Review Article

T-regulatory and T-helper type17 Cells Associated Cytokines (IL-35, IL-17) as Potential Diagnostic and Prognostic Biomarkers in Egyptian Acute Myeloid Leukemia Patients

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Abstract

Background: The etiology of acute myeloid leukemia (AML) is heterogeneous and has been associated with several risk factors; tumor escape mechanism is one of the major factors. In this study, we tried to investigate the role of T-reg cells and T- helper cells associated cytokines mainly IL-35, IL-17 in the pathogenesis of AML by determining IL-35 and IL-17 levels in different groups of AML patients.

Methods: For this purpose, 70 adult AML patients and 20 healthy controls were studied for IL-35 and IL-17 blood level. From total AML patients 35 were newly diagnosed (ND) patients and 35 patients in complete remission (CR).

Results: High levels of IL- 35 and IL-17 were detected in AML patients than the control group and their levels in the ND patients were significantly higher than those patients in CR. There was a significant positive correlation between IL-35 and IL-17 concentration in total AML patients. Also there was a significant positive correlation between IL-35 and IL-17 levels and each of WBCs and bone marrow blasts.

Conclusion: IL-35 and IL-17 levels were significantly higher in newly diagnosed AML patients suggesting their role as a diagnostic potential of the disease, the levels were decreased when patients achieved CR following chemotherapy suggesting that assessment of their levels may be beneficial to evaluate the efficacy of treatment.

Keywords: AML; IL-35; IL-17

Introduction

Acute myeloid leukemia (AML); a clonal malignant disease of hematopoietic tissue is the most common hematological malignancy in adults and its incidence increases with age. AML is characterized by distorted proliferation and development of myeloid cells and their precursors and accumulation of abnormal cells, mainly leukemic blasts in the blood and bone marrow. It has several subtypes; treatment and prognosis varies among subtypes [1].

The etiology of AML is heterogeneous and has been associated with several risk factors. These include age, antecedent hematologic disease, genetic disorders, exposure to viruses, radiation, chemicals, or other occupational hazards and previous chemotherapy [2].

T-regulatory (T-reg) which represent a novel subset of CD4+T cell, play a role in the pathogenesis of AML and sequential measurement of T-reg cells frequency may have clinical value in the evaluation of the therapeutic effects and clinical outcome [3]. T-reg cells prevent autoimmune diseases by suppressing self-reactive cells and host immune responses. T-reg cells are increased in cancer patients, it may also block anti-tumor immune responses and that tumor cells recruit these T-reg cells to inhibit anti-tumor immunity in the tumor micro-environment, thus limiting the efficiency of cancer immunotherapy [1].

Circulating T-reg cells in AML patients mediate vigorous suppression through contact-dependent and contact-independent mechanisms. Patients at diagnosis with lower T-reg cells frequency have a better response to induction chemotherapy. Future therapy aimed to T-reg cells depletion could be beneficial for patients with AML [4].

Particularly in the context of cancer, T-reg cell frequencies and function are important because increased numbers might favor tumor development or growth and influence the course of the disease.

Interleukin (IL)-35 a member of the IL-35 family, is a heterodimeric inhibitory cytokine consisting of Epstein-Barr virus-induced gene protein 3 (EBI3) and the p35 subunit of IL-12 [5]. IL-35 is secreted by T-reg cells play an active role in inflammatory, autoimmune diseases and solid tumors and used for therapeutic manipulation of T-reg cells activity in order to treat cancer and autoimmune diseases [1]. IL-35 is a novel regulatory cytokine that has potent inhibitory effects on T cell responses [6]. IL-35 could be an important factor in the tumor microenvironment that impacts tumor-specific T cell responses and tumor progression [6,7].

T helper 17 cells (TH-17) represent a subset of CD4+ T cells producing a pro-inflammatory cytokine IL-17 that discovered in 2007. They are considered developmentally distinct from Th1 and Th2 cells and excessive amounts of cells are thought to play a key role in autoimmune disease [8,9], other studies showed that they have an impact on solid tumors [10]. IL-17 acts as a stimulatory hematopoietic cytokine by expanding myeloid progenitors and initiating proliferation of mature neutrophils, it acts synergistically with both tumor necrosis factor and IL-1 [11]. Angiogenesis plays important roles in hematological malignancies; IL-17 belongs to a class of indirect angiogenic factors which stimulate angiogenesis *in vivo*.

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The peripheral blood levels of pro-inflammatory TH-17 cells and IL-17 plasma levels are also increased in untreated AML but in contrast to T-reg cells these normalize when complete remission is achieved [10].

This study aimed to investigate the diagnostic potential of T-regulatory associated cytokine (IL-35) T-helper associated cytokine (IL-17) and their role as prognostic markers in Egyptian AML patient.

Subjects and Methods

This study included 70 patients with AML admitted to oncology department - Assiut University Hospital and South Egypt cancer institute from January 2013 to December 2014: 35 newly diagnosed patients and 35 patients in complete morphological remission, after full course of standard induction chemotherapy. Age of the patients ranged from 15-76 years, 46 males and 24 females. In addition 20 apparently healthy individuals served as control group.

Venous blood was collected from patients and control group on plain tube. Samples were clotted for 10-20 min at room temperature before centrifugation for 20 min at the speed of 2000-3000 rpm. Serum samples were obtained and preserved at -20°C in aliquots for IL-35 and IL-17 level assays.

Serum IL-35 and IL-17 concentration was measured by enzyme linked immunosorbent assay (ELISA) using a kit from WKEA MED supplies Corp. according to the manufacture's instruction.

IL- 35 assay range: 3 ng/L- 70 ng/L

IL-17 assay range: 10 pg/ml - 400 pg/ml.

The study was approved by the ethical committee of faculty of medicine, Assiut University. Written consents were taken from the patients before enrollment in this study.

Statistical Analysis

Categorical variables were described by number and percent, but continuous variables described by Mean ± SD. Continuous variables were compared by t-test and Pearson Correlation coefficient was used to assess the association between variables. A two-tailed $p<0.05$ was considered statistically significant and p value <0.001 highly significant. All analysis was performed with the SPSS 20.0 software.

Results

This study included 70 patients and 20 healthy control group. Thirty five of them were newly diagnosed patients and 35 patients in complete remission, after full course of standard induction chemotherapy, Characteristic features of different groups involved in this study summarized in Table 1.

IL-35 level is higher in total patients "ND+CR" (mean ± SD, 74.4 ± 28.5 ng/ml) and in both patients group (mean ± SD, 90.2 ± 25.5 and 58.6 ± 21.9 ng/ml; ND and CR respectively) in comparison to the control group. Serum IL-35 concentrations were found to be higher in ND AML group of patients compared to patients in CR (p value <0.001).

The IL-17 level is also significantly higher in total patients (mean ± SD, 142.4 ± 58.7 pg/ml) and in both patients group (mean ± SD, 176 ± 60 and 108.8 ± 32.6 pg/ml; ND and CR respectively) in comparison to the control group. Serum IL-17 concentrations were found to be higher in AML ND patients compared to patients in CR (p value <0.001) (Table 2).

The correlations between cytokine concentrations (IL-35 and IL-17) and total patients (n=70) laboratory results were analyzed: The data demonstrated that, there were significant positive correlations between IL-35 and IL-17 levels and each of WBCs (r=0.284, 0.268; P=0.017, 0.025 respectively) and bone marrow blasts (r=0.515, 0.466; P ≤ 0.001 respectively). However, IL-35 levels showed significant negative correlations with hemoglobin level (r=-0.324; P=0.006) and no correlation were found between peripheral blood blast cells, platelet count and IL-35 concentration. On the other hand there were no correlation between peripheral blood blast cells, hemoglobin levels, platelet count and IL-17 concentration (Table 3).

There was significant positive correlation between IL-35 and IL-17 concentration in total AML patients (r=0.436; P ≤ 0.001(Table 4).

Table 1: Study population demographic characteristic.

		Patients Total cases N=70		Control N=20
		newly diagnosed N=35	complete remission N=35	
Age	range	17-76	15-63	22-57
	Mean ± SD	45.7 ± 17.1	38.3 ± 13.7	36 ± 10.8
Diagnosis AML	M0	0 (0.0%)	0 (0.0%)	
	M1	3 (8.6%)	0 (0.0%)	
	M2	8 (22.9%)	6 (17.1)	
	M3	6 (17.1%)	5 (14.3%)	
	M4	11 (31.4%)	7 (20.0%)	
	M5	2 (5.7%)	7 (20.0%)	
	M6	5 (14.3 %)	10 (28.6%)	
	M7	0 (0.0%)	0 (0.0%)	
Fever		11 (31.4%)	8 (22.9%)	
Hepatomegaly		16 (45.7%)	10 (28.6%)	
Splenomegaly		16 (45.7%)	13 (37.1%)	
Lymphadenopathy		7 (20.0%)	0 (0.0%)	
Laboratory data	WBCs × 10⁹/L	40.3 ± 67.3	4.2 ± 2.4	6.9 ± 1.6
	Hb g/dl	8.3 ± 2.3	10.7 ± 1.9	13.5 ± 1.2
	Platelet × 10¹²/L	100.4 ± 79.5	113.7 ± 52.6	259.6 ± 59.4
	Pbl. Blast %	29.8 ± 26.3	0	0
	BM Blast %	40.9 ± 25.9	2.1 ± 0.7	0

Table 2: Serum IL-35 and IL-17 level in study population.

		Patients Total cases N=70		Control N=20
		newly diagnosed N=35	complete remission N=35	
IL-35 (ng/ml)	range Mean ± SD	13-130	8-103	6-90
		90.2 ± 25.5**	58.6 ± 25.9**	30 ± 21.4
IL-17(pg/ml)	range Mean ± SD	39-290	30-194	29-96
		176 ± 60**	108.8 ± 32.6**	63.2 ± 20.3

Table 3: Correlation between IL-35 and IL-17 level and patients laboratory data.

		Total patients (70)		Newly diagnosed (35)		complete remisson (35)	
		IL-35	IL-17	IL-35	IL-17	IL-35	IL-17
WBCs	r=	0.284*	0.268*	0.15	0.09	-0.22	0.08
P. Blast	r=	0.479**	0.458**	0.34*	0.29*	-	-
HB	r=	-0.324**	-0.109	-0.03	0.26	-0.14	0.22
Platelet	r=	-0.037	-0.118	-0.14	-0.06	0.31	-0.11
B.M blast	r=	0.515**	0.466**	0.24	0.09	0.19	-0.09
*p<0.05 significant, ** p<0.001 highly significant.							

Table 4: The correlation between both IL-35 and IL-17.

IL-17 level	IL-35 level					
	Total patients N=70		Newly diagnosed N=35		complete remisson N=35	
	r	P-value	r	P-value	r	P-value
	0.436**	<0.001**	0.25	0.151	0.01	0.967
**p<0.001 highly significant.						

Discussion

AML is an aggressive disorder characterized by accumulation of malignant cells in bone marrow. Most adult patients with AML die from the disease. Even high-dose multi-agent chemotherapy and allogeneic stem cell transplantation often fail to prevent relapses. The heterogeneous phenotype of AML is based on cytogenetic mutations and molecular aberrations. Based on analyzing large cohorts of patients, most have a defined prognostic significance with direct impact on treatment strategy [12].

Cytokines are soluble molecules carrying specific information for target cells. Acting through a surface receptor, they provide target cells with specific information about conditions inside the organism, and cause a specific response [13]. The response may be stimulating and activating in the case of inflammation or in the case of tissue damage, causing proliferation or apoptosis [14].

Under abnormal conditions, this physiological role of cytokines is maladaptive. The influence of inflammation and altered cytokine signaling on oncogenesis, leading to tumor progression, has been documented [15], and is still a site of interest in several solid tumors [16,17] .

Blood cells and their marrow based progenitors are exquisitely responsive to their environment, and cytokines are an essential part of it.

Aberrant cytokine levels in AML and abnormal responsiveness to them is well-documented. The over-expression of cytokines in leukemia patients declines in complete remission [18], suggesting that these events are dependent on AML activity, possibly due to autonomous blast cytokine secretion [19].

The present study was carried out on 70 patients with AML and 20 controls which aimed to determine the role of T regulatory and T-helper type 17 cells associated cytokines (IL-35, IL-17) in the pathogenesis and prognosis of AML.

IL-35 is secreted by T-reg cells and contributes to their suppressive activity [5]. In turn, treatment of naive human T cells with IL-35 induces a regulatory population, known as Inducible T-reg-35 cells. Inducible T-reg-35 cells constitute a key mediator of infectious tolerance and contribute to T-reg cell mediated tumor progression [20,21].

In this study, we found that the levels of IL-35 in the ND AML patients were significantly higher than those AML patients in CR and control group, suggesting that the measurement of IL-35 concentrations may be valuable in the evaluation of therapeutic effect.

These results are accepted with WU et al. [1] who demonstrated for the first time that the serum concentrations of IL-35 in AML ND patients were significantly higher compared to control group. Increased frequency and enhanced suppressive activity of T-reg cells in peripheral blood as well as bone marrow in patients with AML, might play a crucial role in suppressing the host immune responses to tumor [22]. In addition, the circulating T-reg frequencies decreased in patients with AML who achieved CR and increased when relapsed. These suggest that sequential measurements of T-reg frequencies can monitor the disease status and predict the clinical outcome in patients with AML [22]. The suppressive activities of T-reg cells were significantly higher in patients with AML than normal controls [23]. Studies showed that T-reg frequencies are greater in patients with AML compared with control at diagnosis; however, they remain high throughout induction therapy. Moreover, some of these studies indicated that increased T-reg cells at diagnosis of AML is poor prognostic marker [24,25].

These results corresponding with the role of IL-35 in AML, which in the tumor microenvironment, Foxp3+ T-reg and other T-regs are common [26]; they provide a source of IL-35. In addition, tumor-infiltrating dendritic cells [27], could be an additional source of IL-35. Taken together, IL-35 could be an important factor in the tumor microenvironment that impacts tumor-specific T cell responses and tumor progression [6].

Human Th17 cells release the pro-inflammatory cytokine IL-17; one important function of IL-17 seems to be the coordination and regulation of local inflammation through up-regulation of other pro-inflammatory cytokines and chemokines [28].

In this study, although the level of IL-17 was in the reference range supplied by the kit, the level was found to be higher in ND AML patients when compared with CR patients and control group. These results

were in agreement with other studies, who found that, the frequency of Th17 cells and the concentrations of IL-17 were higher in untreated patients than those in complete remission and control groups [10]. Also assessment of Th17 cells and IL-17 expression showed higher expression in AML patients compared with the control group and a decrease in Th17 cells in patients who achieved CR after chemotherapy [29].

Our results were in agreement with Han et al. who found elevated frequencies of Th17 cells with increased IL-17 levels in peripheral blood and bone marrow of AML patients compared with those of healthy donors; suggesting that increased Th17 cell frequency might be an unfavorable prognostic marker for AML patients [30].

However, these results were not consistent with other studies that found the frequency of Th17 cells and the serum level of IL-17 were not elevated in AML patients compared with controls; they suggested that angiogenesis in AML is not mediated by T cells [31]. This contradiction in the results could be because of several factors including patients' selection, the small number of samples used from control group, which may not have been adequate to show a difference, and the differences in the treatment regimens used for the patients.

Pre-therapeutic and post-therapeutic assessment of Th17 cell frequency by estimation of serum level of IL-17 may be valuable as an evaluation of the therapeutic effect [10] and the number of Th17 cells may relate to tumor burden, which is an important prognostic factor in a variety of tumor types [31].

From this study, we concluded that determination of serum level of IL-35 and IL-17 can be used as indicator for therapeutic response and may play an important role in the occurrence and development of AML; also their increased levels might be an unfavorable prognostic marker for AML patients. Future studies should stress upon their modulation to provide a new immunotherapy for AML.

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