

Significance of aberrant CD82 expression and their Clinical Impact in Acute Lymphoblastic Leukemia among Sudanese Patients in Khartoum state

Ayman .E. Elnour Atia¹, Tarig A. M. Hamid², Samah Saif Eldin M. Mohamed^{3*}, Mubarak Mustafa Elkarsany¹, Osama A. Altayeb⁴, Amro B. E. Bashir¹

¹ Department of Hematology and Immunohematology, College of Medical Laboratory Science, Karary University, Khartoum, Sudan

² Department of Hematology and Immunohematology, Sharq El Nile College, Khartoum, Sudan

³ Collage of applied medical sciences /Alqwyiyah, Shaqra University, Riyadh, Saudi Arabia

⁴ Flow Cytometry Consultant at Flow Cytometry Laboratory for Leukemia & Lymphoma Diagnosis, Khartoum, Sudan

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ABSTRACT

Acute lymphoblastic leukemia(ALL) is a malignant disease that arises from several genetic mutations in a single B- or T-lymphoid precursor. The risk of developing (ALL) is more common in children younger than 5 years of age. Aberrant phenotype expression due to genetic defects may be associated with unfavorable outcomes. This study was aimed to determine the significance of aberrant CD82 and CD 45 expressions in Sudanese Patients with Acute lymphoblastic Leukemia, and their clinical significance in Khartoum state. Eghity-eight newly diagnosed patients with ALL were randomly selected as case groups, and 12 aberrantly healthy controls. 3.0 ml of EDTA blood and other 3.0 ml of bone marrow aspirate was drawn from each patient and control subjects. The laboratory investigations included that were a Complete blood count by using Automated hematology analyzer and CD45 and CD82 marker detection by flowcytometer. All patients were Sudanese with acute lymphocytic leukemia (ALL), their average age was (15.7) and the stander deviation(SD) was (17.4±1.8). The frequency of the aberrant markers concerning control groups was significantly associated with patients in CD82 expression with a *P value* (0.001). Also significant association of expression of CD82 marker and presence of immature cells in B.cell leukemia was found with *P value* (0.016), while no difference between childhood (70.1%) and Adult (67.8%) in immature cell ratios. The current study confirms the previous studies in which aberrant antigens CD45 and CD82 were significantly associated with childhood and adult ALL and may be considered as important prognostic factors.

1. Introduction

CD82 is a human protein encoded by the CD82 gene(1). The expression of this gene is downregulated in the tumor development of human tumors and can be triggered by p53 through a consensus binding sequence in the promoter. Its expression and that of p53 are strongly correlated, and the loss of appearance of these two proteins is related with poor existence for prostate cancer patients(2).

Also, CD82 is related with integrin's on the surfaces of various malignant cells, and its appearance is associated with metastasis suppression, this metastasis suppressors can potentially serve as prognostic markers, therapeutic targets, and predictors for treatment response (3). CD82 (gene) has been shown to interact with CD19,CD63,and CD234(4, 5).CD82 plays a key role in the progress of endometriosis (6).

Acute lymphoblastic leukemia (ALL) is a type of cancer that caused by different genetic mutations in a single B- or T-lymphoid precursor, resulting in altered blast cell survival, proliferation, and maturationPui (7). The ALL type of lukaemia risk is more common in children under 5 years of age. The risk then declines slowly until the mid-20s and begins to rise again slowly after age 50. Overall, about 4 out of every 10 cases of ALL are in adults(8).

ALL is not a common malignancy, 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(9). Children may do better than adults because of differences like childhood and adult ALL, variances in treatment (children's bodies can often accept aggressive treatment better than adults), or some combination of these(10, 11). The incidence of ALL is measured at 1.6 per 100000 population, 6590 new cases were diagnosed in 2016 alone, with over 1400 deaths due to ALL(12). The incidence of

* Corresponding authors at Shaqra University

E-mail addresses: smerghani@su.edu.sa (Samah S. Mohamed)

ALL follows a bimodal distribution, with the first highest occurring in childhood and the second-highest occurring around the age of 50(13). While dose intensification policies have led to significant progress in consequences for pediatric patients, the prognosis for the aged remains very poor. Even with a high frequency of response to induction chemotherapy, only 30–40% of adult patients with ALL will achieve long-term reduction(14).

CD45 is a pan-leukocyte protein with tyrosine phosphatase activity involved in the regulation of signal transduction in hematopoiesis. which was originally called leukocyte common antigen (LCA)(15, 16). CD45 does not colocalize with lipid rafts on murine and human non-transformed hematopoietic cells, but CD45 arranging within lipid rafts is adapted during their oncogenic alteration 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(17).

One of the most significant current health problems in Sudan is Leukemia in many cases may be associated with certain a large proportion of the causes of death and disease. A considerable amount of literature has been published on Acute Leukemia(18). Most of these studies consider CD82 it as a good risk Factor and associated with a high complete remission rate and long-term disease-free survival (1, 3, 19, 20). So far, however, there has been little discussion about the effect of aberrant markers in acute lymphoblastic leukemia in Sudan and no data available about CD82 marker for acute lymphoblastic leukemia in Sudanese patients.

2. Materials and Methods

The current study was a case-control study done in Khartoum State attending the flowcytometry laboratory for leukaemia and lymphoma diagnosis in the period from 2019 to 2021. Out of the one hundred participants, 88 newly diagnosed ALL patients and any patients who had another form of other types of dysplastic disorders and

tumors were excluded. 3.0 ml of bone marrow aspirate and 3.0 ml of blood was drawn in separate EDTA tube for each participants . The laboratory investigations included that were a Complete blood count by using Automated hematology analyzer (Sysmex) and detection of CD45 and CD82 markers by flowcytometer (XP300C JAPAN).

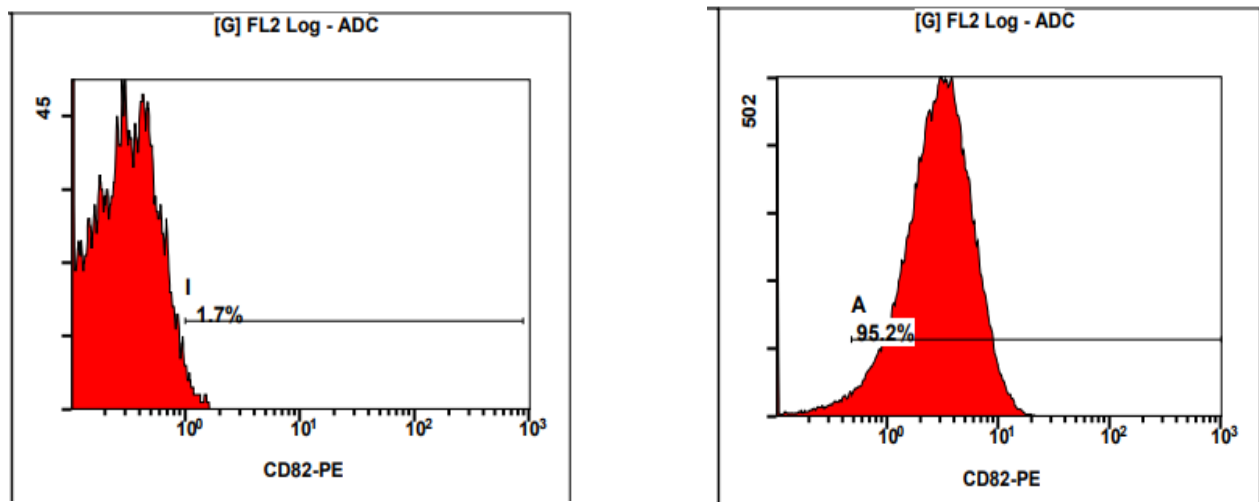
All samples and apparently healthy controls were analyzed using a flow cytometer (FC500 Beckman Coulter, Miami – USA) following the instructions of immune phenotyping kits offered by IMMUNOSTEP Company.

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) that 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.

The data was analyzed using the Chi-square test and Fisher exact test. The numerical variables were presented in mean and stander deviation (SD), while 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.

3. Results

Eighty-eight acute lymphoblastic lukaemia patients were stratified in this study by detection of abberent CD markers, the mean and SD of the patients age was 15.7 and (17.4 ±1.8) years old, while the control was 15.9 and (11.8±3.4). The aberrant CD markers results were obtained in histograms representing the concentration of each aberrant antigens, an example of patient's and control results are shown in the figure (1) below.



A: Control sample (negative result in CD82)

B: ALL sample (positive results for CD82)

Fig. 1: Example of positive and negative results in flowcytometer

Frequency of each marker was calculated and around 83% of the patients was CD45 positive while only 73% positive for CD82.

The distribution of the aberrant markers concerning control groups was significantly associated with All patients in CD 45 and CD 82 with *P values* (0.0001 and 0.001) respectively and shown in figure (2) for expression of CD82.

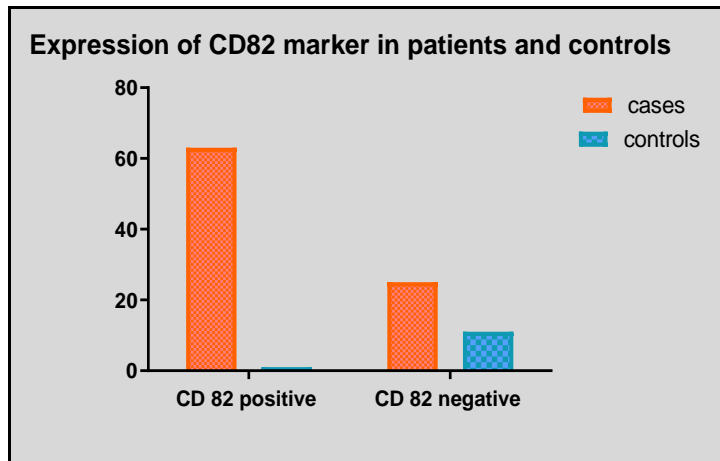


Fig. 2: CD 82 marker expression in cases and controls with *P value* (0.0001)

The most common group in age distribution was less than 20 that represent 79 % of the whole study group, while the age distribution according to CD markers

expression (CD45 and CD82) is shown in figure (4) with no significant between age groups in the expression of different aberrant antigens.

The current study includes males more than females in ratio 1:0.5 for cases while the parallel ratio for normal controls is 1:0.7. There was no significant difference in aberrant CD markers (CD 45 and CD82) expression between males and females where the male expressed CD 45, CD 82 (94.8% and 68.9% respectively) while the females expressed the markers as (93.3% and 76.7% respectively)

CBC was done for all samples of ALL patients using an automated Hematological cell counter. The basic cells parameters TWBCs, RBCs, platelets counts, and hemoglobin levels were calculated and the average of them was (54.5, 2.8 50.9, 7.9 respectively) while their SD is (70.3±7.5, 0.8±0.09, 58.3±6.2 and 2.4±0.25 respectively) and no significant difference in these parameters between patients with or without expression of Aberrant CD markers (CD 45, CD82) that summarized in table (1)

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 CD3, CD7 for T-ALL, CD10, CD19, cCD79a for B-ALL CD13, CD33, and cMPO, for myeloid lineage, while using HLA-DR and CD34 for non-lineage and no different between adult and childhood lukaemia.

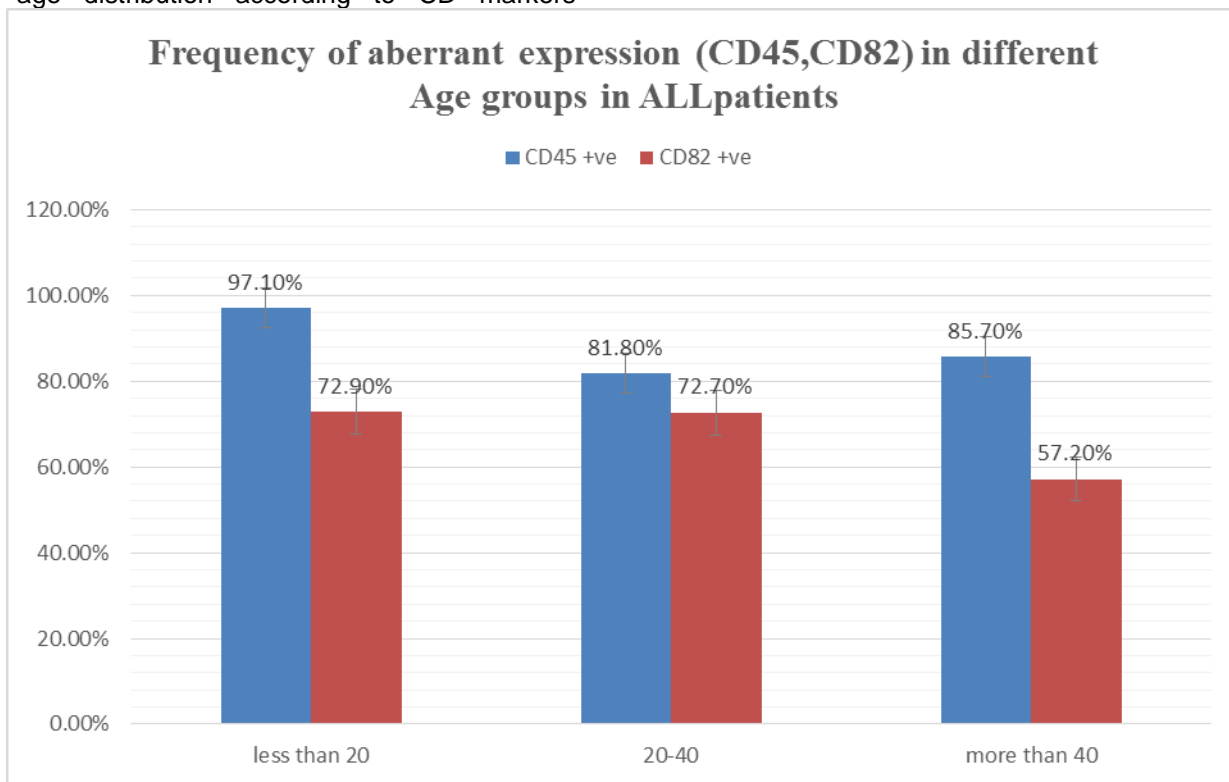


Fig. 3: Frequency of aberrant markers expression (CD45, CD82) in different Age groups in ALL patients

Table 1: Distribution of complete blood count Basic parameters between positive and negative patients to aberrant CD45 and CD82 markers:

		CD45		P. value	CD82		P.value
		-ve	+ve		-ve	+ve	
RBCs	Low	4	71	0.618	22	53	0.121
	Normal	1	12		3	10	
TWBCS	High	2	51	0.559	15	38	0.898
	Low	2	18		7	13	
	Normal	1	14		3	12	
PLTs	Low	5	77	1.000	24	58	0.848
	Normal	0	6		1	5	

The study group were classified according to their origin into B-cell leukemia represents 84.1% (74 out of 88) while T-cell leukemia 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 T-cell leukemia groups where expressed in 94.6% and 92.8% respectively of each group and the frequency distribution of each maker(CD45 and CD82) in two types were illustrated in figure (5)

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) according to attended patients to the flowcytometry centre , where the B-ALL were classified into (pro, early preB, common and preB), each sub-classification of B.cell and T.cell leukemia were compared with aberrant CD markers expression.

We found a strong association between the presence of early pre-stage in childhood B.cell leukemia rather than Adults one shown in figure (6)

The patients were stratified depend on their co-expression of these two markers and compared with gender and type of ALL. There was no difference between male and female or between T.cell and B.cell leukemia in co-expression of both CD45 and CD82 markers.

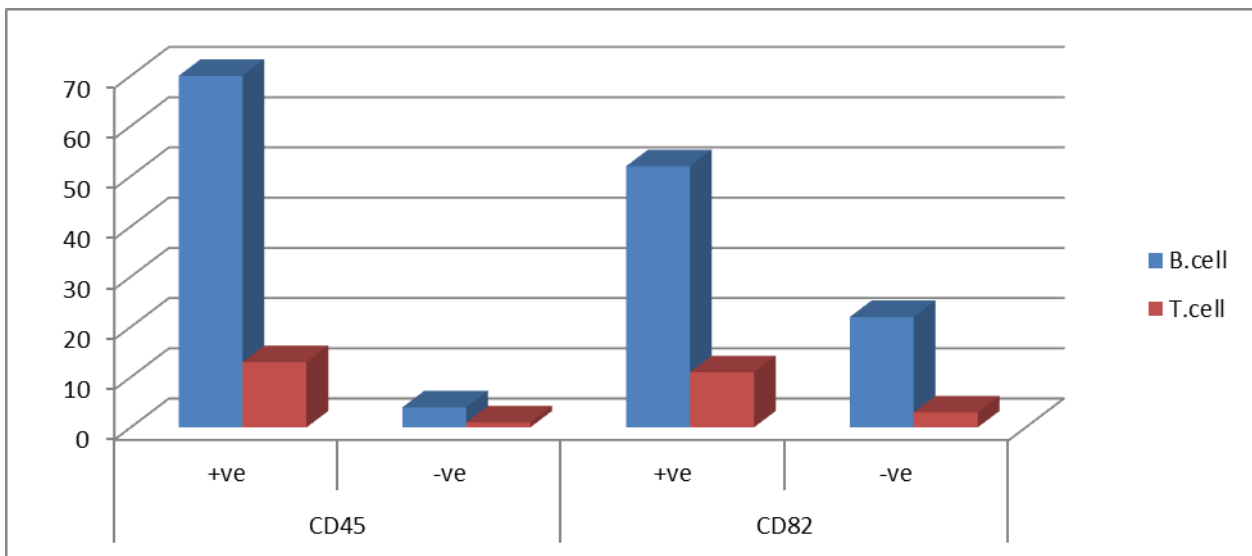


Fig. 5: Frequency of aberrant antigens among different types of ALL

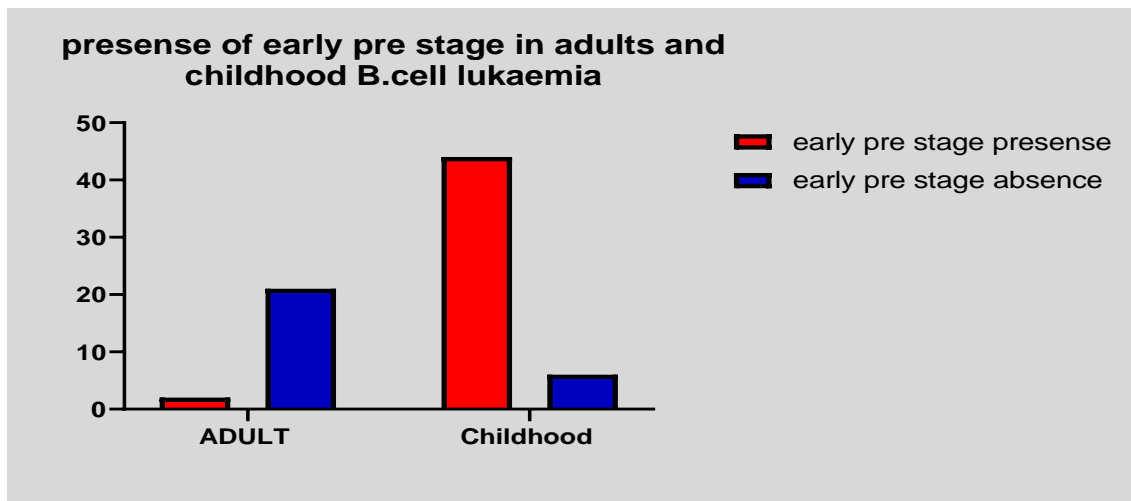


Fig. 6: Significant presence of early pre-stage in childhood leukemia rather than adult B.cell leukemia

4. Discussion

The frequency of aberrant myeloid antigen expression in acute lymphoid leukemia and their clinical significance is still not clear around the world(21). Also, there are no published studies about the expression of these aberrant CD markers (CD45 and CD82) in acute lymphoblastic leukemia in Sudan. The current study proved that the expression of aberrant markers CD45, CD82 was highly significant in cases of ALL when compared to the control group. CD45 was expressed in 94.3 % of the patients, while CD82 only expressed 71.6 %. Earlier studies have also reported a frequency of different aberrant myeloid antigens in ALL patients, whereas independent studies have varied from 10 to 100% (21, 22) Similar to our results, the percentage of CD45 in the previous study was 78% in B.cell leukemia and 100% in T.cell leukemia (22). Thus agreeing with other previous studies, this indicates the important role of CD45 in ALL.

CD82 expression on ALL were a few studies reported in literature, the most recent study was done in 2017. This study had similar results and reported a strong expression of CD82 in ALL patients when compared to the control group(20). That may confirm our hypothesis about the important role of CD82 in ALL.

Additionally, the expression of this aberrant CD marker between childhood and adult (using ≤ 14 as the cut-off for children) was done. There was no significant difference between the expression of CD45 and CD82 in childhood and adult lymphoid leukemia. The expression was slightly more common in childhood than an adult, which agrees with a similar study for other aberrant markers in ALL.(21) and (23).

There is also a stronger association of the presence of early pre-stage in childhood B.ALL leukemia than Adults. This association may indicate a good prognosis for patients which reported in other studies(24)

In this study, the classification of ALL into B-ALL and T-ALL were also compared with expression of aberrant myeloid antigen (CD45 and CD82). The frequency of these

antigens was found in (94.8% and 70.25%, respectively) of samples with B-ALL and (92.8% and 78.5%, 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(25). Whilst disagreeing with other studies which reported 100% in samples with T-ALL and 28.3 % samples with B-ALL are positive for aberrant markers(26). This difference may be due to different study population and age factor since they carry their studies only on childhood patients.

In agreement with a previous study from Oman(27), the current study includes more males than females. This results in agreement with the facts that was previously mentioned in most papers(28-30).

The age destitution is an important role in the diagnosis and prognosis of acute lymphoid leukemia. In the current study, we stratified the patients into four groups and the most common group was ages less than 20 represent 79%, this confirms the previous facts that Acute lymphoblastic leukemia is more common in children than adults(31, 32). Alternatively, no clinical significant correlation between age groups and expression of aberrant CD markers confirm the results from different earlier studies (20, 24, 33)

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 [38]. Alternatively, different immunophenotyping profiles were detected in adult B.ALL than in childhood profile. While in little similar profiles for both adult and childhood profile in T.ALL were the same as what was reported in other studies (21, 23, 34)

The frequency of co-expression of aberrant CD markers was obtained, which represent 68.2% of the study groups. but no significant difference between male and females or between T.cell and B.cell leukemia. In co-expression of aberrant CD markers, these results about co-

expression disagreed with another study that reports co-expression of CD82 with other markers were associated with B.ALL only(20).

Conclusion

Aberrant CD45 and CD82 in ALL may be considered an important prognostic markers due to their significant expression in both childhood and adult acute lymphoid leukemia, as well as the current study was found significant association between the presence of early pre-stage in childhood B.cell leukemia rather than Adults.

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