

# Tissue oxidant, antioxidant and TNF-alpha levels in ureteropelvic junction obstructions

Oxidative stress in ureteropelvic junction obstructions

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## Abstract

**Aim:** In this study, it was aimed to determine the role of some oxidant, antioxidant agents and tumor necrosis factor alpha (TNF-alpha) in the pathophysiology of ureteropelvic junction obstructions because of their effect on cell life, development and death.

**Material and Methods:** A total of 56 patients who applied to the Urology Clinic of Firat University Hospital between 2015-2018 were included in the study. The patients were divided into two groups: 'patients' and 'controls'. In the 'patients' group, 30 patients with ureteropelvic junction obstruction were evaluated. In the 'control' group, 26 patients who were diagnosed with a kidney tumor or non-functional kidney were evaluated. TNF-alpha, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) levels were measured in the tissue samples taken from the uretero pelvic junction segments of the study groups.

**Results:** MDA and TNF-alpha levels were detected to be significantly higher in the patient group than in the control group. SOD, CAT and GPx enzyme levels were lower in the patient group than in the control group ( $p < 0,05$ ). In the Pearson correlation graph, TNF-alpha revealed a negative correlation with SOD, CAT and GPx and a positive correlation with MDA. Also, a negative correlation was detected between SOD and MDA.

**Discussion:** In our study, it was concluded that oxidative stress and antioxidant mechanisms may have a role in the pathophysiology of ureteropelvic junction obstructions. In this regard, antioxidants were thought to be useful in the prevention and treatment of ureteropelvic junction stenosis.

## Keywords

Oxidant; Antioxidant; TNF-alpha; Ureteropelvic obstruction; MDA

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## Introduction

Ureteropelvic junction obstruction (UPJO) is defined as insufficient urine flow from the renal pelvis to the ureter as a result of functional or anatomical obstruction. Obstruction in the kidney and urinary system can lead to recurrent urinary tract infection (UTI) and permanent renal parenchymal damage if left untreated [1]. The most common cause of UPJO is the presence of aberrant or accessory lower pole vein. UPJO secondary to inflammation, neoplasms, kidney stones and cysts can be seen [2]. In the kidney with obstruction at the ureteropelvic junction (UPJ), a decrease in glomerular volume, an increase in tubular dilatation, glomerular sclerosis, and tubular atrophy and interstitial fibrosis occur [3].

The diagnosis of UPJO is made by evaluating the results of various imaging methods or tests together. Currently, most UPJOs are diagnosed with antenatal USG [1, 4]. The use of biochemical markers in UPJO is important because they are not invasive tests, can be detected with simple urine test, and their costs are low. Although many markers have been studied in clinical studies and animal studies until now, none of them have entered routine clinical use. This is due to the insufficient number of studies or a limited number of patients. In the current literature, there are several markers defined such as tumor growth factor beta-1 (TGF- $\beta$ 1), monocyte [1, 5, 6].

Free radicals are constantly formed in living things, but these molecules are regularly destroyed by the antioxidant defense system. There is a balance between the formation of free radicals and their destruction through the antioxidant system, which is called oxidative balance [7, 8]. The harmful effects of free radicals can be eliminated with substances known as antioxidants. Living cells contain numerous types of low molecular weight antioxidants (e.g. vitamins E and C, carotenoids, flavonoids, etc.) and larger molecular weight antioxidant enzymes, both of which serve to prevent or repair damage caused by free radicals [9]. The endogenous antioxidant defense system consists of enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (KAT), and nonenzymatic antioxidants with glutathione and bilirubin.

In studies conducted with experimental models, radical oxygen species (ROS) have been shown as the primary cause in the pathogenesis of ischemic, toxic and immunologically induced kidney damage. The occurrence of oxidative stress in the kidney tissue as a result of various factors leads to serious kidney disorders [10, 11]. In this study, it was aimed to determine the role of some oxidant, antioxidant and tumor necrosis factor-alpha (TNF-alpha) in the physiopathology of ureteropelvic junction obstruction because of their effects on cell life, development and death.

## Material and Methods

This study was conducted after receiving approval from the Ethics Committee for Non-Invasive Clinical Research, Faculty of Medicine, Firat University (date 17.09.2019, meeting number 13, decision number 10). In this study, it was aimed to determine the role of some oxidant, antioxidant and TNF-alpha in UPJO pathogenesis. A total of 56 patients who applied to Firat University Hospital Urology Clinic between 2015-

2018 and met the study criteria were included in our study. The patients were divided into two groups: "patients" and "controls". Thirty patients who were diagnosed with unilateral UPJO and had undergone pyeloplasty were included in the patient group. The control group included 26 patients who were diagnosed with unilateral kidney tumor or non-functional kidney and who had undergone nephrectomy. UPJ tissue samples taken from the study groups were examined in the biochemistry laboratory. Fifty-six patients included in the study were analyzed retrospectively. Demographic characteristics of the patients, presence of additional renal anomalies, renal parenchymal thickness, scintigraphy and pathology results, blood biochemistry parameters, urine culture results, discharge time and complications information were compiled.

Patients with bilateral hydronephrosis and bilateral UPJO, posterior urethral valve, ureterovesical stenosis, external genitalia anomaly, urinary system stone disease, urinary system infection, neurogenic bladder and vesicoureteral reflux, urea and creatinine values were not included in the study.

TNF-alpha, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) levels were measured in tissues taken from ureteropelvic junction segments of individuals who underwent pyeloplasty and nephrectomy. TNF-alpha levels were determined spectrophotometrically using the Enzyme-Linked Immuno Sorbent Assay (ELISA) method, malondialdehyde level, superoxide dismutase, catalase and glutathione peroxidase activities using appropriate manual methods [7, 12-15].

### Statistical analysis

IBM SPSS Statistics Version 22.0 package program was used for statistical analysis of the data. Categorical measurements were summarized as numbers and percentages, and continuous measurements as mean and standard deviation (median and minimum - maximum where necessary). Whether continuous measurements provided the normal distribution assumption was tested with the Kolmogorov- Smirnov test. In the comparison of continuous measurements between the groups, if the assumptions were met, the T- test was used in independent groups, and the Mann- Whitney U test was used if the assumptions were not met. The statistical significance level in all tests was taken as  $p < 0.05$ .

## Results

In the patient group, 30 patients diagnosed with UPJO were examined. All of these patients had unilateral UPJO. Among these 30 patients, 17 (57%) were male and 13 (43%) were female. The youngest of the patients was 1 year old, while the oldest was 57 years old. Urea and creatinine values of the patient group were within normal limits. Urinary tract infection was excluded by sending a pre-op urine culture from these patients.

Sixteen patients in the patient group had UPJO on the right side (53%) and 14 on the left side (47%). Open pyeloplasty operation was performed in 15 (50%) of these 30 patients, and laparoscopic pyeloplasty operation was performed in 15 (50%) of them. The average length of stay of the patients was determined as 4 days. DJ stents were implanted in all patients during the operation, and the implanted stents were removed

1 month later.

There were 26 patients in our control group. These patients consisted of patients with a diagnosis of kidney tumor (n = 17) or with a non-functional kidney (NFK) (n = 9). Blood urea creatinine values of control group patients were within normal values. Urinary tract infection was ruled out by sending urine culture from these patients in the pre-op period. Sixteen (62%) patients in the control group were male and 10 (38%) were female. Simple nephrectomy was performed in 9 (35%) patients in the control group due to the diagnosis of non-functional kidney (NFK), and radical nephrectomy was performed in 17 (65%) with the diagnosis of renal cell cancer (RCC). Right nephrectomy was performed in 12 (46%) and left nephrectomy in 14 (54%) controls. While 18 (69%) of these 26 patients underwent laparoscopic nephrectomy, 8 (31%) underwent open nephrectomy.

In our study group of 56 patients in total, 28 (50%) had the related disease on the right side and 28 (50%) on the left side. While 33 (59%) of these 56 individuals underwent laparoscopic surgery, 23 (41%) of them underwent open surgery.

TNF-alpha, CAT, GPx, MDA and SOD levels were measured in the tissues taken from the UPJ segment of patients with and without UPJO. The comparison of the levels of TNF-alpha, MDA and antioxidant enzymes with each other is shown in Table 1. In the patient group with UPJO of MDA, the mean in the tissue was  $5.33 \pm 1.45$ , in the control group without UPJO it was  $3.38 \pm 876$ . The MDA level was found to be statistically significantly higher in the patient group compared to the control group ( $p < 0.05$ ). The mean of SOD in the tissue in the patient group with UPJO was  $14.80 \pm 4.31$ , in the control group without UPJO, the mean in the tissue was  $28.03 \pm 7.68$ . SOD level was found to be statistically significantly lower in the patient group than in the control group ( $p < 0.05$ ). In the patient group with UPJO of CAT, the average in the tissue was  $5.51 \pm 3.23$ , in the control group without UPJO it was  $9.70 \pm 5.92$ . The CAT level was found to be statistically significantly lower in the patient group compared to the control group ( $p < 0.05$ ). In the patient group with UPJO of GPx, the mean in the tissue was  $240.46 \pm 58.67$ , in the control group without UPJO it was  $326.79 \pm 125.98$ . The GPx level was found to be statistically significantly lower in the patient group than in the control group ( $p < 0.05$ ) (Table 1).

TNF-alpha in the patient group with UPJO had the smallest value of 9.14, the highest value as 126.64 and the mean value of 32.27, the lowest value in the tissue was 1.22 and the highest value was 35.43 and the average value was determined as 6.47 in the control group without UPJO. It was found that TNF-alpha was statistically significantly higher in the patient group compared to the control group (Table 2).

The correlation graph for the patient group with UPJO with each other and with the renal scintigraphy values of the patients is shown in Table 3. In particular, it was observed that the renal scintigraphy values of the patients were not correlated with any markers. It was found that MDA has a negative relationship with SOD ( $p < 0.05$ ). It was observed that while MDA value increased, SOD value decreased significantly. However, it was determined that MDA had a strong positive relationship with TNF-alpha ( $p < 0.01$ ). While MDA increased, it was observed that TNF-alpha also increased statistically. It was determined that SOD has a

positive relationship with GPx, and GPx decreased while SOD decreased ( $p < 0.001$ ). SOD had a negative relationship with TNF-alpha, while SOD decreased, TNF-alpha increased ( $p < 0.001$ ).

It was observed that there was a negative correlation between glutathione peroxidase (GPx) and TNF-alpha levels, and while GPx decreased, TNF-alpha increased ( $p < 0.01$ ). It was determined that TNF-alpha correlated with all markers except scintigraphy results, whereas scintigraphy results did not correlate with any markers.

**Table 1.** Distribution of enzyme levels according to patient and control groups (T test)

	Group	N	Mean±sd	T	p
MDA (nmol/mg prot)	Patient	30	5,33±1,45	5,952	,001
	Control	26	3,38±,876		
SOD (U/mgprot)	Patient	30	14,80±4,31	-8,080	,001
	Control	26	28,03±7,68		
CAT	Patient	30	5,51±3,23	-3,344	,002
	Control	26	9,70±5,92		
GPx	Patient	30	240,46±58,67	-3,360	,001
	Control	26	326,79±125,98		

MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase.

**Table 2.** Distribution of TNF-alpha level according to patient and control groups (Mann-Whitney U Test)

	Group	N	Mean Rank	Sum of Ranks	MWU	Z	p
TNF - alpha (pg/mg prot).	Patient	30	40,23	1207,00	38,00	-5,783	0,001
	Control	26	14,96	389,00			
	Total	56					

TNF- alpha: Tumor necrosis factor- alpha

**Table 3.** Patient group-Pearson correlation test

		Correlations					
		DTPA Scintigraphy	MDA	SOD	CAT	GPx	TNF-alpha
DTPA Scintigraphy	R	1	,101	,055	-,347	-,317	,009
	P		,594	,771	,061	,087	,960
MDA	N	30	30	30	30	30	30
	R	,101	1	-,282*	-,149	-,156	,381**
SOD	P	,594	,035	,272	,250	,004	
	N	30	56	56	56	56	56
CAT	R	,055	-,282*	1	,233	,611**	-,520**
	P	,771	,035	,083	,000	,000	
GPx	N	30	56	56	56	56	56
	R	-,347	-,149	,233	1	-,009	-,386**
TNF- alpha	P	,061	,272	,083		,945	,003
	N	30	56	56	56	56	56
GPx	R	-,317	-,156	,611**	-,009	1	-,367**
	P	,087	,250	,000	,945		,005
TNF- alpha	N	30	56	56	56	56	56
	R	,009	,381**	-,520**	-,386**	-,367**	1

\*Correlation is significant at the 0,05 level (2-tailed).

\*\*Correlation is significant at the 0,01 level (2-tailed). (N: number of patients P: Significance R: Correlation coefficient)  
MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, TNF- alpha: Tumor necrosis factor- alpha

## Discussion

Today, there is no diagnostic method that can be considered the gold standard in demonstrating UPJO. Diagnosis is usually made by repeated imaging methods such as diuretic scintigraphy and sequential ultrasonography (evaluation of kidney dimensions and anterior-posterior diameter of the kidney) [1]. Therefore, it was necessary to use new methods, especially non-invasive and specific markers, in the diagnosis, treatment and follow-up of patients with UPJO. Most of the studies in this field are studies with tissue, serum and urine samples to understand the underlying pathophysiology of obstruction. However, there are fewer studies on diagnosis, follow-up and treatment options. Although the results of many studies are meaningful and promising, the general opinion is that more comprehensive new studies are needed [1, 4].

Oxidative stress mechanisms and inflammation are thought to play an important role in the pathogenesis and progression of UPJO. There is a limited number of studies in the literature on the relationship between oxidative stress and UPJO. In a study published by Zhe-Ming Xu et al., the levels of thioredoxin, an antioxidant substance in the urine and serum of patients with UPJO were examined. Thioredoxin level was found to be higher in the patient group compared to the control group [14]. In a study conducted by Manucha et al., it was stated that oxidative stress increased twice in obstructive uropathic patients compared to normal individuals, whereas antioxidant levels such as SOD, CAT and GPx decreased [16]. In our study, it was found that oxidative stress increased and antioxidants decreased. However, in our correlation test, scintigraphy results could not detect a relationship between oxidant-antioxidant substance levels.

There are few studies in the literature on the role of TNF alpha in the pathogenesis of ureteropelvic stenosis. In a study conducted by Koca et al. on the histopathogenesis of intrinsic UPJO, they compared the tissue TNF-alpha and Tumor growth factor beta 3 (TGF  $\beta$ 3) levels in the UPJ of 36 patients with UPJO and 14 non-UPJO controls. TNF-alpha levels were found to be statistically significantly lower in the patient group ( $0.53 \pm 0.84$ ) compared to the control group ( $0.86 \pm 0.36$ ). In a study conducted by Shirazi et al., urinary TNF-alpha levels of a patient group of 31 children with UPJO and a control group of 33 children without UPJO were compared. In the study, the level of TNF-alpha in the urine of the patient group ( $80 \pm 26.07$ ) was found to be statistically significantly higher than the TNF-alpha levels of the control group ( $13.90 \pm 5.3$ ) [17]. When the studies conducted were examined, it was observed that Koca et al. have achieved results different from others. It was determined that Madsen and Shirazi achieved results parallel to the results of our study.

In our study, we aimed to investigate the role of oxidant-antioxidant mechanisms and TNF-alpha in the pathophysiology of ureteropelvic junction strictures, which have been associated with many diseases in recent years and have been the subject of intensive research. Our study is of particular importance since the studies in this area are not sufficient, and our study was conducted directly on human UPJ tissues. In the studies we conducted in a study population consisting of 56 individuals in total, it was determined that MDA was statistically significantly

higher in the patient group compared to the control group ( $p < 0.01$ ). Manucha et al. [16], in their similar study, detected oxidative stress markers approximately twice as high in the patient group as compared to the control group and obtained results parallel to our study. Again, regarding urinary system cancers, Piriñçi et al. [13] in patients with kidney tumors, Geçit et al. [7], on the other hand, detected higher levels of MDA in patient tissues with bladder tumors compared to healthy normal tissues. These results support that MDA is mutagenic and carcinogenic.

In our study, SOD, CAT and GPx enzyme levels, which constitute the most important enzymatic defense system against oxidants, were found to be statistically significantly lower in the patient group compared to the control group ( $p < 0.01$ ). In the study by Manucha et al. [16], which was similar to our study in this area, SOD, CAT and GPx levels were found to be lower in the UPJO patient group compared to the healthy control group, and it is seen that the results of this study coincide with our study. When we compare our study with studies on other diseases related to the urinary system in this field, Piriñçi et al. [13] in patients with kidney tumors, Geçit et al. [7], on the other hand, found that SOD, CAT and GPx levels were lower than those of normal healthy tissues in their studies on patients with bladder tumor. It is observed that Murowski et al. also detected lower SOD levels in patients with oligoasthenozoospermia [12]. Contrary to all these studies, it is seen that SOD, CAT and GPx levels were detected higher in kidney adenocarcinoma cells [15].

In our study, TNF-alpha levels were found to be statistically higher in the patient group compared to the control group. When we compared our study with similar studies on TNF-alpha in UPJOs, it was observed that Shirazi et al. [17] obtained results in the same direction as we did, while Madsen et al. [18] did not detect a significant change in TNF-alpha levels between the patient and control groups. Contrary to the results of our study in this area, it was observed that Koca et al. [19] detected lower TNF-alpha in tissues of patients with UPJO than healthy tissues.

## Conclusion

In our study, it was concluded that oxidative stress and antioxidant mechanisms may play a role in the physiopathology of ureteropelvic junction strictures. Based on this, it is thought that antioxidants may be useful in the prevention and treatment of uretero-pelvic junction strictures. However, more comprehensive and more randomized studies are required in order to obtain more accurate and healthy results.

### Scientific Responsibility Statement

*The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.*

### Animal and human rights statement

*All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.*

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### Conflict of interest

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