

# Effects of Adalimumab MTX Combination on Serum (NF- $\kappa$ B) and Caspase3 in Iraqi Patients with Rheumatoid Arthritis

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**Abstract:** *Background:* Rheumatoid arthritis (RA) is a chronic inflammatory disease with variable degrees of bone and joints damage. Treatment with methotrexate (MTX) +/- biological agents such as adalimumab (ADA); may produce dramatic changes in the progression and severity of RA which in turn affect expression of inflammatory pathway; inflammatory cytokines; NF- $\kappa$ B and caspases-3. *Objective:* To assess effects of treatment by MTX and MTX-adalimumab on levels of inflammatory signaling. *Subjects and methods:* Open label three groups prospective study on 100 Iraqi patients with RA plus 50 apparently healthy subjects. Patients were allocated to take MTX and MTX plus ADA. *Results:* Serum (NF- $\kappa$ B) initially; was significantly elevated in RA groups of patients. Which in turn indicate the activation of this transcription factor. MTX-ADA treatment resulted in significant (NF- $\kappa$ B) lowering as compared to MTX. Serum caspases-3 in RA groups; initially were significantly lower than in normal subjects. After 3 months of treatment; A significant elevation in caspases-3 found in ADA+ MTX group as compared with initial value and MTX group. *Conclusion:* Use of adalimumab-MTX combination may result in lowering level of expression of serum NF- $\kappa$ B used in regulation of inflammatory signaling and significant changes in caspase-3 level expressed in serum indicating increase cellular apoptosis.

**Keywords:** Rheumatoid arthritis, adalimumab, MTX, NF- $\kappa$ B and caspase 3

## 1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease of poly articular arthritis in association with serological evidence of auto-reactivity affecting approximately 1% of adults worldwide. It typically leads to deformity and destruction of the joints and systemic disorders throughout the body as well<sup>(1,2)</sup>.

The advent of the biological agents has had a major impact on the treatment of RA, with TNF- $\alpha$  blockers achieving widespread use frequently in combination with MTX. The TNF- $\alpha$  blockers include monoclonal antibody (adalimumab). Well-performed clinical trials indicate that TNF- $\alpha$  blockers can improve the signs and symptoms of RA and can retard radiographic progression. Biomarkers play pivotal roles in disease diagnosis and interventions at early stage and are also helpful in knowing the state of treatment and how body is acting or responding to the medication<sup>(3)</sup>. Therefore, exploring and measuring biologic markers in blood or in joint fluids may serve as indicators of diagnosis and also indicators of prognosis and the subsequent response to therapy.

The nuclear factor- $\kappa$ B (NF- $\kappa$ B) family of transcription factors is essential for the expression of pro-inflammatory cytokines, but can also induce regulatory pathways. It is well established that the activation of NF- $\kappa$ B is essential both in acute inflammatory responses and in chronic inflammatory diseases, including rheumatoid arthritis (RA). Although is less extensively studied<sup>(4)</sup>, NF- $\kappa$ B can be activated via two distinct pathways: the classical or canonical pathway, and the alternative or non-canonical pathway. NF- $\kappa$ B activation via canonical pathway which can be resulted by stimulation

of a variety of cell membrane receptors, including tumor necrosis factor (TNF) receptor, interleukin (IL)-1 receptor, also Activators of the non-canonical NF- $\kappa$ B pathway play an important role in RA synovial inflammation,<sup>(4)</sup>

Caspases (cysteiny aspartate-specific proteases) are a family of important signaling molecules with various tasks depending on the subtype and organ involved. Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins. However, the specific requirements of this (or any other) caspase in apoptosis have remained largely unknown until now. Pathways to caspase-3 activation have been identified that are either dependent on or independent of mitochondrial cytochrome c release and caspase-9 function.<sup>(5,6)</sup> Since the introduction of TNF- $\alpha$  blocking agents, their efficacy and safety have been studied in many randomized controlled clinical trials<sup>(7)</sup> as well as observational studies of RA patients<sup>(8)</sup>. However, data regarding predictors of good clinical response of anti-TNF therapy are still sparse. The aim of this study is to assess the predictors of response to adalimumab in treatment of Iraqi patients with active RA.

## 2. Subjects and Materials

**Study design:** This is an open label three group prospective study that was conducted over 11 month period. Patients: The study was conducted on Iraqi patients with RA who visited the Rheumatology Clinic in Baghdad Teaching Hospital from December 2014 to November 2015. To be included in this study, the patient should meet the 1987 American College of Rheumatology criteria for the classification of RA<sup>(9)</sup>, also he should have history of failed adequate response to conventional DMARDs and his disease

activity score 28 (DAS28)<sup>(10)</sup> should be equal to or greater than 5.1 (severe disease activity).

The exclusion criteria include patients less than 18 years old, patients with a previous history of biologic agent intake and those with other connective tissue diseases overlapping with RA.

One hundred (100) patients with RA completed the follow up. Patients were allocated to take either methotrexate (MTX) and adalimumab plus MTX. In addition to 58 apparently healthy subjects participated in this study as a control group, only 50 patients completed the follow up.

All patients included in the study signed the informed consent form. Ethical Approval was obtained from the scientific Ethics Committee of Baghdad University, College of Pharmacy, and from Baghdad Teaching Hospital (Medical City, Iraq), Rheumatology Medical Department.

### 3. Sample Collection

Venous blood sample were obtained from each participant by veno- puncture before and 12 weeks after initiation of adalimumab therapy.

The blood was transferred to plane tube, and then left at room temperature for at least 30 minute to coagulate, and then centrifuged for 10 min at 4000 rpm to obtain serum. The resultant serum was kept frozen until the time of analysis. After that the serum was used for by using ELIZA technique for; NF $\kappa$ B and caspase 3.

### 4. Statistical Analysis

The results have been expressed as Mean  $\pm$  Standard Deviation (SD), Paired T test was used to test the significance of difference in means of pre and post treatment, One-way analysis of variance (ANOVA) was used to examine the degree of difference among studied groups, Chi Square test was applied for the analysis of some parameters in the study, The results of analysis with (P) values less than 0.05 were considered significant.

### 5. Results:

#### Effects of treatment on the nuclear factor- $\kappa$ B (NF- $\kappa$ B)

In this study; serum(NF- $\kappa$ B) in RA groups of patients where significantly elevated. Which in turn indicate the activation of this transcription factor. Treatment with MTX and MTX-ADA combination did not produced significant changes in serum (NF- $\kappa$ B) as compared with their initial values.

This have some agreement with finding SemmlerM et. al 2007<sup>(11)</sup>MTX group show elevation in level or expression of(NF- $\kappa$ B) although this value was not significant as compare with initials. This may related to over expression of NF- $\kappa$ B induced by methotrexate<sup>(12)</sup>

Use of ADA in combination of MTX resulted in significant (NF- $\kappa$ B) lowering as compared to MTX. Where the postulated mechanism to the explain this lowering NF- $\kappa$ B

may related to therapeutic effect of Adalimumab; where it acts by blocking the interaction of TNF with the p55 and p75 cell surface TNF receptors. That may lower receptor activator of NF- $\kappa$ B ligand (RANKL) expression which in turn lower(NF- $\kappa$ B)<sup>(13)</sup>

### 6. Effects of treatment on the caspase 3

In our study measuring of caspase 3 in serum of the patient with RA was never reported. In this study; adalimumab found to significantly an increase level of serum caspase 3, this finding is agree with reported finding in some studies that demonstrated that adalimumab can activate the caspase 3 pathway, resulting in a higher level of cellular apoptosis.<sup>(14,15)</sup>

### 7. Conclusion

Use of adalimumab in combination with MTX may result in lowering level of expression of serum NF- $\kappa$ B used in regulation of inflammatory signaling and significant changes in caspase- 3 level expressed in serum which indicate increase cellular apoptosis.

### 8. Acknowledgements

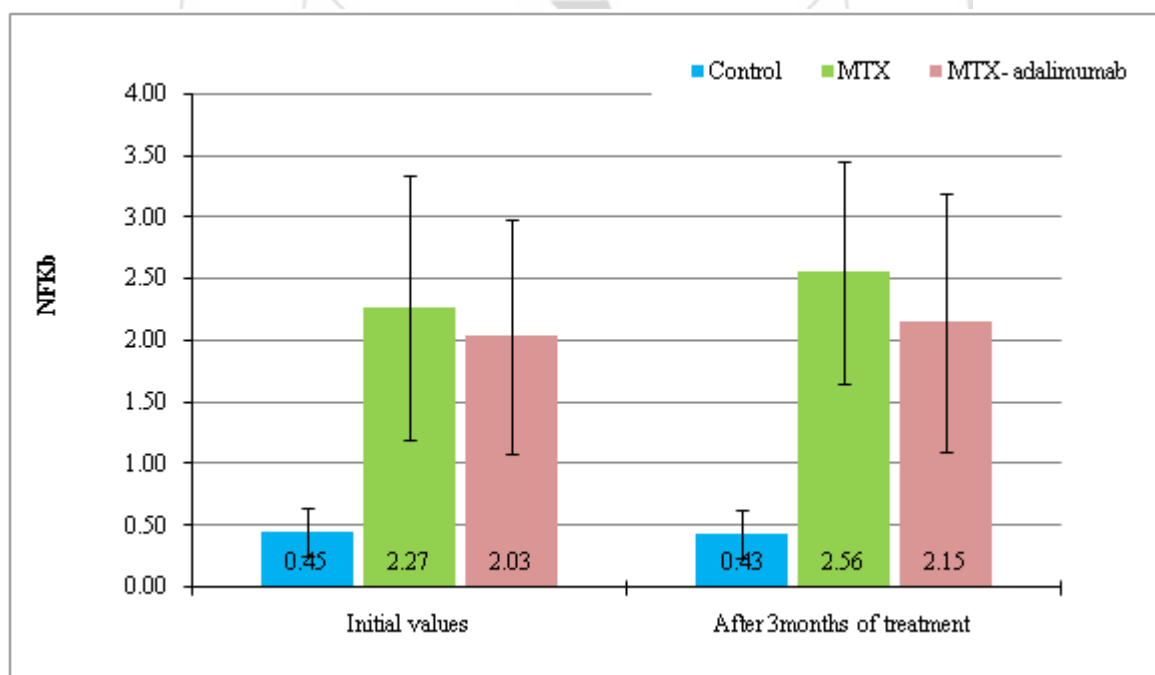
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### References

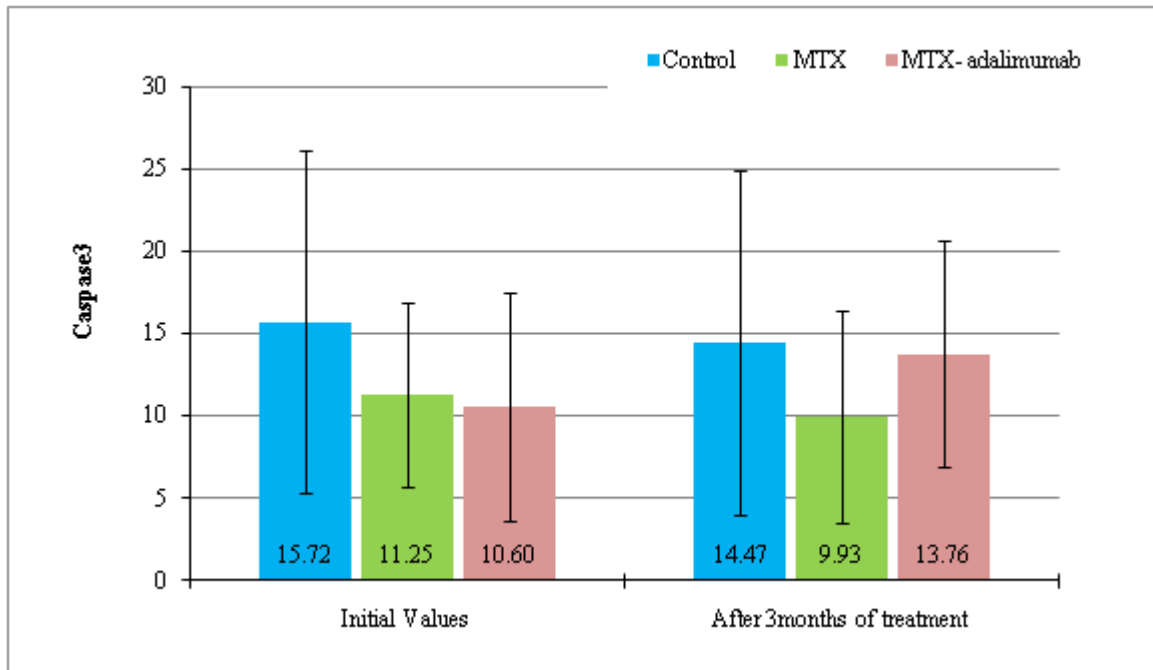
- [1] M. Feldmann, F. M. Brennan, and R. N. Maini, —Rheumatoid arthritis,” *Cell*, vol. 85, no. 3, pp. 307–310, 1996.
- [2] D. L. Scott, F. Wolfe, and T. W. J. Huizinga, —Rheumatoid arthritis,” *The Lancet*, vol. 376, no. 9746, pp. 1094–1108, 2010.
- [3] Xiaoyin Niu , Guangjie Chen.. Clinical Biomarkers and Pathogenic-Related Cytokines in Rheumatoid Arthritis. *J Immunol Res*. 2014; 2014: 698192
- [4] DrJekyll ,MrHyde,Ae R Noort,et.al. REVIEW Non-canonical NF- $\kappa$ B signaling in rheumatoid arthritis. *Arthritis Research & Therapy*.2015;17:15
- [5] Porter AG1, Jänicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ*. 1999;6(2):99-104.
- [6] Caspase-3 Activation: An Indicator of Apoptosis in Image-Based Assays, Application 2012.
- [7] Maini R, St Clair EW, Breedveld F, et al. Infliximab (chimeric 4. anti-tumor necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomized phase III trial. ATTRACT Study Group. *Lancet* 1999;354:1932–9.
- [8] Zink A, Listing JD, Kary Set al. Treatment continuation 5. in patients receiving biologics or conventional DMARD Therapy. *Ann Rheum Dis* 2005;64:1274-9

- [9] Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31(3):315-24.
- [10] Van Der Heijde DM, Van't Hof M, Van Riel PL, and Van De Putte LB. Development of a disease activity score based on judgment in clinical practice by rheumatologists. *J Rheumatol* 1993;20: 579- 81
- [11] Semmler M1, Seeck U, Neustadt B.et.al. No effects of adalimumab therapy on the activation of NF-kappaB in lymphocytes from patients with severe rheumatoid arthritis. *ClinRheumatol*. 2007; 26(9):1499-504.
- [12] Samar F. Darwish, Wesam M. El-Bakly, Hossam M. Arafa.et.al. Targeting TNF- $\alpha$  and NF- $\kappa$ B Activation by Bee Venom: Role in Suppressing Adjuvant Induced Arthritis and Methotrexate Hepatotoxicity in Rats. *Ebtehal El-Demerdash PLOS ONE* 2013; 8 , 11.
- [13] Philip J Mease. Adalimumab in the treatment of arthritis. *TherClin Risk Manag*. 2007; 3(1): 133–148.
- [14] Shen C1, Van Assche G, Rutgeerts P.et.al. Caspase activation and apoptosis induction by adalimumab: demonstration in vitro and in vivo in a chimeric mouse model. *Inflamm Bowel Dis*. 2006;12(1):22-8.
- [15] Urbano PCM, Soccol VT, Azevedo VF. REVIEW Apoptosis and the FLIP and NF-kappa B proteins as pharmacodynamic criteria for biosimilar TNF-alpha antagonists. 2014,8: 211—220.

Table 1 Effect treatment on inflammatory signaling				
Variables	Control N=50	MTX Alone N=50	MTX plus Adalimumab N=50	P value
Serum NF- $\kappa$ B				
Initial values	0.45 $\pm$ 0.2	2.27 $\pm$ 1.1 <sup>a</sup>	2.03 $\pm$ 0.9 <sup>a</sup>	<0.001
After 3 months	0.43 $\pm$ 0.11	2.56 $\pm$ 0.9 <sup>a</sup>	2.15 $\pm$ 1.0 <sup>ab</sup>	<0.001
% change	-4.3	12.7	5.6	<0.001
Serum caspase 3				
Initial values	15.72 $\pm$ 10.4	11.25 $\pm$ 5.6 <sup>a</sup>	10.6 $\pm$ 6.9 <sup>a</sup>	<0.001
After 3 months	14.47 $\pm$ 34.54	9.93 $\pm$ 6.5 <sup>a</sup>	13.76 $\pm$ 6.9 <sup>b*</sup>	<0.001
% change	-7.9	-11.7	29.8	0.1736
* Significant (p value<0.05) as compared with initial values a Significant (p value<0.05) as compared with control values b Significant (p value<0.05) as compared with MTX alone values				



**Figure 1:** Effect of treatments on NF $\kappa$ B in RA patients, treated with MTX alone for 12 weeks compared with ADA +MTX and control .



**Figure 2:** Effect of treatments on Caspase-3 in RA patients, treated with MTX alone for 12 weeks compared with ADA +MTX and control

Abbreviation

RA : Rheumatoid arthritis

MTX :Mehotrexate

ADA : Adalimumab

NF<sub>κ</sub>B :Nuclear factor kappa B

TNF-α : Tumor necrosis factor alpha

DAS 28 :Disease activity score 28

