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Effect of the Biological Drug Etanercept on Tumor necrosis factor-α Levels in Psoriatic Patients

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Abstract

Psoriasis is a common, chronic, immune mediated disorder.The disease is arising as a result of dysregulated interactions of the innate and adaptive immune system in the context of skin epithelium and connective tissue. The biological drug Etanercept(ETN) approved for use in treated psoriasis. ETN is tumor necrosis factor- α (TNF-α) inhibitor. In this study, 48 psoriatic patients were taken before and after treatment who attended to the Dermatology and Venereology Department in Baghdad Teaching Hospital during the period from December 2016 to September 2017and 50 samples were used as healthy control group. The results showed that most psoriatic patients 52.08 % were within the second and third decades 20-35 year, and the majority of psoriatic patients were males 62.5% and the ratio of male to female is 1.67:1. Moreover, the results demonstrated that the males were more expected psoriasis compare with females. Blood samples were collected and TNF- α was estimated in sera of all subjects by using Enzyme Linked Immunosorbent Assay (ELISA). The TNF- α mean levels in psoriatic patients before treatment was 189.5±26.0 ng/ml, and after treatment was 223.6±41.1 ng/ml compar with the healthy control group 93.5±2.4 ng/ml. The results showed significant differences between the studied groups.

Keywords: Etanercept, Tumor necrosis factor-α, Psoriaticpatients.

تأثير العلاج البايولوجي الايتانرسيبت على مستوى عامل التنخر الورمي في مرضى داء الصدفية

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الخلاصة

الصدفية هو من الامراض الشائعة والمزمنة والذي يحدث نتيجة للاختلالات المناعية. ينشأ هذا المرض نتيجة للاضطرابات الوظيفية بالمناعة النوعية واللانوعية قي الجهاز المناعي بالاقتران مع النسيج الظهاري والنسيج الضام في الجلد. العلاج البايولوجي إيتانرسبت ملائم لعلاج الصدفية. وهو مثبط لعامل التنخر الورمي. في هذه الدراسة، تم أخذ ٤٨ مريضا بداء الصدفية قبل وبعد العلاج و ٥٠ عينة كعوامل سيطرة. سجلت النتائج أن معظم مرضى الصدفية ٥٢.٠٨٪ كانوا ضمن العقدين الثاني والثالث ٢٠–٣٥ سنة، وكان غالبية المرضى من الذكور ٦٢.٥٪ ، حيث ان نسبة الذكور إلى الإناث كانت ١.٦٧: ١. أن أكثرية المرضى كانوا من الذكور مقارنة بالاناث. جمعت عينات الدم و قدر عامل التنخر الورمي في مصول جميع المرضى باستخدام تقنية الاليزا. كان معدل مستوى التنخر الورمي في مرضى الصدفية قبل العلاج ١٨٩.٥ ± ٢٦.٠

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نانوجرام / مل، وبعد العلاج كان ٢٢٣.٦ ± ٤١.١ نانوجرام / مل مقارنة مع مجموعة السيطرة ٩٣.٥ ± ٢.٤ نانوجرام / مل، أظهرت النتائج فروق معنوية بين المجاميع قيد الدراسة.

Introduction

Psoriasis is a chronic and wide spread all over the world that mediated the immune response in the skin with systemic pro-inflammatory activation. The environmental and genetic factors are responsible for its pathogenesis[1, 2]. In the second-to-fourth decade of life, the disease begins to appear, the rate of incidence is equal for women and men[3]. Environmental and genetic factors, play an remarkable role in the spread of psoriasis from one region to another[4]. Throughout the world, a systematic review found that predominance of psoriasis ranged from 0.5 to 11.4 percent in adults and 0 to 1.4 percent in juvenile psoriasis[5]. It is characterized by the presence of sharply demarcated, red plaques with adherent silvery-white scales and a tendency for symmetrical distribution over the body [6]. It is actually caused by a combination of both a primary defect in keratinocytes and an inappropriate innate and adaptive immune response– driven type I interferon (IFN- α) and that it is mediated mainly by resident and infiltrating T cells [7]. The cause of the loss of control of keratinocyte turnover is unknown. However, environmental, genetic, and immunologic factors appear to play a role [8]. Psoriasis is arises as a result of dysregulated interactions of the innate and adaptive immune system in the context of skin epithelium and connective tissue [9]. Cytokines, including Th1-related [Tumor Necrosis Factor - alpha (TNF- α)], interferon gamma (IFN-γ), interleukin- (IL-2) and Th17-related (IL-17A, IL-17F, IL-22, IL-26, and TNF-α) proteins, together with IL-23, IL-20, and IL-15 were increased in the sera of psoriatic patients [10]. Individuals with active skin disease have elevated levels of tumor necrosis factor alpha (TNF-α) in both blood and lesional skin [11]. TNF-α is a potent pro-inflammatory cytokine exerting pleiotropic effects on various cell types and plays a critical role in the pathogenesis of chronic inflammatory diseases, such as psoriasis. This cytokine is produced by numerous cell types, including immune cells (B cells and T cells, basophils, eosinophils, dendritic cells, natural killer cells, neutrophils and mast cells), nonimmune cells (astrocytes, fibroblasts, glial cells, granuloma cells and keratinocytes) and many kinds of tumor cells. The biological activity of TNF-αis triggered by binding to one of two structurally distinct receptors: TNF receptor type I (TNFRI [or p55]) and TNF receptor type II (TNFRII [or p75]) [12]. TNFRI and TNFRII are present in all cell types except erythrocytes. Upon binding to TNF receptors, bothtransmembrane and soluble TNF- α mediate pleiotropic effects (apoptosis, cell proliferation and cytokine production) [13]. TNF-α, which is secreted by both T cells and antigen-presenting cells within lesional skin, has emerged as a key mediator in the disease process. Specifically, TNF-α is a pro-inflammatory cytokine that amplifies inflammation through several distinct pathways: facilitating entry of inflammatory cells into lesional skin through induction of adhesion molecules on vascular endothelial cells; stimulating keratinocyte production of other pro-inflammatory mediators [14]; and finally activating dermal macrophages and dendritic cells. Recently, the efficacy of TNF-α inhibitors in treating psoriasis has been attributed to their inhibition of Th17 T cells [11].Etanercept (ETN) (Enbrel trade name) was the first TNF-α inhibitor to be approved for use in Psoriasis. ETN is a dimeric, soluble fusion protein consisting of the extracellular ligand binding portion of the TNF receptor linked to the Fc portion of human IgG1. It is capable of binding and neutralizing soluble TNF and transmembrane TNF[15]. It is a soluble tumor necrosis factor receptor fusion protein that reversibly binds to tumor necrosis factor, [16], furthermore, it alters neutrophil migration, dendritic cell and T-cell maturation and migration, thus decreasing the local and systemic production of pro-inflammatory cytokines and their subsequent effects [17]. This study aimed to investigate the role of biological drug (Etanercept) ETN on psoriasis disease activity by evaluating TNF-α cytokine before and after treatment in patients with different ages and sexes.

Materials and methods:

A total of 48 Psoriatic patients that must be suffering from sever to moderate psoriasis disease were included in the present study, who attended to the Dermatology and Venereology Department in Baghdad Teaching Hospital during the period from December 2016 to September 2017. These patients stopped their response to all other treatments so they were diverted to take biological therapy such as Etanercept (ETN).

Blood sampling

Five milliliters of blood were collected by venipuncture from all patients and control groups. Each collected blood sample was placed in the tubes and then centrifuge was used to obtain serum for immunological measurements.Tumor Necrosis Factor alpha (TNF-α) Human ELISA Kit, Demeditec, Germany.

Statistical analysis system (SAS) program was used for data analysis. Person Chi-square -$\chi 2$ test, and mean ± SE, ANOVA Table by using computer program IBM SPSS version [18] P value <0.05 was considered statistically significant.

Results and Discussion

Demographical distribution of the studied groups according to the age is summarized in Table-(1.1). The results clarified that the age was ranged between 20-60 years and the mean age for psoriasis was 42.8±2.0. The results recorded that most psoriasis patients (52.08 %) were within the second and third decades (20-35) year, while the lowest percentages were in (51-65) year.

Table 1.1-The percentage distribution of the studied groups according to the age:

Studied group		Age range (years)			Total	Mean age (years)±SEM
		20-35	36-50	51-65		
Psoriasis patients	N	25	14	9	48	42.8±2.0
	%	52.08%	29.17%	18.75%	100	
Healthy control	N	16	23	11	50	36.6±2.2
	%	32%	46%	11%	100	

This results were in agreement with [19, 20], who indicated that the age of psoriatic patients was within the second and third decades. The psoriasis disease can occur at any age, and its prevalence increase with age and its peak appeared between the second and third decades usually [21]. Geographic location influences the likelihood of having psoriasis; disease prevalence tends to increase with increasing distance from the equator. A systematic worldwide review found the prevalence of psoriasis ranged from 0.5 to 11.4 percent in adults and 0 to 1.4 percent in children [5].

Table 1.2-The percentage distribution of the studied groups according to the gender

Studied group		Gender		Total	M/F Ratio
		Female	Male		
Psoriasis patients	N	18	30	48	1.67:1
	%	37.5%	62.5%	100	
Healthy control	N	34	16	50	0.43:1
	%	68%	32%	100	

Distribution of studied groups according to their gender showed that the majority of psoriasis patients (moderate or severe) were males (62.5%) with males to females ratio of (1.67:1) Tables-(1.2). It seems that males preponderance among psoriasis patients in comparison females.

This result was higher than previously results by Al-Mokhtar et al., (2017) and Sharquie(Sharquie, 2017) [22, 23] who mentioned that the prevalence of psoriasis among males to females were equal. Other studies also shown that equal incidence of psoriasis in both sexes [24, 20].

In the present study, the high frequency of psoriasis attack was among males rather than females, this may be due to the hormonal differences between them [25, 26], and in turn, their effect on immune response [27], consequently males tend to provoke more T-helper cells which have a pro-inflammatory role in inducing or development of psoriasis to become severe incidence [28].

As demonstrated in the figure below, the results documented that serum TNF-α levels had been elevated significantly (P<0.01) in sera of psoriatic patients (before treatment) which was 189.5±26.0 ng/ml in comparison with healthy control group 93.5±2.4 ng/ml, also the results showed a significant difference (P<0.05) between psoriatic patients before and after treatment 189.5±26.0 ng/ml and 223.6±41.1 ng/ml, respectively, Figure-2.Results were calculated by drawing a standard curve. Plotting on the horizontal axis the TNF-α concentration of the standard, and on the vertical axis the corresponding absorbance. The average absorbance for each sample was located on the vertical axis and the corresponding TNF-α concentration of the standards, and on the vertical axis was read as shown in Figure-1.

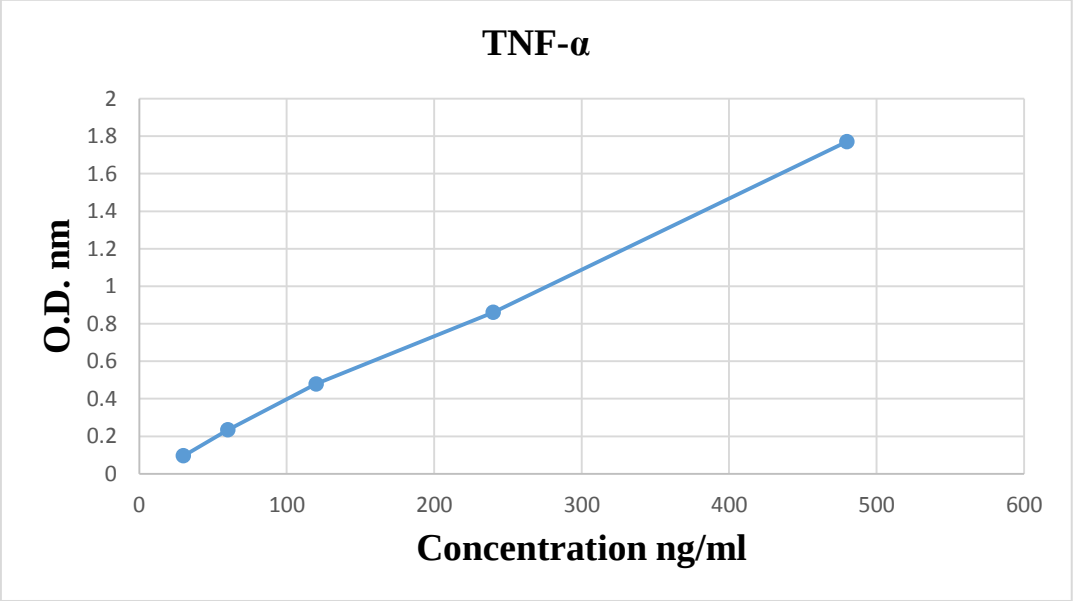

Figure 1-Standard curve of total TNF-α concentration

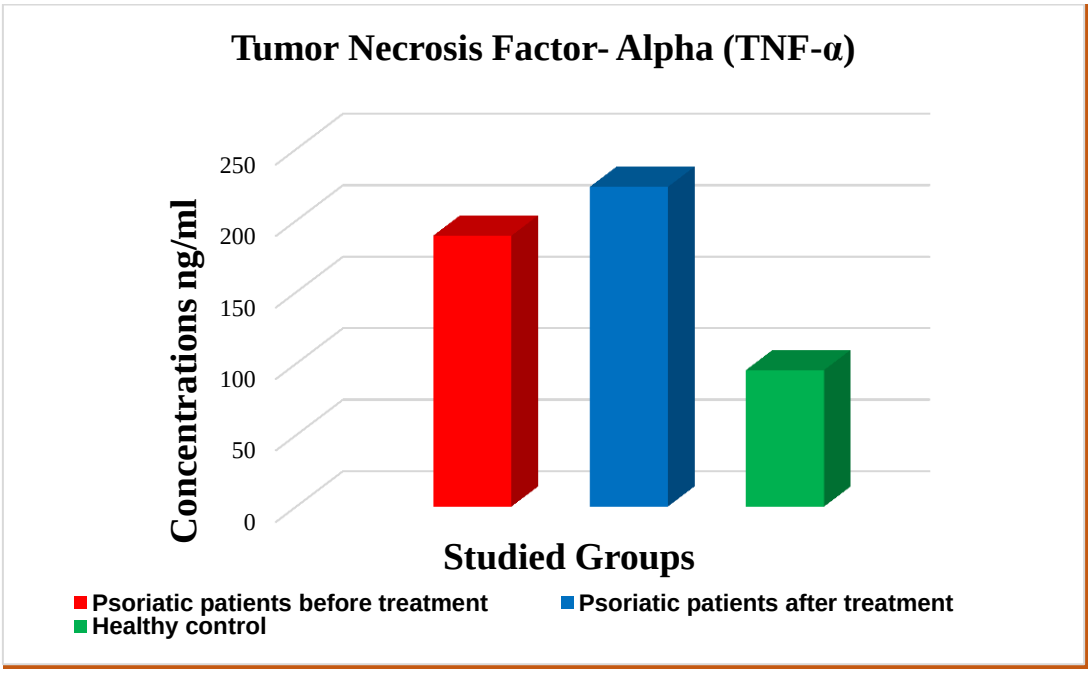

Figure 2- Mean levels of TNF-α in psoriatic patients before and After treatment in comparison with healthy control group

The current study showed that the serum levels of TNF-α in psoriatic patients were significantly increased comparing with healthy control group. In line with this results, most studies have reported that the serum levels of TNF-α are significantly increased in patients with psoriasis compared with those of healthy controls [29, 11, 30-33]. These data confirmed by recent studies which denoted that TNF-α has pivotal role in the pathogenesis of psoriasis [34, 14, 35]. While, Tigalonova *et al*. [36] and Jacob *et al* [37] have found that the serum levels of TNF-α do not significantly differ between psoriatic patients and controls.

Monocytes and macrophages are the main cells related to the production of TNF-α, but other immune cells are also capable of synthesizing it such as NK cells, basophils, eosinophils, neutrophils and T and B lymphocytes, as well as other nonimmune cells - astrocytes, glial cells, neurons, osteoblasts, melanocytes, smooth muscle cells, and spermatogenic and tumoral cells [38]. Constitutionally or by stimulation, it can be produced by almost all cells of the skin, such as keratinocytes, Langerhans cells and other dendritic cells, activated T cells, macrophages, fibroblasts and endothelial cells [39]. So, it can be secreted by both T cells and antigen-presenting cells within lesional skin of psoriasis. Specifically, TNF-α is a pro-inflammatory cytokine that amplifies inflammation through several distinct pathways: facilitating entry of inflammatory cells into lesional skin through induction of adhesion molecules on vascular endothelial cells; stimulating keratinocyte production of other pro-inflammatory mediators [14]. When these cells activation then it will increase the secretion of other cytokines especially TNF-α [40].

The exact etiology of psoriasis remains unclear, current evidence indicates that it is T-cell driven. Individuals with active skin disease have elevated levels of TNF-α in both blood and lesional skin. Based on these findings the serum TNF-α concentrations may be altered by several processes like the production, tissue deposition, degradation, and elimination of these molecules [11]. The origin of circulating TNF-α in blood serum in psoriatic patients is still not completely clear. Huge amounts of free cytokines are required, to achieve the cytokine concentration that can induce biological responses at distant skin lesions [28]. Probably, there are sources other than the skin that contribute to the production of these cytokines; this theory may provide a potential mechanism linking psoriasis with its extra-cutaneous comorbidities [41].

Also the levels of TNF-α had interestingly elevated in psoriatic patients treated with ETN comparing with the concentration of TNF-α to patients before treatment Figures-(3, 2). These data confirmed by recent studies which revealed that ETN increase serum TNF-α levels by ELISA method [42- 44].

The biological activity of TNF-α is triggered by binding to one of two structurally distinct receptors: TNF-α is receptor type I (TNFRI [or p55 or CD120a]) and TNF-α is receptor type II (TNFRII [or p75 or CD120b]) [45, 12]. TNFRI and TNFRII are present in all cell types except erythrocytes. Upon binding to its receptors, both transmembrane and soluble TNF-α mediates pleiotropic effects (apoptosis, cell proliferation and cytokine production). Three anti- TNF-α agents, Infliximab (INF), Adalimumab (ADA) and ETN are approved worldwide for the treatment of psoriasis. INF and ADA are anti- TNF-α, they are monoclonal antibodies. INF is a human-murine chimeric monoclonal antibody with a constant human region (Fc) and a variable mouse region, while ADA is a fully human IgG1 monoclonal anti- TNF-α antibody. Both have two binding sites for TNF-α and present high specificity, affinity and avidity for the cytokine [45, 13]. ETN is composed of the extracellular portion of two human TNFRII linked to a Fc portion (CH2 and CH3 domains) of human IgG1. ETN is supposed to form 1:1 complex with the TNF-α is trimer. INF and ADA form stable complexes with TNF- α, while ETN forms relatively unstable complexes[45]. ETN binds free TNF-α and weakly inhibits TNF-α trimers *in vivo*[46].

The TNF-α is -producing cells temporarily express TNF-α is in their plasma membranes (tTNF) [45, 13]. INF, ADA and ETN bind to transmembrane TNF-α is with similar affinities that are lower (weaker) than for soluble TNF-α [45]. Since INF and ADA are IgG1 antibodies, binding to tTNF, they are capable of complement fixation and also can produce the destruction of the TNF-α is –bearing cell by antibody dependent cell cytotoxicity (ADCC) [45, 13]. ETN possess the Fc portion of IgG1 that can induce ADCC, but it does not carry the CH1 domain of IgG1 which is important for the activation of C3. Thus, differential clinical efficacies of anti- TNF-α is agents may be explained by their different action on transmembrane TNF-α-bearing cells [45].

In the present study, high serum concentrations of TNF-α in psoriatic patients after treatment result from the accumulation of TNF-α in serum patients due to the role of ETN drug in the blocking TNF-α receptors by the ETN drug. Inhibition of its action by ETN reduces the inflammatory response which is especially useful for treating autoimmune diseases. ETN mimics the inhibitory effects of naturally occurring soluble TNF-α receptors, the difference being that ETN, because it is a fusion protein rather than a simple TNF-α receptor, has a greatly extended half-life in the bloodstream, and therefore a more profound and long-lasting biologic effect than a naturally occurring soluble TNF-α receptor [47].

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