THE EFFECT OF PREOPERATIVE FASTING PERIOD ON RENAL ISCHEMIA REPERFUSION INJURY IN RATS

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ABSTRACT

Introduction: *Preoperative fasting is a traditional method used to avoid pulmonary aspiration and related complications. However, with the enhanced recover after surgery (ERAS) protocols, preoperative fasting periods have started to decrease. Ischemia reperfusion injury (IRI) has an important place in the etiology of acute kidney injury. We aimed to observe the effect of preoperative short fasting periods on renal IRI.*

Materials and Methods: *21 wistar albino rats were included in the study. Rats were randomized as sham A group fasted for 12 hours preoperatively, group B fasted for 12 hours preoperatively, group C for 2 hours preoperatively. Firstly blood samples were taken for neutrophil gelatinase-associated lipocalin and interlukin-6. After laparotomy, in groups B and C, renal ischemia applied by clamping left renal pedicle 20 min. Then 20 minutes reperfusion was allowed. After then left and right nephrectomy was performed. After blood samples were taken to postoperative values, rats were sacrificed.*

Results: *In the histopathological examination, proximal tubular epithelium brush-border loss, apoptosis and tubular cast formation were examined. For apoptosis, the score was significantly higher in the left kidney with ischemia in group B compared to the right kidney (p=0.008). In tubular cast formation scores, statistically significant increases were observed for both right and left kidneys in group B (p=0.015, p=0.017).*

Conclusion: *In our study, we found that the preoperative short fasting period in rats has a positive effect in the case of mild renal ischemia reperfusion injury. However, we think that many more studies should be done on this subject.*

Keywords: *Neutrophil gelatinase-associated lipocalin (NGAL), preoperative nutrition, renal ischemia reperfusion injury, preoperative fasting, interleukin-6.*

DOI: 10.19193/0393-6384_2023_4_137

Received *January 15, 2023;* ***Accepted*** *April 20, 2023*

Introduction and aim

Preoperative fasting is a traditional method used by surgeons and anesthesiologists for years, especially to avoid pulmonary aspiration and related complications[1]. However, studies have shown that long preoperative fasting periods increase insulin resistance, inflammatory response to surgery, and oxidative stress[2-5]. With the implementation of enhanced recovery after surgery (ERAS) protocols, which have been put into clinical practice in the last 20 years, complication and morbidity rates have decreased in the perioperative period and hospitalization times have been shortened. An important step of ERAS protocols is shortening the preoperative fasting period. Oral fluids with carbohydrate content are given to the patients 2-3 hours before the operation. This procedure reduces insulin resistance and acute phase response ((C-reaction protein and interleukin-6) in the perioperative period, and also reduces oxidative stress in patients undergoing surgery[1, 6-8].

Acute kidney injury (AKI) can be observed at a high rate of 18-47% during and after surgery in the perioperative period[9]. When the etiology of AKI is examined, it is seen that ischemia-reperfusion (IR) injury has an important place[10].

Apoptosis occurs in cells in the nephron structure as a result of insufficiency in oxygen and glucose delivery to the tissue due to ischemia. During reperfusion, both free radical levels increase and the inflammatory process begins. The result is devastating damage to the nephron structure[11, 12]. In some experimental studies, it has been shown that preoperative fasting for a shorter time protects cardiac functions and reduces oxidative damage in rats which exposed intraoperative mesenteric ischemia[3]. However, the effects of the length of the preoperative fasting period on renal ischemia-reperfusion injury are unclear in the literature. In this study, it was aimed to investigate the effects of preoperative fasting times in ERAS protocols, which are mostly applied in abdominal surgery procedures, on modeling of renal ischemia-reperfusion injury. In order to evaluate the effects of different preoperative fasting periods, interleukin-6 (IL-6) and neutrophil gelatinase-associated lipocaine (NGAL), which is one of the important biomarkers for acute kidney injury in recent years, were used as biochemical parameters. At the same time, histopathologically, tissue pathologies were evaluated objectively.

Materials and methods

Ethics committee approval was obtained from the Animal Experiments Local Ethics Committee of Health Sciences University Ankara Training and Research Hospital with the date of 26.11.2020 and number 0063. After the planned study was completed in Health Sciences University Ankara Training and Research Hospital Experimental and Clinical Research Laboratories, the evaluation of blood and tissues was carried out in Gazi University Faculty of Medicine, Department of Medical Biochemistry and Pathology Laboratories.

In the study, 21 male Wistar albino rats with an average weight of 250±25 grams were used. The room temperature was 22-25 ºC and the environment including 12 hours light / 12 hours dark cycles was provided before the experiment. Rats were fed ad libitum (23% protein, 5% fat, 15% fiber, 50% carbohydrate) standard rat chow and drank tap water. Before the study, 3 experimental groups were formed, 7 in each group (n=7).

At the beginning of the study, all rats were anesthetized with Ketamine 90 mg/kg intraperitoneally (Ketalar Vial, 50 mg/ml Pfizer, Istanbul, Turkey) and Xylazine 10mg/kg intraperitoneally (Rompun 2% Vial Bayer, Istanbul, Turkey). First blood samples were taken from all rats in order to determine the preoperative biochemical basal value. All rats underwent laparotomy with an incision of approximately 3 cm from the midline.

• Group (Group A): Sham group: After 12 hours of fasting and thirst preoperatively, after abdominal laparotomy, bilateral nephrectomy was performed without creating renal ischemia.

• Group (Group B): After 12 hours of preoperative fasting and thirst, abdominal laparotomy was performed. The left kidney pedicle was clamped. Ischemia was created for 20 minutes. Afterwards, the clamp was opened and reperfusion was allowed for 20 minutes. Bilateral nephrectomy was performed after reperfusion.

• Group (Group C): Abdominal laparotomy was performed after 2 hours of preoperative fasting and thirst. The left renal pedicle was clamped. Ischemia was created for 20 minutes. Afterwards, the clamp was opened and reperfusion was allowed for 20 minutes. Bilateral nephrectomy was performed after reperfusion (Figure 1).

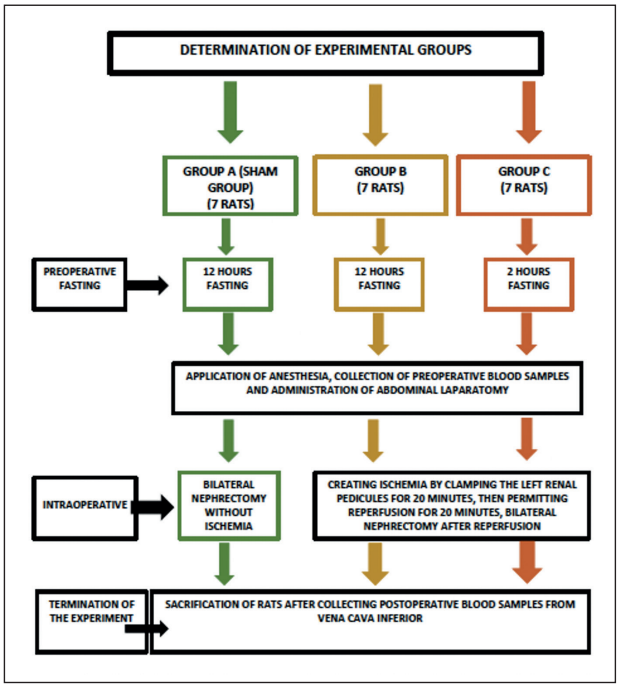

Figure 1: Experiment study plan.

Renal samples were taken into containers containing 10% formaldehyde solution, which were previously labeled for histopathological examination during the experiment. After bilateral nephrectomy

was performed for each subject, second blood samples were taken from the inferior vena cava for biochemical analysis of postoperative values. Afterwards, the rats were sacrificed.

Histopathological examination of the kidneys

All tissues were fixed in 10% formaldehyde for 12-36 hours, routinely deparaffinized with xylene and graded alcohol solutions, and 4 μm sections were obtained by embedding in paraffin. Hematoxylin and eosin staining protocol and histochemical PAS staining protocol were applied to these sections.

At least 10 areas in each kidney at x200 magnification were evaluated and scored in terms of loss of proximal tubule epithelium brush border, apoptotic cells, and tubular cast formations. Scoring was made as 0= 0, 1= 0-10%, 2=11-25%, 3=26-45%, 4=46-75%, 5=76-100%.

Biochemical analysis

Enzyme-Linked Immunosorbent Analysis Measurement Method (ELISA): It is based on the antigen-antibody relationship, it is a method that measures the amount of antigen according to the amount of enzyme that binds to the antibody and the intensity of the color change that occurs. NGAL, IL-6 levels in serum samples were measured by solid-phase sandwich ELISA using the NGAL Elisa Kit (USCN, Lot: L201013972), Human IL-6 Elisa Kit (USCN, Lot: L201013952) according to the manufacturer's instructions.

Statistical analysis

The Shapiro-Wilk test was used to determine whether continuous numerical variables were distributed close to normal, and whether the assumption of homogeneity of variances was provided was investigated by Levene's test. Descriptive statistics; For continuous numerical variables, the median (25th-75th) percentile was expressed. Sortable variables; displayed as both the median (25th-75th) percentile and the number of subjects and (%).

Wilcoxon sign test was used to determine whether there was a statistically significant difference in biochemical measurements after surgery compared to pre-operatively, and whether there was a histopathologically significant difference between the right and left kidneys.

The significance of the differences between the groups in terms of biochemical measurements and histopathology scores was evaluated with the Kruskal Wallis test. If the Kruskal Wallis test statistic results were found to be significant, the group(s) causing the difference were determined using the Dunn-Bonferroni test. Whether there was a statistically significant correlation between biochemical measurements and histopathological indicators was investigated using Spearman's rank-order correlation test.

Data analysis was done in IBM SPSS Statistics 17.0 (IBM Corporation, Armonk, NY, USA) package program. Unless otherwise stated, results for $p<0.05$ were considered statistically significant. However, Bonferroni Correction was performed to control for Type I error in all possible multiple comparisons.

Results

In this study we performed in rats, biochemical NGAL and IL-6 values and histopathologically morphological findings of ATN, proximal tubule brush border loss, apoptosis and tubular cast formation were examined. It was observed that the groups were homogeneous in terms of basal biochemical values characteristics. No rats were lost during the study.

Biochemical results

There was no statistically significant difference between the groups in terms of preoperative NGAL levels (p=0.489).

When the results within the groups were compared, there was no statistically significant difference between preoperative and postoperative NGAL levels according to Bonferroni correction (p>0.0167). There was no statistically significant difference between the groups in terms of NGAL levels after surgery (p=0.297). The amount of change in NGAL levels after surgery compared to preoperative values was similar between the groups (p=0.437) (Table 1) (Figure 2).

There was no statistically significant difference between the groups in terms of preoperative IL-6 levels (p=0.385). When the results within the groups are compared; There was no statistically significant difference between preoperative and postoperative IL-6 levels according to Bonferroni correction (p>0.0167). There was no statistically significant difference between the groups in terms of IL-6 levels after surgery (p=0.320). The amount of change in IL-6 levels after surgery compared to pre-surgery was similar between the groups (p=0.409) (Table 1) (Figure 3).

	Preopertive	Postoperative	p-value†	Change
NGAL (ng/ml)				
Group A	9,8 (7,0-16,4)	11,2 (8,2-35,0)	0,310	3,2 (-5,2-26,4)
Group B	16,9 (9,1-20,0)	7,5 (5,8-10,0)	0,398	-5,9 (-13,3-4,5)
Group C	10,8 (6,9-12,9)	15,4 (5,7-23,1)	0,398	3,1 (-1,4-10,8)
p-value‡	0,489	0,297		0,437
IL-6 (pg/ml)				
Group A	150,5 (118,8-158,2)	152,6 (143,4-158,8)	0,933	0,6 (-18,1-45,0)
Group B	155,9 (153,8-200,5)	155,7 (119,0-184,0)	0,310	-23,3 (-41,7-24,6)
Group C	156,3 (70,9-203,8)	158,2 (155,5-190,7)	0,237	27,7 (-52,1-84,6)
p-value‡	0,385	0,320		0,409

Table 1: Biochemical measurements before and after surgery by groups.

Descriptive statistics; the median (25th-75th) was expressed as a percentage, †Comparisons within the groups before and after surgery, Wilcoxon sign test, and Bonferroni correction for p<0.0167 were considered statistically significant, ‡Comparisons between groups, Kruskal Wallis test, comparisons made within the pre- and post-op periods were considered statistically significant for p<0.025 according to Bonferroni correction. In comparisons made in terms of the amount of change in the postoperative period according to the preoperative results, the results were considered significant if p<0.05.

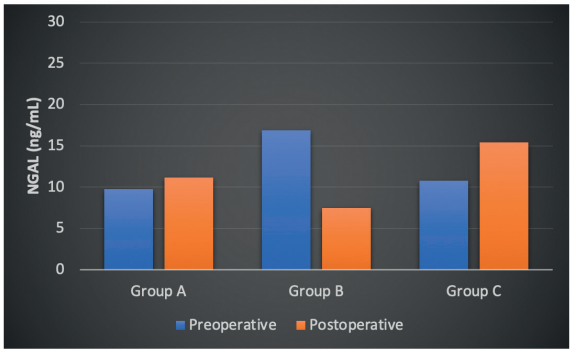

Figure 2: Preoperative and postoperative comparison of serum NGAL values between groups.

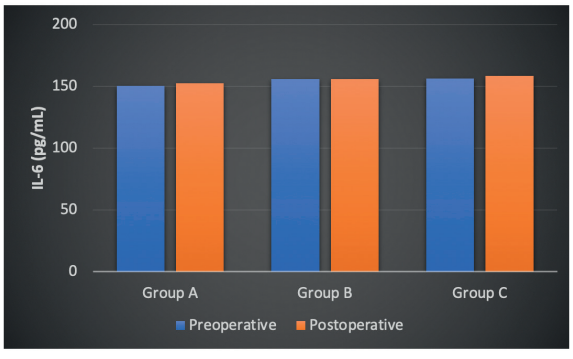

Figure 3: Preoperative and postoperative comparison of serum IL-6 values between groups.

Histopathological results

In order to examine the histopathological changes, pathological examination was performed in terms of ATN, where the effects of renal ischemia-reperfusion can be seen most clearly. For this purpose, the brush border of the proximal tubule epithelium (Figure 4), apoptotic cells and tubular cast formations were examined. For quantitative evaluation, scoring was done as 0= none, 1= 0-10%, 2=11-25%, 3=26-45%, 4=46-75%, 5=76-100%. A score of 4 and above was not observed in any sample. No score of 2 or more was observed in any of the subjects in group A.

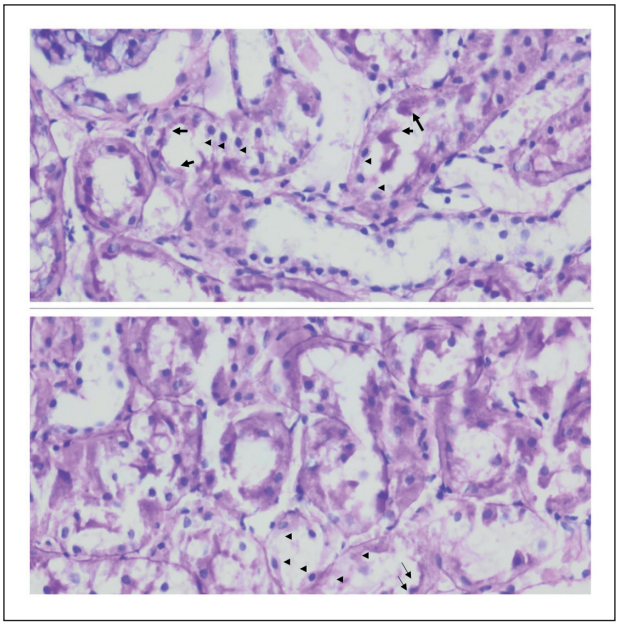

Figure 4: Proximal tubule brush border and loss of proximal tubule brush border.

In the proximal tubules, the brush border is shown with an arrow, and the loss of the brush border is shown as a triangular arrowhead. PAS dye x400.

In group B, especially in the left kidney, the scores were generally 2 (Table 2). In the pathological examinations of the subjects, it was observed that the formation of apoptotic cells and cast formation was higher in group B than in group C (Figure 5). There was no statistically significant difference between the right and left kidneys within the groups in terms of proximal tubule epithelium brush border loss scores according to Bonferroni correction (p>0.0167). There was a statistically significant difference between the groups in terms of the loss of the right proximal tubule epithelium brush border scores (p<0.001). The situation that caused the said difference; The scores of groups B and C were higher than group A (p<0.001 and p=0.019).

There was a statistically significant difference between the groups in terms of left proximal tubule epithelial brush border loss scores (p<0.001), and the reason for this difference; The scores of groups B and

C were higher than group A (p<0.001 and p=0.006) (Table 3). There was no statistically significant difference between the right and left kidneys within the groups (except group B) in terms of apoptotic cell scores according to Bonferroni correction (p>0.0167). In Group B, the apoptotic cell score in the left kidney was statistically significantly higher than in the right kidney (p=0.008).

GROUP	RIGHT			LEFT		
	A	B	C	A	B	C
Loss of brush border of proximal tubule epithelium						
0	7 (%100,0)	-	-	7 (%100,0)	-	-
1	-	2 (%28,6)	6 (%85,7)	-	1 (%14,3)	3 (%42,9)
2	-	5 (%71,4)	1 (%14,3)	-	6 (%85,7)	4 (%57,1)
Apoptotic cell						
0	7 (%100,0)	-	-	7 (%100,0)	-	-
1	-	7 (%100,0)	7 (%100,0)	-	-	3 (%42,9)
2	-	-	-	-	7 (%100,0)	4 (%57,1)
Tubular cast formation						
0	4 (%57,1)	-	-	2 (%28,6)	-	-
1	3 (%42,9)	6 (%85,7)	7 (%100,0)	5 (%71,4)	-	6 (%85,7)
2	-	1 (%14,3)	-	-	6 (%85,7)	1 (%14,3)
3	-	-	-	-	1 (%14,3)	-

Table 2: Frequency distribution of the subjects according to the scores of the histopathological indicators of the right and left kidneys within the groups.
**0=0, 1= 0-%10, 2=%11-25, 3=%26-45.*

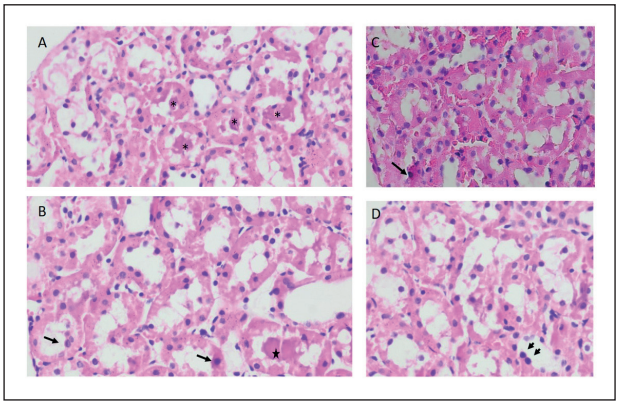

Figure 5: Apoptotic cells and tubular cast formation. Pictures A and B show group B left kidney, pictures C and D show group C left kidney. Apoptotic cells in the proximal tubules are shown with an arrow, and tubular castes with an asterisk.
(*). H&E x400.

There was a statistically significant difference between the groups in terms of right apoptotic cell scores (p<0.001). The situation that caused the said difference; The scores of groups B and C were higher than those of group A (p<0.001). There was a statistically significant difference between the groups in terms of left apoptotic cell scores too (p<0.001). The reason for this difference; the scores of groups B and C were higher than those of group A (p<0.001 and p=0.008) (Table 3). There was no statistically significant difference within the groups in terms of tubular cast formation scores between the right and left kidneys according to Bonferroni correction (p>0.0167).

There was a statistically significant difference between the groups in terms of right tubular cast formation scores (p=0.011); The scores of group B were higher than group A (p=0.015). There was a statistically significant difference between the groups in terms of left tubular cast formation scores too (p<0.001). The situation that causes this difference; The scores of groups A and C were lower than those of group B (p<0.001 and p=0.017) (Table 3).

	Right	Left	p-value†
Loss of brush border of proximal tubule epithelium			
Group A	0 (0 - 0)[a,b]	0 (0 - 0)[a,b]	>0,999
Group B	2 (1 - 2)[a]	2 (2 - 2)[a]	0,564
Group C	1 (1 - 1)[b]	2 (1 - 2)[b]	0,083
p-value‡	**<0,001**	**<0,001**	
Apoptotic cell			
Group A	0 (0 - 0)[a,b]	0 (0 - 0)[a,b]	>0,999
Group B	1 (1 - 1)[a]	2 (2 - 2)[a]	**0,008**
Group C	1 (1 - 1)[b]	2 (1 - 2)[b]	0,046
p-value‡	**<0,001**	**<0,001**	
Tubular cast formation			
Group A	0 (0 - 1)[a]	1 (0 - 1)[a]	0,157
Group B	1 (1 - 1)[a]	2 (2 - 2)[a,c]	0,020
Group C	1 (1 - 1)	1 (1 - 1)[c]	0,317
p-value‡	**0,011**	**<0,001**	

Table 3: Histopathological scores of the subjects according to the groups.
Descriptive statistics; The median (25th-75th) was expressed as a percentage. †Comparisons between right and left kidneys within the groups, Wilcoxon sign test, Bonferroni correction for p<0.0167 were considered statistically significant. ‡Comparisons between groups, Kruskal Wallis test, Bonferroni correction for p<0.025 were considered statistically significant. [a]: The difference between Group A and B is statistically significant (p<0.025), [b]: The difference between Group A and C is statistically significant (p<0.025), [c]: The difference between Group B and C is statistically significant (p=0.017) .

Correlation analysis

As a result of the existing correlation analyzes, no statistically significant correlation was found between histopathological indicators and both preoperative biochemical measurements and postoperative measurements, according to Bonferroni Correction (p>0.025). Likewise, no statistically significant correlation was found

between the change in biochemical measurements after surgery compared to pre-surgery and histopathological indicators according to Bonferroni Correction (p>0.025). There was no statistically significant correlation between the biochemical measurements according to Bonferroni Correction (p>0.025). (Tables 4, 5, 6, 7).

	NGAL	IL-6
Right proximal tubule epithelium brush border loss		
Correlation coefficient	0,122	0,314
p-value†	0,598	0,166
Left proximal tubule epithelium brush border loss		
Correlation coefficient	0,139	0,306
p-value†	0,549	0,178
Right apoptotic cells		
Correlation coefficient	0,150	0,234
p-value†	0,516	0,308
Left apoptotic cell		
Correlation coefficient	0,145	0,271
p-value†	0,531	0,234
Right tubular cast formation		
Correlation coefficient	0,314	0,419
p-value†	0,166	0,059
Left tubular cast formation		
Correlation coefficient	0,065	0,223
p-value†	0,781	0,331

Table 4: Correlation coefficient and significance levels between preoperative biochemical measurements and histopathological indicators among all subjects.
†Spearman's rank correlation test; According to Bonferroni correction, the results were considered statistically significant for p<0.0125.

	NGAL	IL-6
Right proximal tubule epithelium brush border loss		
Correlation coefficient	-0,380	0,003
p-value †	0,089	0,991
Left proximal tubule epithelium brush border loss		
Correlation coefficient	-0,285	0,238
p-value†	0,210	0,299
Right apoptotic cells		
Correlation coefficient	-0,317	0,300
p-value†	0,162	0,186
Left apoptotic cell		
Correlation coefficient	-0,378	0,230
p-value†	0,091	0,316
Right tubular cast formation		
Correlation coefficient	-0,144	0,325
p-value†	0,533	0,150
Left tubular cast formation		
Correlation coefficient	-0,317	0,109
p-value†	0,162	0,637

Table 5: Correlation coefficient and significance levels between post-surgical biochemical measurements and histopathological indicators among all subjects.
†Spearman's rank correlation test; According to Bonferroni correction, the results were considered statistically significant for p<0.0125.

	NGAL	IL-6
Right proximal tubule epithelium brush border loss		
Correlation coefficient	-0,288	-0,231
p-value †	0,206	0,314
Left proximal tubule epithelium brush border loss		
Correlation coefficient	-0,164	-0,140
p-value†	0,477	0,545
Right apoptotic cells		
Correlation coefficient	-0,184	0,017
p-value†	0,426	0,943
Left apoptotic cell		
Correlation coefficient	-0,210	-0,123
p-value†	0,362	0,594
Right tubular cast formation		
Correlation coefficient	-0,189	-0,112
p-value†	0,412	0,629
Left tubular cast formation		
Correlation coefficient	-0,150	-0,100
p-value†	0,517	0,665

Table 6: Correlation coefficient and significance levels between histopathological indicators and changes in the postoperative period according to preoperative status in biochemical measurements among all subjects.
†Spearman's rank correlation test; According to Bonferroni correction, the results were considered statistically significant for p<0.025.

	IL-6	
	r	*p†*
Preoperative		
NGAL	0,421	0,057
Postoperative		
NGAL	-0,051	0,827
Change		
NGAL	0,223	0,330

Table 7: Correlation coefficient and significance levels of biochemical measurements among all subjects in terms of the postoperative change compared to preoperative, postoperative, and preoperative.
†While Spearman's ordinal number correlation test, the results for p<0.025 were considered statistically significant according to Bonferroni correction in the analyzes performed within the pre- and post-op periods. In the analyzes made in terms of the amount of change that occurred in the post-op period compared to the pre-op, the results were considered significant if p<0.05.

Discussion

In our experimental study, in which we evaluated the effects of preoperative fasting duration in rats, we found that short fasting duration had more protective effects in ischemia-reperfusion injury cases. Although the biochemical parameter data evaluated in the study did not statistically differ between the groups, the pathological data were effective in determining the outcome of the

study. ATN findings due to ischemia-reperfusion injury were statistically significantly higher in rats with a longer preoperative fasting period than in shorter ones. Acute kidney injury (AKI) is a common condition in hospitalized patients and is associated with increased medical costs, prolonged hospital stay, and increased morbidity and mortality. Ischemia-reperfusion injury is one of the most common causes of AKI in clinics[13]. Knowing that it can be delayed in the diagnosis of AKI by measuring serum creatinine and glomerular filtration rate (GFR), which are traditional methods, led to the search for new biochemical markers[14].

NGAL has been used as an early biochemical marker of AKI in many experiments with renal IR injury in the literatüre[15-20]. Compared to serum creatinine, NGAL is more prominent in the diagnosis of AKI due to its more significant increase, especially in the early period. Similarly, serum and urine NGAL measurements show parallelism[21, 22]. Similar to animal experiments, in clinical studies (patients undergoing cardiac surgery, acute decompensated heart failure), NGAL is considered an early sensitive and specific marker of AKI. It was concluded that NGAL is an important marker in monitoring short-term graft function after kidney transplant surgeries[23-25]. Akpinar et al. reported that urinary NGAL measurements may be more significant than GFR measurement in the early diagnosis of AKI in patients who underwent partial nephrectomy for renal tumor, but they did not find a correlation between urinary NGAL elevation and clinical severity of AKI[20].

Arakawa et al. They found that the amount of NGAL protein increased in the renal tissue by using immunohistochemical staining method in their study in which they used 20 min ischemia to subjects. According to their experiments, the amount of urine NGAL increasing is observed by 20 min. and longer ischemia, but serum NGAL increasing observed by 30 min. and longer ischemias[15]. Dong et al.; investigated the relationship between tubular biomarkers and ischemia duration and frequency in IR injury. As a result They found that both serum and urine NGAL levels increased significantly after 10-15 min ischemia[18]. Similarly in the literature, Woodson et al. in their studies in which they caused renal IR damage in rats; They proved that NGAL is effective in demonstrating renal damage. Although they observed an effect with 15 minutes of ischemia and then 30 minutes of reperfusion, they reported that the maximum effect was seen with 30 minutes of ischemia and 45-60 minutes of reperfusion, and that longer ischemia times resulted in a decrease in NGAL values.[26]. We did not observe a statistically significant difference in serum NGAL values of our subjects, in which we applied 20 minutes of reperfusion after 20 minutes of ischemia, in groups with sham and different fasting periods, within themselves and also between groups. This may be related to both our ischemia and reperfusion time. Since we could not find any study in the literature on the relationship between preoperative fasting and serum NGAL, we unfortunately interpreted this issue only on our own data.

IL-6 is a pleiotropic cytokine, primarily involved in the immune and inflammatory response. It is produced not only from T and B lymphocytes, but also from fibroblasts, vascular smooth muscle cells, endothelial, mesangial cells and renal tubule epithelial cells[27]. This pleitropism complicates the role of IL-6 in ischemia-reperfusion injury. For example, IL-6 levels increase in cerebral IR injury. This increase turns into a positive protective picture[28]. Nimesh et al. In an experimental study consisting of 3 groups: normal rats, IL-6 deficient rats and IL-6 antibody administered; When reperfusion is applied for 24 hours after 30 minutes of bilateral renal ischemia; determined that renal damage, inflammation and loss of function were seen minimum in IL-6 deficient rats[29].

De Vries et al. reported that the amount of IL-6 increased from the 5th minute in the samples they took from the greft veins after renal transplantation, but they also showed that survival decreased when IL-6 antibody was given to the rats who underwent renal transplantation in the same study[30]. Chen et al. In their study based on the anti-inflammatory activity of IL-6 when bound to membrane receptors and pro-inflammatory properties when bound to the receptor dissolved in serum; reported that less fibrosis was observed in rats given antibodies against gp-130, a IL-6 receptor dissolved in serum, on the 14th day after 45 minutes of renal ischemia compared to rats that were not given antibodies[31].

When seeking an answer to the question of whether the reason for the increase in IL-6 in renal IR damage is the decrease in renal filtration or the increase in local or systemic inflammation; It has been reported that after 45 minutes of renal ischemia, IL-6 expression increases in both the kidney and liver at both the 1st and 6th hours, and that renal ischemia reperfusion causes a systemic response[32]. In our study, we wanted to evaluate

the effect of preoperative fasting time on IL-6, a biochemical marker of renal ischemia-reperfusion injury. Similar to serum NGAL values, we did not detect any difference between and within groups. When the relationship between IL-6 and NGAL in renal ischemia-reperfusion injury was examined, it was shown that the major factor in the increase in NGAL was caused by liver NGAL production and the IL-6 cytokine was the active stimulator in this situation[33]. We did not observe a statistically significant association between IL-6 and NGAL correlation in our study. This may be related to the relatively short duration of ischemia. Because the studies in the literature; It shows that IL-6 is increased especially in cases where severe damage is caused by prolonged ischemia. The rise of IL-6 is especially associated with fibrosis formation and progression to chronic kidney damage[31, 32].

While acute kidney injury is a 5% mortal condition when faced in isolation without any additional pathology in patients, it is an important problem with a mortality rate of 50% or more, especially in intensive care patients, if there is an accompanying organ failure. The most common cause appears to be ATN due to ischemia. Proximal tubule cells are also the most susceptible to ischemia, as they are the most energy-demanding part with high mitochondria[34]. Renal ischemia reperfusion animal models in the literature; warm ischemia reperfusion, cold ischemia-warm reperfusion, and isolated renal reperfusion. Heyman et al. warm ischemia reperfusion model among these models; They stated that it is the most preferred model because of simple, functional damage and pathology being correlated with humans, and the inflammatory response being compatible with humans[35] Hesketh et al. In their study examining the effect of renal ischemia time on IR damage in rats, they proved histopathologically that 20 min ischemia with mild injury, 22 min ischemia with moderate damage, 24 min ischemia with severe tubular necrotic injury[36]. In our study, we used the warm ischemia reperfusion model in accordance with the literature, instead of applying a drug or substance, we applied ischemia for 20 minutes to allow mild damage instead of severe necrotic damage in order to compare the effects of a condition on ischemia reperfusion damage. Due to the technical inadequacies of the laboratory where the experiment was conducted, we ended the experiment after 20 minutes of reperfusion.

It has been shown in studies that shortening the preoperative fasting period reduces insulin resistance and acute inflammatory response[4, 5, 37]. Preoperative nutrition has an important place in ERAS protocols. Rege et al. retrospectively examining the effects of ERAS protocols on kidney donors; preoperative carbohydrate-containing fluid consumption; argued that it contributes to early postoperative recovery due to the ability to keep blood glucose under control and muscle preservation mechanisms[38]. Fleming et al. when they examined the effects of ERAS protocols in cardiac surgery; with many postoperative complications, including acute kidney injury, atrial fibrillation, cardiac tamponade; They found that they encountered less in patients who applied the ERAS protocol[39].

Although the positive effects of ERAS protocols in renal ischemia-reperfusion situations were observed in these studies, the role of preoperative nutrition in this effect has not been fully explained. van Hoorn et al. examined the relationship between preoperative fasting time and ischemia-reperfusion injury. In their study, ischemia was created by clamping the superior mesenteric artery for 60 minutes in rats that were fasted for 13 hours and 2 hours preoperatively, and then reperfusion was allowed for 180 minutes. They showed that cardiac functions were better preserved and oxidative stress-related damage was less observed in rats with a short preoperative fasting period. The results of their studies, when preoperative shorter fasting periods in rats are compared with long periods of fasting, They reported that it reduces IR damage and preserves organ function[3]. When we examined the histopathological effects of renal IR injury in our study, we observed negative high scores in terms of all histopathological evaluations in rats exposed to a long preoperative fasting period.

This result was consistent with the targeted level of mild ischemic injury. In terms of brush border loss, we found statistically significant higher scores in both left and right kidneys in the other groups compared to the sham group. In terms of apoptosis, a statistically significant increase was observed in the left kidney compared to the right kidney in rats with a long preoperative fasting period, while this increase was not observed in the other groups.

In terms of tubular cast formation, a statistically significant increase was observed in rats that were fasted longer preoperatively in both the left and right kidneys (without ischemia), compared to the other groups. This made us think that a short preoperative fasting period may have protective effects in cases of renal ischemia-reperfusion injury.

Conclusion

General practices during the preoperative fasting period have begun to change in recent years, unlike the traditional protocol. In the formation of this result, the decrease in insulin resistance and inflammatory response due to surgical stress due to the consumption of preoperative carbohydrate-containing fluids 2-3 hours before the operation, which is an important step of ERAS protocols, has an important role. In our study, we concluded that the short preoperative fasting period has a protective effect on renal ischemia perfusion injury.

In our study, we observed the effects on renal ischemia-reperfusion injury by fasting a group of rats for 12 hours and a group for 2 hours. In rats with shorter preoperative fasting periods, histopathological damage is seen less in the case of mild renal ischemia reperfusion. We did not observe significant changes in IL-6 and NGAL parameters, which may be because we aimed to cause mild ischemic damage in our study. In this respect, it does not contradict other studies in the literature which longer ischemia reperfusion times and severe ischemic injury. We believe that future studies that will use histopathological criteria and different biochemical markers on moderate and severe renal ischemia-reperfusion injury will be even more useful to fully explain the effects of preoperative fasting time on renal ischemia-reperfusion injury.

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