UNDERSEA & HYPERBARIC MEDICINE

Hyperbaric oxygen mitigates KIM-1 and inflammatory cytokine levels in kidney transplantation

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ABSTRACT

Büyük B, Öztopuz Ö. Hyperbaric oxygen mitigates KIM-1 and inflammatory cytokine levels in kidney transplantation. Undersea Hyperb Med. 2023 Third Quarter; 50(3):271-282.

Background: The aim of this study was to investigate the effect of hyperbaric oxygen (HBO_2) administration applied during cold ischemic time to organs removed from donors before kidney transplantation. A total of 24 rats were divided into three groups: Group 1 was the control group, Group 2 received 60 minutes of HBO_2 at 2.5 atmospheres absolute, and Group 3 received 120 minutes of 2.5 ATA HBO_2. The renal artery was entered with a polyethylene catheter and perfused with a standard organ preservation solution. Falcon tubes containing organs obtained from rats in Groups 2 and 3 were placed in a box supported by ice blocks. The temperature was kept constant at 4 °C and the box was placed in a pressure tank with 2.5 ATA HBO_2. HBO_2 was applied for 60 and 120 minutes, respectively. Organ samples were harvested at the end of 24 hours for histopathological evaluation, immunohistochemical analysis of TNF-α and IL-18, TUNEL analysis for apoptosis, and gene expression levels of kidney injury molecule-1 (KIM-1) and caspase-3. In histopathological examinations, hematoxylin and eosin staining was performed and samples were evaluated for tubular necrosis and vacuolization criteria. Group 2 and Group 3 had significant decreases compared to Group one in this regard. Immunohistochemical staining was performed for TNF-α, IL-18, and apoptosis levels; significant decreases were found in Groups 2 and 3. There were significant decreases in Groups 2 and 3 for KIM-1 and caspase-3 gene expression levels compared to Group 1, as well. Thus, it was demonstrated that during the cold ischemic period before kidney transplantation, HBO_2 administration to organs removed from donors can reduce apoptotic cell numbers, inflammatory cytokine release, and histopathological damage to the organs as well as decreasing the expression of the KIM-1 gene, which is an indicator of kidney damage.

Keywords: cold ischemia; hyperbaric oxygenation; interleukin-18; kidney; organ transplantation; KIM-1; rat

INTRODUCTION

Compared to other methods, transplantation is the treatment that offers the highest quality of life for patients with end-stage renal disease [1]. As the number of organs obtained is insufficient to meet the demand for transplantations, it is of paramount importance to optimize the transplant procedure to achieve the best possible outcome for each donated kidney [2].

After a kidney is surgically removed for transplantation, it is stored in a cold preservation solution at 4 °C to preserve the viability of the cells [3]. The purpose of hypothermic preservation is to lower the temperature below 10 °C to slow the metabolism and

oxygen demand. During hypothermic preservation, adenosine triphosphate (ATP) is gradually depleted, leading to the accumulation of toxic substances inside the cells. Mitochondria play an important role in cellular homeostasis and potential membrane changes induced by hypothermic conditions, promoting apoptosis and necrosis. With the loss of aerobic respiration, large amounts of free radicals are produced, causing further damage. Preservation solutions are predominantly designed to counteract these processes by preventing swelling and changes in the cell membrane [4].

Cold ischemia time (CIT) in kidney transplantation begins with the clamping of the renal artery and perfusion of the organ with a preservation solution, and it elapses with the first vascular anastomosis after transplantation. This time is used for donor tissue typing, cross-matching, organ transport, and recipient and operation preparation [5]. Cold preservation for solid organs slows the anaerobic metabolism, preventing metabolic waste production and reduction of ATP levels [5]. However, while cold ischemia can alleviate cellular damage, it cannot completely prevent it [3].

Cold ischemia triggers several harmful effects, including the production of inflammatory and immune responses that can potentially result in delayed graft function, increased alloimmune reactivity, and the development of progressive pathological changes [3]. Studies have demonstrated that prolonging the CIT in kidney transplantation may increase the risk of allograft failure and death [6]. Increased CIT is the most important factor contributing to delayed graft function after kidney transplantation [7,8].

Hyperbaric oxygen (HBO_2) therapy is the delivery of oxygen under high atmospheric pressure. It is used to promote wound healing and to treat decompression sickness, carbon monoxide poisoning, gas embolisms, and circulatory disorders. Under normal atmospheric pressure, there is a limit to the amount of oxygen that can be carried in the blood. Increasing the atmospheric pressure enhances the amount of dissolved oxygen in the plasma, allowing it to penetrate tissues better. Therefore, with HBO_2 therapy, tissues can be adequately oxygenated in the absence of blood flow, providing an advantage in the protection of organs [4].

Free radical scavenging activities, and especially that of superoxide dismutase, may increase in tissues after HBO_2 treatment. HBO_2 has also been shown to increase antioxidant gene expressions in human endothelial cells, which may protect against oxidative damage seen in cases of ischemia-reperfusion (I/R) injury. In this way, HBO_2 appears to produce beneficial effects for ischemic tissue by both reducing the production of reactive oxygen species (ROS) and increasing their degradation [9]. Studies of rats with renal I/R injury treated with HBO_2 prior to ischemia demonstrated reduced oxygen radical-induced lipid peroxidation [10]. In addition, the inhibition of apoptosis achieved with HBO_2 and improvement in cellular proliferation following renal I/R injury has been demonstrated [11].

While researchers have previously investigated the effects of HBO_2 under various conditions, we were unable to find any study in the literature about the effects of HBO_2 on the preservation of the kidneys of donors in transplantation procedures. In addition, we could not find any studies about differences in the levels of kidney injury molecule-1 (KIM-1), an important marker of kidney injury, after HBO_2 administration for kidney tissues.

The aim of this study was to investigate the effects on organ viability and cold ischemic injury of 60 and 120 minutes of HBO_2 therapy administered during CIT to organs removed from donors before kidney transplantation.

MATERIALS AND METHODS

Materials and experimental design

This study was approved by the Institutional Animal Use and Care Committee of Canakkale Onsekiz Mart University (COMU) (Approval No: 2019/04-07) and performed in accordance with the recommendations of the Declaration of Helsinki for animal studies.

A total of 24 adult female Wistar albino rats were obtained from COMU Experimental Research Application and Research Center with a mean age of 2 months and mean weight of 250-310 grams. The rats were housed in standard rat cages in an animal room maintained with standard humidity (45 to 55%) and temperature of 22 ± 2 °C with 12-hour

light/dark cycles. All animals were fed standard food and water. Twelve hours before the study procedure began, feeding was stopped and the rats were only allowed to drink water. The entire experiment was conducted under half-sterile conditions.

The 24 female Wistar albino rats were randomly divided into three groups with eight animals in each group:

Group 1 (n=8): Control group

Group 2 (n=8): Administration of 60 minutes of HBO_2 at 2.5 atmospheres absolute (ATA)

Group 3 (n=8): Administration of 120 minutes of 2.5 ATA HBO_2

Experimental procedure

Kidney perfusion was performed as previously described [12]. An abdominal midline incision was made in rats under anesthesia with ketamine hydrochloride (50 mg/kg, Ketalar®, Pfizer, Turkey) and xylazine (15 mg/kg, Rompun®, Bayer, Canada). The proximal renal artery was ligated and entered with a polyethylene catheter, and a standard organ preservation solution (Cold Storage Solution BEL-GEN, Institut Georgez Lopez, France) stored at 4 °C was administered. Perfusion was continued until clear fluid came from the renal vein. After perfusion, the rats were killed by cervical dislocation. The removed organs were placed in Falcon tubes containing 15 mL of standard organ preservation solution and stored at a constant temperature of 4 °C.

HBO_2 administration

Organs obtained from rats in Group 1 were kept at a constant temperature of 4 °C for 24 hours. HBO_2 administration to animals in Groups 2 and 3 was performed as previously described [13]. Falcon tubes containing the organs obtained from rats in Groups 2 and 3 were placed in a box supported by ice blocks. The temperature was kept constant at 4 °C, and the box was placed in a pressure tank with 2.5 ATA HBO_2. The HBO_2 was applied for 60 minutes for Group 2 and 120 minutes for Group 3. Temperature monitoring was performed with a digital thermometer (Berlin Fridge Tag Memory Digital Thermometer, Berlinger, Switzerland). The temperature changes in the tank over time were followed, and it was confirmed that the tank did not exceed 4 °C. It was kept at a constant temperature of 4 °C for 23 hours (60 minutes of HBO_2 + 23 hours of storage for a total of 24 hours) for Group 2 and 22 hours (120 minutes of HBO_2 + 22 hours of storage for a total of 24 hours) for Group 3.

Tissue samples from the perfused and preserved organs were taken after 24 hours for histopathological and molecular analysis.

Histopathological analysis

At the end of 24 hours, kidney tissue samples were quickly added to 10% neutral buffered formalin and left for fixation for 48 hours. After fixation, a routine tissue processing procedure was followed, and samples were embedded in paraffin blocks. A routine hematoxylin and eosin (H&E) staining protocol was performed using 5-µm sections from paraffin-embedded tissues.

Tissue sections were evaluated by one histologist who was blinded to the groups. Evaluations were performed under a camera-attached light microscope (Olympus CX43), and samples were graded in terms of tubular necrosis and vacuolization parameters as follows [12,13]:

0: No injury
+: Mild injury
++: Moderate injury
+++: Severe injury

Immunohistochemical staining

Sections of 4 µm in thickness obtained from tissues were placed on poly-L-lysine-coated slides. Immunohistochemical (IHC) staining for tumor necrosis factor-α (TNF-α; Clone 13F9.1, Lot #Q2573230, EMD Millipore Corporation, USA) and interleukin-18 (IL-18; Cat. No. orb107403, Biorbyt Ltd., UK) was performed. After the sections were deparaffinized, an antigen retrieval method was applied, and tissue sections were incubated separately with primary antibodies. DAB (3,3-diaminobenzidine tetrahydrochloride) was used as the chromogen and counterstaining was performed with Harris's stain. The stained sections were examined under an Olympus CX43 camera-attached light microscope (Olympus Corporation, Japan) by one histologist who was

blinded to the groups. The findings were scored as follows [12]:

Grade 0: <10% of cells were stained
Grade I: 10-25% of cells were stained
Grade II: 25-50% of cells were stained
Grade III: >50% of cells were stained

In situ apoptosis detection by TUNEL method (terminal deoxynucleotidyl transferase dUTP nick end labeling)

To assess apoptosis, 4-µm-thick sections were obtained from each paraffin block and stained with the ApopTag Apoptosis Detection Kit (S7100, Merck Millipore, Germany) following changes of xylene and reducing alcohol. Evaluations were performed by one histologist who was blinded to the groups. The sections were examined under a light microscope (Olympus CX43) with 400× magnification, and brown/black-stained nuclei of apoptotic cells were noted. The apoptotic cell ratio was calculated as the apoptotic index (AI) in percentage by counting 500 brown/black-stained cells:

Apoptotic index (AI) = (Number of positive cells/ Total number of cells counted) × 100

Gene expression analysis

Changes in the expressions of KIM-1 and caspase-3 (Casp-3) were determined in kidney tissues after 60 and 120 minutes of HBO_2 administration. At the end of the experimental period, approximately 10-30 mg of kidney tissue was taken and homogenized in a homogenizer (Mixer Mill MM 400, RETSCH, Germany). Total RNA isolation (Ambion PureLink RNA Mini Kit, Thermo Fisher Scientific, USA) was performed with the obtained homogenates. The concentrations of the samples were then equalized by measuring the RNA concentrations and purity with a NanoDrop photospectrometer. cDNA synthesis (High-Capacity cDNA Reverse Transcription Kit, Thermo Fisher Scientific) was determined from the obtained RNA samples. Polymerase chain reaction (PCR) conditions were as follows: Step 1: 25 °C, 10 minutes; Step 2: 37 °C, 120 minutes; and Step 3: 85 °C, 5 minutes. The obtained cDNAs were amplified using the StepOne real-time PCR system (Thermo Fisher Scientific) in accordance with the TaqMan qPCR Master Mix protocol. β-Actin was used as the housekeeping gene. Gene expressions were determined using cycle threshold (Ct) values and fold changes were evaluated by the 2-ΔΔCT method. The primary ID numbers of Casp-3, KIM-1, and β-actin were Rn00563902_m1, Rn01525859_g, and Rn00667869_m1, respectively.

Statistical analysis

IBM SPSS Statistics 22 (IBM Corp., USA) was used for statistical analysis. Bivariate comparisons were conducted with paired samples t-tests and one-way analysis of variance (ANOVA). For gene expression analysis, groups were compared using ANOVA followed by the Tukey test and values of $p<0.05$ were accepted as statistically significant. To evaluate the expression levels of the genes, the 2-ΔΔCT method was used [2-ΔΔCT = (Ct target gene - Ct reference gene)]. The statistical significance level (p) was set to <0.05.

RESULTS

Histopathological evaluation

The kidney tissues obtained from the experimental groups were evaluated in terms of tubular necrosis and vacuolization, as described in the Materials and Methods section. Tubular necrosis was decreased in Groups 2 and 3 compared to the control group (p-values were 0.001 and <0.001, respectively). It was thus understood that 60 and 120 minutes of HBO_2 administration were effective in preventing damage to the tubular epithelial tissue. The decrease was significantly greater in Group 3 compared to Group 2 (p=0.002). This further showed that 120 minutes of HBO_2 administration was more effective than 60 minutes of HBO_2 administration.

Vacuolization levels were decreased in Groups 2 and 3 with HBO_2 administration compared to the control group (p-values were 0.05 and <0.001, respectively). This decrease was significantly greater in Group 3 compared to Group 2 (p=0.004). This finding reveals that HBO_2 administration may be a viable treatment choice for protecting kidney tissues from vacuolization. In addition, 120 minutes of HBO_2 administration was more effective. H&E-stained sections of the study groups are shown in Figure 1. The results of H&E-stained sections are shown in Figure 2.

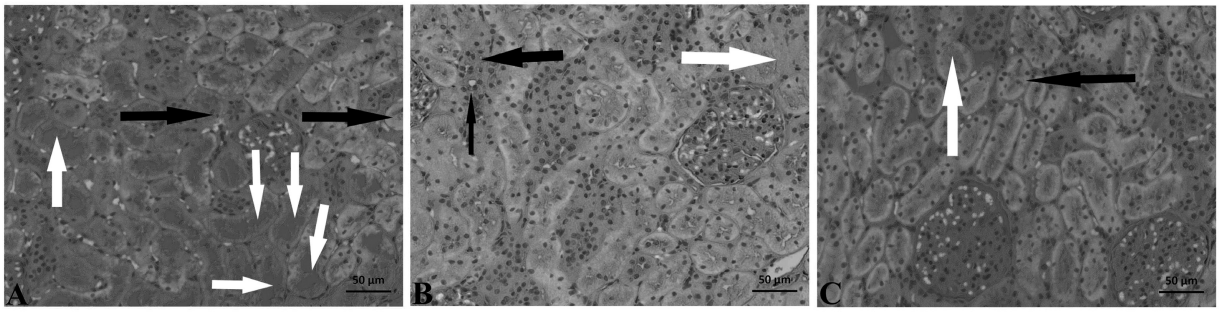

Figure 1. H-E-stained sections of the study groups have given in Figure 1. The sections belong to Control, HBO-60 and HBO-120 groups can be seen in A, B and C, respectively. White arrows sign tubular necrosis, and black arrows sign vacuolization areas in figure. Magnification x200.

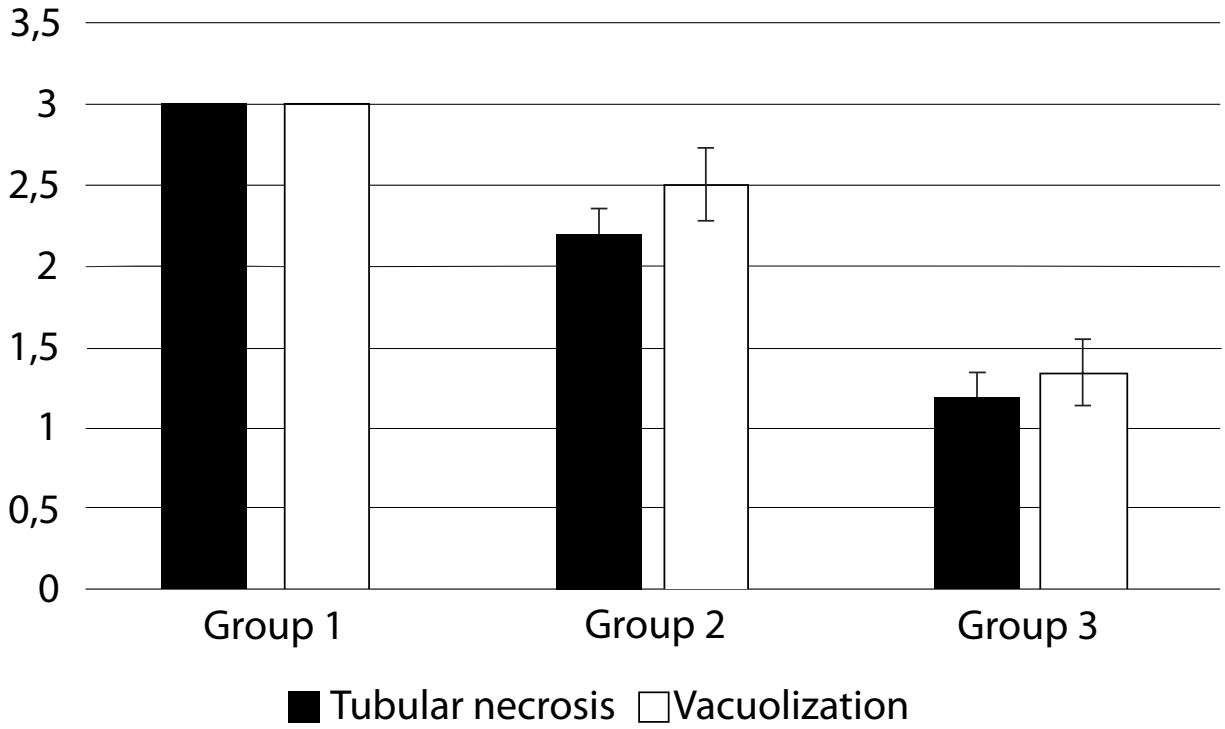

Figure 2. Evaluation results of H-E-stained sections (α: compared to Group 1, β: compared to Group 2 $p< 0.05$).

Immunohistochemical evaluation

For the kidney tissues obtained at the end of the study, IHC staining was performed and evaluated in terms of TNF-α and IL-18.

IHC evaluations were first performed for TNF-α. A significant decrease was detected in Groups 2 and 3 compared to the control group (p-values were 0.001 and 0.004, respectively). However, there was no significant difference between Groups 2 and 3 ($p=0.36$). Thus, it was understood that HBO_2 decreased the expression of TNF-α, but this effect was not dose-dependent.

We also immunohistochemically evaluated IL-18 levels, and the release of IL-18 was found to be significantly decreased in Groups 2 and 3 compared to the control group (p-values were 0.04 and 0.01, respectively). However, no significant difference was found between Groups 2 and 3 ($p=0.60$). IHC-stained sections of the study groups are shown in Figure 3. Evaluation results of IHC-stained sections are shown in Figure 4.

Apoptosis assessment by TUNEL method

AI values reflect the apoptotic cell percentages of each tissue section. It was found that the AI values

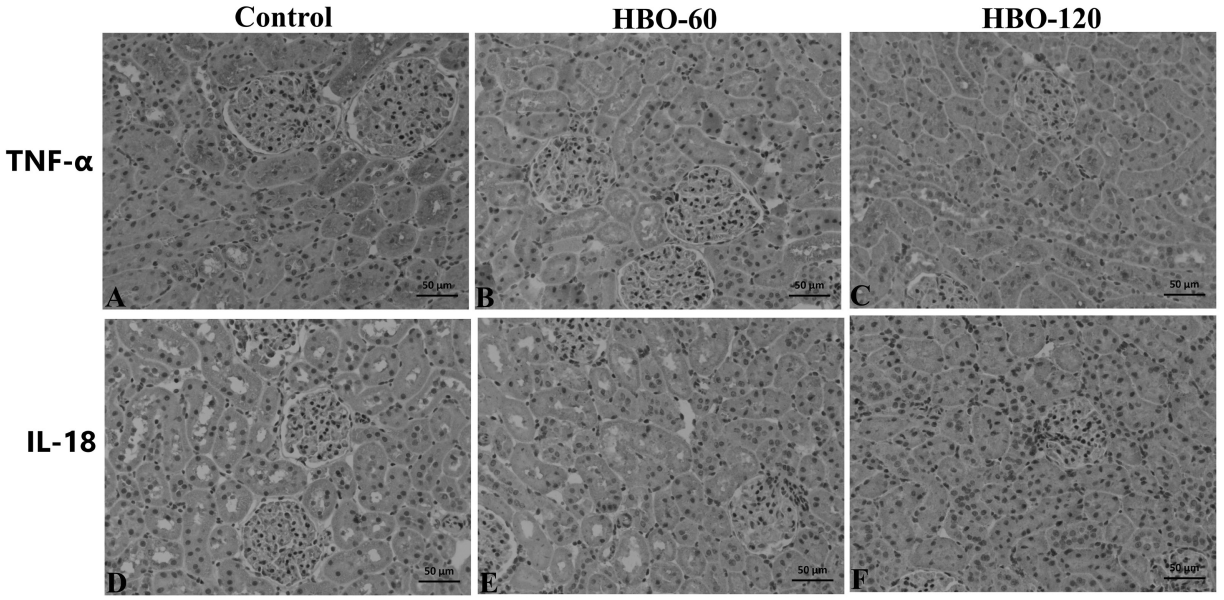

Figure 3. IHC stained sections of the groups can be seen. Sections A and D belong to Control group, section B and E belong to HBO-60, sections C and F belong to HBO-120. DAB was used as chromogen during IHC staining process. Areas seen in brown color can be defined as immunohistochemical intensive areas. Magnification x200.

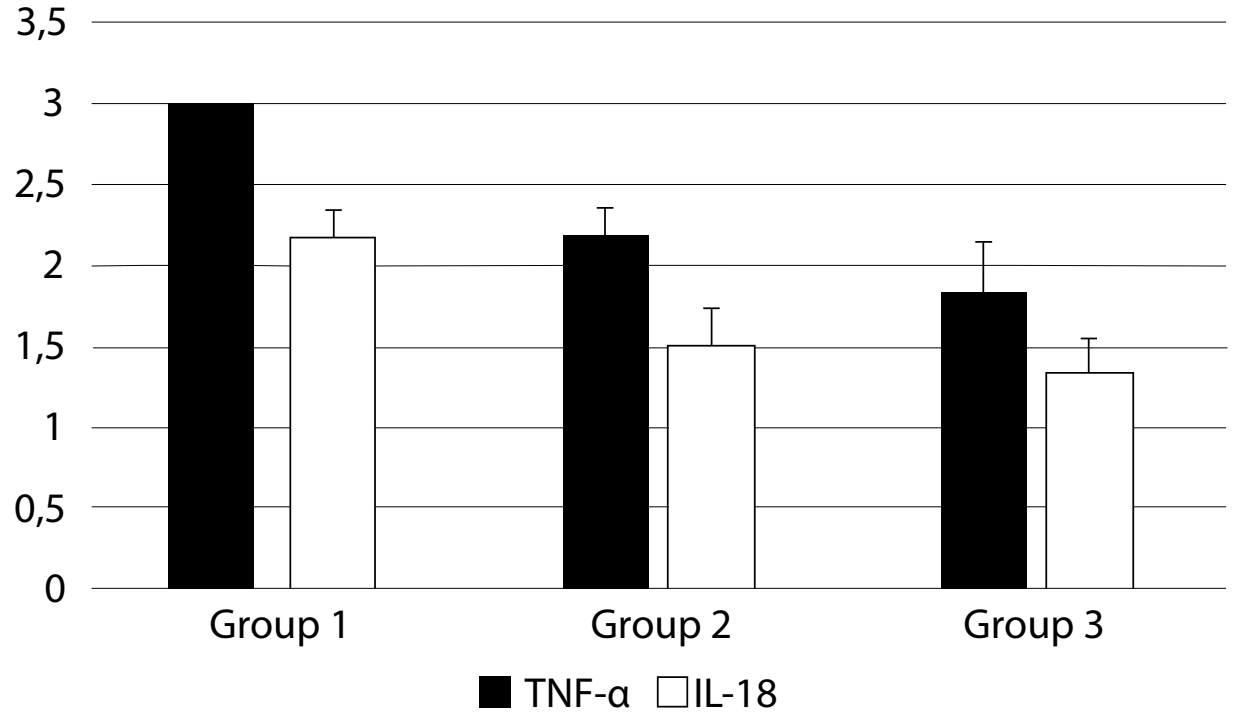

Figure 4. Evaluation results of IHC-stained sections (α: compared to Group 1 $p < 0.05$).

of Groups 2 and 3 were significantly decreased compared to the control group (p-values were 0.001 and <0.001, respectively). Furthermore, the AI values of Group 3 were significantly more reduced compared to Group 2 (p=0.03). It was thus demonstrated that HBO_2 reduced the apoptotic cell counts, and this effect was enhanced with increased exposure time. TUNEL-stained sections of the study groups are shown in Figure 5. TUNEL evaluation results are shown in Figure 6.

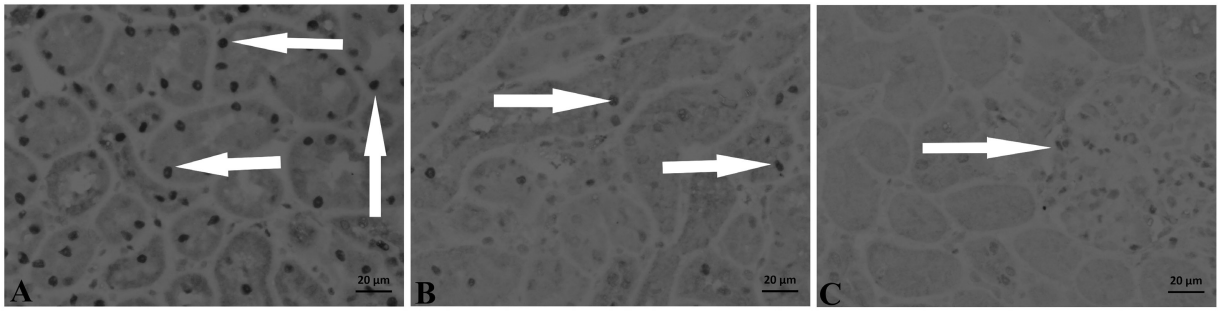

Figure 5. TUNEL-stained sections of control, HBO-60 and HBO-120 groups can be seen in A, B, and C, respectively. White arrows mark apoptotic cell nuclei. Magnification x400.

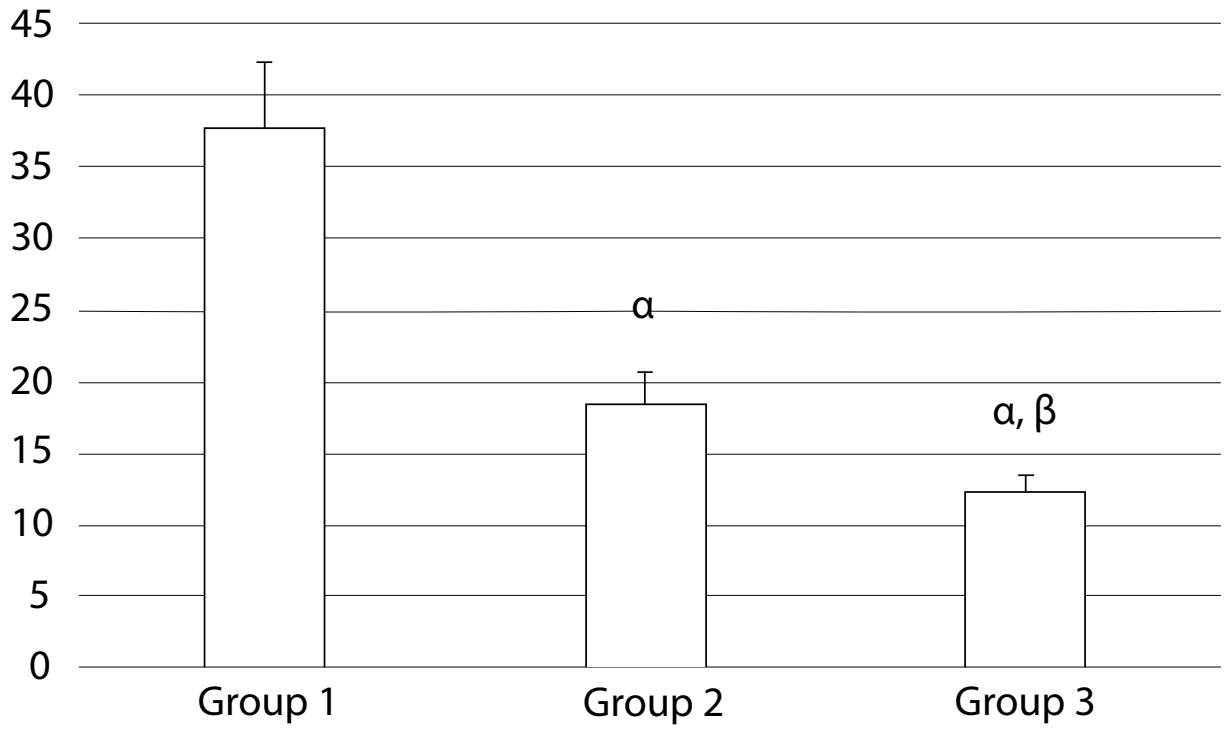

Figure 6. TUNEL evaluation results (α: compared to Group 1, β: compared to Group 2 $p< 0.05$).

Gene expression results

In the evaluation of KIM-1 gene expression, there was no significant difference between the control group and Group 2 ($p=0.10$), while a significant decrease was observed in Group 3 compared to the control group ($p=0.03$). Moreover, no significant difference was found between Group 2 and Group 3 for KIM-1 gene expression ($p=0.83$).

It was observed that the administration of HBO_2 significantly reduced Casp-3 gene expression in Groups 2 and 3 compared to the control group (p-value was <0.001 for both groups). However, the Casp-3 gene expression level did not significantly change with exposure time; expression levels were found to be similar in Groups 2 and 3 ($p=0.35$). A comparison of the KIM-1 and Casp-3 expression levels in the kidneys of the study groups is shown in Figure 7.

DISCUSSION

In our study, in order to demonstrate the efficacy of HBO_2 in the cold ischemia process before kidney transplantation, HBO_2 was applied to kidneys perfused with organ preservation fluid at 4 °C for 60 and 120 minutes. Less histopathological damage was observed in the kidney tissues of the experimental groups than in the control group. In addition, in IHC evaluations and KIM-1 gene expression analysis, it

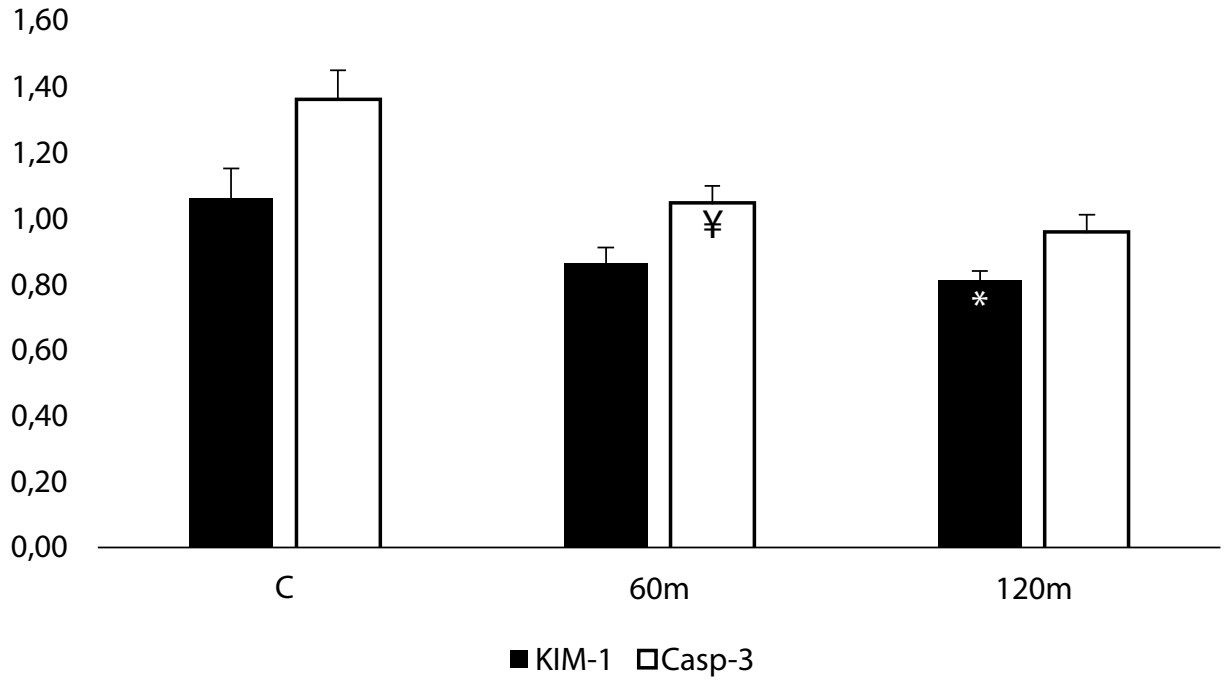

Figure 7. **Comparison of KIM-1 and Casp-3 expression levels in kidney between groups.**
***: p<0.05 compared to control group for KIM-1 gene expression.**
¥: p<0.05 compared to the control group for Casp-3 gene expression.

was observed that damage was reduced in the experimental groups that had undergone treatment with HBO_2.

Considering the number of apoptotic cells and Casp-3 gene expression, which are important parameters in demonstrating organ viability, it was observed that apoptosis values were decreased in the HBO_2-administered groups compared to the control group.

Kidney transplantation is an important treatment option for patients with organ failure [12]. In the process of kidney transplantation, it is of crucial importance to protect the organ until the time of transplantation. For this purpose, the organ to be transplanted is subjected to cold preservation. Cold ischemia in the kidney slows anaerobic metabolism [5]. Cold ischemia can also alleviate cellular damage, although it cannot completely prevent it [3]. For this reason, efforts to develop strategies that can provide better protection of organs to be transplanted during the CIT are ongoing [12,14-16]. Preservation of the organ during the CIT is a very important factor affecting the success of the transplantation. Damage to the organ to be transplanted must be kept to a minimum during this process.

With the clamping of the renal artery before kidney transplantation, hypoxia begins in the organ. The release of ROS from the mitochondria increases in response to hypoxia. During ischemia, cells tend to provide ATP through anaerobic respiration, and the resulting lactic acid from this pathway leads to intracellular acidosis. This anaerobic energy production ceases after the glycolytic substrates are depleted. Due to weak ATP production, the activity of the Na/K-ATPase pump is disrupted, and this results in cell swelling by disrupting the sodium-potassium balance in the cells. These changes lead to cell-cycle arrest and, ultimately cell death [3]. Cellular apoptosis may reduce the viability of the organ after transplantation. For this reason, there are many studies in the literature undertaken with the aim of preventing such damage by reducing the CIT [8,17,18]. However, it is not always possible to reduce the CIT. In particular, the donor and recipient being in different geographical areas may cause the CIT to be prolonged. Therefore, reducing the rate of apoptosis that may develop in the organ without reducing the CIT may allow an increased rate of successful transplantations.

HBO_2 treatment entails the application of oxygen to tissues under high atmospheric pressure. It is now used in the treatment of many different diseases [4]. Studies have shown that the application of HBO_2, especially in cases where ischemia plays a role, can reduce tissue injury. HBO_2 also has a mitigative effect on tissues during I/R [19]. The amount of oxygen available in the blood at normal atmospheric pressure is limited. As the pressure increases, however, the amount of soluble oxygen in the plasma also increases and more oxygen is delivered to the tissues. This is advantageous during organ preservation when the blood flow is cut off [4]. A previous study demonstrated HBO_2-induced inhibition of apoptosis and improvement in cellular proliferation following renal I/R injury [11]. According to Solmaz et al. [20], HBO_2 was administered at 2.5 ATA for 60 minutes in a 24-hour reperfusion model following 30 minutes of ischemia, and it was shown to protect against I/R damage. The data obtained in these studies highlight the protective effect of HBO_2 on tissues, particularly in ischemic processes. In addition, HBO_2 has been administered to donors for kidney transplantations, but there are no data available in the literature to date about the use of HBO_2 in the CIT [21]. Therefore, in our study, 60- and 120-minute HBO_2 treatments were used to protect kidney tissues during cold ischemic storage. This minimized the hypoxic ischemic damage to the tissues and reduced the number of cells entering apoptosis in this hypoxic environment. Parameters signifying damage were significantly reduced in the groups for which cold ischemia was applied for 24 hours at 4 °C together with HBO_2 treatment compared to the control group, the tissues of which were routinely stored without the application of HBO_2. In particular, the decrease in the number of apoptotic cells as detected by the TUNEL method compared to the control group represents increased post-transplant viability of these organs. In our study, the fact that the number of apoptotic cells was found to be lower in the group receiving 120 minutes of HBO_2 treatment compared to the group that received 60 minutes of HBO_2 treatment suggested that the protective effect of HBO_2 increased as the duration of its administration increased. Casp-3 gene expression levels further supported the apoptosis values obtained by the TUNEL method. HBO_2 administration significantly decreased Casp-3 gene expressions in both the 60-minute and the 120-minute groups. These findings support the conclusion that apoptosis levels were reduced by HBO_2 administration. However, there was no difference between the 60-minute and the 120-minute groups for Casp-3 gene expression, while the AI value obtained by the TUNEL method was significantly reduced for the 120-minute group. This finding may be the result of the methods used for obtaining the data. Methods that detect gene expression generate data about the protein synthesis levels of genes. IHC methods, such as TUNEL, reveal the impact of the related genes. For instance, in this study, while the Casp-3 levels were not different between the 60-minute and 120-minute groups, AI values differed, being lower in the 120-minute group. Casp-3, produced by gene expression, affects the cells, leading them to undergo apoptosis. After that effect, the molecules are broken down, and apoptosis can be observed under light microscope at the end of the molecular pathway. For this reason, we could observe the effects under a light microscope but not in gene expression data at some points in the present study. Perhaps we would not have seen the difference between the AI values of these two experimental groups if we had obtained the tissues at a time point later than 24 hours.

IL-18 is a proinflammatory cytokine belonging to the IL-1 family [22]. IL-18 increases IL-1β and TNF-α secretion by stimulating T-helper lymphocytes [23]. The role of IL-18 in acute kidney injury has been previously demonstrated [22]. It has been shown that IL-18 is particularly increased in blood and urine in cases of acute kidney injury that develop after liver transplantation [24]. In a rat model of kidney allograft rejection, it was shown that IL-18 mRNA expression increased threefold [25]. It has also been shown that the amount of IL-18 in the urine is clinically increased in patients with acute tubular necrosis [26]. These data confirm that IL-18 as a proinflammatory cytokine is increased significantly in cases of hypoxia and subsequent processes that may occur in the kidneys. Accordingly, kidney damage may be diagnosed by monitoring IL-18 levels. It was previ-

ously demonstrated that HBO_2 administration decreased the IL-1 and TNF-α levels in tissues [19, 27, 28]. In the current study, IL-18 was examined immunohistochemically to ascertain whether HBO_2 administered during the CIT has protective effects on kidney tissues. Based on the immunohistochemical evaluation results, levels of IL-18 were significantly lower in Groups 2 and 3 compared to the control group. This demonstrates that HBO_2 administered to kidneys to be transplanted during the CIT can reduce inflammation and, thus, the damage that may occur in kidney tissues.

Nitric oxide (NO) is a free radical that is crucial in I/R injury. There are three forms of NO synthase (NOS), one of which is inducible NOS (iNOS). iNOS is produced by neutrophiles and macrophages, which are induced by TNF-α. iNOS is related to vascular leakage and tissue injury. HBO_2 exerts a beneficial effect by reducing iNOS levels [19]. In addition, in the present study, IHC staining was also performed for TNF-α, and it was shown that the expression of TNF-α, like that of IL-18, was significantly decreased in Groups 2 and 3 compared to the control group. Considering that TNF-α secretion is induced by IL-18 [23], these findings support each other. These results show that HBO_2 administration to kidneys to be transplanted during the CIT can reduce the ischemic damage in the kidney tissues and thus increase the likelihood of a successful transplantation.

KIM-1 is a type of membrane protein that is expressed mainly in the kidneys, with lower levels of expression in the liver [29]. KIM-1 expression in the kidneys is associated with renal inflammation and fibrosis. KIM-1 levels are high in biopsy materials taken from individuals with kidney damage [30]. After damage to the kidney for any reason, apoptosis and necrosis occur in the tubule epithelium. Eliminating apoptotic and necrotic cells, relieving inflammation, and promoting tissue repair are essential for successful healing. KIM-1 contributes to eliminating those apoptotic and necrotic cells [31]. Therefore, KIM-1, which is expressed at very low levels in normal kidney tissue, is increased in cases of primary and secondary kidney diseases [32]. Ischemic damage also occurs in preserved kidney tissues during the CIT, and KIM-1 expression is increased in pre-transplant kidneys due to ischemia. Therefore, in the present study, KIM-1 expressions were investigated in kidney tissues during the CIT. The measurement of lower KIM-1 expression levels in Groups 2 and 3 compared to the control group confirms that HBO_2 protects kidneys to be transplanted from damage during the CIT period. Considering that the expression of KIM-1 is especially high in the proximal tubule epithelium, it is thought that the tubular epithelium can be better protected with HBO_2 administration, thus preventing the acute tubular necrosis and reperfusion injury that may develop after transplantation. The fact that there was no difference between 60 and 120 minutes of administration suggests that HBO_2 can exert its beneficial effects in shorter periods of time. However, according to the histopathological evaluations and TUNEL staining results, 120 minutes of HBO_2 administration is more beneficial for the protection of kidneys to be transplanted than 60 minutes. Although there was no difference between the 60- and 120-minute groups in terms of KIM-1 expression, both the decrease in the number of apoptotic cells and the histopathological data confirm that increased HBO_2 administration times can better protect kidney tissues from cold ischemic damage. Therefore, if the CIT will be prolonged before transplantation, it will be helpful to increase the duration of HBO_2 application to 2 hours.

CONCLUSION

In this study, it has been demonstrated that in the CIT before kidney transplantation, HBO_2 administration to the organs removed from donors can reduce apoptotic cell numbers, inflammatory cytokine release, and histopathological damage in the organs, as well as decrease the levels of KIM-1 gene expression, which is an indicator of kidney damage. Therefore, it is concluded that post-transplant success may be increased if HBO_2 can be administered to kidneys removed from donors, especially during a prolonged CIT.

■

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