Role of CD99 in Adult Patients with Acute Myeloid Leukemia

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ABSTRACT

Background: When it comes to acute myeloid leukemia (AML), the large proportion of patients usually have relapses. Acute myeloid leukemia, Lymphoblastic lymphoma/leukemia, as well as Ewing sarcoma all have high CD99 expression.

Objective: The aim of the current work was to assess CD99 expression frequency in patients with AML, and also to evaluate its association with different clinical and laboratory data.

Patients and Methods: This comprehensive study included a total of 40 AML patients and 20 matched healthy subjects, attending at Departments of Clinical Pathology and Medical Oncology, Faculty of Medicine, Zagazig University Hospitals. Clinical and laboratory data were correlated with CD99 expression frequency in patients with AML in order to examine its usefulness as a predictive and prognostic factor.

Results: Examination of CD99 expression in AML patients revealed that 80% of patients are CD99 positive, while (20%) are negative.

Conclusion: CD99 expression in acute myeloid leukemia patients is of good prognostic value.

Keywords: AML, CD99, Oncology.

INTRODUCTION

Immature myeloid blasts and inefficient blood cell generation are hallmarks of acute myeloid leukemia (AML), which is a clonal hematopoietic tumor [(1)]. The therapy and prognosis of AMLs differs depending on the subtype. If left untreated, AML can advance swiftly and lead to death within a matter of weeks or months [(2)].

There is a limited chance of long-term survival in most patients of AML, despite high rates of remission after induction and consolidation chemotherapy [(3)].

Ewing sarcoma and lymphoblastic lymphoma/leukemia have both been found to have high CD99 expression [(4)].

As well as myeloid malignancies [(5)], tumors of the thymus and other cancers. There is evidence that CD99 is acting as an oncogene in each of these cancers [(5)].

Anti-CD99 monoclonal antibodies could be used to distinguish leukemic stem cells from functionally normal hematopoietic stem cells in AML, highlighting the therapeutic potential of CD99 monoclonal antibodies [(6)].

Immunophenotypic AML leukemic stem cells (LSCs) and MDS hematopoietic stem cells (HSCs) often express more CD99 compared to hematopoietic stem and progenitor cell counterparts (HSPCs) in the normal state [(7)].

For the treatment of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), CD99 has been identified as a potential therapeutic target [(8)].

The aim of the current work was to assess CD99 expression frequency in patients with AML, and also to evaluate its association with different clinical and laboratory data.

SUBJECTS AND METHODS

This comprehensive study included a total of 40 AML patients and 20 matched healthy subjects, attending at Departments of Clinical Pathology and Medical Oncology, Faculty of Medicine, Zagazig University Hospitals. This study was conducted between December 2018 to October 2020.

***Ethical Consideration*:**

This study was ethically approved by Zagazig Medical Research Ethics Committee. all participants signed an informed consent form. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion criteria: Diagnosis as de novo AML before recieving induction chemotherapy, not having any other malignancy, and from 18 to 60 years, of both sexes.

Exclusion criteria: Age < 16 years, previously treated AML patients, promyelocytic leukemia (M3), and other malignancy.

All patients were subjected to the following:

a) History taking with special attention paid to the occurrence of leukemia-related symptoms in the medical history (easy fatigability, fever, bone aches, and bleeding tendency).

b) Thorough clinical examination: focusing on leukemia involvement, as the existence of lymphadenopathy, size of lymph nodes, pallor, liver and spleen sizes, and purpuric eruptions.

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c) Laboratory investigations:

A-Routine laboratory Investigations including: (a) As part of a complete blood count (CBC), Leishman-stained peripheral blood (PB) smears were examined for differential leukocyte count and blast cell percentage using sysmex XN. (b) Assessment of kidney function tests, LDH as well as Liver function tests using automated analyzer "Cobas 8000 platform-702c module"

B- Specific laboratory investigations including: (a) Leishman-stained smears were used to determine the percentage of BM blast cells in the blood. Smears of bone marrow were examined for myeloperoxidase using a cytochemical method. (b) Immunophenotyping of blast cells in BM aspirates samples using (FACS Calibur Flow Cytometry, BD, USA).

CD 99 marker analysis was done as the following steps:

There were sets of tubes for each sample that had been labelled for all of the MoAbs that were to be utilized. Each tube contained 50 µl l of diluted samples. Tubes were injected with a total of 5 µl of each MoAb. For fifteen minutes, the tubes were vortexed and incubated at room temperature in the dark. Each tube received 1 mL of 1x10 lysing solution. In the dark and at room temperature, the tubes were vortexed and incubated for 5 to 10 minutes. To get rid of the supernatant, the tubes were centrifuged at 1500 rpm for five minutes. Each tube received 2 ml of PBS as a wash buffer and was carefully mixed. Twice wash steps were done. The supernatant was removed after 5 minutes of centrifugation at 1500 rpm. 500 l of PBS was used to prepare the cells for FCM data acquisition. Analysis of CD99 expression in a CD34+ cell population more than 11.25 percent. Results of cytogenetic analysis based on patient data.

Statistical Analysis

We used SPSS version 24 to tabulate and analyze data. The independent t-test (t) and the Mann-Whitney (MW) tests were employed to compare parametric and non-parametric data, respectively, in the analysis of the differences between the groups. When there was a difference between two groups of non-parametric data, the Kruskal-Wallis test (KW) was employed to determine. Proportions were compared using the Chi-square test (x2). P value 0.05 was considered statistically significant (S). Kaplan Meier curve analysis was used to compare the effect of a factor on different patients' population It was judged highly significant (HS) when the P value was 0.001 and non-significant (NS) when the P value was >0.05.

RESULTS

The current study included 40 adult patients with newly diagnosed AML. They are 21 males and 19 females and mean age of cases was 42.08±14.23years old. As regards the clinical symptoms of the AML patients, fever was found in 17 patients (42.5%), followed by purpura (37.5%) and organomegaly (17.5%). Bleeding was found in 16 patients (40%), while lymphadenopathy was found in 10 patients (25%). **(Tables 1, 2)**

As regards the hematological data of AML patients. A triad of high WBCs count, anemia and thrombocytopenia were observed. **(Table 3)**

As regards the CD99 expression in AML patients. 80% were positive while 20% were negative **(Table 4).**

As for the FAB classification of the AML patients. M2 was found to be the commonest among them (42.5%), followed by M4 (27.5%) then M5 (22.5%) while M1 (7.5%). **(figure 1)**

Regarding the cytogenetic risk categories of AML patients. cytogenetic abnormalities were detected in 27.5% of patients, while other patients were CN-AML **(Table 5).**

As regards the relation between CD99 and response to induction therapy at day 28 in the AML patients. There was a significant relation between the CD99 positivity and the occurrence of remission, and also there was a significant relation between CD99 negativity and occurrence of relapse and shorter overall survival **(Table 6).**

Table (1): Demographic characteristics of the studied Acute Myeloid Leukemia group

Item	AML group (N=40)	
Age (years) Mean ± SD Range	42.08 ± 14.23 17–60	
Sex	**no**	**%**
• Male	21	52.5
• Female	19	47.5

Table (2): Present history and clinical data of the studied AML patients

Item	AML group (N=40)	
	No.	**%**
Fever		
• No	23	57.5
• Yes	17	42.5
Purpura		
• No	25	62.5
• Yes	15	37.5
Bleeding		
• No	24	60.0
• Yes	16	40.0
Soft tissue infiltration		
• No	32	80.0
• Liver	4	10.0
• Lymph nodes	10	25.0
• Spleen	2	5.0
• spleen +portohepatic LNs	1	2.5

Table (3): Laboratory investigation among the studied AML patients

Item	AML patients (N=40)
BM blasts (%)	
Mean ± SD	61.57 ± 18.63
TLC ($\times 10^9$/L)	
Mean ± SD	49.02 ± 40.02
HB (g/dl)	
Mean ± SD	8.01 ± 1.47
Platelets ($\times 10^3$/dL)	
Mean ± SD	40.7 ± 31.6
ESR (mm/hr)	
Mean ± SD	81.3 ± 35.4
LDH (IU/L)	
Mean ± SD	636.4 ± 535.9

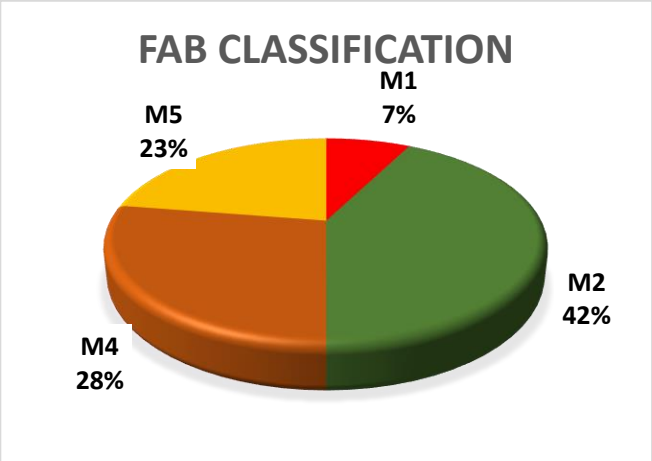

Figure (1): FAB classification of AML group.

Table (4): CD99 expression of the studied cases:

CD99	No.	%
Positive	32	80.0
Negative	8	20.0

Table (5): Cytogenic risk categories of AML patients in relation to CD99 expression.

Cytogenic risk categories	CD 99				p-value
	Negative (N=8)		Positive (N=32)		
	No.	%	No.	%	
Normal	6	75.0	23	71.9	0.859
Abnormal	2	25.0	9	28.1	(NS)

Table (6): The response to induction therapy and follow up of the AML patient group in relation to CD99.

Variable	CD 99				p-value
	Negative (n=8)		Positive (n=32)		
	No.	%	No.	%	
Complete remission (CR)	2	25.0	22	68.8	0.024*
No remission	6	75.0	10	31.3	

DISCUSSION

One third of all leukemias globally are caused by acute myeloid leukemia (AML) [8]. Most patients with acute myeloid leukemia die of their disease despite recent improvements in converting AML biology into clinically approved therapies. There hasn't been a major breakthrough in the treatment of AML for decades. CD99 is a cell surface molecule that could be targeted with antibodies for treatment. Antitumor efficacy of monoclonal antibodies targeting CD99 in preclinical cancer models has been reported. An anti-leukemic antibody (moAb) that targeted CD99 was found to be effective. Anti-CD99m Ab significantly decreased leukemic cell proliferation and migration and boosted apoptosis and differentiation in leukemic patients who received it [9].

To achieve our aim, immunophenotyping and cytogenetic analysis were done for all the patients together with detection of CD99 expression by flow cytometry.

This study was conducted on forty de novo AML patients with males to female's ratio 1:1. Their age ranged between 18 and 60 years with mean ± SD of 42.08± 14.23 years, while **Vaikari *et al.*** [9]. which reported that median age 57.8 years.

Among the newly diagnosed AML patients, fever was found in (42.5%) of patients, followed by purpura (37.5%) and organomegaly (20%). Gum bleeding was found in (40%) of patients, while lymphadenopathy was found in (25%) 0f patients. These findings are in agreement with **Asif and Hassan** [10] who stated that fever is the most common initial clinical presentation. Symptoms related to AML are caused by replacement of bone marrow and failure of normal hemopoiesis.

In this study, the median value of WBC count in the AML group at the time of diagnosis was 44.2 x10^3/dl**, Renneville *et al.*** [11] **and Pezzi *et al.*** [12] reported that the median WBC count of the patients at the time of diagnosis was 11 and 6.6 x10^3/dl; respectively.

The median haemoglobin level of the AML group was 8.25 gm/dl. **Hou *et al.*** [13] reported that the median hemoglobin level was 8 g/dl. Inflammatory cytokines may play a role in the development of anemia in cancer patients (e.g. tumor necrosis factor, interleukin-1, and interferons). Also, The thrombocytopenia of the AML patients is in concordance with **Hou *et al.*** [13] **and Elkerdany *et al.*** [14] who reported thrombocytopenia at time of diagnosis. This finding was expected as these are directly attributable to the leukemic infiltration of bone marrow with resultant cytopenia [14].

In this study, Examination of CD99 expression in AML patients revealed that (80%) of patients are CD99 positive, while (20%) are negative. This in accordance with **Tavakkoli *et al.*** [15] who reported that most of his AML cases were CD 99 positive, while **Kang and Dunphy** [16] reported that half of their AML cases were CD 99 positive. Differences between research may be due to differences in ethnic groups, the distribution of FAB subtypes in AML patients, and different detection technologies, such as flowcytometry or

immunohistochemistry, which are used in different studies.

According cytogenetic study, we found that there is no significant difference between CD99 expression and cytogenetic normal and abnormal in AML patients. These finding in accordance with **Zhang *et al.* [17],** who reported that CD99 expression does not correlate with any specific karyotyping, and also **Vaikari *et al.* [18]** reported that there is no significant difference in CD99 expression between cytogenetic normal AML and cytogenetic abnormal AML cases.

In the current study CR rate was statistically higher in CD99 positive, while relapse was statistically higher in negative CD99. This in accordance with **Zhang *et al.* [17]** who reported that CD99 was negative in relapsed cases. Larger series of relapsed AML with proper follow-up and controls are necessary to evaluate further the significance of CD99 expression in AML with relapse.

The mortality rate was statistically higher in negative CD99 cases. Thus, CD99 expression has favorable outcome in adult patients with AML. This finding in accordance with **Vaikari *et al.* [9]** who reported that high CD99 was associated with better outcome.

In this study, one-year OS for the positive CD99 expression and Negative CD99 expression groups was estimated. Overall survival varied significantly between the two groups which longer with CD99 positive expression. This findings in accordance with **Vaikari *et al.* [18]** who found that the median overall survival (OS) of the CD99 high group was significantly longer than that of the CD99 low patients and also **Zhang *et al.* [17].**

Further studies are recommended with more number of cases and longer duration follow up in addition to evaluation of P53 mutation to confirm our results.

CONCLUSION

There are many factors that influence the prognosis of AML including presence of certain markers on the blast cells e.g.CD34 percentage, karyotyping analysis and molecular studies e.g. FLT3-ITD gene mutation, Although the relation between CD99 expression and occurrence of complete remission in Acute myeloid leukemia patients proved to be significant in our study, CD99 expression proved to be important prognostic factor in AML patients.

The good prognosis of CD99 is the most effective factor in determination of the line of treatment due to high incidence of complete remission and long OS in CD99 positive cases while high incidence of relapse and short OS in CD99 negative cases.

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