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(CD34) PROPERTIES IN SUDANESE PATIENTS WITH ACUTE LEUKEMIA BY FLOWCYTOMETRY

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Abstract

Background: CD34 a 110- to 115-Kda transmembrane sialoglyco pro- V twin, is expressed by early hematopoietic (myeloid and lymphoid) progenitor cells, endothelial cells, murine embryonic fibroblasts, and bone marrow (BM) stromal cells and their precursors. CD34 expression has great importance in diagnosis, classification and management of acute leukemia.
Aim of the study: To evaluate the expression of CD34 (percentage and meanfluorescence intensity) in Sudanese patient with acuteleukemia.
Materials and Methods: This is descriptive cross-sectional study, conducted in Flowcytometry centre in Khartoum state, 50 new cases of acute leukemia analyzed by flowcytometry for CD34expression. The flowcytometery lysing procedure for bone marrow aspiration and peripheral blood monoclonal antibody combination was done as the follows: All tubes were labeled then pipette into each tube 20 µL of CD34 monoclonal antibody and added 100 µL of sample containing no more than 1 x 10^4 leukocytes / ml. The tubes were vortex for 5 seconds, then incubated at room temperature (18-25 °C) for 10 minutes, then added 1 ml of the "fix-and-lyse" mixture to the tube and vortex immediately for two seconds, after incubated at room temperature for at least 10 minutes, all tubes were run on flowcytometer. Statistical analysis was performed using statistical package for social science (SPSS) software (version 16 for windows 7). Evaluations of patients' data were performed using the *Chi-square* and *One way Anova*. Results with p.value < 0.05 were considered statistically significant.
Result and discussion: In this study we found that most case of AML were positive for CD34 while some cases of ALL were positive. All cases of biphenotypic and undifferentiated were positive so we found that (P.value = 0.012) of CD34 remark was significant between the groups of acute leukemia, also we found (69%) of B cases was positive and all cases of T cell was negative with significant (P.value = 0.013), so we can use the positivity of CD34 in exclusion of Tcases. This result was agree with Ching-Hon Pui [(9)] which found (70%) of B case were positive, also CD34 was reported by Mansoura[(10)] study to have the highest positivity in AML-M0 followed by AML-M1/M2 subtypes, this result were agreement with our study (CD34 positive in M0, M1, M2) therefore, we can use the negativity of CD34 in exclusion of these three type.
Conclusion: CD34was positive in most cases of leukemia and have important role in differentiation between acute leukemia.

Keywords: Transmembrane, Sialoglyco, Meanfluorescence, Undifferentiated, Expression.

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INTRODUCTION

Acute leukemia is a cancer of blood cells and bone marrow that characteristically come on suddenly and lead to rapid increase in the number of immature blood cells (Jemal *et al*., 2002). Classified into Acute myeloid leukemia (AML), also known as acute myelogenous leukemia oracutenonlymphocytic leukemia, is a cancer of the myeloid line of blood cell scharacterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells (Hoffman and Ronald, 2005). Acute lymphoblastic leukemia (ALL) or acute lymphoid leukemia is an acute form of leukemia, or cancer of the white blood cells, characterized by the overproduction of cancerous, immature white blood

cells—known as lymphoblasts in persons with all lymphoblasts are overproduced in the bone marrow (Seiter, 2014). CD34 a 110- to 115-Kda transmembrane sialoglyco pro- V twin, is expressed by early hematopoietic (myeloid and lymphoid) progenitor cells, endothelial cells, murine embryonic fibroblasts, and bone marrow (BM) stromal cells and their precursors found on 1% to 5% of normal BM cells the CD34+ population contains virtually the entire hematopoietic colony-forming cell. Levels of CD34 expression are highest on the most immature hematopoietic progenitors and decrease progressively with cell maturation 13 Parallelling the hierarchy of CD34 expression on normal hematopoietic cells, a large percentage of acute leukemia are CD34+, whereas the chronic leukemia, which involve more mature cell types, are uniformly CD34 negative (9).

Expression of CD34 in diagnosis of acute leukemia CD34 antigen is expressed on all colony forming cell and lymphocyte progenitors of either T or B lineage. The degree of positivity for CD34 decrease with cell differentiation and dis appears in more mature bone marrow precursors From the beginning of its production, monoclonal antibodies (McAbs) directed against CD34 antigen, have been extensively explored in different hematological malignancies. Accordingly, evidence progressively accumulated that reactivity for CD34 was almost exclusive to acute leukemia blast cells while absent in chronic mature disorders. Since then, CD34 expression has been included in most panels of McAbs used to characterize the immunophenotype of immature leukemic cells as a useful marker in discriminating between mature and immature hematopoietic leukemic cells (8).

The importance of CD34 cell quantification has been clearly demonstrated in autologous or allogeneic peripheral blood hemato- poietic stem cell transplantations (11/12). Studies of CD34+ cell dose in the setting of allogeneic BMT have been mainly restricted to T-cell–depleted related or unmanipulated unrelated BMT. Mavrou- dis *et al* (13) were the first to suggest in only 28 patients who received a T-cell–depleted BM graft that CD34+ cell dose predicted survival, post transplantation morbidity, and rate of hematologic recovery. More recently, the same group showed that a CD34+ cell dose greater than 3 * 106/kg correlated with a better survival rate, lower TRM, and less relapses. (14). In previous study CD34 was expressed on 10% of blast cells and the frequencies of CD34 expression was highest 83% in the early pre _B cases and lowest 46% in the T cells Cases.(9). Flowcytometry is a power full technique for the analysis of multiple parameters of individual cell within heterogeneous population.In this study we analyzed the expression of CD34 in Sudanese patient

MATERIALS ANDMETHODS

This descriptive cross-sectional study was conducted in Khartoum state at flowcytometry laboratory, Khartoum,Sudan in the period from February to April 2015. This study included 50 patients with acute leukemia. The samples were fresh representative sample (more than 20% blast cells) from venous blood or bone marrow aspiration .Two ml of blood samples were received in the hematology unit after bone marrow collection in EDITA vacutte (5ml) and were mixed gently. Samples analysis was performed at the Flowcytometry Laboratory using COULTER EPICS XL-MCLTM Flowcytometer (Miami - USA). The flowcytometrylysing procedure for bone marrow aspiration monoclonal antibody combination was done as the follows: All tubes were labeled then pipette into each tube 20 µL of CD34 monoclonal antibody (Salamanca, Spain) was added and 100 µL of sample containing no more than 1 x 10 leukocytes / ml was added. The tubes were vortex for 5 seconds, then incubated at room temperature (18-25 °C) for 10 minutes, then added 1 ml of the "fix-and-lyse" mixture to the tube and vortex immediately for two seconds, after incubated at room temperature for at least 10 minutes, the tubes were centrifuged at 150 x g for 5 minutes and the supernatant was discarded by aspiration, then 1 ml of PBS was add, then all tubes were run on flowcytometer. Statistical analysis was performed using statistical package for social science (SPSS) software (version 16 for windows 7). Evaluations of patients' data were performed using the *Chi-square* and *One way Anova*. Results with p.value < 0.05 were considered statistically significant.

Quality control

Depending up on pilot study in the quality control results (that saved in the Q.C system II software file) of EPICS XL Flowcytometer, which adjusted the cut off points between negative and positive scale for every marker, Positivity was considered when =30% of the population expressed the marker. The percentages, mean fluorescence intensity were also recorded for most of the markers

RESULTS

In this study we analyzed 50 patients newly diagnosed as acute leukemia for CD34 expression. The patient's ages were as follows: (28.6%) of patients were in the group of (1-12) year, (38.8%) of patients were in the group of (13-45) years, (32.7%) of patients were in the group of (46-77) years (Figure 1).

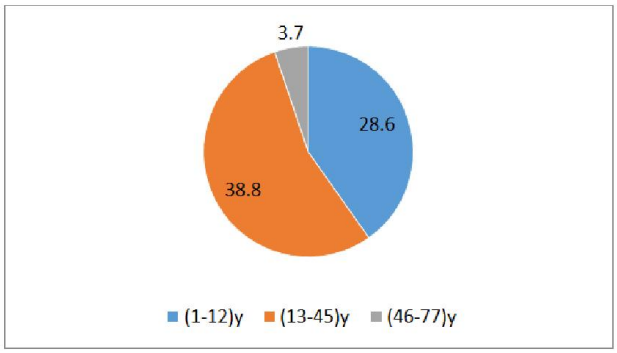

Figure 1. Showed the age groups of the study population

Concerning gender of the study population: 27 (55.1%) were male and 22(44.9%) were female. The type of the samples used: 36 (73.5%) were bone marrow aspiration sample and 13 (26.5%) were peripheral blood sample (Figure 2). Ourstudy showed that out of 17 cases of ALL, (53%) of patients were CD34 positive and(47%)were negative,27 (74%) cases of AML were positive and (26%)were negative,1 case of biphenotypic acute leukemia was positive, and 4 (100%) of undifferentiated acute leukemia cases were positive (Figure 3).

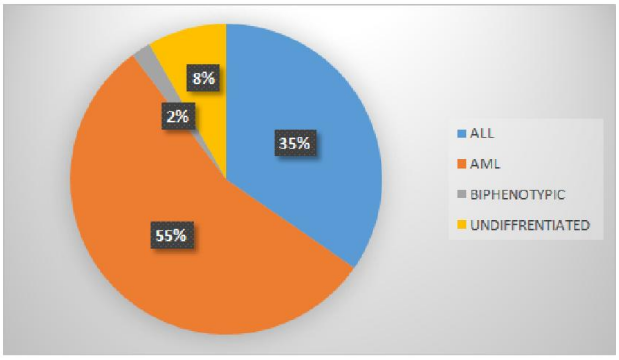

Figure 2. Showed the study population among the cases diagnosis

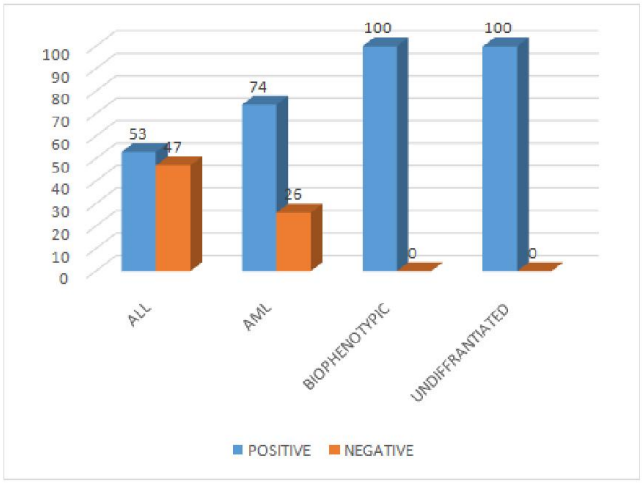

Figure 3. Showed the expression of CD34 among the study population

The CD34 mean fluorescence intensity (MFI) for ALL was : (60%) were dim MFI , (29%) were moderate and (11%) werebright. For AML: (37%) weredim MFI, (48%) were moderate and (15%) were bight. For biphenotypic acute leukemia (100%) of cases were moderate, and for undifferentiated cases (25%) were dim, (25%) were moderate and (50%) were bright (Figure 4).

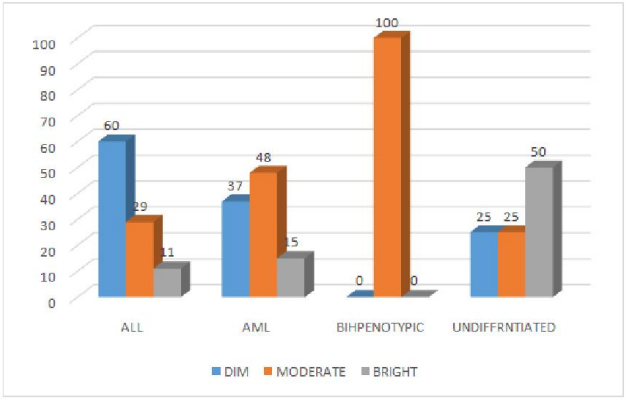

Figure 4. Showed the expression of CD34 MFI among the study population

Regarding the level of CD34 percentage, (47%) of ALL cases were negative, (23%) were low percentage, (67%) were moderate percentage, (23%) were high percentage.

For AML, (30%) of cases were negative, (15%) showed low percentage, (25%) were moderate, and (30%) were high. For biphenotypic, all case (100%) showed moderate percentage level and for undifferentiated AL, (75%) were high and (25%) were moderate. According to the sample diagnosis: (100%) of M0 and M1 cases were positive, (86%) of M2, (50%) of M4 and M5, (100%) of M6, and (67%) of M7, while all M3 cases (100%) were negative. (Figure5).

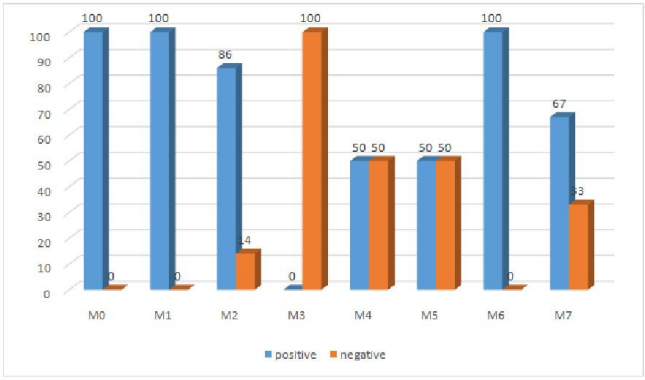

Figure 5. Showed the expression of CD34% among the AML cases

The result of the CD34 percentage level among the cases diagnosis were as follow: For M0 cases (42%) were high, (29%) were moderate, (29%) were low percentage, all case of M1 (100%) were high, For M2 (14%) were low percentage, (14%) were moderate and (43%) were high, For M4 (25%) were low percentage, (25%) were high. For M5 (50%) were high expression. All of M6 cases (100%) were moderate percentage. For M7 (67%) were moderate percentage. The result of the CD34 Mean Fluorescence Intensity (MFI) expression among the cases diagnosis were as follow: All M0 and M1 cases (100%) showed moderate MFI. (57%) of M2 were moderate MFI and (29%) were dim and (14%) were bright. (50%) of M5 cases were dim MFI and (50%) were bright. All M6 cases (100%) were dim MFI. (67%) of M7 cases were dim MFI and (33%) were moderate (Figure6).

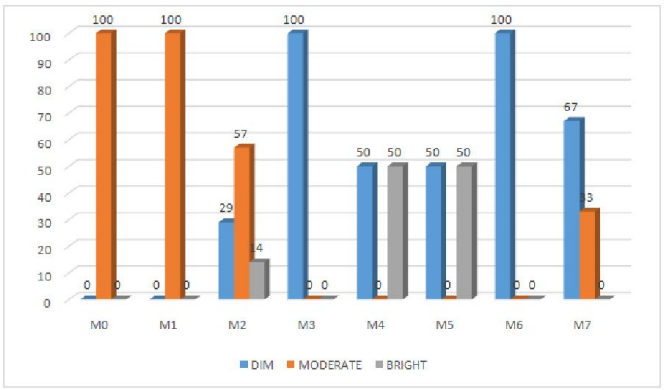

Figure 6. Showed the expression of CD34 MFI among the AML cases

Regarding to the T and B ALL cases, All T cell cases were negative for CD34 while (69%) of B cells were positive and (31%) were negative, (Figure 7). (31%) of B cases showed weak MFI, (7%) were moderate and (31%) were strong.

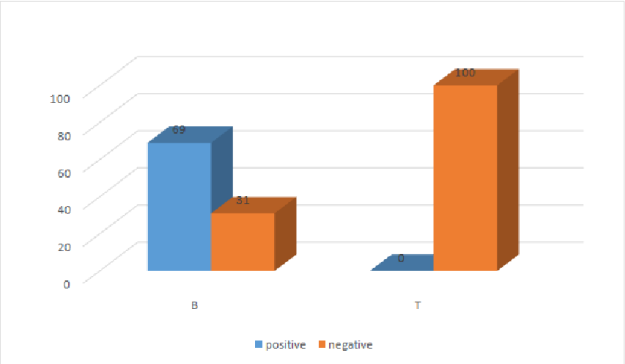

Figure 7. Showed the expression of CD34% among the ALL cases

DISCUSSION

In this study we found that most cases of AML were positive for CD34 while some cases of ALL were positive. All cases of biphenotypic and undifferentiated acute leukemia were positive, so we found that the remark of CD34 was significant in differentiation between the groups of acute leukemia (P.value = 0.012), also we found that (69%) of B-ALL cases were positive while all cases of T-ALL were negative with significant differentiation (P.value = 0.013), so we can use the positivity of CD34 in exclusion of T-cell cases. This result agreed with Ching-Hon Pui (Ching-Hon Pui, ?) which found 70% of B-ALL cases were positive, also CD34 was reported by Mansoura (Salem, 2011) study to have the highest positivity in AML-M0 followed by AML-M1/M2 subtypes , this result was agreed with our study so we can use the negativity of CD34 in exclusion of these three types of cell. One of the most important finding that CD34 was completely negative in all cases of M3, this feature is very remarkable to identification or exclusion of M3 especially with the highly aggressive situation of M3 requiring prompt diagnosis and specific early intervention. Also we can use the CD34 expression to differentiate between M2 and M3 due to high positivity with M2 cases especially when the morphology findings and cyto-chemical stain (Like Sudan Black B) were comparable.

Conclusion

CD34 have an important role in differentiation and classification of acute leukemia and subtype especially in AML (AML M0, AML M1, AML M3 and T-ALL).

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