The role of IL-2 in the pathogenesis of multiple myeloma "a study in Iraqi patients"

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الخلاصة :
يلعب الانترلوكين٢ (IL-2) دوراً محوريا في حثّ الاستجابات المناعية للمضيف ضد نمو الأورام حيث يمتلك نشاط مضاد للورم. ولذلك هدفت هذه الدراسة إلى تسليط الضوء على مجموعة من المرضى العراقيين المصابين بمرض الورم النخاعي المتعدد بتقييم مستوى هذا النوع من الحركيات الخلوية. وقد أُخذعاملي االعمر و الجنس في نظر الاعتبار. وايضا تمَّ التحقيق فيما إذا كانت هناك أية رابطة بين مستوى (IL-2) ومستوى البروتين المسمى بيتا ٢ مايكروكلوبيولين(β2-m) والغلوبيولين المناعي من نوع (IgG) . حيث شملت هذه الدراسة ٤٩ مريض و ١٢ متطوعا كمجموعة ضابطة حيث تمّ تقييم كمية هذا الانترلوكين في مصولهم بأستخدام تقنية المُمْتَزٌ المناعي المُرْتَبِطِ بالانزيمELISA . وتم تقسيم المرضى إلى مجموعتين: المجموعة الأولى شملت (١٧) مريض المشخصين حديثاً والذين لم يتلقوا أي علاج في وقت جمع العينات، بينما تضنمت المجموعة الثانية (٣٢) من المرضى الذين تلقوا العلاج.
النتائج: وقد لوحظ انخفاضا كبيرا في مستوى (IL-2) في مصول المرضى (٣,٨٩ ± ٣,٦٤ مقابل ١١,٦١ ± ٢٣,٣٧ وحدة/ مليلتر) بالمقارنة مع المجموعة الضابطة . كمالايوجد فرق معنوي في تركيز (IL-2) بين المرضى الذين تلقوا العلاج وأولئك الذين لم يتلقوا ،ايضا توصلت الدراسة الى الى انه لايوجد علاقة بين انتاج هذا الانترلوكين وانتاج كل من ال (β2-m) من جهة والغلوبيولين المناعي من نوع (IgG) من جهة اخرى ، اخيرا لايوجد تأثير لعاملي العمر والجنس على انتاج هذا الانترلوكين عند المرضى كما لايوجد فرق في انتاجه بين الذكور والاناث .

Abstract:
IL-2 plays a pivotal role in the induction of host immune responses against tumor growth and it has anti-tumor activity. Therefore the present study aimed at shedding light on a group of Iraqi patients with multiple myeloma by evaluating the level of this cytokine in their sera . The age and gender were also taken into consedration. and investigate if there was any association between IL-2 and the level of β2-m. A group of 49 patients and ١٢ healthy volunteers were included in the study. The quantitative determination of IL-2 serum level was performed by ELISA. The patients were divided into two groups: the first group included (17) patients who were recently diagnosed and not received any treatment at the time of collecting samples, while the second group included (32) patients who received treatment.

Results: A significant decrease in the serum level of IL-2 was observed in MM patients (3.89 ± 3.64 vs. 11.61 ± 23.37 U/ml) as compared with controls. There were no significant difference in level of IL-2 between the patients who received treatment and those who did not as well as there was no association between production of IL-2 one hand and production of β2-m and IgG on the other hand .

Finally there was no association between age and IL-2. The serum level of this interleukin showed no gender-related variations between males and females and there was no correlation between it (IL-2) and gender factor.

INTRODUCTION

Multiple myeloma (MM) is a malignant disorder characterized by the proliferation of a single clone of plasma cell derived from B cells in the bone marrow whose etiology is not well understood. Frequently, there is invasion of the adjacent bone, which destroys skeletal structures and results in bone pain and fractures **(1),(2)**. Occasionally, myeloma cells infiltrate multiple organs and produce a variety of symptoms **(3),(4),(5).** Some studies have shown that repeated or chronic stimulation of the immune system may lead to MM (**6),(7).**Others have observed elevated risks for categories of immune-stimulating medical conditions e.g., autoimmune conditions, infections, and allergies or for specific immune-stimulating medical conditions e.g., rheumatoid arthritis and eczema**(8).**The characteristic feature of the disease is a clonal proliferation of malignant plasma cells, which produces a monoclonal protein (M protein) **(9)**. These M-proteins invade adjacent bone structures, causing skeletal destruction. By crowding outside the normal bone marrow cells, myeloma cells also cause other secondary complications, including infections, renal dysfunction, blood disorders (i.e, anemia, neutropenia, thrombocytopenia), neuropathy, or multiorgan involvement **(10)**.The diagnosis of myeloma is based on the presence of at least 10% clonal bone marrow plasma cells and one of the following:

1) Monoclonal protein (M- protein) in the serum.
2) Bence Jones protein (M- protein) in the urine.
3) Reveal lytic bone lesion in the skull, spine, or ribs, detected by using radiological examination **(11),(12),(13).**

Monoclonal immunoglobulin free light chains (FLCs) are frequently found in the urine of patients with MM, Waldenström macroglobulinemia, AL amyloidosis, light chains deposition disease, and, occasionally, in non-Hodgkin lymphomas, chronic lymphocytic leukemia.

In MM, a number of cytokines and growth factors are produced either by myeloma cell or by stromal cells, due to interactions between them, **(14).** These cytokines have been implicated in the stimulation of bone resorption by enhancing osteoclast formation.However, the most important of these cytokines are insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), tumor necrosis factor alpha (TNF-α), transforming growth factor-beta (TGF-β), IL-10 and stromal-cell-derived factor-1α (SDF-1α) **(15),(16),(17).**

IL-2 is a 15,000-kDa α-helical cytokine produced predominately by activated CD4+ and CD8+ T cells, the latter to a lesser extent. Activated dendritic cells (DCs), natural killer (NK) cells, and NKT cells also produce IL-2 **(18),(19)**. IL-2 is a member of the family of cytokines that includes IL-4, IL-7, IL-9, IL-15, and IL-21 **(20).** 76 Data suggest that high serum levels of IL-2 correlate with longer survival in patients with multiple myeloma. Animal and laboratory studies indicate that IL-2 has anti-tumor activity**(2).**Therefore ,We aim to characterise IL-2 pattern involved in the group of Iraqi patients with MM before and after treatment. The identification of the cytokine profile will lead to possible new therapeutic options to treat those patients.

MATERIAL AND METHODS

A total of 49 Iraqi patients with MM were recruited for the study, 27 (55.10%) males and 22(44.91%) females. The mean age of the total group of MM patients was 55.83 ±12.25 years (ranging from 31 to 85 years).These were suffering from MM and were referred to the Hematology Consultation Clinic in each of the teaching hospitals

at Al-Najaf governorate (Alsader), Babil governorate (Marjan) and Baghdad governorate (Baghdad) during the period from June 2010 to April 2011 for diagnosis and/ or treatment. Those MM cases then have been diagnosed by a specialized haematologist. Diagnosis was based on bone marrow aspiration, biopsies reports and other diagnostic criteria mentioned previously for MM according to the International Staging System (ISS).For the purpose of comparisons, 12 Iraqi subjects, comparable to MM patients regarding their age (40-55 years) and gender (3 females and 9 males), were included in the study as a control group.Approximately 5 ml of blood was collected from the antecubital vein of each patient using a 5 ml gauge syringe. The blood was put in a plain test tube, left to clot at room temperature (20-25°C) for 15-30 minutes, centrifuged at 3,000 RPM for 10 minutes to separate the serum. After that, the serum was aliquoted and preserved in tightly closed Eppendorf tubes at -20^{o} C for future usage. The quantity determination of IL-2 in sera was performed by ELISA kit (DRG company ,USA).Statistical analysis was carried out using the SPSS base 16 (SPSS Inc‹.Chicago, IL) statistical software package. All the data were presented as the mean ± SD. Chi-square test was used to compare the gender of patients between the two groups. Independent sample t-test was used to compare the means of age of patients and levels of measured factors between the two groups. Correlations were calculated by Spearman's rank correlation. A P-value of <0.05 was considered to be statistically significant.

Results

- ***Patients with multiple myeloma versus Controls***

From Table (1) we see that the level of IL-2 in the entire patients group ranged from undetectable values (0 U/ml) to (15.12U/ml) ,while the values of this cytokine in the sera of the control group ranged from undetectable values (0U/ml) to (84.85U/ml). This table also shows that a significantly ($P \leq 0.05$) decreased serum level of IL-2 was observed in MM patients (3.89 ± 3.64 SD *vs*. 11.61 ± 23.37 SD U/ml) as compared with controls.

Table 1:Concentration of IL-2 in patients and controls.

Groups	Number	Serum level of IL-2 (U/ml)		
		Mean ± SD	Minimum	Maximum
Patients	49	3.89 ± 3.64	0	15.12
controls	12	11.61 ± 23.37	0	84.85
$P \leq 0.05$ (significant)				

- ***Level of IL-2 and β 2 microglobulin in patients with multiple myeloma***

Table (2) shows that although the levels of β2-m in the sera of patients showed a slightly increased mean in comparison with concentration level of IL-2 (5.13± 3.53 SD vs.3.89 ±3.65 SD), there were no significant differences in correlation patterns ($r = -0.123$; $P > 0.05$ (not significant). In addition to that, we can observe that the values of β2-m also ranged from undetectable values (0 µg/ml) to (15.65 µg/ml).

Table (2): Serum level of IL-2 and β 2 -microglobulin in patients with MM.

Groups	Mean ± SD	Minimum	Maximum
IL-2	3.89 ± 3.65	0	15.12
β 2 Microglobulin	5.13± 3.53	0	15.65
r = - 0.123 , P = 0.401 (not significant)			

- ***Level of serum IL-2 in patients with multiple myeloma before and after treatment.***

  Table (3) shows that no significant differences were observed in the mean (3.4 ±3.4 SD vs 4.82± 4 SD) concentration of IL-2 in patients with multiple myeloma who received treatment, when compared with those who did not take the treatment.

Table (3): Concentration of IL-2 in patients before and after treatment.

Groups	Number	Serum level of IL-2 (U/ml) in patients before and after treatment.			P value
		Mean ± SD	Minimum	Maximum	0.197 (not significant)
Before	17	4.82 ± 4	0	13.66	
After	32	3.4 ±3.4	0	15.12	

- ***Level of IL-2 and IgG in sera patients with MM***

  Table (4) shows that although the levels of IgG, as compared with IL-2 (1485.9±1113.03 S.D vs.. 3.89±3.65 SD), in the sera of patients with MM increased, the correlation between IL-2 and IgG levels in MM patients was not significant (r =.016 , p> 0.05).Furthermore we can see that the values of IgG also ranged from undetectable values (0 µg/ml) to (3218 µg/ml)

Table (4): Concentration of IgG and IL-2 in patients with MM

Group	Number	Mean ± SD	Minimum	Maximum
IL2	49	3.89±3.65	0	15.12
IgG	49	1485.9±1113.03	0	3218
r = .016 ,P = 0.914 (not significant)				

- ***The correlation between IL-2 concentration in sera of patients with MM and the age of patients.***

As shown in figure (3-2A), no correlation was found between IL-2 concentration in patient's sera and the age of patients (r = - 0.118, P = -0.225).

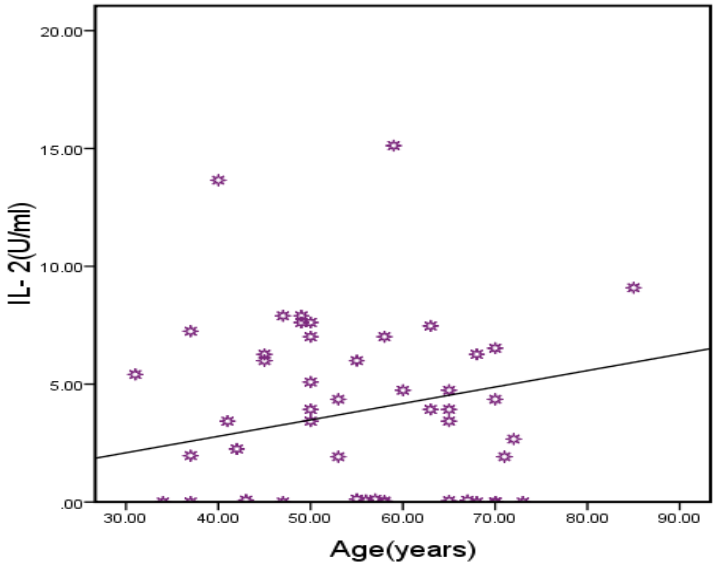

Figure 1: The correlation between the age and the concentration of IL-2 in the sera of patients with MM

- ***The gender and IL-2 concentration in the sera of patients with MM.***

Table (5) shows that no significant differences were observed in the mean (3.51±3.71 SD vs 4.21± 3.63 SD) concentration of IL-2 in the female group of patients with multiple myeloma, when compared with that male group of patients with MM. Figure (3-2B) shows that there was no evidence of a statistical correlation between IL-2 in the sera of patients and the gender (r = 0.096 ; P = 0.513).

Table (5): Distribution of IL-2 concentration in male and female patients with MM.

Group	Gender	Number	Mean ± SD	Minimum	Maximum
IL2	**Female**	22	3.51±3.71	0	13.66
	Male	27	4.21± 3.63	0	15.12
P value > 0.05 (not significant)					

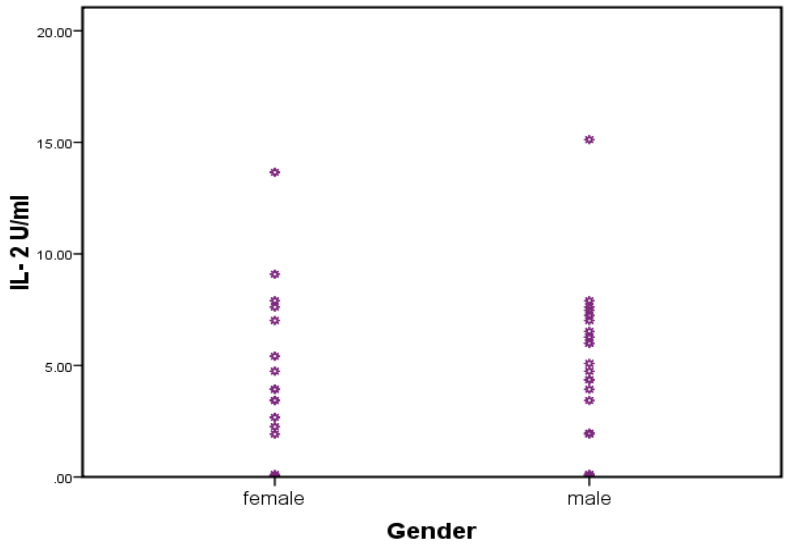

Figure 2: The correlation between the gender and the concentration of IL-2 in the sera of patients with MM.

Discussion

Multiple myeloma patients showed a significant decline in the levels of IL-2, as compared with the control group (see Table 1). These results are in accordance with previous findings reported by **(21),** which indicated that IL-2 was found to decrease significantly in patients with stage III MM when compared to stages I and II and healthy individuals. Other studies confirmed that the lowest serum IL-2 values were found in patients with active multiple myeloma **(22)**. However, releasing of IL-2 from T helper lymphocytes promotes the generation, proliferation, and differentiation of T lymphocytes,enhances the activity of natural killer cells,induces the generation of lymphocyte-activated killer cells,and promotes the production of antibodies by B lymphocytes. Through these mechanisms, it plays an important role in anti-tumor immune responses **(23)**.

The current study unexpectedly demonstrated that,although serum β2- m and IL-2 levels were both associated with MM disease, they were not correlated with each other (Table 2). The respective biological origins of serum IL-2 and β2-m may account for this discrepancy. However these results were in disagreement with those suggesting that IL-2 was found to be the major inducer of β2-m and soluble interleukin-2 receptor (sIL-2R) directly, or via mitogen-induced IL-2 production**(24)**. On the other hand it is in agreement with other studies that confirmed that collectively high-risk patients with MM were associated with high level of β-2-microglobulin **(25); (26).** Other studies indicated that IL-2 has anti-tumor activity **(2)**.

Interleukin-2 level was measured in two groups of MM patients: the first group (including 17 persons) represents patients at the stage of the early diagnosis and before conducting the treatment course. The second group (including 32 persons) represents the patients at the stage after taking the treatment (table:3). This investigation shows that no significant differences were observed between patients of the two groups. This result was compatible to previous findings: (**27**). found no alteration in serum IL-2 and sIL-2R levels in patients with stages I and II MM as compared to normal individuals.

The present study (Table 4) demonstrated that in spite of the high level of IgG in the sera of patients in comparison with levels of IL-2, there was no correlation between these two groups .Such findings might not be expected because the previous studies stated that IL-2 could have up-regulated CD8 cell function and thus depressed immunoglobulin production by CD19 cells **(28).** However, the low level of IL-2 and high level of IgG in the present study indicated that the disease is very severe because augmented levels of IL-2 in the MM microenvironment can potentiate T-cell and NK cell proliferations, while an increment in their potency seems to be attributable to both enhanced phagocytic function and antibody-dependent cell-mediated cytotoxicity**(29).**

In the present study we found no evidence to suggest an age-related decrease in circulating IL-2 (see figure 1). This result differs from previous findings of many studies such as **(30)**, which mentioned that aging is associated with reduced T cell function, as demonstrated by decreased T-cell proliferation and IL-2 production. This difference can be attributed to the variation in population structure between Iraq and Western countries. Elderly individuals in Western countries live longer than those in Iraq **(31)** cited in **(32)**.The difference in sample size may play a role in this difference, as well.

The current study indicated that there was no effect of gender on level of IL-2 (Table: 5) and there was no correlation between IL-2 and gender (Figure 2). Although this finding was compatible with studies done by **(33)**,which stated that there were no

significant differences for the cytokine (IFN-γ, interleukin (IL)-2, TNF-α, IL-4,IL-10 and IL-13) production between male and female multiple sclerosis subjects. These results, however, differ from other previous studies which indicated that in males, as compared with females, greater Th2 and less Th1 cytokine production had been observed **(34)**.

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