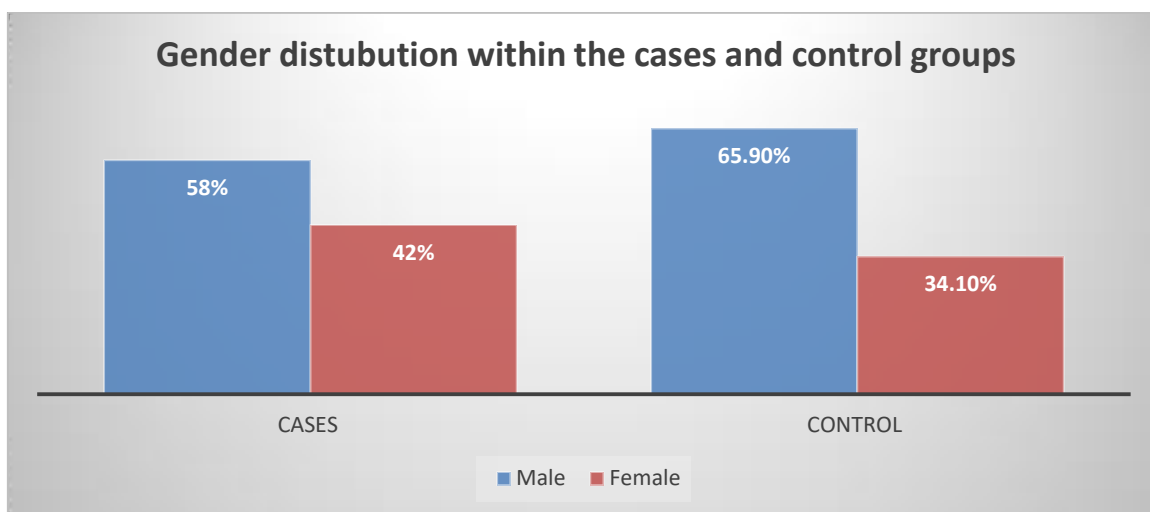**Figure 5: Frequency of aberrant myeloid expression in different Age**

Figure 6: The gender distribution within case and control groups

The RBCs and platelets counts were insignificant decreased in most of CD 45+ patients with p.value (0.618 and 1.000, respectively) while the TWBCs was insignificant increased in most of CD 45+ patients with p.value (0.559). The RBCs and platelets counts were insignificant decreased in most of CD 133+ patients with p.value (and 1.000 and 0.128, respectively) while the TWBCs was insignificant increased in most of CD 45+ patients with p.value (0.833).

The cases samples of the study group were also stratified for determined the immunologic classification into B or T-phenotypes. The panel of monoclonal antibodies that used was CD2, cytoplasmic (c) CD3, CD7 for precursor T-ALL, CD10, CD19, cCD79a for precursor B-ALL CD13, CD33, and cMPO, for myeloid lineage, while using HLA-DR and CD34 for non-lineage

On the other hand, the different profile was detected for childhood and adult B.cell and T.cell leukemia were summarized in figure (7 and 8).
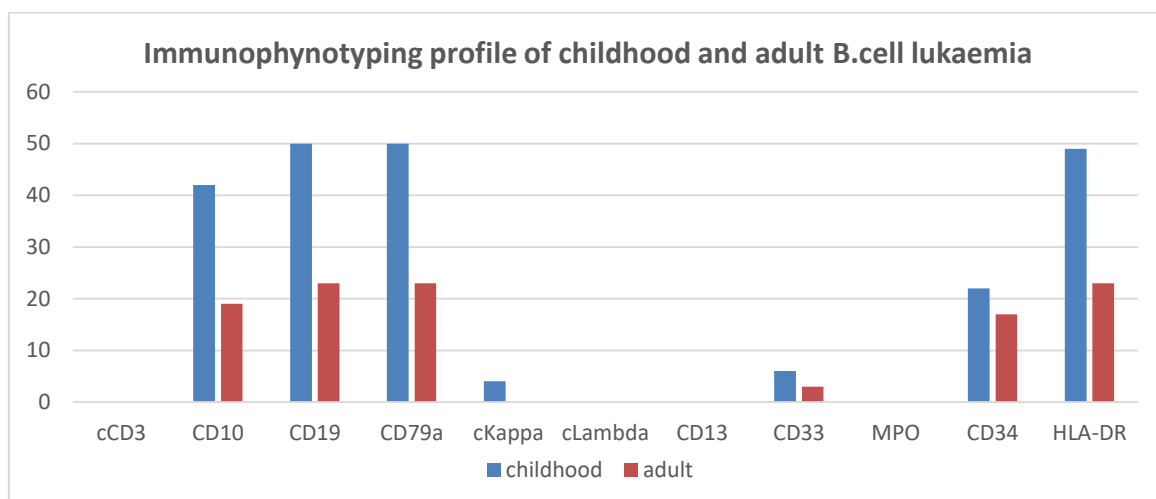

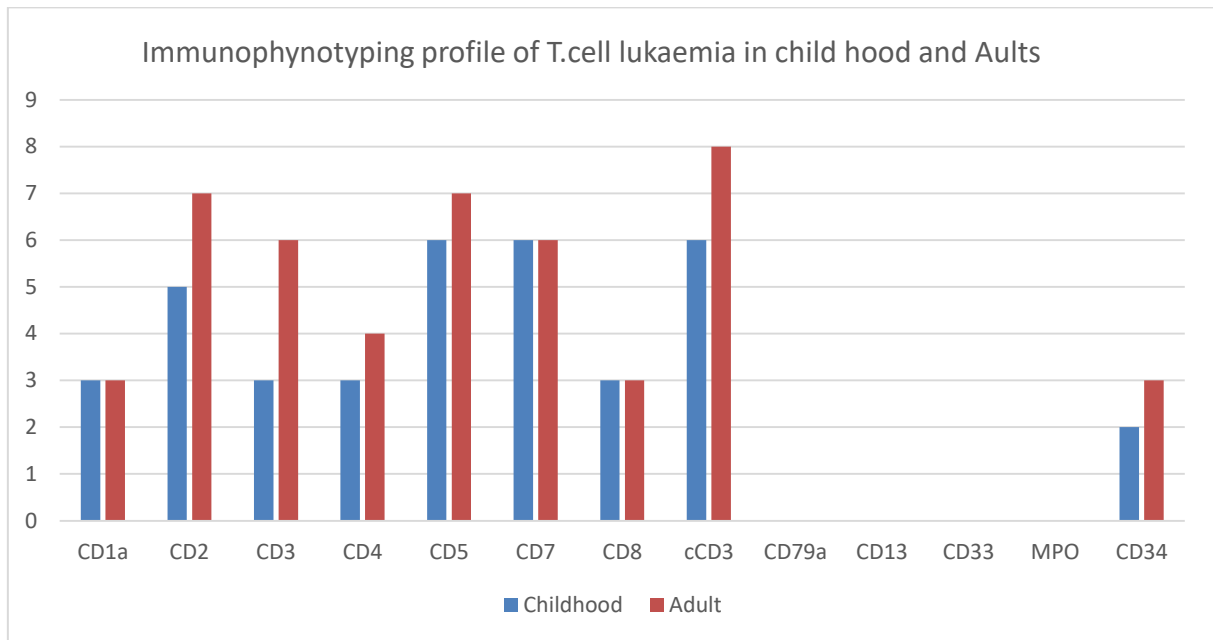**Figure 7: Frequency of aberrant antigens among different types of ALL**

Figure 8: Frequency distribution of aberrant antigens among different types of T.ALL

The study group again were classified according to their origin into B. cell leukemia represents 84.1% (74 out of 88) while T. a cell represents 15.9% (14 out of 88) of the study group.

No significant difference in expression of all aberrant CD markers between B.cell leukemia and Tell leukemia groups where expressed in 94.6% and 92.8% respectively of each group and the frequency distribution of each maker in two types were illustrated in figure (9)
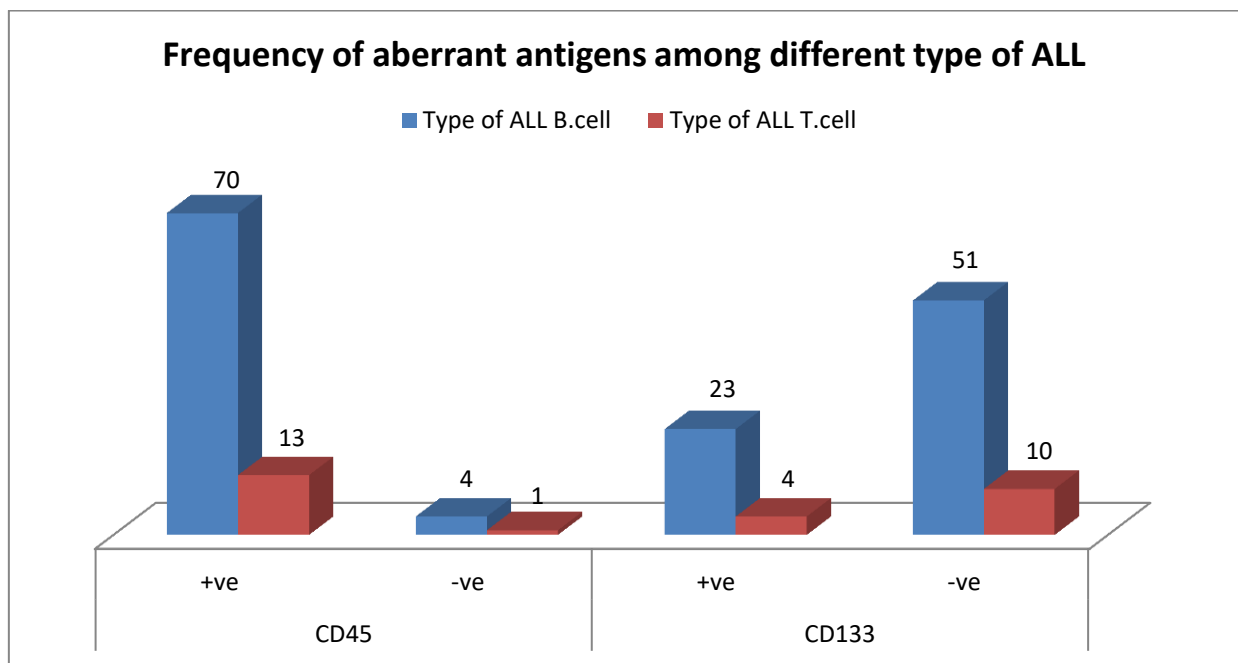
Figure 9: Frequency of aberrant antigens among different types of ALL

Further sub-classification of T.ALL was observed according to the result of flow cytometer and blood film in which they classified into (cortical and mature), where the B.ALL were classified into (early pre, pre, pro, and common), each sub-classification of B.cell and T.cell leukemia were compared with aberrant CD markers expression and summarized in tables (1 and 2 respectively).

Table 1: CD markers among different sub-classification of B.cell leukemia

B. cell Stage	CD45 count		CD133 count	
	+ve	**-ve**	**+ve**	**-ve**
EarlyPre B	45	1	15	31
Pre B	6	0	0	6
Pro	6	0	2	4
Common	13	3	4	12
Total	70	4	21	53

Table 2: CD markers among different sub-classification of T.cell leukemia

T. cell Stage	CD45 count		CD133 count	
	+ve	-ve	+ve	-ve
cortical	6	0	3	3
Mature	2	1	0	3
Total	8	1	3	6

There is no statistical significance of any clinical feature and aberrant CD 45 and CD 133 marker expression, the analysis results were shown in table (3)

Table 3: Association of common clinical feature and aberrant CD marker expression among the patient group:

Clinical features in ALL patients		CD45		P.value	CD133		P. value
		-ve	+ve		-ve	+ve	
Fever	yes	3	70	0.428	59	23	0.196
	No	2	13		2	4	
Lymphadenopathy	yes	1	48	0.234	59	23	0.828
	No	4	35		2	4	
bleeding tendency	yes	0	12	0.632	59	23	0.710
	No	5	70		2	4	
Hepatomegaly	yes	2	22	0.888	59	23	0.333
	No	3	61		2	4	
Splenomegaly	yes	2	38	0.801	59	23	0.441
	No	3	45		2	4	
Joint pain swelling	yes	1	28	0.885	59	23	0.238
	No	4	55		2	4	
Cough	yes	0	7	1.000	59	23	0.248
	No	5	76		2	4	

The present study showed the frequency of patients with positive with CD 45 and CD 133 together when correlated to gender were insignificant more frequent in males than a female with p.value (0.236) and no significant more frequent positive CD45 and CD133patients with B.cell ALL with p.value (0.689) as shown in table (5).

Table 5: Frequency of gender and type of ALL in CD45+ and CD133+

		CD45+ and CD133+	P. value
Gender	Male	21	0.236
	Female	4	
Type of ALL	T cell	4	0.689
	B cell	21	

Also, these groups of co-expressed markers were compared with clinical feature and clinical feature severity and the result was illustrated in the table (6). The lymphadenopathy feature was also significantly related to co-expression with a P value (0.036), then again significant association between clinical feature and clinical feature severity with P.value (0.025) was detected.

Table 6: Frequency of clinical features and clinical feature severity

Clinical features		CD45+ and CD133+	P.value
Fever	**No**	2	0.337
	Yes	8	
Lymphadenopathy	No	1	0.036[*]
	Yes	9	
Bleeding tendency	No	10	0.112
	Yes	0	
Hepatomegaly	No	7	0.532
	Yes	3	
Splenomegaly	No	5	0.491
	Yes	5	
Joint pain swelling	No	8	0.594
	Yes	2	
Cough	No	8	0.376
	Yes	2	
Clinical feature severity	No	1	0.025[*]
	Medium	9	

Discussion

The frequency of aberrant myeloid antigen expression in acute lymphoid leukemia and their clinical significance is still not clear around the world. Currently, there are no published studies about the expression of these aberrant CD markers (CD45 and CD133) in acute lymphoblastic leukemia in Sudan. The current study found that the expression of aberrant markers CD45 was highly significant in cases of ALL. In comparison, the control group where CD45 was expressed in 94.3 % of samples while only 30.7 % of samples expressed CD133. Earlier studies have also reported the frequency of different aberrant myeloid antigens in ALL in independent studies has varied from 10 to 100% [14,15] Similar to our results, the percentage of CD45 in the previous study was 78% in B.cell leukemia and 100% in T.cell leukemia [15]. While similarly agreeing with other previous studies, this indicates the important role of CD45 in ALL.

In contrast, our result of CD133 expression agrees with Fetnat M. Tolba and his colleague who reported no correlations were elicited between CD133 expression and ALL [16], but disagreed with other studies that found strong expression of CD 133 in ALL [17]. These differences may be due to the detection of mRNA expression in their studies rather than flow cytometer.

Furthermore, the current study found the expression of CD 133 significantly expressed in males more than females, and no previous published study report that.

Additionally, the expression of this aberrant CD marker between childhood and adult (using ≤ 14 as the cut-off for children) was done, and no significant difference between the expression of the three marker CD45 and CD133 in childhood and adult lymphoid leukemia was found. The expression agrees with a similar study that other aberrant markers in ALL have been reported an aberrant expression of CD133. The reported 39 and 23% cases were adults and children, respectively [14,18].

Furthermore, a strong association of the presence of early pre-stage B.ALL leukemia in childhood rather than Adults. This association may indicate a good prognosis for patients which reported in other studies [19]

In this study, the classification of ALL into B-ALL and T-ALL also were compared with expression of aberrant myeloid antigen (CD45 and CD133), the frequency of these antigens was found (94.8% and 31.2 %, respectively) of samples with B-ALL and (92.8% and 28.5%, respectively) in samples with T-ALL. Our results for CD45 were slightly higher than earlier reports where 83.2 % samples with B-ALL and 28.3 % samples with T-ALL were CD 45 positive [20] while disagreeing with other studies where report 100% samples with T-ALL and 28.3 % samples with B-ALL are positive for aberrant markers [21], this difference may be due to different study population and age factor since they carry their studies only on childhood patients.

Although there was no significant difference between the presence of B.ALL and T-ALL in their expression of these aberrant markers, with different clinical symptoms we found a strong association between expression of CD45 and lymphadenopathy in B-ALL, while in T-ALL, the cough was significantly associated with expression of CD133. These results disagree with the poor prognosis for CD 133 which was reported in a previous systemic review and meta-analysis by Wesley Messias Santos and et.al in Europe [22]. Other studies also report no clinical significance between CD133 and clinical feature presentation [16].

Regarding the presentation of the clinical feature in our patients with ALL [23,24], the most common clinical feature was fever (82.9%) while the other feature represents (55.7%, 45.5%, 33%, 27.3%, 13.6%, and 7.9%) for lymphadenopathy, splenomegaly, joint pain, hepatomegaly, bleeding tendency, and cough respectively. Similar findings were observed where Fever and hepatomegaly were the most common signs and symptoms in patients [25-27]. While in another study different results were reported hepatomegaly was the most common abnormality in almost 80%, and 60% for both lymphadenopathy and splenomegaly [28].

In agreement with a previous study from Oman [29], the current study includes more males than females, this results in agreement with the facts previously mentioned in most papers [30-32].

Age destitution is an important role in the diagnosis and prognosis of acute lymphoid leukemia. In this study, we stratified the patients into four groups and the most common group between ages 0-20 represent 79 %, this confirms the previous facts that Acute lymphoblastic leukemia is more common in children than adults [33,34]. Alternatively, no clinical significant correlation between age groups and expression of aberrant CD markers were found, which confirm the results from different earlier studies [17,19,35].

B.ALL and T.ALL were detected in childhood and adult which occur in 68.9% and 31.1 % of cases, while 42.8. % were B.ALL and 57.2% were T.ALL, respectively. Thus the B.ALL are more common in childhood than adults while the T.cell are more common in adults, an almost similar result was reported in an earlier study [36]. Alternatively, different immunophenotyping profiles were detected in adult B.ALL than in childhood profile. While in little similar profiles for both adult and childhood profiles in T.ALL were the same as what was reported in other studies [14,18,36].

In the present study, patients with positive CD 45 and CD133 were compared according to the gender and type of ALL with no significant difference between male and female or between T.cell and B.cell leukemia in co-expression of aberrant CD markers were found. These results about co-expression disagreed with another study that reports co-expression of CD133 was associated with B.ALL [17].

The co-expressed markers were compared with a clinical feature in addition to the significant association with lymphadenopathy as well as severe clinical remarks in B.ALL. Also, coughing was significantly associated with CD133 in T.ALL, this result confirms the hypothesis that co-expression of CD marker is associated with severe ALL that was reported in different studies [14,17].

Conclusions

Ultimately, the present study concluded that there are no correlations between CD133 expression and ALL. Thus, we found the expression of CD 133 is significantly expressed in males more than females.

Acknowledgements

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