

# The Effects of Increased Humidity and Weather Conditions on Inflammatory and Oxidative Stress Markers in Healthy Rats

## Nem ve Hava Koşullarının Subklinik İnflamasyon ve Oksidatif Stres Markırları Üzerine Etkileri

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**Abstract:** Although it is known that the symptoms of rheumatoid arthritis patients increase in cold, humid and rainy days but its mechanism is unclear. It was aimed to investigate the effects on some inflammatory and oxidative stress markers in different weather conditions in rats. Some inflammatory, oxidant and antioxidant markers were measured in sera of healthy rats in sunny, cloudy and rainy days. The levels of reduced glutathione (GSH) and glutathione reductase (GR) were found to be lower in both cloudy and rainy days with respect to sunny days ( $p = 0.001$  and  $0.004$  respectively). Similarly,  $\alpha$ -glutathione-S-transferase levels were found to be lower on rainy days than on sunny days ( $p = 0.017$ ). Malondialdehyde (MDA) was found to be higher ( $p = 0.044$ ) in rainy days compared to sunny days. Nitric oxide (NO) and interleukin-1 $\beta$  (IL-1 $\beta$ ) were found to be higher in cloudy and rainy days compared to sunny days ( $p = 0.003$  and  $0.025$ , respectively). The findings indicate that oxidative stress-related inflammation is triggered in rainy and cloudy days.

**Keywords:** inflammatory process; bad weather condition; oxidative stress; chronic rheumatic disease.

**Özet:** Romatoid artritli hastaların semptomlarının soğuk, nemli ve yağmurlu günlerde arttığı bilinmekle birlikte, mekanizması açık değildir. Bu çalışmada, farklı hava koşullarında, bazı inflamatuvar ve oksidatif stres markırlarının üzerine etkilerinin araştırılması hedeflenmiştir. Bazı inflamatuvar, oksidan ve antioksidan markırların düzeyleri, güneşli, bulutlu ve yağmurlu günlerde sağlıklı ratların serumunda ölçüldü. Glutasyon redüktaz (GR) ve indirgenmiş glutasyon hem bulutlu hem de yağmurlu günlerde, güneşli güne kıyasla daha düşük olarak bulundu ( $p = 0.001$  ve  $0.004$  sırasıyla).  $\alpha$ -glutasyon-S-transferaz yağmurlu günde güneşli güne kıyasla daha düşük olduğu bulundu ( $p = 0.017$ ). Malondialdehit (MDA) yağmurlu günde güneşli güne kıyasla daha yüksek olarak bulunmuştur ( $p = 0.044$ ). Nitrik oksit ve interlökin-1  $\beta$  (IL-1 $\beta$ ) bulutlu ve yağmurlu günde güneşli güne kıyasla daha yüksek bulunmuştur ( $p = 0.003$  ve  $0.025$ , sırasıyla). Bulgular, yağmurlu ve bulutlu günlerde oksidatif stres ilişkili inflamasyonun tetiklendiğini göstermektedir.

**Anahtar Kelimeler:** inflamatuvar süreç; kötü hava koşulları; oksidatif stres; kronik romatizmal hastalık.

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## 1. Introduction

It is known that the intensity of complaints in patients with chronic inflammatory diseases such as osteoarthritis and rheumatoid arthritis (RA) varies depending on different weather conditions (1,2). Especially in cold, humid, and rainy days, the symptoms of these patients aggravate (3,4). In addition, non-steroidal anti-inflammatory agents (NSAIDs), which are frequently used in the treatment of rheumatic diseases, have been proven to be less effective in rainy days (5). Oxidative stress and inflammation have been reported to play an important role in etiopathogenesis of rheumatic diseases (6,7). Pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-6 have also been reported to be effective on the induction of the inflammation and exacerbation of rheumatic diseases (8–11).

TNF- $\alpha$ , IL-1 $\beta$  and IL-6 are cytokines used to assess anti-inflammatory activity. It has been reported that oxidant molecules increase and antioxidant molecules decrease in parallel with the rise of cytokines in inflamed tissue (12). Increased production of reactive oxygen species (ROS) causes oxidative stress, which is potentially detrimental to DNA structure, lipid and protein structures. There are many defense mechanisms to protect against ROS effects (13). Antioxidant defense systems can be evaluated under two principal categories (7). Non- enzymatic antioxidant defense systems (vitamins A and C, reduced glutathione (GSH)), and enzymatic defense systems such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST). In an organism, there is a balance between free radicals and antioxidant defense systems. It is called oxidative stress when this balance is disturbed in favor of free radicals (14).

In evaluating oxidative stress, malondialdehyde (MDA), myeloperoxidase (MPO) and nitric oxide (NO) are used as oxidant parameters, while GSH, GR and GST are used to evaluate antioxidant enzyme activity (7,15–19).

Previous studies have suggested a possible relationship between certain weather conditions and worsening symptoms, mainly based on predictions without evidence. The present study is different in that it offers an explanation on the role of certain weather conditions in the induction of inflammation through oxidative stress in individuals with certain predispositions to the inflammatory process.

## 2. Materials and Methods

*Animals:* Male albino Wistar rats ( $n = 21, 258 \pm 22$  g) were obtained from the Medical Experimental Research Center, Ataturk University. Before the experiments, the rats were housed and fed as divided groups, under standard conditions at 20–24 °C and the moisture at 50–55% in the laboratory. The animals were not fed the night before the surgical operation. All experiments were performed in the same laboratory under standard conditions. Experiments on the rats were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by Ataturk University's local animal care committee (Number: E.1700212428, Date: 28.07.2018). A total of 21 rats were divided into 3 equal groups. Group I; It was identified as the group of rats receiving blood in full sunny open air (temperature 33.9 °C, pressure 1005.9 hPa, humidity 13%). Group II; the group of rats receiving blood in cloudy weather (temperature 29.1 °C, pressure 1002.2 hPa, humidity 28%). Group III; the group of rats receiving blood on a rainy day (temperature 22.1 °C, pressure 1000.8 hPa, humidity 63%). All rats were kept in a covered environment for 5 hours under the specified weather conditions. All rats were removed from the lab at 8 am and blood was taken at 1 pm o'clock.

*Preparation of sera:* Blood samples were taken from all rats and were collected into separate gel vacutainer serum tubes. All blood samples were incubated for 15 min in room temperature, then serum layers were separated by centrifugation at 1500xg for 10 min. All

serum samples were stored at  $-80^{\circ}\text{C}$  until biochemical analysis was conducted.

**Biochemical analyses:** GR, GSH,  $\alpha$ -GST, MDA, MPO, NO, TNF- $\alpha$ , IL-6, IL-10 tests were measured using the ELISA method (Hangzhou East-Biopharm Co., Ltd., China).

**Statistical Analysis:** Statistical analysis was performed using SPSS 18.0 (SPSS Inc., Chicago, IL) software program. The conformity of the quantitative data to the normal distribution was examined using the Kolmogorov-Smirnov test. The descriptive statistics were conducted as a mean  $\pm$  95 % Confidence interval. Comparisons between groups were made by one way ANOVA test. Student's t-test was used for binary comparisons between groups at different values. Correlations between the parameters were evaluated by the Pearson test. The statistically significant difference was assumed as  $p < 0.05$  value.

### 3. Results

GR and GSH were found to be lower in both cloudy and rainy days compared to sunny days ( $p = 0.001$  and  $0.004$  respectively).  $\alpha$ -GST was lower on rainy days than on sunny days ( $p = 0.017$ ). MDA was found to be higher in rainy days than on sunny days ( $p = 0.044$ ). NO and IL-1 $\beta$  were higher in cloudy and rainy days compared to sunny days ( $p = 0.003$  and  $0.025$ , respectively). The differences between MPO, TNF- $\alpha$ , IL-6 and IL-10 groups were not significant (Table 1).

As far as the correlation between inflammatory and oxidant / antioxidant markers is concerned, there was an opposing, moderate correlation between IL-1 $\beta$  and GR. A moderate positive correlation was also found between IL-1 $\beta$  and MDA, MPO and NO. The correlations were found to be moderately positive between TNF- $\alpha$  and NO and, between IL-6 and GSH. There was no other significant correlation between the other values (Table 2).

**Table 1.** Comparison of inflammatory markers and oxidant/antioxidant markers between groups.

Parameter	Group 1 (Sunny day) Mean (95 % CI)	Group 2 (Cloudy day) Mean (95 % CI)	Group 3 (Rainy day) Mean (95 % CI)	P value	Multiple comparisons	
					Group	p-Value
GR (ng/ml)	59.3 (50.6-68.0)	45.0 (41.1-48.9)	42.3 (34.9-49.7)	0.001**	1-2 1-3	0.003** 0.003**
GSH (mg/L)	2705 (2527-3355)	2244 (2172-2844)	1908 (1178-2390)	0.004**	1-2 1-3	0.025* 0.004**
$\alpha$ -GST (ng/ml)	189 (185-284)	125 (73-210)	123 (111-130)	0.017*	1-3	0.004**
MDA (nmol/ml)	7.69 (5.73-8.90)	8.16 (7.63-10.06)	9.68 (8.04-10.91)	0.044*	1-3	0.016*
MPO (ng/mml)	133.1 (99.2-165.8)	153.8 (138.9-188.8)	160.4 (142.0-207.2)	0.134		
NO ( $\mu\text{mol/L}$ )	231 (143-284)	335 (252-658)	447 (361-552)	0.003**	1-2 1-3	0.010* 0.004**
TNF- $\alpha$ (ng/L)	1113 (1002-1322)	1405 (1213-1591)	1367 (1095-1687)	0.142		
IL-1 $\beta$ (pg/L)	7841 (5917-9994)	9630 (8938-13752)	10586 (8798-15053)	0.025*	1-2 1-3	0.025* 0.016*
IL-6 (ng/L)	1577 (1229-2093)	1334 (1161-1514)	1422 (969-1809)	0.109		
IL-10 (pg/ml)	929 (752-1132)	813 (291-1290)	969 (740-1180)	0.611		

\*:  $p < 0.05$ , \*\*:  $p < 0.01$  CI: Confidence interval GR: Glutathione reductase, GSH: reduced glutathione,  $\alpha$ -GST:  $\alpha$ -glutathione-S-transferase, MDA: malondialdehyde, MPO: myeloperoxidase, NO: nitric oxide, TNF- $\alpha$ : tumor necrosis factor  $\alpha$ , IL: interleukin.

**Table 2.** The correlations between inflammatory markers and oxidant / antioxidant markers.

		GR	GSH	$\alpha$ - GST	MDA	MPO	NO
IL- 1 $\beta$	r	-0.635	-0.240	-0.433	0.501	0.610	0.623
	p	0.005**	0.338	0.073	0.035*	0.007**	0.006**
TNF - $\alpha$	r	-0.316	-0.374	0.452	0.452	0.243	0.619
	p	0.201	0.127	0.060	0.060	0.332	0.006**
IL - 6	r	0.224	0.501	0.265	-0.198	-0.262	-0.138
	p	0.372	0.034*	0.289	0.431	0.293	0.586
IL - 10	r	0.027	-0.124	0.120	0.190	0.0915	0.298
	p	0.918	0.635	0.648	0.466	0.727	0.246

\*:  $p < 0.05$ , \*\*:  $p < 0.01$  GR: Glutathione reductase, GSH: reduced glutathione,  $\alpha$ -GST:  $\alpha$ -glutathione-S-transferase, MDA: malondialdehyde, MPO: myeloperoxidase, NO: nitric oxide, TNF- $\alpha$ : tumor necrosis factor  $\alpha$ , IL: interleukin.

#### 4. Discussion

The results found suggest that inflammatory markers and oxidative stress markers are higher in rainy and cloudy days, while antioxidant molecules decrease. Therefore, oxidative stress and inflammatory process are intensified in rainy and cloudy days. All these results suggest that the molecular mechanism of this deterioration is mediated by increased inflammatory markers and oxidant molecules and reduced antioxidant enzymes.

Savage et al. (20) in a recent study suggested that the disease activity score fell in sunny and under less humid conditions in patients with RA. Another study showed that the symptoms of patients with RA were associated with low air temperatures (21). Joint pain has been shown to worsen in patients with osteoarthritis at low atmospheric pressure (22), in low air temperatures and with high humidity (3,4,23). It is also found that patients with ankylosing spondylitis, another rheumatic disease, have increased pain in cold and humid days (24). Suleyman et al. (5) demonstrated a decreased NSAID effects in rainy days, which was related to the decreased level of adrenaline and cortisol in rainy days.

MDA, a marker of lipid peroxidation, and MPO, a marker of neutrophil activation, were found to be higher in RA than those of the healthy controls (15). Increased ROS in chronic inflammatory diseases cause lipid peroxidation and consequently, MDA levels are found to rise (16).

Antioxidant molecule GSH, antioxidant enzymes SOD, catalase and GR were reported to decrease while MDA and NO increased in RA (7). GSH is an important antioxidant molecule and it protects cellular structures from reactive oxygen species. It is pointed out that GSH decreases in RA and OA.  $\alpha$ -GST is a detoxifying enzyme that increases the water solubility of harmful molecules, both in RA and in osteoarthritis (17). In this study, decreased GSH levels in rainy and cloudy days suggest increased GSH consumption due to increased oxidative stress. Decreased  $\alpha$  - GST levels in rainy days may also be one of the causes of increased oxidative stress these days. GR plays an antioxidant role in the conversion of oxidized glutathione (GSSG) to GSH and has been found to decrease in many diseases (17,18). Our findings reveal that reduced GR levels in cloudy and rainy days are important biomarkers that increase oxidative stress these days. NO is an oxidative stress marker, reported increasing in RA (19). In this study, we found that NO levels raised in rainy and cloudy days, suggesting another cause of oxidative stress.

It is confirmed that inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6 and anti-inflammatory cytokines such as IL-10 play an important role in the pathogenesis of inflammatory rheumatic diseases (8–11). In this study, we found IL-1 $\beta$  levels in rainy and cloudy days, suggesting that this cytokine is particularly responsible for the increased inflammation during these days. In addition,

positive correlation between oxidative markers MDA, MPO and NO, and between TNF- $\alpha$  and NO, and a negative correlation between IL-1 $\beta$  and antioxidant enzyme GR, suggest that the increased oxidative stress is mainly caused by these cytokines. No other association was found significant.

Previous studies have reported that increased inflammation is associated with oxidant markers. Increased oxidative stress-induced inflammation, suggesting that both inflammation and oxidative stress, are two interrelated processes that affect each other (12).

An account of worsening the symptoms under bad weather conditions includes mechanical changes such as those in expansion and

contraction of the musculoskeletal system due to cold and humidity, and increase in the viscosity of the synovial fluid (25,26).

## 5. Conclusion

In conclusion, previous studies have suggested an assumption of a possible relationship between certain weather conditions and worsening rheumatoid symptoms which were mainly based on predictions without evidence. The present study has demonstrated that oxidative stress and inflammation increase as a result of the correlation in rainy and cloudy days. The study is the first to demonstrate the relationship at the molecular level. However, further research is needed to investigate the function of such a relationship

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