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SIGNIFICANCE OF THE EXPRESSION OF CD123 AND THEIR CLINICAL IMPACT IN ACUTE MYELOID LEUKEMIA AMONG SUDANESE PATIENTS

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Abstract
Background: Acute myeloid leukemia (AML) is a heterogeneous clonal disorder characterized by immature myeloid cell proliferation and bone marrow failure. This study was prospective study conducted in Khartoum state during period from October 2018 until 2021
Objective: This study was aimed to determine the significance of the expression of CD123 in Sudanese Patients with Acute Myeloid Leukemia, and their clinical impact.
Method: The study population was selected as 100 AML patients as study group. 2.5 ml of venous blood were collected and poured into ethylene di-amine tetra acidic acid (EDTA) for examination of peripheral Blood smears and complete blood count, aspirate and trephine biopsy of bone marrow were collected for smearing and staining and detected CD markers by using flow cytometry
Results: The result showed that 57.6% of AML patients with positive CD123. The study was revealed insignificant difference in CD123 in correlated to age with p.value=0.509, and also insignificant difference in CD123 when correlated to gender male and female with p.value=0.705, and the result showed that CD123 expression was significant decreased in bone marrow sample compared to peripheral sample with p.value=0.019, also the finding showed that CD123 expression was strongest being in M3 subtype (56.8±36.8) with P-value (0.099).
Conclusion: This study was concluded that 57.6% of AML patients were positive for CD123 and also reflect thatCD123 expression was strongest being in M3 subtype.
Keyword: Acute Myeloid Leukemia, CD123 ,Sudan .

Introduction

Acute Myeloid Leukemia (AML) is a life-threatening haematopoietic disease (Jordan CT *et al*, 2000), it comprises a heterogeneous group of neoplastic disorders characterized by increased production of myeloid cells and their accumulation both in peripheral blood and bone marrow. AML results from transformation of hematopoietic stem or progenitor cells through the acquisition of genetic defect concerning chromosomal rearrangements and multiple gene mutations (Estey E, 2012).

Immunophenotyping is regularly used to distinguish acute lymphoblastic leukemia (ALL) from acute myeloblastic leukemia (AML) (Qadir M *et al*, 2006; Ratei R *et al*, 2007). This is done by using markers common to AML (CD13, CD33) or to ALL. Also there are correlation between immunophenotyping, diagnosis and prognosis (Chang H *et al*, 2004; Legrand O *et al*, 2000). Cluster of differentiation (CD) molecules are markers on the cell surface, recognized by specific sets of antibodies, used to identify the cell type, stage of differentiation and activity of a cell (Leong KG *et al*, 2008). CD 123 is a molecule found on cells which helps transmit the signal of interleukin-3. It is found on pluripotent progenitor cells, induces tyrosine phosphorylation within the cell, and promotes proliferation and differentiation within the

hematopoietic cell lines. (Edling CE and Hallberg B, 2007).

Materials and methods:

This was prospective study done in Khartoum State in period fromOctober 2018 to January 2021. One hundred AML patients with high blast were involved in our study. Patients had other form of myeloproliferative disorders were excluded. Two and half ml of Blood was collected in EDTA container to investigate peripheral Blood smears stained with Leishman's stain, bone marrow aspirate smears, CBC by using Sysmex kx2, and Immunophenotyping by flowcytometry

Immunophenotyping:
usingMiltenyiBiotecMACSQuant TM flowcytometry analyzer.

Procedure:

Cell surface staining

Were analyzed by Attune Cytometer Software (Life Technologies, Carlsbad, CA) version 2.1.0.

Phenotypic characterization of blood cells:

Immunophenotyping of the leukemic blast cells was performed on PB or BM samples using MiltenyiBiotecMACSQuant TM flowcytometry analyzer equipped with MACS Quantify software version 2.4.Monoclonal antibodies (DAKO-USA) labeled with Fluorescein isothicyanate (FITC) or phycoerythrin (PE) were used for immunophenotyping ,the antibodies were arranged in a primary panel that was applied to all cases consisting of CDs

Data was analyzed by statistical package for social science (SPSS), version 16. Qualitative date was presented as mean and SD.

Results:

One hundred patients with Acute Myeloid Leukemia (AML) were included in our study. The majority of the patients were adults (71.0%) and about (29%) patients were children. The most of patients were males (52.0%) and about (48.0%) of patients were females.

The distribution of cases in correlated to clinical presentation were 17% of patients with lymphadenopathy, (21.0%) of cases with hepatosplenomegaly, (8.0%) of cases with splenolmegaly, (90.0%) with constitutional symptoms which defined as fever, fatigue and any common symptoms, and about (34.0%) of cases with others clinical signs.

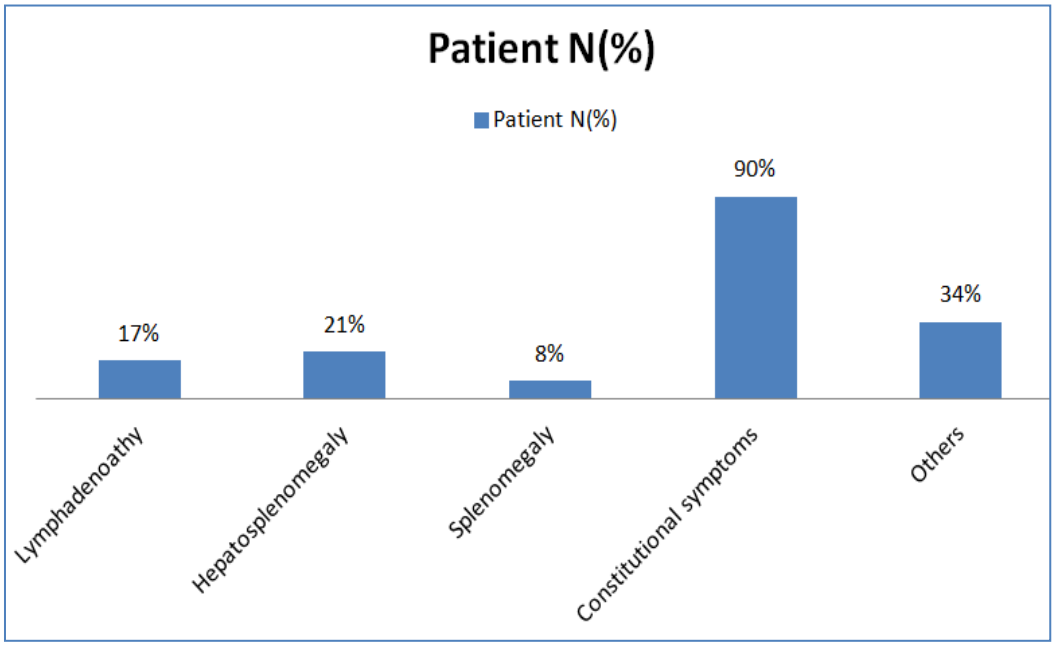

Figure 1: Clinical presentations of the AML group

As in table (1), the frequency of patients with positive CD123 were 57.6% with median expression was 61.0 ranged from 31-100

Table 1: Frequency and median of CD123 expressions among AML patients

Expression	**Patient N (%)**	Median(range)
CD 123	57(57.6)	61.0(31-100)

In our study, the mean±SD of CD123 was insignificant different in Children (45.3±34.8) when compared to adult (40.4±32.9) with p.value=0.509, and themean±SD of CD123 in correlated to gender was insignificant increased in female (43.2±32.3) compared to male (40.6±34.6) with p.value=0.705. Also the mean±SDof CD123 was significant increased in Peripheral blood (51.9±35.6) compared with bone marrow sample (35.8±30.7) with p.value= 0.019 (table 2).

Table 2: Association of CD123 expressions with baseline patient characteristics

Characteristics		CD 123 (mean±SD)
Age	Children	45.3±34.8
	Adults	40.4±32.9
	P-value	0.509
Gender	Male	40.6±34.6
	Female	43.2±32.3
	P-value	0.705
Sample type N (%)	Bone marrow	35.8±30.7
	Peripheral blood	51.9±35.6
	P-value	0.019*

The mean of WBCs count in positive CD123 AML patients was insignificant increased ($55.3x10^3/\mu L$) with p.value=0.195. The mean of RBCs count was insignificant decreased ($2.8\times10^{12}/L$) with p.value=0.753. The mean of platelets count in positive CD123 AML patients was insignificant decreased ($73.2\times 10^9/L$) with p.value=0.703. The mean of Hb in positive CD123 AML patients was insignificant decreased (8.1 g/dl) with p.value=0.357.

Table 3: Association of CD123 expressions with haematological parameters

		CD 123
TWBCs	Mean	55.3
	P-value	0.195
RBCs	Mean	2.8
	P-value	0.753
PLTs	Mean	73. 2
	P-value	0.703
Hb	Mean	8.1
	P-value	0.357

According to the clinical remarks, CD 123 was found to be low in lymphadenoathy of AML patients and this association was statistically significant (P-value=0.037).

Table 4: Association of CD123 expressions with clinical presentation

Clinical presentation	CD 123	
	mean±SD	P-value
Lymphadenoathy	26.5±28.6	0.037*
hepatosplenomegaly	30.2±30.1	0.073
Splenomegaly	37.7±36.5	0.721
constitutional symptoms	43.3±33.8	0.184
Others	34.4±34.4	0.111

As shown in table (5), CD123 expression was strongest being in AML-M3 (56.8±36.8) followed by AML-M1 (49.5±38.1), then M0 (48.7±33.4), M5 (48.5±35.5) and weak expression in M2 (30.0±27.9), M6(27.2±33.1) and M4 (20.3±20.8). CD123 was found to be statistically insignificant with (P-value=0.099).

Table 5: Expression of CD45, CD117 and CD123 according to FAB subtype

Sub-diagnosis	CD 123 (mean±SD)
M0	48.7±33.4
M1	49.5±38.1
M2	30.0±27.9
M3	56.8±36.8
M4	20.3±20.8
M5	48.5±35.5
M6	27.2±33.1
P-value	0.099

Discussion:

Immunophenotyping has a recognised role in the diagnosis, evaluation, classification and diagnostic of acute leukaemia. based on highly standardized immunophenotyping techniques allowed us to prospectively evaluate CD123 expression in 1000 acute leukemia patients.

In this study reported about 57.6% of AML patients with positive CD123 with median expression was 61.0 ranged from 31-100.

This finding was consistent with study done by Bras AE *et al*, CD123 expression was high in AML compared to Normal RBC (Bras AE *et al*., 2019). Also analogous with study reported by Fu Li *et al,* in South San Francis study for AML. CD123 expression was highly expression among AML patients (Fu Li *et al*., 2018).That also consistent to result of Al-Fatlawi and Musa showed 40% of patients with positive CD123 (Al-Fatlawi and Musa., 2016). In another study conducted by Salah Aref *et al*, the CD123 expression was 35/80 (43.75%) (SalahAref*et al*., 2020). In another study conducted by Ehninger*et al*, CD123 was positive in 77.9% (232/298) of AML patents (Ehninger*et al., 2014*). Also in similar study conducted by Leila Mekkaoui*et al*, reported that (65.2%) of patients had CD123 over-expressed .The mean value (+/- SD) of the %CD123 in the CD123 positive cases of AML was 35.1 +/- 30.5%.(Leila Mekkaoui*et al*., 2015)

In our study revealed the mean±SD of CD123 was insignificant different between children (45.3±34.8) and adult (40.4±32.9) with p.value=0.509.

This finding was agreed with results of Bras AE *et al* reported there were no significant difference in CD123 was present between pediatric and adult AML (Bras AE *et al*., 2019).

The present study showed that the mean result of WBCs count in positive CD123 AML patients was insignificant increased

($55.3x10^3/\mu L$) with p.value=0.195. The mean of RBCs count in positive CD123 AML patients was insignificant decreased (2.8×10^{12}/L) with p.value=0.753. The mean of platelets count in positive CD123 AML patients was insignificant decreased (73.2×10^9/L) with p.value=0.703. The mean of Hb in positive CD123 AML patients was insignificant decreased (8.1 g/dl) with p.value=0.357.

This finding was in agreement with study done by Salah Aref *et al*. The mean±SDof Hb in AML patients with positive CD123 was significant decreased (9.97±2.66 g/dl) with p.value=0.014, the median of WBCs count in positive CD123 AML patients was significant increased ($20.8\times 10^3/\mu L$) ranged (1.11–315) with $P<0.05$, and the median of platelet count in positive CD123 AML patients was significant decreased (25.6×10^3 /uL) ranged from 4.55–249 with p.value=0.043. (SalahAref et al., 2020). In Iraq study was conducted by Al-Fatlawi and Musa. The mean±SDof Hb in AML patients with positive CD123 was insignificant decreased (8.19±2.66 g/dl) with p.value=0.6.The mean of WBCs count in possitive CD123 AML patients was significant increased (62.34×10^9/L) with $P=0.002$. The mean±SDof platelet count in possitive CD123 AML patients was insignificant decreased ($57.01\pm43.53 \times 10^9$ /L) with p.value=0.7. (Al-Fatlawi and Musa., 2016)

The finding also showed that CD123 expression was strongest being in AML-M3 (56.8±36.8) followed by AML-M1 (49.5±38.1), M0 (48.7±33.4), M5 (48.5±35.5) and weak expression in M2 (30.0±27.9), M6(27.2±33.1) and M4 (20.3±20.8) respectively . CD123 was found to be statistically insignificant with (P-value=0.099). This finding was analogous to study conducted by Ehninger *et al*, reported the CD123 was highly expressed in M3(Ehninger*et al., 2014*). In recent study was reported by Bras AE *et al*, CD123 was significantly lower in patients with the (M6) or (M7) (Bras AE *et al*., 2019). This finding was disagree with study conducted on Iraqi AML patients by Al-Fatlawi and Musa, CD123 was expressed more in M5 (80%) subtype cases, while expression was poor in M3 (Al-Fatlawi and Musa., 2016).

Conclusion:

In current study we found that 57.6% of AML patients were positive for CD123 and also reflect thatCD123 expression was strongest being in M3 subtype.

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