Study the IL-10 serum level in acute myeloid leukemia patients before &after chemotherapy

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Abstract

Interleukin-10 (IL-10) is commonly regarded as an anti-inflammatory. has pleiotropic effects in immunoregulation and inflammation and influences many aspects of the immune response ,and is cytokine that stimulates various hematopoietic cells,. and is primarily produced by monocytes and, to a lesser extent, lymphocytes, namely type 2 T helper cells (T_H2), mastocytes, regulatory T cells.The function of IL-10 has been detected in the leukemic cells of most ALL and AML cases and it suppresses the immune reactions. The aim of this study to evaluate serum IL-10 concentration of patients before and after treatment ,and comparative with control subjects . The other aims is to associate this protein with age groups stage and the gender .

A direct ELISA was used to quantify serum IL-10 concentrations in 60 patients with acute myeloid leukemia (AML)and 15 healthy subjects (control) ,and so there was significant effect of age on the level of IL_10 at AML patients,where no significant effect of gender on the level of IL_10.

We found serum concentrations of IL-10 were significant decrease in patients with AML after treatment in compared with patients before treatment ($P< 0.5$) .

Key words : IL-10, AML ,ELISA

Introduction :

Acute Myeloid Leukaemia (AML) is a cancer of the bone marrow, the organ which produces the majority of blood cells. AML is the most common subtype of leukaemia in adults and accounts for 15-20 % of childhood leukaemia [1]. AML is

characterised by continued proliferation and suppressed differentiation of haemopoietic progenitors in the bone marrow with disease cells characterised by enhanced survival and self-renewal.

Cytarabine, also known as Arabinofuranosyl Cytidine (AraC), is chemotherapy drug that is used primarily for the treatment of acute myeloid leukaemia (AML). because AraC is used to target the white cell compartment and anthracyclines (eg, idarubicin, daunorubicin) [2]. that interfere with DNA replication and induce apoptosis primarily in replicating cells. it is known to have immunosuppressive effects. A number of studies have shown that the degree of cancer-induced immune suppression can be correlated to tumour size [3],[4]

interleukin-10 (IL-10) is commonly regarded as an anti-inflammatory The IL-10 is encoded by the *IL10* gene, which is located on chromosome 1 and comprises 5 axons, [5]. and is primarily produced by monocytes and, to a lesser extent, lymphocytes, namely type 2 T helper cells (T_H2), mastocytes, $CD4^+CD25^+Foxp3^+$ regulatory T cells, and in a certain subset of activated T cells and B cells Interleukin 10 (IL10) is a pleiotropic cytokine that stimulates various hematopoietic cells

Interleukin-10 (IL-10) has pleiotropic effects in immunoregulation and inflammation and influences many aspects of the immune response [6]. The function of IL-10 has been detected in the leukemic cells of most ALL and AML cases and it suppresses the immune reactions, suggesting that IL-10 could be associated with escape of leukemia cells from immune surveillance [7]. The significance of IL-10 production within the tumor microenvironment, which can be sustained by malignant cells and tumor-infiltrating macrophages (TIM) and lymphocytes ,including natural killer (NK) and T cells, [8].IL-10 can favor tumor growth in vitro by stimulating cell proliferation and inhibiting cell apoptosis [9]. More recent studies have clarified that the IL-10 immunosuppressive activity on T cells is mainly indirect and is mediated by other two-immune cell types dendritic cells (DC) and T regulatory (Treg) cells. High systemic levels of IL-10 correlate with poor survival of some cancer patients [10]. The aim of this study to evaluate serum IL-10 concentration of patients before and after treatment ,and comparative with control subjects and so association of this protein level with the age and the gender .

Material and methods

The blood sample were collected from the (60) acute myeloid leukemia patients(AML) from Baghdad Teaching hospital in Medicine city tube, where (33) sample before treatment and (27) after treatment, in addition to(15) healthy subjects were as a control groups

The period of study from May-2011 to May-2012 were eligible for this study. The cases were diagnosed clinically by consultant hematologist at Baghdad Teaching Hospital. blood samples were centrifuged at 1500 rpm, for 5 minutes ,the serum was frozen at -20ºC until the (IL-12/P70) measurement by ELISA . IL-12/P70 concentrations was quantitatively determined in serum of patients and healthy control subjects by means of ELISA (Enzyme Linked Immunosorbent Assay) using ready kits manufactured by the (R&D system).

Statistical Analysis :

The Statistical Analysis System [11] was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means this study

Result &discussion :

Our results explained a significant ($P \leq 0.05$) increase serum level of IL-10 in patients pre- and post-treatment (51.24 ± 15.52and 41.78 ± 3.44 pg/ml, respectively) compared with healthy group ,so our result explained the differences between the two patients group (before and after treatment) but it is not significant . This result agreed with Park and his group about cytokine levels of bone marrow T cells (bm T cells) at the time of complete remission (CR) after chemotherapy was decreased significantly, compared to the cytokine levels of the bmT cells before the start of chemotherapy [12],and there was significant ($P \leq 0.05$) increased serum level of IL-10 was observed inpatients when compared with health group,and this result disagreed with Panoskaltsis and his group's result when measured the intracellular cytokine levels of circulating lymphocytes derived from AML patients, They did not find any significant changes in the IL-4, IL-10, IL-12 or IFN_γ levels for the cell subsets derived from patients compared with healthy individuals, suggesting normal TH1 and TH2 profiles[13], and so when we compared the level of IL_10 for patients with

controls (14.43 ± 2.41pg/ml),where the intracellular cytokine-producing T lymphocyte subsets in the peripheral blood have been reported to be increased in patients with ALL, AML, and malignant lymphoma [13]. The T lymphocytes showed release of IL-4, IL10, and IFN-γ in the presence of ALL and AML blasts that act as accessory cells [14]

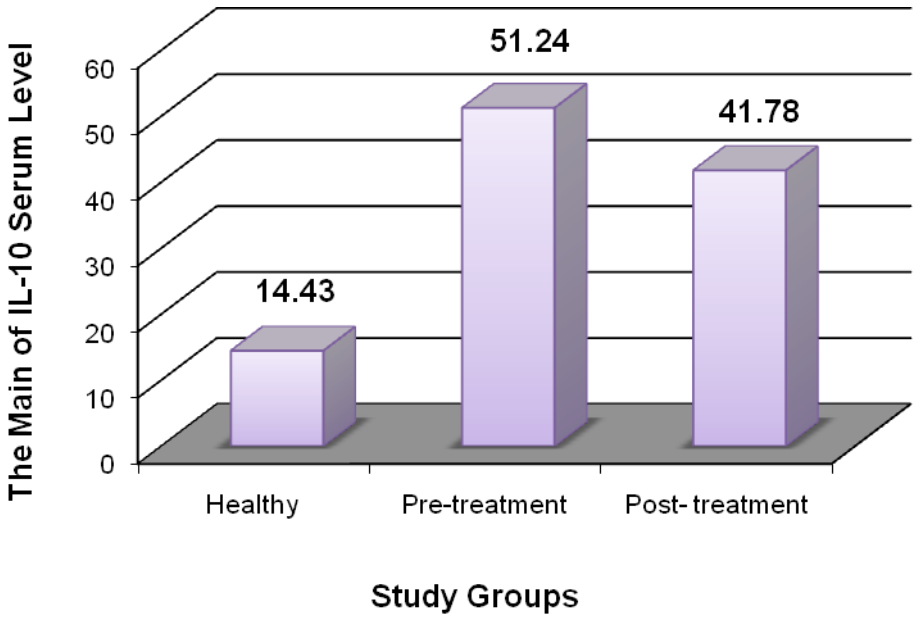

Figure 1. Comparative Among Groups Study in IL-10 Serum Level

Table 1. Compare Between Groups Study in IL-10 Serum Level

Group	No.	Mean ± SD
		IL-10
Healthy	15	14.43 ± 2.41
Pre-	33	51.24 ± 15.52
Post-	27	41.78 ± 3.44
P-Value	--	0.054
LSD Value	--	36.089 *
* (P<0.05), ** (P<0.01), NS: Non-significant.		

Other researchers showed that the T lymphocytes release of IL-4, IL-10, and IFN-γ in the presence of ALL and AML blasts that act as accessory cells (14).IL-10 has been detected in the leukemic cells of most ALL and AML cases and it suppresses

the immune reactions, suggesting that IL-10 could be associated with escape of leukemia cells from immune surveillance (15).

The result of this study explain, there was significant effect of age on the level of IL_10 at AML patients, as show in table (2) a significant increase was observed in the age group (40_50) years(75.57 IU/ml) as compared to patients in the age group ≥ 40 years (29.55 IU/ml),and patients in the age group ≤ 40 years (43.89 IU/ml). Where Gupta and his group refer Leptin activates B cells from aged humans via increased intracellular signaling to secrete IL-6, TNF-α, and IL-10 to a greater extent than B cells in young subjects, which may contribute to chronic inflammation associated with human aging[16].

Table 2. Effect of age of patients in IL-10 Serum Level

Age group (year) No.	Mean ± SE IL-10
Least than 40	43.89 ± 6.65
40-50	75.57 ± 33.74
More than 50 year	29.55 ± 4.55
P-Value	0.051
LSD Value	43.714 *
* (P<0.05), NS: Non-significant.	

Our study explain there was no significant difference in releasing IL-10 between males and females, and this agreed with other studies ,which conformed no significant difference in releasing IL-10 between males and females in circulating blood after ex vivo stimulation with lipopolysaccharrides [17]. Another study showed that IL-10 secretion did not differ in males and females of healthy blood volunteers [18]. No difference in IL-10 production between both males or post-menopausal women and premenopausal women could be demonstrated (19). Also during the menstrual cycle lymphocyte IL-10 production after stimulation is stable (20), suggesting no effect of sex hormones on IL-10 production.

The effects of gender and reproductive conditions on lymphocytes are not very obvious. However, in males the decreased T lymphocyte count as compared to females may play a role in the differences in immune responses between sexes(21). Thus far no differences in Th2 cytokine production (IL-4 and IL-10) could be found

between gender and within reproductive phases, which is in-line with lack of effect of the sex hormones in vitro on the production of these cytokines(21)

Table 3. Effect of gender of patients in IL-10 Serum Level

Gender	Mean ± SE
	IL-10
Male	50.42 ± 13.18
Female	41.04 ± 6.37
P-Value	0.599
LSD Value	35.558 NS
* (P<0.05), NS: Non-significant.	

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دراسة المستوى المصلي للبروتين الحركي 10 لدى مرضى ابيضاض الدم النخاعيني قبل وبعد العلاج

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الخلاصة:

المقدمة :

البروتين الحركي 10 يعد برتيناً مضاداَ للالتهابات ذا تأثيرات مناعية متعددة وفعالة في تنظيم الاستجابة المناعية عند حدوث الالتهاب،وكذا يعمل على تحفيز مختلف مكونات الدم الخلوية. وينتج بصورة رئيسية من الخلايا اللمفاوية وحيدة النواة وبدرجة اقل من قبل الخلايا اللمفاوية التائية المساعدة نوع 2 والخلايا البدينة والخلايا اللمفاوية المنظمة . تم تحديد وظيفة البروتين الحركي لخلايا الابيضاض السرطانية في معظم حالات ابيضاض الدم النخاعيني واللمفاوي على انه بروتيناً مثبطاً للتفاعلات المناعية.الهدف من الدراسة تقييم مستوى تركيز البروتين الحركي 10 لدى مرضى ابيضاض الدم النخاعيني قبل وبعد العلاج ومقارنة مستوى الانتاجية مع الاصحاء هذا فضلاً عن معرفة مدى علاقة الانتاجية بالعمر وجنس مريض اللوكيميا.

تم استخدام اختبار الاليزا المباشر لتقدير مستوى تركيز البروتين الحركي 10 لمصل 60مريض يعانون من مرض ابيضاض الدم النخاعيني بواقع 33 عينة قبل العلاج و 27 عينة بعد اخذ العلاج و15 أشخاص أصحاء

وجدت الدراسة إن مستوى تركيز البروتين الحركي 10 في مصل المرضى قبل العلاج اعلى من تركيزه بعد العلاج وبفرق معنوي واضح مع القيمة الإحصائية ($P< 0.05$) وكذا يعد الفرق واضحا عند مقارنته بمجموعة الاصحاء . اذ يعتقد ان الخلايا السرطانية ذات تاثيرا محفزا لانتاج هذا النوع من الحركي كما اظهرت الدراسة وجود فرقا معنويا بين المجاميع العمري موضوع الدراسة و عدم تاثيراً للجنس على انتاجية هذا البروتين.