

## Research Article

# Does non-steroidal anti-inflammatory drugs increase tumor necrosis factor-alpha levels?

Eylem Çağiltay<sup>1\*</sup>, Mustafa Kaplan<sup>2</sup>, Selim Nalbant<sup>3</sup>,  
Yaşam Kemal Akpak<sup>4</sup>, Burak Sahan<sup>2</sup>, İbrahim Akmaz<sup>5</sup>

<sup>1</sup>Department of Endocrinology and Metabolism, GATA Haydarpaşa Teaching Hospital, Istanbul, Turkey

<sup>2</sup>Department of Internal Medicine, GATA Haydarpaşa Teaching Hospital, Istanbul, Turkey

<sup>3</sup>Department of Internal Medicine, Maltepe University, Istanbul, Turkey

<sup>4</sup>Department of Obstetrics and Gynecology, Ankara Mevki Military Hospital, Ankara, Turkey

<sup>5</sup>Department of Orthopedics, GATA Haydarpaşa Teaching Hospital, Istanbul, Turkey

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### \*Correspondence:

Dr. Eylem Çağiltay,

E-mail: [eylem723@yahoo.com](mailto:eylem723@yahoo.com)

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## ABSTRACT

**Background:** The study was designed to investigate the possible effects of the non-steroidal anti-inflammatory drugs on the levels of TNF-alpha in osteoarthritis patients.

**Methods:** Three different non-steroidal anti-inflammatory drug; diclofenac sodium (100 mg), indomethacin (25 mg) and nabumethon (500 mg) have been included to the study. Twenty osteoarthritis patients who do not have another inflammatory disease for each group of NSAID were recruited from outpatients' clinics of our hospital. Blood samples of these patients were collected before (at 0 hour) and after (at first and sixth hours) one of these NSAIDs was given orally for the sequential measurements of the serum levels of TNF- $\alpha$ .

**Results:** In all groups, levels of TNF-alpha were similar at the beginning of the study before the placebo and drugs. One hour later, after the NSAIDs were given, increased levels of TNF-alpha were obtained in diclofenac and nabumethone ( $55.4 \pm 52.4$  and  $35.7 \pm 27.9$ ) groups. In contrast to diclofenac group, increased levels of TNF-alpha was insignificant in nabumethone group ( $P > 0.05$ ). However, at six hours later after drugs have been given, TNF-alpha levels decreased to basic levels at the beginning in only nabumethone group. Levels of the TNF-alpha in diclofenac group ( $101.9 \pm 142.2$ ) increased significantly at sixth hour, too. In contrast to this result, insignificant increase has been found in indomethacin and nabumethone groups ( $39.6 \pm 34.6$  and  $30.2 \pm 15.9$ ). No significant changes were obtained in control group.

**Conclusions:** This study shows that levels of TNF-alpha may increase after NSAIDs have been taken orally. NSAIDs effects in our study, increasing TNF-alpha levels may cause unexpected results and may also alter the inflammation.

**Keywords:** TNF-alpha, Non-steroidal anti-inflammatory drugs, Diclofenac, Nabumethone, Indomethacin

## INTRODUCTION

Despite the cardiovascular risk attributable to the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), they are one of the most prescribed treatments in the world.<sup>1</sup> These drugs target the enzyme cyclooxygenase (COX) thus

affording relieve from pain, inflammation or fever. As COX-dependently formed prostanoids not only mediate signals involved in inflammation and pain, but also regulate important physiological cardiovascular functions. Some NSAID have recently been reported to be associated with arterial thrombosis or hypertension.

This is in contrast to the well-known antiplatelet effects of low-dose aspirin, but in coherence with the specific effects of some NSAID on prostanoid formation in the vasculature. A correlation between the intake of selective inhibitors of the cyclooxygenase 2 (COX-2) isoform and atherothrombotic events has also been established.<sup>2-6</sup>

However, it is impossible to explain all negative effects of NSAID on cardiovascular system with only the prostanoids. For instance, in our previous animal study, it has been shown that selective COX-2 inhibition may cause increases in the levels of Tumor Necrosis Factor-alpha (TNF-α) which may lead to the progression of left ventricular dysfunction.<sup>7</sup> So, we have suggested to investigate the serum levels of TNF-α in patients who have been orally given different kinds of NSAIDs.

**METHODS**

Patients: All procedures adhered to the tenets of the Declaration of Helsinki, and local approval was taken from the GATA Military Training Hospital ethic committee. Informed consent was obtained from all patients with osteoarthritis<sup>8</sup> after explanation of the research purposes. Twenty patients for each group were recruited. Patients who have Erythrocyte Sedimentation Rate (ESR) more than 30 mm/h or C-Reactive Protein (CRP) more than 7 mg/dl according to our laboratory standards or taking any other anti-inflammatory drugs or taking acetylsalicylic acid or with any other systemic disease have been excluded from the study due to possible effect on TNF- α levels.

Three different NSAID have been included to our study, diclofenac sodium (100 mg) and indomethacin (25 mg) and nabumethon (500 mg). The dose of the NSAIDs have been chosen according to recommendations of the manufacturers that declared minimum dosage needed for anti-inflammatory effect.

Control group: Twenty patients with osteoarthritis were included to this group by applying the same procedure. The patients of this group were given placebo.

Detection of serum TNF-alpha concentrations: Blood samples of these patients were collected three times before (at 0 hour) and after (at first and sixth hours) NSAID was given orally in the morning. After peripheral blood (10 ml) samples were obtained from each patient

and centrifuged at 3500 rpm to get serum serums were deposited in an eppendorf tube at -70°C for subsequent processing until assayed for cytokines by Enzyme-Linked Immuno-Sorbent Assay (ELISA). Tumor Necrosis Factor alpha (TNF-α) in the serum were measured with commercially available sandwich ELISA kits (Biotrak Amersham Bioscience, USA). To reduce the effect of other proteins in the specimens that might be inhibitory, every sample was diluted five to twenty fold, depending on the cytokine. The minimum detectable concentrations of TNF-α were 1.5 pg/ml. In all assays, OD450 nm values obtained from diluted controls were subtracted to construct standard curves. Each experiment was performed in duplicate, and mean values of the determinations were used for the final results.

Statistical analysis: Statistical analyses were performed by SPSS software version 12.0 (SPSS Inc, Chicago, Illinois, USA). Comparison of levels of TNF-alpha at each time period for the same NSAID was done with the Friedman test. P values less than 0.05 were considered significant. All results and demographic features of the patients were summarized in the Table.

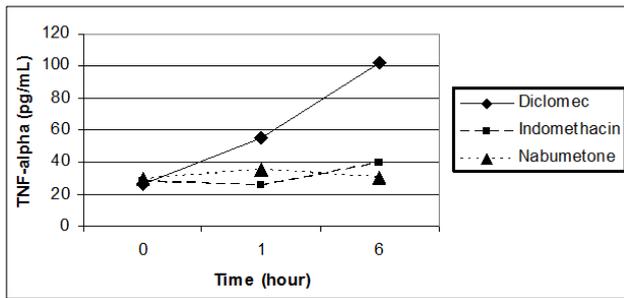
**RESULTS**

No significant changes were obtained in control group during the study (26.9 ± 12.3, 22.3 ± 11.1, 25.5 ± 10.9, respectively). In all groups of patients (control, diclofenac, indomethacin and nabumethone), levels of TNF-α (26.9 ± 12.3, 26.2 ± 20.1, 27.6 ± 16.7 and 29.3 ± 16.6, respectively) were similar at the beginning of the study before the drugs were given (P>0.05). One hour later, after the NSAIDs were given, increased levels of TNF-α were obtained in diclophenac and nabumethone (55.4 ± 52.4 and 35.7 ± 27.9) groups.

The difference between the first hour and the basic levels of TNF-α in diclofenac group was significant (P<0.05), in contrast to nabumethone group (P>0.05). However, six hours later, after drugs have been given, TNF-α levels decreased to basic levels at the beginning levels in only nabumethone group (29.3 ± 16.6 vs. 30.2 ± 15.9, P>0.05). TNF-α levels in diclofenac group (101.9 ± 142.2) were increased significantly again, at sixth hour too (P<0.05), Figure. In contrast to this result, insignificant increase has been found in indomethacin and nabumethone groups (39.6 ± 34.6 and 30.2 ± 15.9) six hours later.

**Table 1: Demographic features of the cases and results of the study.**

	n	M/F	Age	Erythrocyte sedimentation rate (mm/h)	C-reactive Protein (mg/dL)	TNF-alpha levels (pg/dL)			P
						Time (hour)			
						0	1	6	
Control group	20	10/10	54.10 ± 4.7	18.7 ± 8.1	2.7 ± 1.4	26.9 ± 12.3	22.3 ± 11.1	25.5 ± 10.9	0.9
Diclofenac	20	7/13	53.02 ± 4.5	18.7 ± 5.6	3.6 ± 1.2	26.2 ± 20.1	55.4 ± 52.4	101.9 ± 142.2	0.03
Indomethacin	20	9/11	56.76 ± 5.2	22.3 ± 4.9	4.1 ± 1.8	27.6 ± 16.7	25.5 ± 5.8	39.6 ± 34.6	0.9
Nabumethone	20	8/12	51.27 ± 4.8	17.3 ± 7.8	2.3 ± 1.1	29.3 ± 16.6	35.7 ± 27.9	30.2 ± 15.9	0.6



**Figure 1: The correlation of the TNF- $\alpha$  levels with the time after NSAID given.**

## DISCUSSION

According to the results of this study, NSAIDs may cause increases at the levels of TNF- $\alpha$  in patients with osteoarthritis. Actually, this result has been reported by different ways in two studies that have been published before. One of these studies was our previous animal study. We found that selective COX-2 inhibition can cause increased levels of TNF- $\alpha$  in monosodium urate induced inflammation in rat air pouch model.<sup>7</sup> In the second study, Pinhitero and coworkers showed that excessive inhibition of prostaglandins also may cause increasing in the levels of TNF- $\alpha$ .<sup>9</sup>

The decision about the selection of NSAIDs depends mostly on the effectiveness and also on the side effects of these important kind of drugs. Elevation at the serum levels TNF- $\alpha$  because of NSAID usage is very important because elevated levels of TNF- $\alpha$  may contribute to the deterioration of cardiovascular function through various mechanisms.<sup>10-13</sup> TNF- $\alpha$  also has been shown to exert pro-inflammatory vascular effects (e.g., induction of oxidative stress, endothelial apoptosis, up-regulation of adhesion molecules and chemokines).<sup>12</sup>

Besides direct chronic effects of the TNF- $\alpha$  on the cardiovascular system, it may effect as a component of insulin resistance. Insulin resistance is increasingly recognized as a chronic, low-level, inflammatory state. Hyperinsulinemia and insulin action were initially proposed as the common preceding factors of hypertension, low high-density lipoprotein cholesterol, hypertriglyceridemia, abdominal obesity, and altered glucose tolerance by linking all these abnormalities to the development of coronary heart disease. The similarities of insulin resistance to another inflammatory state, atherosclerosis, have been described only in the last few decades. Atherosclerosis and insulin resistance share similar pathophysiological mechanisms, mainly due to the actions of the two major pro-inflammatory cytokines, TNF- $\alpha$  and IL-6.<sup>14,15</sup>

On the other hand, NSAID can suppress the inflammation in the treatment of patients with Rheumatoid Arthritis (RA). However, NSAID treatment in RA patients cannot prevent the bone and joint from the inflammatory

destruction in contrast to corticosteroids. The reason of this consequence could not be explained entirely. Prostaglandins are one of the components of the inflammation and are believed to be one of the reasons about the symptomatic effects of the inflammation. They can be effectively suppressed by NSAID treatment. However, even though symptomatic relief is obtained, destructive complications of the RA still can continue. In fact, the reasons of the joint and the bone destruction in RA are because of IL-2 and TNF- $\alpha$ . So, the levels of the TNF- $\alpha$  can be still high during NSAID treatment without other disease modifying agent in RA patients and destructive complications of RA can be on going.<sup>16-20</sup>

Another interesting result of the study is the differences among the effects of these three non-steroidal drugs on the levels of TNF- $\alpha$ . In our opinion, this may be related to nature of their effects. When compared together, the minimum concentration of NSAID that is required for inhibiting the activity of cyclooxygenase-1 and cyclooxygenase-2 is the least for diclofenac, the little for indomethacin and the most for nabumethone.<sup>21</sup> To conclude, diclofenac caused the most increased the levels of the TNF- $\alpha$  in this study which is the strongest non-selective inhibitor of the COX among these three NSAIDs. So, it can be suggested that the degree of the COX inhibition is correlated with increasing levels of TNF- $\alpha$ .

Despite some limitations (sample size, pitfalls in measuring the serum levels of TNF- $\alpha$ ) our study shows that NSAIDs may cause to increases at the serum levels of TNF- $\alpha$ . So, the entire discussion above can be very important to explain some mechanisms related with NSAID and their effects. However, these results still need further studies to confirmations.

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## REFERENCES

- Schaeffer T, Heloire F, Deray G. How to watch over a patient treated with a NSAID in relation to the cardiovascular and renal risk? *Presse Med.* 2006;35:41-6.
- Huang WF, Hsiao FY, Wen YW, Tsai YW. Cardiovascular events associated with the use of four nonselective NSAIDs (etodolac, nabumetone, ibuprofen, or naproxen) versus a cyclooxygenase-2 inhibitor (celecoxib): a population-based analysis in Taiwanese adults. *Clin Ther.* 2006;28:1827-36.
- Zarraga IG, Schwarz ER. Coxibs and heart disease: what we have learned and what else we need to know. *J Am Coll Cardiol.* 2007;49:1-14.
- Oshima K, Takeyoshi I, Tsutsumi H, Mohara J, Ohki S, Koike N, et al. Inhibition of cyclooxygenase-2 improves cardiac function

- following long-term preservation. *J Surg Res.* 2006;135:380-4.
5. Hermann M, Ruschitzka F. Coxibs, non-steroidal anti-inflammatory drugs and cardiovascular risk. *Intern Med J.* 2006;36:308-19.
  6. Dogne JM, Hanson J, Supuran C, Pratico D. Coxibs and cardiovascular side-effects: from light to shadow. *Curr Pharm Des.* 2006;12:971-5.
  7. Nalbant S, Akmazı I, Kaplan M, Aysar K, Solmazgul E, Sahan B. Does rofecoxib increase TNF- $\alpha$  levels? *Clin Exp Rheumatol.* 2006;24:361-5.
  8. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. (Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association). Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. *Arthritis Rheum.* 1986;29:1039-49.
  9. Pinheiro RM, Calixto JB. Effect of the selective COX-2 inhibitors, celecoxib and rofecoxib in rat acute models of inflammation. *Inflamm Res.* 2002;51:603-10.
  10. Balakumar P, Singh M. Anti-tumour necrosis factor-alpha therapy in heart failure: future directions. *Basic Clin Pharmacol Toxicol.* 2006;99:391-7.
  11. Krotz F, Hellwig N, Schiele TM, Klauss V, Sohn HY. Prothrombotic potential of NSAID in ischemic heart disease. *Mini Rev Med Chem.* 2006;6:1351-5.
  12. De Biase L, Pignatelli P, Lenti L, Tocci G, Piccioni F, Riondino S, et al. Enhanced TNF alpha and oxidative stress in patients with heart failure: effect of TNF alpha on platelet O<sub>2</sub>- production. *Thromb Haemost.* 2003;90:317-25.
  13. Yu X, Patterson E, Huang S, Garrett MW, Kem DC. Tumor necrosis factor alpha, rapid ventricular tachyarrhythmias, and infarct size in canine models of myocardial infarction. *J Cardiovasc Pharmacol.* 2005;45:153-9.
  14. Aguilera AA, Diaz GH, Barcelata MI, Guerrero OA, Ros RM. Effects of fish oil on hypertension, plasma lipids, and tumor necrosis factor-alpha in rats with sucrose induced metabolic syndrome. *J Nutr Biochem.* 2004;15:350-7.
  15. Fernandez-Real JM, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr Rev.* 2003;24:278-301.
  16. Fowler PD. A double-blind comparison of diclofenac sodium (Voltarol) and placebo in inpatients with rheumatoid arthritis. *Rheumatol Rehabil.* 1979;2:75-7.
  17. Doreen MS, Boardman PL, Fowler PD, Poole PH. Diclofenac (Voltarol) in rheumatoid arthritis: a report of a double-blind trial. *Rheumatol Rehabil.* 1979;2:78-80.
  18. Bijlsma A, ten Pas JG. A study of the anti-inflammatory effect of voltaren in patients with rheumatoid arthritis. *Scand J Rheumatol Suppl.* 1978;22:46-50.
  19. Wafin F, Valindas E, Wuolijoki E. Comparison of diclofenac and indomethacin suppositories in rheumatoid arthritis. *Clin Rheumatol.* 1984;3:67-70.
  20. Kajander A, Martio J. Diclofenac sodium (Voltaren) and naproxen in the treatment of rheumatoid arthritis: a comparative double-blind study. *Scand J Rheumatol Suppl.* 1978;22:57-62.
  21. FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl J Med.* 2001;345:433-42.

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