The role of TNF-α in the pathogenesis of multiple myeloma "a study in Iraqi patients"

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Abstract:

During recent years, there has been an increasing interest in the investigation of the cytokines roles in pathogenesis of cancer, thus the study aimed at evaluating the level of tumor necrosis factor-alpha(TNF-α) in sera of Iraqi multiple myeloma (MM) patients. Beta 2-microglobulion (β2-m) was assessed to determine if there was any association between this cytokine and the level of β2- m, as the latter is related to the stage of the disease. In addition, the age and gender were also taken into consideration. Furthermore, we investigated the relationship between IgG and TNF-α in sera of patients. 49 Iraqi patients (27 males and 22 females).The patients were also 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. A further group was also investigated which included (12) apparently healthy individuals (9 males and 3 females), who were regarded as a control group. Serum TNF-α and β2- m were determined using enzyme-linked immunosorbent assay (ELISA).while the concentration of IgG was measured by radial immune diffusion plates

The study reached to the following results: TNF-α levels were not significantly elevated in the patients with MM compared to control group (5.98 ± 8.47 SD vs. 4.85 ± 12.1 SD) and no significant differences (P > 0.05) were observed in the mean (6.02 ±8.1SD vs 5.9 ±9.4SD) concentration of TNF-α in patients with MM who received treatment, when compared with those who did not take the treatment .In addition, there are positive significant correlations between TNF-α and β 2 Microglobulin (r = 0.316, P = 0 .027), and no relationship between IgG and TNF-α (r = - 0.032, P = 0.829).Furthermore, the study observed that, there was no correlation between TNF-α on the one hand and factors of age and gender on the other hand.

Key words: TNF-α ;Pathogenesis ; Multiple myeloma; Iraqi patients

Introduction:

Multiple myeloma (MM) is a neoplasm of post–germinal center, terminally differentiated B cells. It is characterized by a multifocal proliferation of clonal, long-lived plasma cells within the bone marrow (BM) and associated skeletal destruction, serum monoclonal gammopathy, immune suppression, and end organ sequelae. Recent studies have defined the importance of interactions between the MM cells and their BM microenvironment, dysregulation in signaling pathways and in a specialized subpopulation of cells within the tumor (termed myeloma cancer stem cells) for tumor cell growth and survival, and the development of resistance to therapy (1).Pathophysiology of multiple

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myeloma is still poorly understood and its cause remains unknown (2). Many scientific reports suggest that, Several cytokines have been implicated in the pathogenesis of MM. Interleukin-6 (IL-6) is considered the major growth and antiapoptotic factor for myeloma cells. (3) .However, other cytokines may substitute for IL-6 as a growth factor in vitro, including tumor necrosis factor (TNF),IL-10, insulinlike growth factor-1 (IGF-1), interferon alpha, andIL-15 (4,5,6).Freshly isolated cells from patients with MM generally grow poorly in vitro despite the presence of known growth promoting factors. This may imply that myeloma cells depend on still unknown growth factors in vivo. Finding these factors is important for identification of new therapeutic targets (7). However, although there are many studies shows that TNFin combination with IL-4 has been implicated in myeloma precursor cell differentiation. as well as , Iit is able to trigger the secretion of IL- 6 from BMSc, which constitutes a major growth factor for the tumor cells (8,9) in addition to other roles in patients with MM ,but there is no Iraqi study investigate the role of this cytokine in MM patients ,therefore this study aim to quantitative TNF-α in the sera of group of Iraqi patients with MM.

Material and Methods:

A total of 49 Iraqi patients with MM (27 male, 22 female; age (mean ± SD) = 55.83 ±12.25 years (ranging from 31 to 85 years) were enrolled in the study group.These patients were suffered 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 . The control group comprised apparently healthy individual (9 male, 3 female; age (mean ± SD) = 49.17± 4.49 (mean ±SD) years, (range 40 to 55).Serum levels of TNF-α were evaluated using an ELISA kit (DRG company, USA) designed to measure human TNF-α in serum. Blood samples were collected from each studied subject; approximately 5 mL of blood were placed into a dry clean plain tube and left to clot at room temperature, then separated by centrifugation for 15 minutes , The serum was removed and stored at −20°C until required. Repeated freeze–thaw cycles were avoided to prevent loss of bioactive substances. 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 also 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 (10).

Results:

1. Patients with multiple myeloma versus Controls

Table (1) shows that the level of TNF-α (pg/ml) in the patients group ranged from undetectable values (0 pg/ml) to (33.65 pg/ml), while the values of this cytokine in the sera of the control group ranged from undetectable values (0 pg/ml) to (41.36 pg /ml). The table also displays that

although the levels of TNF-α in the sera of patients with MM showed a slightly increased mean in comparison with the concentration level of TNF-α in the control group (5.98 ± 8.47 SD vs. 4.85 ± 12.1 SD) , these increments in the mean were not statistically significant (p > 0.05).

Table 1: Concentration of TNF-α (pg/ml) in patients with MM and control

Groups	Number	Serum level of TNF -α (pg/ml)			P ≤
		Mean ± SD	Minimum	Maximum	
Patients	49	5.98 ± 8.47	0	33.65	0.704
Controls	12	4.85 ± 12.1	0	41.36	

2. Level of TNF-α and β 2-microglobulin in patients with multiple myeloma.

Table (2) shows the mean (5.98 ± 8.47SD) concentrations of TNF-α secreted in the sera of patients with MM in comparison with the mean (5.13± 3.53SD) concentration of β 2 Microglobulin to the same patient group. In addition, there are positive significant correlations between TNF-α and β 2 Microglobulin in the sera of patients with MM (r = 0.316, P = 0 .027).

Table (2): Serum level of TNF-α (pg/ml) and β 2 Microglobulin (µg/ml) in patients with MM.

Immunological parameter	Mean ± SD	Minimum	Maximum
TNF-α(pg/ml)	5.98 ± 8.47	0	33.65
β 2 Microglobulin(µg/ml)	5.13± 3.53	0	15.65
r = 0.316, P = 0 .027 (significant)			

3. Level of serum TNF-α in patients with multiple myeloma before and after treatment.

Table (3) shows that no significant differences (P > 0.05) were observed in the mean (6.02 ±8.1SD vs 5.9 ±9.4SD) concentration of TNF-α in patients with multiple myeloma who received treatment, when compared with those who did not take the treatment.

Table (3):Concentration of TNF -α(pg/ml) in patients before and after treatment

Group	Number	Serum level of TNF -α (pg/ml) in patients before and after treatment.			P ≤
		Mean ± SD	Minimum	Maximum	
Before	17	5.9 ±9.4	0	27.52	0.965
After	32	6.02±8.1	0	33.65	

4.Level of TNF -α and Immunoglobulin concentration (IgG) in sera of patients with MM

Table (4) shows that although the levels of IgG as compared with TNF –α (1485.9±1113.03 S.D vs 5.98±8.5 SD) in the sera of patients with MM increased, there is no correlation between the two groups (r = - 0.032, P = 0.829) in the presence of these increments.

Table (4): Correlation between of IgG (µg/ml) and TNF -α (pg/ml) in sera of patients with MM

Immunological parameter	Number	Mean ± SD
TNF -α(pg/ml)	49	5.98±8.5
IgG(µg/ml)	49	1485.9±1113
r = - 0.032, P = 0.829		

5.The correlation between TNF -α concentration in sera of the patients with MM and the age.

As shown in figure (1) there is no significant correlation of TNF -αwith age (r = 0.243, P = 0.092).

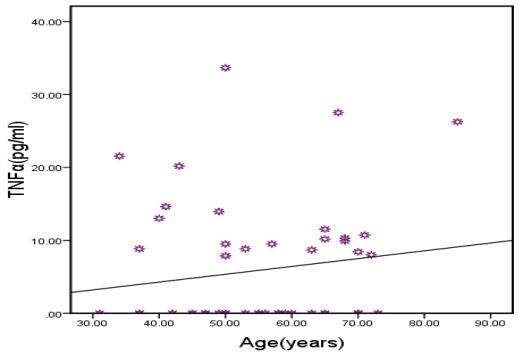

Fig. 1: Scatter plot displays no correlation between the age and the concentration of TNF-α in the sera of patients with MM

6. The gender and TNF-α concentration in sera of the patients with MM.

Table (6) shows,that no significant differences were observed in the mean (6.87 ± 9.67 SD vs 5.26 ± 7.47 SD) concentration of TNF-α in female patients with MM, when compared with the male ones. Figure (2) shows that there was no evidence of a statistical correlation between TNF-α in the sera of patients and the gender (r = - 0.095, P= 0.514).

Table (6): Distribution of TNF-α (pg/ml) concentration in male and female patients with multiple myeloma

Immunological parameter	Gender	Number	Mean ± SD	Minimum	Maximum
TNFα (pg/ml)	Female	22	6.87 ± 9.67	0	33.65
	Male	27	5.26 ± 7.47	0	27.52
P > 0.05 (not significant)					

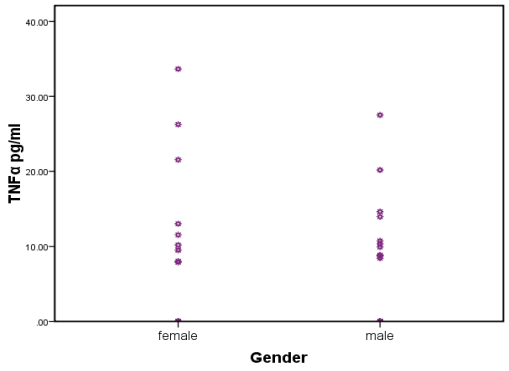

Fig. 2: Scatter plot displays no correlation between the gender and the concentration of TNF-α in the sera of patients with MM

Discussion:

TNF-α represents the last cytokine subjected to study in this research. Although TNF is a necessary growth factor for the expansion and maintenance of MM cells (11), its role in pathogenesis of malignant tumors is not clearly understood Depending on these contrasting views, the levels of TNF-α in patients with MM have been measured and compared with those of the control group. The present study showed that there was no difference in the level of this cytokine between patients with MM and control group. This outcome came identical with that found by (12), who stated that TNF-α levels in patients with MM were similar to those detected in the controls. They were in agreement with what was confirmed by (13) who mentioned that serum TNF-α levels of patients with MM did not differ from those found in healthy control subjects. It is worth mentioning that in order to confirm practically the evaluation of TNF-α in patients with MM, a larger group for study is needed.

The current study also demonstrated that there was positive correlation between β2-m and the level of TNF-α in the sera of MM patients. This finding came identical with what was found by Jurisićand Colović (14) , who studied the TNF-α value which was compared with the serum levels of β2-m,Lactate dehydrogenase, the percentage of plasma cells in the bone marrow , fibrinogen and sedimentation rate in patients with MM .They observed significant positive correlations between TNF-alpha and these values.

Although TNF-α is an important factor in the promotion of the growth and survival of the malignant cells, studies have shown that an elevated levels of TNF-α in myeloma patients correlated with aggressive disease(15).Moreover, the current

study confirmed that TNF-α level in the sera of MM patients, who were newly diagnosed but did not receive any therapy yet, did not differ significantly from those in MM patients who were subjected to treatment . This finding can be attributed to the fact that TNF-α has a bimodal role in cancer(16). It may serve as either apoptotic or a survival signal depending on the cell type and the state of activation of the cell. These two apposite findings arise from the selective activation of different signal transduction pathways **.** (17,18,19)

The above researches may lead us to conclud that TNF-α is an undependable marker for the follow up of treatment regimens on curative response in any type of therapy. That is the reason why this study could not elicit any difference in the level of TNF-α before and after treatment.

The circulatory levels of immunoglobulin and TNF-α were analyzed and no significant correlation was found between them in patients with MM . Although no previous study investigated the relationship between them and the results of this study give a non-significant correlation, the biological origin of serum TNF-α and immunoglobulin (IgG) may account for this result.

The binding of MM cells to marrow stromal cells through vascular cell adhesion molecule-1 (VCAM-1) on stromal cells and α_4 β_1 integrin on MM cells, (20,21) results in increased production of TNF- α, receptor activator of NF-κB ligand (RANKL), and interleukin-6 (IL-6) by marrow stromal cells (22,23).These factors, in turn, increase both osteoclast (OCL) formation and the growth of MM cells**,** (24) **.**Immunoglobulin was produced by malignant plasma cells which accumulate in bone marrow and produce immunoglobulin fragments or immunoglobulin, usually monoclonal IgG or IgA (25,26).

Age is an important prognostic factor, which critically influences treatment options, such as high-dose therapy (27).Thus, we wanted to analyze whether the TNF-α production was affected by patients' age or not. The result of this investigation gave no correlation between these two factors .This finding is combatable with previous reports which confirmed that there was no association between production of TNF-α and age and that there was no difference in the production of TNF-α between the young and elderly groups(28).It is also in agreement with what was found by (29) who confirmed that reduced TNF-α levels were not influenced by age.

Although the level of TNF-α in female patient group is higher than that of the males in this study , there were no statistically significant differences between them ($p>0.05$) there was no significant association between TNF-α and gender, either . This means that the TNF-α could not be affected by gender .This result is in agreement with (30),who stated that no difference in TNF-α level was found between males and females, it is also in agreement with what was found by Bertin(31) ,who confirmed that the plasma TNF-α level was similar in men and women and is not related to age in type 2 diabetic patients. In addition to that, this result was acceptable by Sewell *et al*. (29), who also mentioned that TNF-α was not affected by gender.

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دورعامل نخر الورم الفا (TNF-α) في إمراضية الورم النخاعي المتعدد " دراسة لمرضى عراقيين "

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الخلاصة:
تزايد الأهتمام خلال السَنَوات الأخيرة، في التحرّي عن أدوارِ الحركيات الخلوية في تولّد مرض السرطان ومنشأه ، وهكذا إستهدفتْ الدراسة الحالية تَقييم مستوى عاملِ نخرِالورمِ الفا (TNF-α) في مصول مجموعة من مرضى الورم النخاعي المتعدد في المرضى العراقيين. كما تمّ تقييم مستوى β2-m والتحري عن العلاقة بين عامل نخر الورم الفا TNF- α وβ2-m لإرتباط الأخير بمرحلة المرض.إضافة الى ذلك أُخذت الدراسة بنظر الاعتبار عاملي العمر والجنس.فضلا عن ذلك تمّ التحري عن العلاقة بين تركيز عامل نخر الورم الفا TNF-α من جانب والغلوبيولين المناعي من نوع (IgG) من جانب آخر.شملت الدراسة 49 مريض (27 ذكر و22 انثى)، هؤلاء المرضى قسموا الى مجموعتين تضمنت المجموعة الاولى (17 مريض) من المرضى المشخصين حديثا والذين لم يتلقوا اي علاج في وقت جمع العينات ، في حين تضمنت المجموعة الثانية (32 مريضاً) كانوا خاضعين للعلاج. ايضاً تضمنت الدراسة مجموعة اخرى وهي المجموعة الضابطة والتي تشمل 12 شخص من الاصحاء(9 من الذكور و3 من الاناث).تمَّ قياس مستوى عامل نخر الورم الفا TNF- α و-β2 mبأستخدام تقنية المُمْتَزِّ المناعي المُرْتَبِطِ بالانزيم ELISAفي حين استخدمت طريقة اطباق الانتشار المناعي الشعاعي لقياس تركيز الغلوبيولين المناعي نوع (IgG).توصلت الدراسةالى النتائج التالية :
لايوجد اختلاف في مستوى تركيز عامل نخر الورم الفاTNF- α بين مجموعة المرضى والمجموعة الضابطة (8.47 ±5.98 مقابل 4.85 ±12.1 بيكو غرام \ مليلتر) ،كما لايوجد اختلاف ذو دلالة معنوية بين مجموعة المرضى اللذين تلقوا علاج مقابل مجموعة المرضى المشخصين حديثاً (6.02 ± 8.1 مقابل 4.85 ± 12.1).أضافة الى ذلك لوحظ وجود علاقة معنوية موجبة بين عامل نخر الورم الفاTNF- α و β2-m حيث كان معامل الارتباط (r =0.316 ، P =0.027) كما توصلت الدراسة الى عدم وجود علاقة بين عامل نخر الورم الفاTNF- α و الغلوبيولين المناعي من نوع (IgG) حيث كان معامل الارتباط (r = - 0.032، P = 0.829) ،أخيراً أثبتت الدراسة عدم وجود علاقة معنوية بين انتاج TNF- α من جهة وعاملي العمر والجنس من جهة اخرى كما لايوجد تأثير للجنس على انتاج هذا النوع من الحركيات الخلوية.