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Evaluation of interleukin-35 and interleukin-10 in adult acute myeloid leukemia patients before and after induction chemotherapy

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Abstract:

BACKGROUND: Acute myeloid leukemia (AML) is a clonal proliferation of hemopoietic cells. Interleukin-35 (IL-35) is a pro-inflammatory cytokine expressed in T regulatory (Treg) and B regulatory cells. IL-35 promotes the proliferation of AML blasts and lessens apoptosis. Hence, IL-35-derived from Tregs promotes the growth of adult AML blasts, suggesting that IL-35 has an important role in the pathogenesis of AML. IL-10 is anti-inflammatory cytokines with immune-stimulatory activities and is formed by CD4 and CD8 T-cells and activated B-lymphocytes.

OBJECTIVES: The aim of th study was to estimate the levels of IL-10 and IL-35 in the sera of patients with AML before and after chemotherapy induction and to correlate levels with blast cells percentage and other hematological parameters.

PATIENTS, MATERIALS AND METHODS: This study was conducted on thirty newly diagnosed (ND), *de novo* adult AML patients, 15 males and 15 females with a age range between 19 and 75 years for a period from September 20, 2017, to March 15, 2018. It included 18 healthy (9 males and 9 females) individuals who were taken as a control group. Diagnosis of AML was established according to the morphology, cytochemistry and flow cytometry study of both peripheral blood and bone marrow aspiration as well as biopsy reports. ILs-35 and 10 levels were measured at diagnosis and after induction chemotherapy when achieving complete remission based on Cheson *et al*. definition.

RESULTS: Serum IL-35 and IL-10 were significantly higher in ND AML patients than controls and were reduced after induction chemotherapy ($P < 0.001$). In patients with remission, IL-10 was significantly reduced compared with non-remission group, while the reduction in IL-35 level in remitted compared to nonremitted patients did not reach the level of significance ($P > 0.001$). No correlations were found between hemoglobin, platelet counts, white cell count, and blast percentage with IL-35 and 10 levels in *de novo* AML patients.

CONCLUSIONS: Both ILs-35 and 10 levels were elevated in *de novo* AML patients and lowered following induction chemotherapy. Both ILs have no correlation with hemoglobin level, platelet count, white blood cell count, and blast percentage.

Keywords:

Acute myeloid leukemia, interleukin-10, interleukin-35, induction chemotherapy

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Introduction

Acute myeloid leukemia (AML) is defined as a neoplastic clonal disorder that is characterized by low production and alterations of normal hematopoietic cells; such changes may constrain the differentiation of cells and trigger the proliferation or buildup of blasts. These blasts will substitute normal hematopoietic elements, eliciting the appearance of cytopenias. The buildup of immature cells

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starts in the bone marrow (BM), but in most cases, rapidly, it builds up in the peripheral blood (PB).[1]

AML has an immune suppressive microenvironment, represented by defects in the number and function of T-cells.

These defects are described to influence the effect of regulatory T regulatory cells (Tregs), which suppress the proliferation and function of T helper (Th) cells.[2]

Both accurate diagnosis and classification in AML are essential for both treatment plans and evaluation of prognosis. Preliminary assessment necessitates a careful history, physical examination, complete blood count with PB smear review, BM examination, flow cytometry (FC), cytogenetics, and selected molecular genetic analyses.[3]

Interleukin 35 (IL-35) was lately recognized heterodimeric inhibitory cytokine secreted by Treg cells and contributes to their immune suppressive activity by limitation of antitumor effect and contribution in dysfunction of T-cell.[4]

IL-10 is an anti-inflammatory cytokine secreted by immune cells including macrophages, natural killer (NK) cells, and T-lymphocytes. IL-10 still has an unclear role in the pathogenesis of cancer; some studies exhibited that IL-10 participates in both development and progression of cancer in humans as a tumor promoting (promote cancer potentially) with immune stimulating and immune suppressive (inhibit cancer potentially).[5]

The leukemias signify exceptional models regarding the effect of cancer on the host immune system. Both immune cells and cancer cells in BM and in PB are in close relation and originate from the same hematopoietic elements.[6]

The malignant clone in AML is promptly expanded and seemingly controlled by cellular immunity, including subsets of cytotoxic (CD8+) T–cells, and NK (NK) cells. In patients with AML, cytokines can be produced by both cells of the immune system and leukemic blasts. Despite that, an increased level of cytokine is a feature of leukemia that may subsidize blast survival, proliferation, resistance to chemotherapy, and patients' prognosis.[7]

Patients, Materials and Methods

This case–control study was conducted on thirty newly diagnosed (ND), *de novo* AML patients; they included 15 males and 15 females with an age range between 19 and 75 years. Along with 18 healthy age and sex-matched individuals including 9 males and 9 females were taken as a control group.

The study was carried out at the National Hematology Center at Al Mustansiriyah University/college of medicine and Baghdad Teaching Hospital for a period from September 20, 2017, to March 15, 2018.

Ethical approval to conduct the research was obtained from the National Center of Hematology and Medical City as well as verbal consent from all participants in this study was attained.

Diagnosis of AML was established according to the morphology, cytochemistry, and immunophenotype (cluster of differentiation or CD profile) measured by FC of both PB and BM aspiration as well as studying biopsy reports. Since there is no genetic study done for classification of AML, therefore according to the WHO 2016 classification, the "AML not otherwise specified" subgroup was adopted for classification of AML cases included in this study.

Patients included in our study were assessed for complete remission (CR) achievement within 14–28 days from the onset of their induction chemotherapy. CR was based on Cheson *et al.* definition (<5% blasts in BM aspirate samples, with no persistence of extramedullary disease, no blast cells with Auer rods, and have a platelet count of $\geq 100 \times 10^9$/L and an absolute neutrophil count of $>1 \times 10^9$/L).[8]

Four milliliters of blood was taken by a clean, aseptic venipuncture from each patient and controls.

The drawn sample was then put in serum separator tube and samples were permitted to clot at room temperature for about 2 h or overnight at 4°C prior to centrifugation at 1000 ×g for 15 min.

The serum was collected in Eppendorf tubes in 4 aliquot for each sample. The serum stored at −80°C for a maximum of 5 months and used for measuring serum IL-35 and IL-10 level for control subjects and for AML patients (on two occasions: at diagnosis and after the patients had completed their remission induction therapy) by enzyme-linked immunosorbent assay using the CUSABIO Human IL-35 and IL-10 Immunoassay Kit, China.

Hematological parameters including hemoglobin level, white cell count, platelet count, and blast percentage in the PB and BM were obtained from the patients' records.

Statistical analysis

Data were analyzed using SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA: IBM Corp.

Categorical data represented by percentages and frequencies and their relations were evaluated using the Pearson's Chi-square test. Data were evaluated for

normality of distribution and then tested by Student's *t*-test for differences between cases and controls as well as the response to treatment. On the other hand, ILs were skewed data thus represented by median and interquartile range, then tested by Mann–Whitney nonparametric test and also tested by the Wilcoxon rank test for differences according to the treatment. Spearman's correlation was used.

P value considered significant at 0.05 level.

RESULTS

This study was conducted on 30 AML patients along with 18 healthy age- and sex-matched individuals who served as a control group. The mean age and range with gender incidences are shown in Table 1.

Classification according "AML not otherwise specified" subgroup showed that 2 patients had M0 subtype (6.6%); 6 patients had M1 (20.0%); 6 patients had M2 (20%); 7 patients had M3 subtype (23.3%); 5 patients had M4 (16.6%); 3 patients had M5a (10.0%), and 1 patient had M5b (3.3%), as shown in Figure 1.

At presentation, there was a highly significant increase in the mean level of IL 35 and IL 10 in AML patients compared to their mean level in the control group ($P < 0.001$) [Table 2].

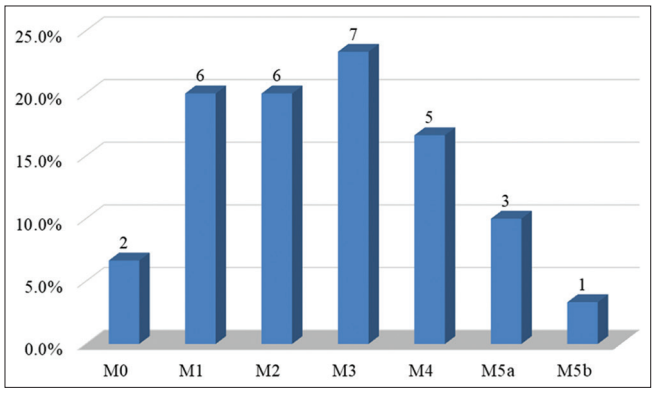

Figure 1: Subtypes of acute myeloid leukemia cases *n* = 30

Moreover, the mean level of IL-10 decreased significantly among patients who had a remission after induction chemotherapy as compared to non-remitted patients and the majority of non-remitted patients showed even increased IL-10 levels ($P < 0.001$), whereas IL-35 level showed similar changes after induction therapy but did not reach the level of significance, as shown in Table 3.

The current study revealed that there were no significant correlations between white blood cell count (WBC) count, platelet count, hemoglobin concentration, and blast percentage in PB and BM and the mean level of both ILs before treatment [Tables 4 and 5].

Discussion

The mean age of patients with AML included in this study was 46.9 ± 14.6 years with a range of 18–70 years. Those results were comparable to other Iraqi studies in 2009,[9] 2017,[10] and an Egyptian study in 2016.[11]

Of the studied 30 cases, M3 was the most frequent (23.3%) followed by M1 (20%) and M2 (20%). This result was comparable with other studies done in Baghdad by Almohsen and Al-Mudallal[12] and by Hussein *et al*.[13] in which M3 was the most frequent subtype (26% and 28%, respectively).

However, other studies done by Poul.[14] conducted in Erbil over a period from 2006 to 2009 showed that M2 was the most common subtype (24%), this may be attributed to our smaller sample size and shorter period of observation.

Hemoglobin level estimation in this study primarily showed low mean hemoglobin concentration 7.2 ± 1.7 g/dL, which are analogous with other Iraqi studies reported by Al-Maaroof *et al*.[15] and Hasan *et al*.[16]

The mean WBC count in this study was $35.9 \pm 23.2 \times 10^9$/L which was closely comparable to the result reported by Assem *et al*.[17] ($38.6 \times 10^{[9]}$/L) and less than that reported by Colovic *et al*.[18] (53×10^9/L).

Table 1: Age and sex comparison according to the study groups

Variables	Mean±SD		*P*
	Cases (*n*=30), *n* (%)	Control (*n*=18), *n* (%)	
Age (years), mean±SD (range)	46.9±14.6 (18-70)	39.5±16.7 (17-75)	0.131[t]
Age groups (years)			
<40	9 (30)	10 (55.6)	0.178[x]
40-59	13 (43.3)	6 (33.3)	
60+	8 (26.7)	2 (11.1)	
Sex			
Male	15 (50)	9 (50)	1
Female	15 (50)	9 (50)	

SD=Standard deviation, *t*=Student's *t*-test (two-tails), x=Pearson's Chi-square test (two-tails)

Table 2: Comparison of laboratory markers according to the study groups before induction chemotherapy

Parameters	Median (IQR)		*P*
	AML patients (*n*=30)	Controls (*n*=18)	
IL-10	379.2 (261.3-878.1)	35.5 (6.4-69.5)	<0.001*
IL-35	1639.3 (842.7-3452.2)	53.2 (13.9-126.2)	<0.001*

*Significant at 0.05 level by Mann-Whitney U-test (two-tails). IQR=Interquartile range, AML=Acute myeloid leukemia, IL=Interleukin

Table 3: Comparison of interleukins difference after induction chemotherapy according to response

Parameters	Median (IQR)		*P*
	Complete remission (*n*=22)	Nonremission (*n*=8)	
IL10 (pg/mL)	309.5 (747.5-118.6)	157 (18.2-801.1)	<0.001*
IL35 (pg/mL)	853 (3211.9-476.5)	164.4 (1352.1-1843.6)	0.097

*Significant at 0.05 level by Mann-Whitney U-test (two-tails). IQR=Interquartile range, IL=Interleukin

Table 4: Spearman's correlation between interleukin 35 and hematological parameters of acute myeloid leukemia cases (*n*=30)

Parameters	IL35	
	Correlation coefficient (*r*)	*P*
WBC (×10^9/L)	−0.127	0.505
Hemoglobin (g/dl)	0.133	0.484
Platelet (×10^9/L)	0.225	0.232
Blast percentage in PB	0.182	0.336
Blast percentage in BM	−0.026	0.891

IL=Interleukin, WBC=White blood cell count, PB=Peripheral blood, BM=Bone marrow

Table 5: Spearman's correlation between interleukin 10 and hematological parameters of acute myeloid leukemia cases (*n*=30)

Parameters	IL-10	
	Correlation coefficient (*r*)	*P*
WBC (×10^9/L)	−0.087	0.649
Hemoglobin (g/dl)	0.002	0.992
Platelet (×10^9/L)	0.028	0.885
Blast percentage in PB	0.275	0.141
Blast percentage in BM	0.223	0.235

IL=Interleukin, WBC=White blood cell count, PB=Peripheral blood, BM=Bone marrow

The mean platelet count in this study was 53.1 ± 27.6 × 10^9/L comparable to Hasan *et al.'s*[16] result (51 × 10^9/L) and Thomas *et al.'s*[19] result (60 × 10^9/L).

The PB blast percentage mean was 58% ± 22.1% (range: 11–97) and the BM blast percentage mean was 70.6% ± 18.8% (range: 30–95). These results were consistent with those described by other studies in 2011 and 2016.[14,20]

Both ILs showed a significantly higher level in ND AML patients compared with their level in the control group. These findings totally agree with studies done by Binder *et al.*[21] in 2018 and Wu *et al.* in 2012[4] which showed higher levels of serum IL-35 and IL-10 in ND adult AML patients compared with the healthy control group.

According to the response to induction chemotherapy, IL-10 was significantly reduced in patients with remission, compared with nonremission (NR) group, while the level of IL35 was lower among those with CR compared to those with NR, but this reduction did not reach the level of significance. Whereas Tao *et al.*[22] reported that IL-35 level was high in ND and NR AML groups as compared to control and CR AML groups; moreover, it was believed that IL-35 positively correlated to adult AML occurrence and development. This disparity in findings may be due to the differences in sample sizes.

Conclusions

In conclusion, both IL-35 and IL-10 were elevated in ND adult Iraqi patients with AML. There is propensity for serum level of IL10 to be reduced following induction chemotherapy. There was no significant correlation of IL-35 and IL-10 with platelet count, hemoglobin level, blast percentage, and WBC count.

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Conflicts of interest
There are no conflicts of interest.

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