Study the IL-12/P70 serum level in acute myeloid leukemia patients before &after chemotherapy

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Abstract

IL-12 P70 is a key immunoregulatory cytokine with a molecular weight of 70 kDa . is produced mainly by DCs, MΦs, monocytes, neutrophils, microglia cells and, to a lesser extent, by B cells IL-12 exerts potent anti-tumor activity through activation of immune effector mechanisms or direct targeting of tumor cells. The aim of this study to evaluate serum IL-12 P70 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-12 P70 concentrations in 60 patients with acute myeloid leukemia (AML)and 15 healthy subjects (control) .

We found serum concentrations of IL-12 P70 were significant increase in patients with AML after treatment in compared with patients before treatment ($P< 0.5$) . Experimental data suggest that they may suppress apoptosis and thus promote tumorigenesis

Key words : IL-12/P70, 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]

The physiologically most important target cells of IL-12 are: haematopoietic progenitors, for which, in synergy with other colony-stimulating factors, IL-12 induces increased proliferation and colony formation; NK cells, NK T cells and T cells, for which IL-12 induces proliferation, enhancement of cytotoxicity and of the expression of cytotoxic mediators, and the production of cytokines, particularly IFN-γ, as well as favoring differentiation to cells that produce type-1 cytokines (Th1, TC1 and NK1 cells) [5]; and B cells, for which IL-12, directly or through the effects of type-1 cytokines such as IFN-γ, enhances the activation and production of Th1-associated classes of immunoglobulin

Neutrophils are well-known first responders to infection, are able to produce IL-12 and IL-18 [6], and are thought to be essential for the innate immune response to many infectious challenges of mice [7].IL-12 exerts potent anti-tumor activity through activation of immune effector mechanisms or direct targeting of tumor cells. IL-12 has multiple biological functions and importantly, it bridges the early nonspecific innate resistance and the subsequent antigen-specific adaptive immunity [5]. IL-12 was previously known as T cell differentiation factor (TCDF) or natural killer cell stimulatory factor (NKSF). The three major effects of IL-12 on NK cells are to induce cytokine production, proliferation and enhance cytotoxic functions. In

addition to its direct stimulatory effects on innate immunity, IL-12 also enhances antigen-specific T cell responses [8].

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 ABO Switzerland kit.

Statistical Analysis :

The Statistical Analysis System [9] 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 and discussion :

Acute myeloid leukemia patients before treatment showed a significant decreased level of IL-12 (12.65 ± 1.15IU/ml), as compared with the control (13.95 ± 1.67IU/ml), while there was significant increased in patient after treatment groups (16.65 ± 1.45 IU/ml).

Table 1. Compare between group study in IL-12

Group	No.	Mean ± SD
		IL-12
Healthy	15	13.95 ± 1.67
Pre-treatment	33	12.65 ± 1.15
Post- treatment	27	16.65 ± 1.45
P-Value	--	0.0315
LSD Value	--	3.152 *
* (P<0.05), ** (P<0.01), NS: Non-significant.		

when Panoskaltsis and his group 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[10].

It is well known that AML patients are immunocompromized and have an increased risk of infections. These patients often have neutropenia initially due to the disease and later eventually due to intensive chemotherapy.

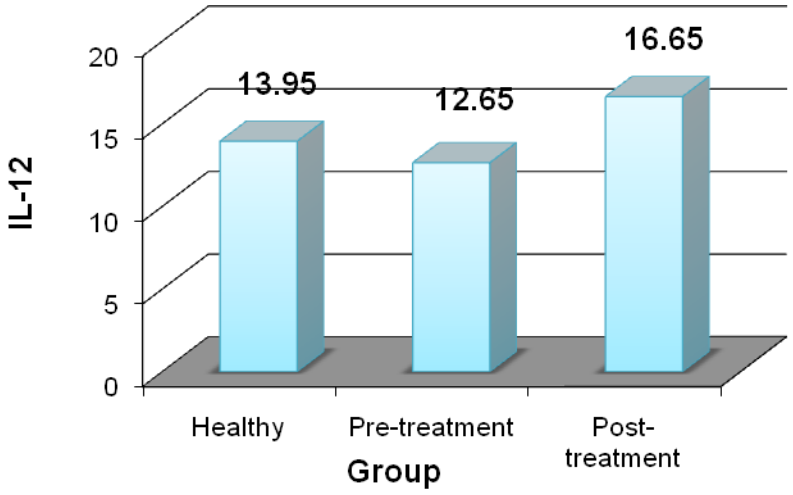

Figure 1. Compare between group study in IL-12

AML cells can regulate and evade the immune system, and thus avoid destruction, in several ways. By decreased expression of MHC molecules as well as decreased expression of co-stimulatory molecules, AML cells circumvent specific cytotoxicity exerted by T cells [11]. Tumor-associated macrophages (TAMs) play an important role in tumor cell invasion, proliferation and survival[12]. TAMs have phenotype and functions more similar to alternatively activated (M2) macrophages, characterized by IL-10 high IL-12 low profile, poor antigen-presenting capacity, immunosuppressive and pro-angiogenic properties [13]. Several studies correlated high TAM number to reduced survival in solid tumors[14].

Our study explain there was no significant difference in releasing IL-10 among age groups, and so no significant difference between males and females as show in table (2 and 3 respectively),and this agreed with Cozen and his group where found no differences in IL-12 levels between male and female controls[15]

Table 2. Effect of age of patients in IL-12

Age group (year)	Mean ± SE
No.	IL-12
Least than 40	14.17 ± 1.42
40-50	14.96 ± 1.60
More than 50 year	14.49 ± 1.92
P-Value	0.984
LSD Value	4.916 NS
* (P<0.05), NS: Non-significant.	

Table 3. Effect of gender of patients in IL-12

Gender	Mean ± SE
	IL-12
Male	13.93 ± 1.15
Female	15.35 ± 1.62
P-Value	0.379
LSD Value	3.999 NS
* (P<0.05), NS: Non-significant.	

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دراسة المستوى المصلي للبروتين الحركي 12 \70 لدى مرضى ابيضاض الدم النخاعيني قبل وبعد العلاج

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الخلاصة:

يعد الحركي 12 من الحركيات المنظمة مناعياً ذات وزناً جزيئياَ 70 كيلو دالتون ينتج بصورة رئيسية بوساطة الخلية الشجيرية،الخلية البلعمية،الخلية الوحيدة،الخلية العدلة وتنتج بكمية اقل من قبل الخلايا اللمفاوية البائية. تظهر الحركية 12 فعالية واضحة ضد الاورام الخبيثة من خلال تنشيط الميكانيكي المناعي المؤثر او من خلال التأثير المباشر ضد الخلايا الورمية. الهدف من الدراسة تقييم مستوى تركيز الحركي 12 ذا الوزن 70 كيلو دالتون لدى مرضى ابيضاض الدم النخاعيني قبل وبعد العلاج ومقارنة مستوى الانتاجية مع الاصحاء هذا فضلاً عن معرفة مدى علاقة الانتاجية بالعمر وجنس المريض

تم استخدام اختبار الاليزا المباشر لتقدير مستوى تركيز البروتين الحركي12لمصل 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 12 في مصل المرضى بعد العلاج اعلى من تركيزه قبل العلاج وبفرق معنوي واضح مع القيمة الإحصائية ($P< 0.05$) بين المجاميع . اذ يعتقد ان الخلايا السرطانية ذات تاثيرا مضادا لانتاج هذا النوع من الحركي كما اظهرت الدراسة عدم وجود فرقا معنويا بين المجاميع العمري موضوع الدراسة وكذا عدم تاثيراً للجنس على انتاجية هذا البروتين.