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Effects of Methotrexate and Etanercept on C-Reactive Protein and Anti-Cyclic Citrullinated Peptide Antibodies level In Rheumatoid Arthritis Patients

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Abstract: Rheumatoid Arthritis (RA) is an autoimmune disease. The treatment of this disease by taking Etnercept and Methotrexate (MTX) drugs. This study was conducted to detect C-Reactive Protein (C-RP) and Anti- Citrullinated Peptide Antibodies (Anti-CCP Abs) level among 126 rheumatoid arthritis (RA) patients treated with Etanercept (ETN) and methotrexate (MTX), 94 RA patients treated with MTX, 80 RA patients without treatment and 100 samples as healthy control. Blood samples were collected and the presence of C-RP antibodies was determined by using Latex agglutination test (LAT). Anti-CCP Abs was also estimated in serum of all subjects by using Enzyme linked immunosorbent assay (ELISA) method too. The seroprevalence of C-RP in RA+ETN+MTX was 57(45.24%), in RA+MTX was 62(65.96%), in untreated RA patients was 53(66.25%) while, it was 5(5%)in healthy group. The mean levels of Anti-CCP Abs in RA+ETN+MTX patients were (18.53± 1.70 pg/ml), in RA+MTX patients was (47.61± 6.24 pg/ml), and in untreated RA patients was (167.98 ± 8.67 pg/ml)while, they were (10.3 pg/ml ± 0.87) in healthy group. The results showed significant difference ($P<0.05$) for both C-RP prevalence and Anti-CCP Abs serum levels was found between studied subjects.

KEY words: Rheumatoid Arthritis, Etanercept, methotrexate, C-Reactive Protein, Anti- Citrullinated Peptide Anti-bodies and Latex Agglutination Test.

Introduction:

Rheumatoid arthritis (RA) is a common autoimmune disease that is associated with progressive disability, systemic complications, early death, and socioeconomic costs (Firestein, 2003). RA is one of the complex immune-mediated diseases for which an understanding of the etiology is dependent on the definition of environmental triggers that, in a restricted genetic context, may initiate immune reactions having the potential to contribute the disease development (Stolt *et al.*, 2003).The release of specific cytokines into the systemic circulation had been observed in a variety of inflammatory disease, including RA. Their concentration levels usually reflect disease severity and prognosis (Goronzy and Weyand, 2009). Interlukin-1(IL-1) and tumor necrosis factor- alpha (TNF-α) have been characterized as the major pro-inflammatory cytokines in the inflamed joint in RA. They have overlapping actions including local inflammation, enhancing adhesive properties of the inflammatory cells, causing angiogenesis, and bone resorption (Zwerina *et al.*, 2005). Also RA has been associated with several auto antibodies; it can be present before the disease manifestation (Silman *et al.*, 1992). The diagnosis of RA is easily made in persons with typical established disease. Constitutional features indicative of the inflammatory nature of the disease, such as morning stiffness, support the diagnosis. Demonstration of subcutaneous nodules is a helpful diagnostic feature. Additionally, the presence of rheumatoid factor and anti-CCP antibodies. Inflammatory synovial fluid with increased numbers of polymorphonuclear cells and radiographic findings of juxta articular bone demineralization and erosions of the affected joints substantiate the diagnosis (Fauci, 2010). Disease modifying drugs are the most effective means of improving the signs and symptoms of RA as well as reducing radiological progression (Donahue *et al.*, 2008). Agents in this class fall into two categories: non-biologics - disease modifying anti-rheumatic drugs such as methotrexate (MTX) (Weinblatt, 1996), and biologic, such as anti-TNF-α antagonists ETN (Zalevsky *et al.*, 2007). MTX is a folic acid analogue originally synthesized in the 1940s which is designed to inhibit dihydrofolate reductase, (Seeger *et al.*, 1949). MTX is used in high doses (100–1000/mg/m^2) to treat malignancies (Jolivet *et al.*, 1983) and in low doses (5–25 / mg/week) for RA and other inflammatory conditions (Weinblatt, 1996). It is well absorbed when given orally or intra muscularly. Intramuscular administration may help reduce side effects, especially nausea, which is commonly associated with oral ingestion (Chan and Cronstein, 2002). Etanercept (ETN) (Enbrel trade name) is a

tubes and allowed to clot at room temperature, before They centrifuged at 3000 round per minute (rpm) for 10 minutes and then sera were dispensed into 5 eppendrof tubes by using micropipette and stored at -20 ^{O}C. C-RP-Latex agglutination test, kit is providing from Leaner Chemical company – Spain, the principle of the test is based on antigen – antibody reaction directly. The sensitively of the test is 64 IU/ml (Young, 1995). Immunoscan CCPlus® test kit (Euro Diagnostica, Sweden) is an enzyme-linked immunosorbent assay (ELISA) used for qualitative and semi-quantitative determination of IgG Antibodies to Cyclic Citrullinated Peptide (CCP) in human sera.

Calculation and statistical analysis:

Results were calculated by drawing a standard curve. Plotting on the horizontal axis the Anti-CCP Abs concentration of the standards, and on the vertical axis the corresponding absorbance. The average absorbance for each sample was located on the vertical axis and the corresponding Anti-CCP Abs concentration on the horizontal axis was read as shown in fig (1).

dimeric human tumor necrosis factor receptor (TNFR) p75-Fc fusion protein made of 2 extra-cellular domains of the human 75 kD (p75) TNFR linked by the constant Fc portion of human immunoglobulin 1 (IgG1) fig (1.4). It consists of 934 amino acids and has an approximate molecular weight of 150 kD (McDermott, 2001). ETN mimics the inhibitory effects of naturally occurring soluble TNF receptors, the difference being that ETN, because it is a fusion protein rather than a simple TNF 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 receptor (Madhusudan *et al.*, 2005).

Materials and methods

From September 2013 till the end of February 2014, a total of 400 blood samples were collected from studied groups from both gender, their ages between 20 – 80 years. Samples were collected from Department of Rheumatology, Baghdad Teaching Hospital.). Five ml of venous blood were drawn from redial vein from each person by using disposable syringe. They were placed in plain

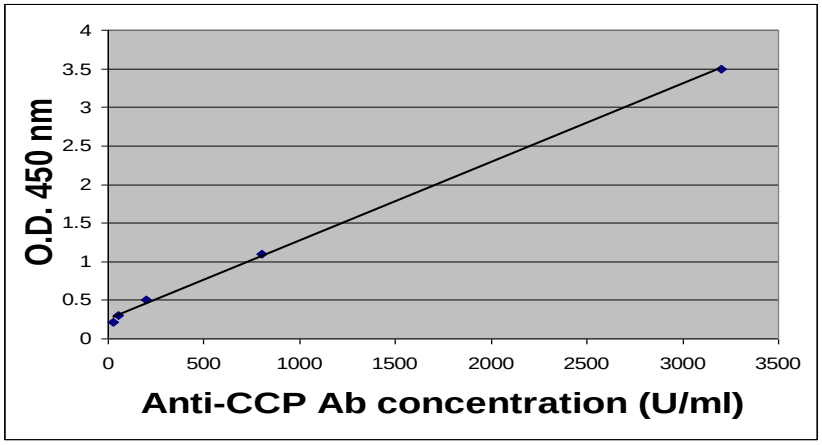

Figure 1: Standard curve of total Anti-CCP Abs concentration

have revealed 62(65.95%) and 53(66.25%) respectively. There was a significant difference (P< 0.05) between RA+ETN+MTX and other studied groups, as shown in table (1). Among RA patients groups, there was a significant difference (P<0.05) between group RA patients treated with MTX & ETN and other groups, while RA patients treated with MTX showed no significant differences (P>0.05) with un treated RA patients. This study was clarified that CRP positivity was decreased in patients who were treated with both drugs (ETN+MTX), because these patients with

Results and Discussion:

Another potential marker for increasing the risk of RA disease is thes C-reactive protein (CRP), since CRP is a sensitive marker of systemic inflammation and it is elevated in patients with RA. Additionally, CRP correlates with response to therapy as CRP levels decrease or normalize in RA patients following effective treatment (Otterness, 1994). Among RA groups, the lower percentage of C-RP was recorded in RA+ETN+MTX patients 57(45.24 %), while RA+MTX patients and untreated RA patients

response in all patients, and response may diminish over time in some patients (Saag *et al.*, 2008), while patients with severe RA treated with MTX often exhibit only partial improvement (Sesin and Bingham, 2005).

well-established RA, biologic agents have been shown to effectively improve clinical, functional and radiographic outcomes. ETN treatment was more effective on CRP level, as is true for any drug, biologic agents do not achieve optimal

Table (1): The percentage distribution of C-RP in studied subjects by Latex agglutination test (LAT).

Test / Subject	C-RP-Latex test		
	Positive No (%)	**Negative No (%)**	**Total**
RA+MTX+ETN	57(45.24%)	69(54.76%)	126
RA+MTX	62(65.96%)	32(34.04%)	94
RA	53(66.25%)	27(33.75%)	80
Healthy	5(5%)	95(95%)	100
P< 0.05* significant differences			

antibodies serum level decreased significantly (P< 0.05) only in those patients whom they received both ETN+MTX drugs (18.53± 1.70 pg/ml) between RA groups. While high level of anti-CCP antibodies revealed in RA patients that have been treated with MTX alone (47.61± 6.24 pg/ml) and RA patients without treatment (167.98 ± 8.67 pg/ml) and in healthy group was (15.26 ± 0.85 pg/ml) table (2).

The anti-cyclic citrullinated peptide (anti-CCP) antibody test has been used for the detection of specific antibody in the sera of studied groups and considered as a diagnostic marker for RA disease. The statistical analysis showed significant differences (P<0.05) between RA groups, table (2). RA patients whom treated with ETN & MTX showed significant differences (P< 0.05) with other studied groups.The mean concentration of anti-CCP

Table (2): the mean concentration of Anti-CCP Abs in studied subjects

Anti-CCP Abs (ELISA)			
Subject	NO	Mean conc. (pg/ml)	±S.E.
RA+ETN+MTX	126	18.53	± 1.79
RA+MTX	94	47.61	± 6.24
RA without treatment	80	167.98	± 8.67
Healthy group	100	15.26	± 0.85

Chen *et al.*, (2006) mentioned that patients treated with the tumor necrosis factor (TNF) blocker (eg. ETN drug) experienced significant reductions in their anti-CCP antibody titers, with a corresponding improvement in disease activity such as DAS28 (Disease activity score 28 joints). Our trial results showed that treatment with both ETN and MTX drugs offered more advantages in terms of clinical symptoms and signs, inflammatory indices, and autoantibody levels

than treating with MTX alone. These finding were close to the results of a previous study done by Klareskog *et al.*, (2004) who showed that the RA patients treated with ETN and MTX is more effective in reducing clinical activity and retarding radiographic progression than MTX alone in RA patients. The clinical response to ETN is rapid, generally occurring within two weeks,

or nearly up to three months after treatment initiation (Keystone *et al.*, 2004). The pathogenesis of anti-CCP antibodies in RA patients has been shown to be attributable to the body's humoral response to citrulline. Citrullination is the post-translational conversion of arginine to citrulline by an enzyme called peptidylarginine deiminase (PADs). This enzyme is normally present as inactive intracellular enzyme (Vossenaar and van Venrooij, 2004). ETN was able to decrease the number of these inflammatory cells in rheumatoid joints (Catrina *et al.*, 2005). During programmed cell death (apoptosis) in the synovial joints of RA patients, PADs may leak out of the dying cells. Once activated (activated by calcium ion), PADs will cause citrullination of extracellular arginine. In the synovium, the citrulline acts as an antigenic stimulant to induce anti-citrullinated protein antibodies (Anti-CCP) locally produced by plasma cells (Vossenaar and van Venrooij, 2004). These anti-inflammatory effects may account for the reduction in acute phase reactants and autoantibody generation. The effect of ETN on anti-CCP production may reflect the importance of therapeutic action of this agent (Chen *et al.*, 2006).

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