



Plasma IL-10 Concentration and Its Role in the Pathogenesis of Acute Myeloid Leukemia: a Prospective Study

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Abstract: Many suggestions support that cytokines have pathogenesis role of acute myeloid leukemia (AML) patients and that may be related with disease progression and patient survival. But their prognostic significance in this disease is unknown. The study aimed to estimate the plasma level of IL-10 before and after treatment in patients with AML. Plasma concentrations of IL-10 were estimated in 26 newly diagnosed patients with AML and follow up 19 of them after treatments, 7 patients were died after induction, matched 16 individuals' healthy donors. IL-10 levels were estimated using the enzyme-linked immunosorbent assay (ELISA) technique. Patients were divided into two groups: 26 before treatment and 19 after treatment. The results showed that plasma concentrations of IL-10 were significantly higher in AML patients compared to control group. Moreover, plasma concentrations of IL-10 were significantly associated with non-responding (NR) AML patient after treatment compare to complete remission (CR). While it observed non-significantly before treatment. The study demonstrated that AML NR patients have increased plasma concentrations of IL - 10 after induction, suggesting that this cytokine is involved in the pathophysiological process of the disease and may be associated with poor prognosis of clinical outcomes.

Key words: Acute myeloid leukemia, IL-10, ELISA.

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Introduction

In USA, each year, approximately 12,330 new cases of acute myeloid leukemia (AML) are reported, and approximately 8,950 patients die from this disease (1). Acute myeloid leukemia increases in incidence with age (2). In Iraq, according to the Iraqi

Cancer Registry 2008 (3), leukemia ranks the third among the ten common cancers in Iraq, constituting 6.77% of all cancers with an annual incidence of 3.01 per 100,000 populations. Acute myeloid leukemia is a malignant disorder characterized by disruption in hematopoiesis and rapid growth of abnormal leukocytes and resulting in

accumulation of immature or blast cells in the bone marrow and the peripheral blood (4). However, the exact causes of AML are still unknown, although, radiation, obesity, smoking, and exposure to chemical carcinogens have been suggested, AML only develops in a small proportion of people exposed to these environmental and lifestyle risk factors, indicating that the host genetic background might play a critical role in its genesis (5,6,7). Additional evidences suggested that patients with immune system disorders or those receiving immunosuppressive therapies may be implicated as risk factors (8,9). Nevertheless, little is known about the immunopathological events, especially the abnormal T helper (Th) subsets, leading to the initiation and progression of this disease (10). Other scientific reports suggested that cytokines secreted by cancer cells have been implicated in the pathogenesis of AML by regulating the disease course and playing a critical role in this disease progress (11); an increment in cancer cells count, survival rate and resistance to chemotherapy had been suggested (12). Although many studies had reported that cytokines have an important role in genesis of this disease, the topic of cytokines effect on the immune system against cancer is remains controversial point (13). One important cytokine is IL-6 which maintains the growth of cancer stem cells (14). Interleukin-10 (IL-10) is predominately secreted by immune cells including macrophages, T lymphocytes, and natural killer (killer) NK cells, and constitutes a major determinant of viral clearance vs. persistent infection (15,16). IL-10 which still having unclear role in cancer pathogenesis, some studies showed that IL-10

involved in the development and progression of cancer in humans, as a tumor promoting (promote cancer potentially) has immune-stimulating (17) and immuno suppressive (inhibit cancer potentially) (18). Dual biological functions of IL-10 makes a controversial role in carcinogenesis in humans, as a tumor promoting and tumor inhibiting factor, may regulate tumor susceptibility and development (19).

As a result for these contradictory suggestions, the present study comes to focus on estimation of the level of IL-10 in the plasma collected from group of Iraqi patients with AML before and after chemotherapy.

Materials and Methods

A total of 26 Iraqi patients with acute myeloid leukemia (12 male, 14 female; age (mean \pm SD) = 34.2 ± 15.7 (ranging from 16 to 72 years) were enrolled in the study group. These patients were suffered from AML and were referred to hematology unit of Baghdad Teaching Hospital in medical city for diagnosis and / or treatment during September 2012 to November 2013. AML cases have been diagnosed by a specialized hematologist. Diagnosis and classification was based on clinical examination, bone marrow aspiration, biopsies reports and other criteria according to the French-American-British (FAB) criteria.

Plasma concentrations of IL-10 were analyzed, using the enzyme-linked immunosorbent assay (ELISA) technique, in 26 newly diagnosed patients with AML and follow up 19 of them after treatments, 7 patients were died after induction. However, patients

were divided into two groups: 26 before treatment and 19 after treatment. For the purpose of comparisons, 16 Iraqi subjects, comparable to AML patients regarding their age (20-55 years) and gender (8 females and 8 males), were included in the study as a control group. Three ml of venous blood were collected using a 5 ml disposable syringe in EDTA tubes. All blood collection tubes were gently shaking for mixing immediately after collected, incubated for 5 min. Plasma was separated at $350 \times g$ for 15 min at room temperature. Separated samples were liquated and preserved in tightly closed Eppendorf tubes at $-20\text{ }^{\circ}\text{C}$ for subsequent batch testing. The quantity determination of IL-10 in samples was evaluated using an ELISA kit (IBL company, Germany) designed to measure human IL-10 in plasma. Repeated freeze–thaw cycles were

avoided to prevent loss of bioactive substances. The Statistical Analysis System- SAS (2010) was used to effect of difference factors in study parameters. P Value was used to significant compare between means this study. (p value $< 0.05^*$ was significant; p value $< 0.01^{**}$ was highly significant).

Results and Discussion

Plasma Levels of IL-10 in AML and Controls

Plasma levels of IL-10 were significantly higher in AML patients (19 ± 1.77 pg/ml; range, 1.94-77.17) compared to the healthy control (8.64 ± 0.62 pg/ml; range, 1.68-23.74; $P < 0.01$) (Figure1).

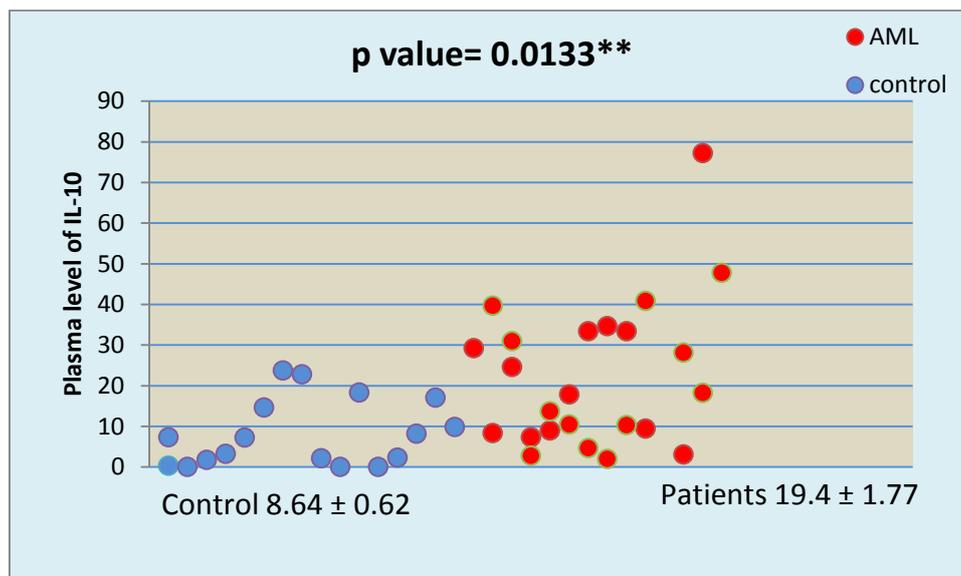


Figure (1): Differences in the plasma concentrations of IL-10 among acute leukemic patients and control

Plasma Levels of IL-10 in AML Patients during Treatment Follow Up

During treatment follow up the plasma concentrations of IL-10 were

significantly higher after induction (23.59 ± 2.09 pg/ml; range,3.93-136.22) compared to the AML patients before chemotherapy (19.4 ± 1.64 pg/ml; range,1.94-77.168; $P<0.01$) (Figure 2).

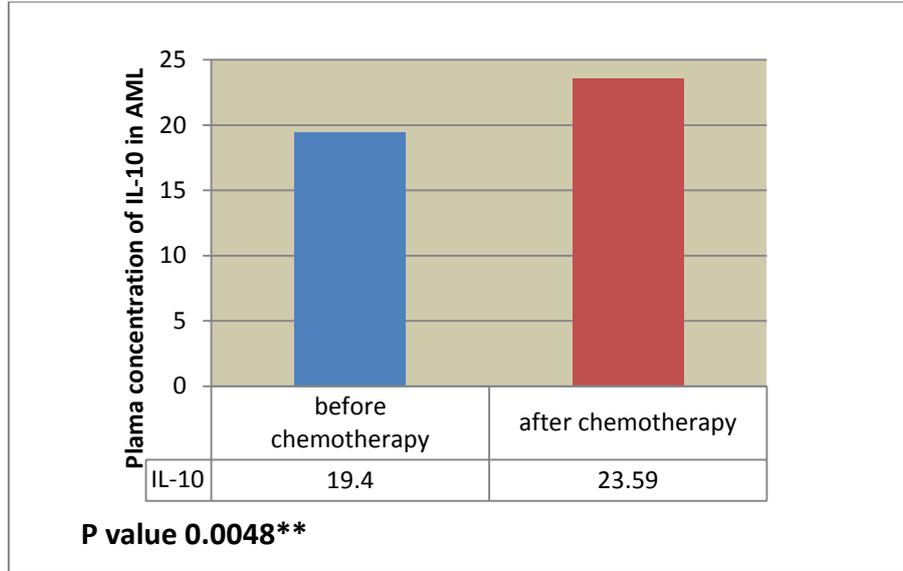


Figure (2): Differences in the plasma concentrations of IL-10 before and after chemotherapy

Additionally, Plasma concentrations of IL-10 were significantly higher in AML NR patients (41.9 ± 3.59 ; range,3.93-136.22) compared to AML CR patients after chemotherapy (13.137 ± 0.87 ;range, 6.17-30.81; $P<0.001$).

While no significant difference was found in the plasma levels of IL-10 between AML NR patients and AML CR group before chemotherapy ($P=0.1477$) (Table1).

Table 1: Relationship between plasma concentration of IL-10 and clinical outcome of AML patients

Before chemotherapy	NR 12 (46.15%)	CR 14 (53.84%)	P-value
	23.927 ± 1.95	19.316 ± 1.44	0.1477 NS
After chemotherapy	NR 10 (52.63%)	CR 9(47.37%)	P-value
	41.9 ± 3.59	13.137 ± 0.87	0.0016 **

The picture is reversed where the concentrations of IL-10 was higher in

those who did not completely remitted in comparison to those who were

completely remit specially after induction.

Interleukin-10 (IL-10) is a broadly acting immune inhibitory cytokine that is mostly thought to support tumor progression (20). IL-10 can favor tumor growth in vitro by stimulating cell proliferation and inhibiting cell apoptosis (21, 22). The main finding of our study suggests that IL-10 participate in the progression of AML more than in its initial development, although IL-10 level significantly high in patients at initial diagnosis compare to healthy control, but it's increased after induction among non-responding AML patients. Consistent with our observations, recent study showed that higher levels of plasma IL-10 were significantly observed in AML newly diagnosed (ND) patients compared with healthy controls or AML CR patients, and IL-10 levels were positively correlated with white blood cell (WBC) and neutrophil (NEU) count (23, 24). Additional observation revealed that IL-10 mRNA expression in AML patients was obvious higher than the non-tumor group and the remission group (25). Several studies revealed that IL-10 is an immunosuppressive molecule secreted by tumors (or tumor-infiltrating immune cells) to allow malignant cells to escape from immune surveillance (26). Other showed that tumor-associated macrophage (TAM) expressed high levels of IL-10, in non-small cell lung cancer (NSCLC), and high levels of IL-10 in TAM significantly correlated with stage, tumor size, lymph node metastasis, lymphovascular invasion or histologic poor differentiation (27). Similar to these results, it has been shown that cancer cells secrete numerous cytokines to preserve their microenvironment,

stimulate certain signal pathways in cancer stem cells (CSCs), and thus increase their survival rate and resistance to chemotherapy (28). Much remains unknown concerning the immune escape mechanisms promoted by AML, and whether efforts to prevent tolerance may affect the progression of this disease (29). Opposite of our observation, recent study showed that high levels of IL-10 represent good prognostic factors for survival in AML patients. These consequences support the idea that cytokine deregulation may be suitable as a marker for predicting clinical outcome in AML patients (30). The inhibitory effect of IL-10 is a multifunctional cytokine with both immunosuppressive and antiangiogenic functions, which may play diverse roles in the pathogenesis and development of cancer (31). The mechanisms that run the cell type- and receptor-specific induction of IL-10 remain unclear. This is due largely to the varied distribution of cellular sources that express IL-10 under various stimulation conditions and in a variety of tissue compartments. Additional confounding is the fact that human IL-10 expression patterns seem to be under genetic effect resultant in differential expression and disease susceptibility (32). Further studies with larger sample size are required to confirm and extend our observations. In addition, more studies should be carried out to the molecular mechanism of IL-10 effects.

In conclusion, we demonstrated that AML NR patients have increased plasma concentrations of IL-10 after treatment, suggesting that this cytokine is involved in the pathophysiological process of the disease and may be associated with poor prognosis of clinical outcomes.

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