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The Expression Pattern of CD45 among Sudanese ALL Patients Using Flowcytometry

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Abstract:

Back ground: *Acute lymphoblastic leukemia is malignancy of immature lymphoid cells, comprises approximately 80% of pediatric acute leukemia and 20% of adult cases. CD45 has potential role in the function of earlier, undifferentiated hematopoietic progenitor cells was*

not identified. CD45 gating strategy plays very important role in diagnosis of acute leukemia and lymphoproliferative disorders as well as many other non haematological diseases. Flowcytometer plays very important role in the diagnosis of acute leukemia. There is need to study the role of CD45 expression pattern and its properties using the flowcytometer in acute lymphoblastic leukemia.

Method: *This is descriptive cross sectional study involving 50 patients newly diagnosed as acute lymphoblastic leukemia (ALL). The tubes were labeled for analysis. 20 µL of monoclonal antibody (CD45) was added into each tube. 100 µL of sample was added containing no more than 1 x 10^4 leukocytes / ml. Each tube was vortexed for 5 seconds. The tubes were incubated at room temperature in dark place for 10 minutes. 1 ml of RBCs lysis was added into each tube then incubated at room temperature in dark place for 10 minutes. All tubes were run by flowcytometer. The flowcytometric parameters (Percentage and mean fluorescence intensity) were recorded by SYSTEM II software.*

Result: *Out of the 50 cases diagnosed as ALL, the most frequent age group was (0.5-12) y. The male was (56.5%) while the female was (43.5%) of all cases. (60.9%) of the samples were from the bone marrow and (39.1%) from peripheral blood. (80.4%) of the samples were B cells and (19.6%) were T cell. CD45 percentage degree among B-ALL sub types were as follow: pro B-ALL, (18%) showed low percentage, (18%) moderate, and (62%) high, common B-ALL: (5%) showed negative CD45, (22%) low percentage, (22%) moderate and (560%) high. Pre B-ALL: (50%) showed moderate percentage and (50%) high. Mature B-ALL: All cases (100%) showed moderate percentage.*

Conclusion: *In our study, we found that CD45 showed a significant role in the diagnosis and differentiation of sub types of acute lymphoblastic leukemia cases in Sudanese patients. These importances were multiply with using of other flowcytometric parameters like percentage level and mean fluorescence intensity. Multi-parametric analysis of CD45 highlighted a lot of advantages assist in the early and accurate differentiation. These benefits may help in the minimization of monoclonal antibodies panel especially with countries of limited recourses like Sudan.*

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Key words: CD45protein; flowcytometry; leukemia.

Introduction:

Acute lymphoblastic leukemia is malignancy of immature lymphoid cells, comprises approximately 80% of pediatric acute leukemia and 20% of adult cases [1]. The current World Health Organization (WHO) classification for malignancies of hematopoietic and lymphoid tissues [2] recognizes two diagnostic entities: precursor B-cell ALL and precursor T-cell ALL, both of which encompass a spectrum of clinical, morphologic, immunophenotypic, cytogenetic, and molecular features. Although Burkitt's lymphoma/leukemia is considered a mature B-cell neoplasm in the WHO classification [2], in the earlier French-American-British (FAB) system [3], this tumor was included among the ALLs (designated L3 ALL) .In current clinical practice, flowcytometric immunophenotyping (FCI) plays an important role in the diagnosis of patients who have ALL. The morphologic characteristics of lymphoblasts may be indistinguishable from those of the myeloblasts in many cases of acute myeloid leukemia (AML), and treatment protocols for ALL and AML differ significantly. Because FCI enables rapid (within hours), typically unambiguous characterization of leukemic blasts with respect to lineage this modality has become a routine component of the diagnostic work-up of patients who have suspected acute leukemia. Moreover, complete immunophenotypic characterization of the lympho blasts at diagnosis may predict prognosis [4].

CD45 is a structurally heterogeneous, transmembrane proteintyrosine phosphatase found exclusively on nucleated cells of hematopoietic origin. The heterogeneity results from differential RNA splicing of 6 exons in the extracellular domain. The isoforms are expressed in a cell type–specific pattern. B cells predominantly express the isoform containing all exons.4,5

Thymocytes express the lowest molecular weight isoforms resulting from the removal of 3 and 4 exons. T cells have a varied isoform expression pattern that is dependent upon the differentiation state, function, and prior antigenic exposure [5]. All leukocytes, including hematopoietic stem and progenitor cell populations, are characterized by unique cell surface expression of CD45. It can serve as both a positive and negative regulator in a cell type and context-dependent manner. CD45 was shown to regulate different stages of lymphocyte maturation, especially their activation and proliferation. However, its potential role in the function of earlier, undifferentiated hematopoietic progenitor cells was not identified. CD45 gating strategy plays very important role in diagnosis of acute leukemia and Lymphoproliferative disorders as well as many other non hematological diseases [6]. Flowcytometry is a technology that simultaneously measures and then analyzes multiple physical characteristics of single particles, usually cells; as they flow in a fluid stream through a beam of light. The properties measured include a particle's relative size, relative granularity or internal complexity, and relative fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence [7]. There is need to study the role of CD45 gating strategy and its properties using flowcytomtery in acute lymphoblastic leukemia because this study not done in Sudan before.

Materials and methods:

This is a descriptive cross-sectional study conducted in Khartoum state, patients attended in the Flowcytometry laboratory in the period from April 2015 till Jun-2015.

This study involving 50 patients newly diagnosed as acute lymphoblastic leukemia (ALL). The tubes were labeled for

analysis. 20 µL of monoclonal antibody (CD45) was added into each tube. 100 µL of sample was added containing no more than 1 x 10^4 leukocytes / ml. Each tube was vortexed for 5 seconds. The tubes were incubated at room temperature in dark place for 10 minutes. 1 ml of RBCs lysis was added into each tube then incubated at room temperature in dark place for 10 minutes. All tubes were run by flowcytometer. The flowcytometric parameters (Percentage and mean fluorescence intensity) were recorded by (SYSTEM II) software.

Results:

CD45 out of the 50 cases diagnosed as Acute Lymphoblastic Leukemia (ALL) analyzed by flowcytometer at flowcytometry laboratory in Khartoum, Sudan during period from April to June 2015. The distribution of age groups was: (56.5%) for (0.5 – 12) y, (23.9%) for (13 – 45) y and (19.6%) for (46 – 70) y. The distribution of gender was: (56.5%) for males and (43.5%) for females. The distribution of sample type was: (60.9%) for bone marrow samples and (39.1%) for peripheral samples. The distribution of sample diagnosis was: (80.4%) were B-ALL and (19.6%) were T-ALL. The distribution of sub type diagnosis was: (34.8%) was pro B-ALL, (39.1%) common B-ALL, (4.3%) pre B-ALL, (2.2%) mature B-ALL, (2.2%) pro T-ALL, (2.2%) pre T-ALL, and (15.2%) cortical T-ALL (Figure 1).

CD45 percentage degree of B-ALL cases: negative (2.7%), low (19%), moderate (24%), high (54 %). CD45 percentage degree of T-ALL cases: negative and low (0%) moderate (11%), high (89%) (Figure 2).

CD45 mean fluorescence intensity level of B-ALL cases: dim (50%), and moderate (29%). CD45 mean fluorescence intensity level of T-ALL cases: dim (22%), moderate (77%) (Figure 3). CD45 percentage degree among B-ALL sub types were as follow: pro B-ALL, (18%) showed low percentage, (18%)

moderate, and (62%) high, common B-ALL: (5%) showed negative CD45, (22%) low percentage, (22%) moderate and (560%) high. Pre B-ALL: (50%) showed moderate percentage and (50%) high. Mature B-ALL: All cases (100%) showed moderate percentage (Figure 4). CD45 mean fluorescence intensity level of B-ALL sub types: pro B-ALL: dim (37%), moderate (50%). common B-ALL: dim (55%), moderate (11%). Pre B-ALL: dim (50%), moderate (50%). Mature B-ALL: dim (100%) (Figure 5). CD45 percentage degree among T-ALL sub types were as follow: Pro T-ALL: high (100%), Pre T-ALL: high (100%). Cortical T-ALL: moderate (15%) and high (85%) (Figure 6). CD45 mean fluorescence intensity level of T-ALL sub types: Pro T-ALL: high (100%), Pre T-ALL: high (100%). Cortical T-ALL: moderate (14%) and high (86%) (Figure 7).

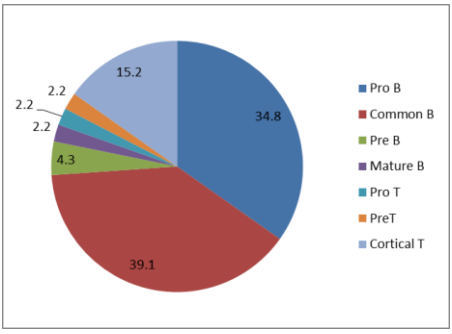

Figure 1: Showed the distribution of sub types of ALL cases in the study population

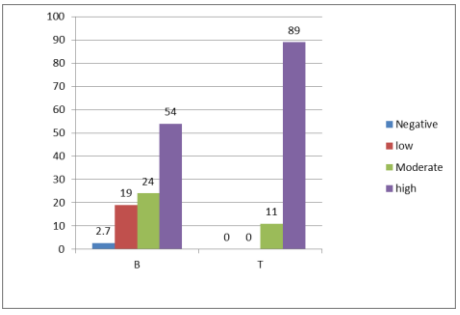

Figure 2: Showed the expression of CD45 percentage level among sub types of ALL cases in the study population

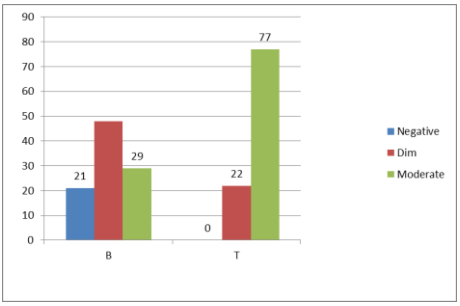

Figure 3: Showed the expression of CD45 mean fluorescence intensity among sub types of ALL cases in the study population

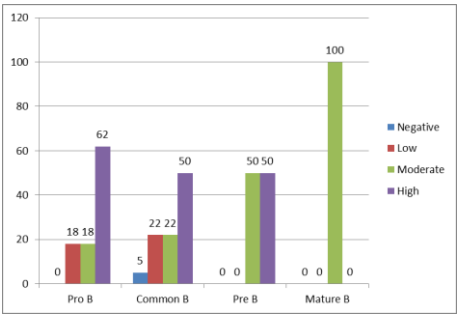

Figure 4: Showed the expression of CD45 percentage level among sub types of B-ALL cases in the study population

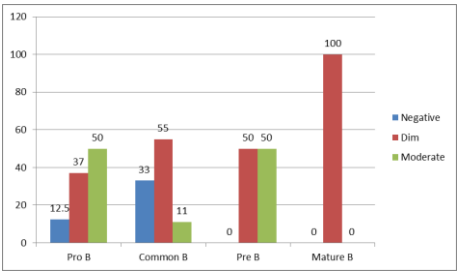

Figure 5: Showed the expression of CD45 mean fluorescence intensity among sub types of B-ALL cases in the study population

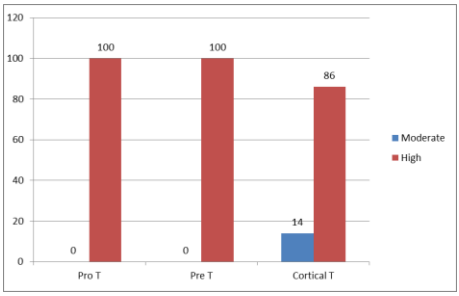

Figure 6: Showed the expression of CD45 percentage level among sub types of T-ALL cases in the study population

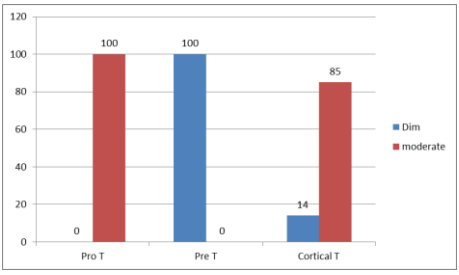

Figure 7: Showed the expression of CD45 percentage level among sub types of T-ALL cases in the study population

Discussion

Our study showed that age, gender and sample types were insignificant differences of positive CD45 expression and negative among B and T acute lymphoblastic leukemia cases. This finding was agreed with M. Y. Shafini *et al* study[8]. T-ALL is more frequent than B- ALL in the positive expression of CD45, this finding is agree with F. G. Bethm *et al* who showed that expression of the CD45 antigen was higher in T- than in B-lineage cases [9], however our result was not concordant with M. Y. Shafini *et al* [8] in CD45 expression of the sub diagnosis results of both B and T lineage. From all cases, the most cases of T- ALL expressed high percentage of CD45 and there are no result with negative and low CD45 percentage, therefore we can use the negative and low percentage of CD45 to exclude the

T-ALL cases and their relation was significant different (P.value = 0.05). We observed that most cases of B-ALL showed dim mean intensity of CD45 while most cases of T-ALL showed moderate mean intensity of CD45 (P.value = 0.008). CD45 percentage degree of sub B-ALL types showed that negative CD45 results only appear with common B-ALL cases. All mature B-ALL cases have moderate percentage of CD45. We observed that there were no negative or moderate mean intensity with mature B-ALL .Also there were no negative CD45 mean intensity with pre B-ALL, therefore the negative CD45 mean intensity should exclude pre and mature B-ALL and cases (significant p.value = 0.017) . Sub diagnosis of T-ALL (pro, pre and cortical T-ALL) showed high level of CD45 expression (insignificant P.value = 0.897). Finally, we found that in CD45 mean intensity group with sub T-ALL types, when the result showed dim mean intensity, we can use this finding to exclude pro T-ALL cases (insignificant P.value = 0.167). we found that in CD45 pw group sub diagnosis of T-ALL the most are heterogeneous, that mean when the result are homogeneous we can exclude pro T and pre T (insignificant P value = 0.630).

Conclusion:

In our study, we found that CD45 showed a significant role in the diagnosis and differentiation of sub types of acute lymphoblastic leukemia cases in Sudanese patients. These importances were multiply with using of other flowcytometric parameters like percentage level and mean fluorescence intensity. Multi-parametric analysis of CD45 highlighted a lot of advantages assist in the early and accurate differentiation. These benefits may help in the minimization of monoclonal antibodies panel especially with countries of limited recourses like Sudan.

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