

# Fellow Eye Involvement in Retinal Ischemia Reperfusion Injury

## Retinal İskemi Reperfuzyon Hasarında Diğer Göz Tutulumu

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### ABSTRACT

**Purpose:** To evaluate histologic changes in the fellow non-ischemic eye retina in ischemia reperfusion (IR) injury and compare with control group retina.

**Materials and Methods:** Sixteen male Wistar-Albino rats weighing 200-250 mg were kept in a stable environment at a constant room temperature and humidity and were divided into study (n=8) and control (n=8) groups. IR injury is induced in right eye of the study group via increasing intraocular pressure to 110 mmHg for 60 minutes. Both eyes of the study and the control group were enucleated and analyzed with hematoxyline-eosin (H&E) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) stainings. Retinal thickness measurements performed in H&E sections were compared between and within groups. TUNEL staining was used to evaluate apoptosis.

**Results:** There was no significant difference between right and left eye of the control group (143.9±4.2 µm and 143.3±3.5 µm respectively, p=0.74). IR had resulted in increment of retinal thickness in the IR eye (228.7±13.1 µm) and fellow non-ischemic eye (166.72±9.7 µm) of the study group and the change was statistically significant when compared to the control group (p<0.01). Compared to the control group, TUNEL staining revealed increased number of apoptotic cells in the IR eye but not in the fellow non-ischemic eye.

**Conclusion:** IR resulted in involvement of fellow eye demonstrated with increased retinal thickness but not caused enough changes to develop apoptosis. Setting the fellow eye in retinal IR injury model is not appropriate.

**Key Words:** Ischemia reperfusion, animal, retinal thickness, apoptosis.

### ÖZ

**Amaç:** İskemi reperfuzyon (İR) hasarında diğer iskemik olmayan gözdeki histolojik değişiklikleri değerlendirmek ve kontrol grup retinası ile karşılaştırmak.

**Gereç ve Yöntem:** Onaltı adet 200-250 mg ağırlığında Wistar-Albino sıçanı çalışma (n=8) ve kontrol (n=8) olarak iki gruba ayrıldı. İR hasarı çalışma grubunda sağ göz içi basıncı 60 dakika 110 mmHg' a çıkarılarak oluşturuldu. Çalışma ve kontrol grubunda her iki göz de enükle edildi ve hematoksilin-eosin (HE) ve terminal deoksinucleotidyl transferase dUTP nick end labeling (TUNEL) boyası ile değerlendirildi. Retinal kalınlık ölçümleri HE kesitlerinde yapıldı, grup içinde ve gruplar arasında karşılaştırıldı. TUNEL boyaması apoptoz değerlendirmesi için kullanıldı.

**Bulgular:** Kontrol grubunda sağ ve sol göz arasında anlamlı fark yoktu (143.9±4.2 µm and 143.3±3.5 µm sırasıyla, p=0.74). İR retinal kalınlıkta IR gözünde (228.7±13.1 µm) ve iskemik olmayan diğer gözünde (166.72±9.7 µm) retina kalınlık artışına yol açmıştır ve bu değişim kontrol grubu ile karşılaştırıldığında istatistiksel olarak anlamlıydı (p<0.01). Kontrol grubu ile karşılaştırıldığında, TUNEL boyaması İR gözünde apoptotik hücre sayısında artışa yol açmış ancak diğer iskemik olmayan karşı gözde yol açmamıştır.

**Sonuç:** İR diğer iskemik olmayan karşı gözde de artmış retina kalınlığıyla ortaya çıkan tutulumla sonuçlanmış ancak apoptoz gelişebilecek kadar ciddi değişikliklere yol açmamıştır. Retinal İR modelinde diğer gözün kontrol olarak kabul edilmesi uygun değildir.

**Anahtar Kelimeler:** İskemi reperfuzyon, hayvan, retina kalınlığı, apoptoz.

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## INTRODUCTION

Retina turns the photon energy to electrical energy for transmittance to brain via optic nerve. The endless hit of photons creates heat and oxidative stress that necessitates a flawless blood supply. Due to these requirements similar to some vital organs, retina gathers nearly the 20% of the cardiac output. Apart from strict dependence to blood supply, the high lipid content of the membranes of photoreceptors makes it more susceptible to ischemic damage.<sup>1</sup> Ischemia develops because of a disturbance of the blood supply to the tissues but the damage inflicted during the reperfusion is much greater than the ischemia itself.<sup>2,3</sup> Because of ischemia reperfusion (IR) injury, the perfect balance between oxidants and antioxidants is disturbed in favor of oxidants. The resultant overproduction of free radicals with depletion of antioxidants creating a vicious cycle with further production of free radicals through chain reactions,<sup>4</sup> damage tissue morphology via protein oxidation, lipid peroxidation and DNA adducts and ultimately leads to cell death.<sup>5</sup> IR injury might yield systemic inflammatory response in addition to the local inflammatory response via different mechanisms such as oxidant production, complement activation, leucocyte-endothelial cell adhesion, trans-endothelial leucocyte migration, platelet-leucocyte aggregation, increased microvascular permeability and decreased endothelium dependent relaxation. Conditions like systemic inflammatory syndrome or multiple organ dysfunction syndrome may develop in the severest form of IR injuries in which remote non-ischemic organs are affected.<sup>6</sup> Some studies, published in respected journals, performed to evaluate ischemia reperfusion injury in rat retina models, had a tendency to set the fellow eye as the control group.<sup>7,8</sup> However, in the light of the above mentioned mechanisms, it may not be appropriate to set the fellow non-ischemic eye as the control. To clarify this issue, we tried to demonstrate the histological changes in the fellow eye retina in rat retinal IR injury model.

## MATERIALS AND METHODS

Institutional ethics committee approval for animal studies was obtained prior to the study. All animals used in the study received care in compliance with the guidelines established by the committee. All experiments were conducted in accordance with the Animal Care and Use Committee and The Association for Research in Vision and Ophthalmology (ARVO) guidelines. Sixteen male Wistar-Albino rats weighing approximately 200-250 mg were kept in a stable environment at a constant room temperature and humidity. Study group (n=8) received IR injury and both eyes were enucleated after 24 hours. Control group (n=8) is the group without any intervention and again both eyes were enucleated.

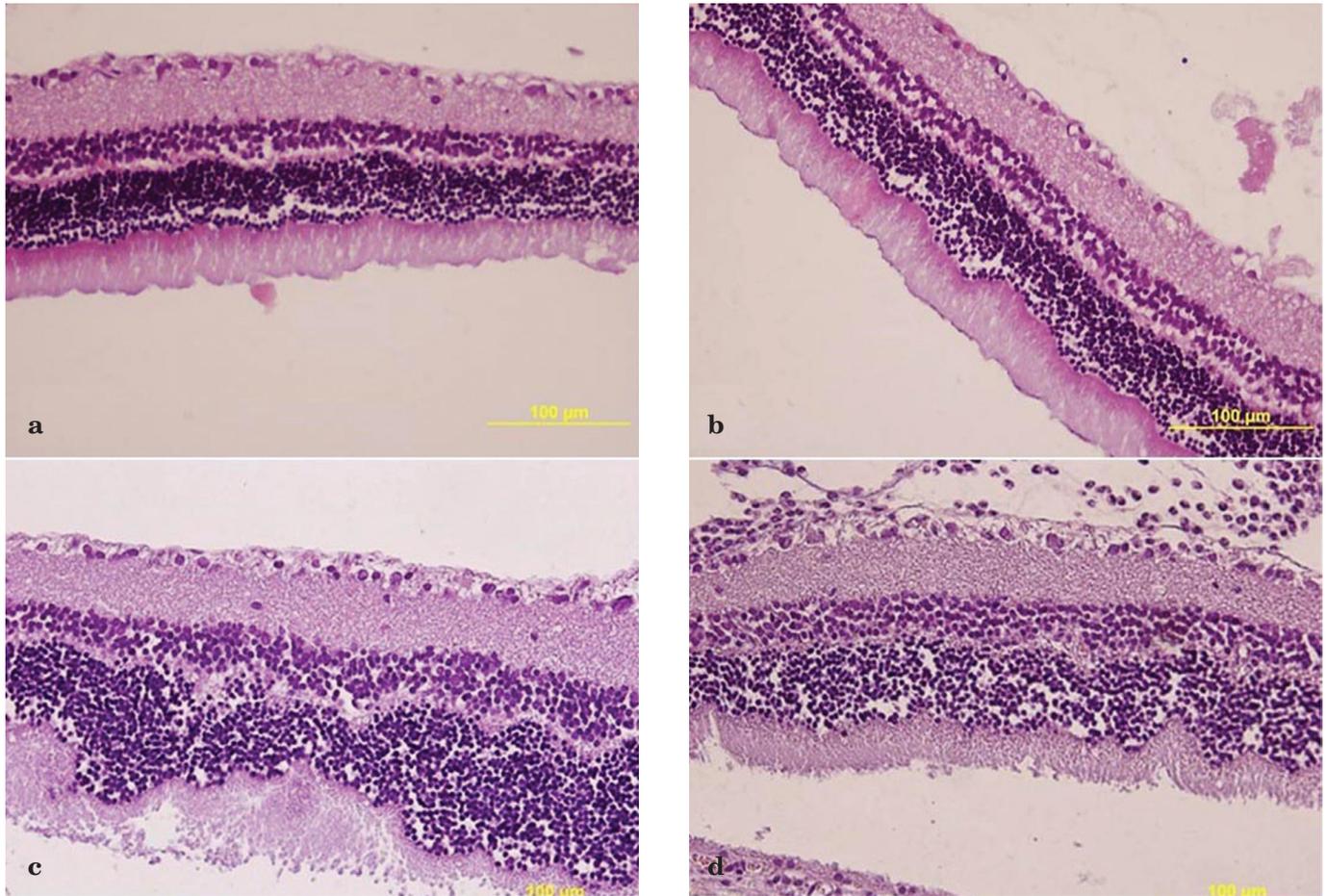
Ischemia was induced by elevating intraocular pressure. After induction with 50 mg/kg of ketamine (Ketalar®, Eczacıbasi, Turkey) and 5 mg/kg xyzaline (Rompun®, Bayer, Turkey), the anterior chamber of the rats right eyes were cannulated with a 30G needle which was then connected to a saline bottle. The bottle was elevated to 150 cm to reach 110 mmHg of intraocular pressure. Ischemia was confirmed by whitening of the anterior segment of the globe and blanching of the episcleral veins.<sup>8</sup> All eyes were enucleated 24 hours after the IR injury and the specimens were fixed in 10% neutral buffered formalin. Sections were stained with hematoxylin-eosin (H&E) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) stainings. The thickness was measured on sections stained with hematoxylin and eosin in the magnified images (×400). Three visual fields under a light microscope (Olympus BX51, Tokyo, Japan) per section were randomly chosen to measure the thickness of the total retina and data are expressed as mean±standard deviation. Quantitative histomorphometry were performed with Image J software in the carefully defined reference fields. The retinal thickness results were statistically analyzed with Spss version 15.0 (Spss Inc., Chi, IL, USA). Wilcoxon test and Mann-Whitney U tests were used for statistical comparison. All data were given as the mean±SD (standard deviation) and p<0.05 was considered as statistically significant.

## RESULTS

IR injury resulted in increased infiltration of inflammatory cells and retinal thickness in ischemia induced eyes. Figure 1 demonstrates H&E stained sections of studied eyes. There was no significant difference in retinal thickness values of right and left eye values of the control group (143.9±4.2 µm and 143.3±3.5 µm respectively, p=0.74). The retinal thickness in IR eye (228.7±13.1µm) was statistically higher compared to the fellow non-ischemic eye (166.7±9.7 µm) (p=0.03). The difference between IR eye and the control group was statistically significant (p<0.01) and TUNEL staining demonstrated the increased number of apoptotic cells. We demonstrated that compared to the control group (143.3±3.5 µm), IR injury also had resulted in significant inflammation and increased retinal thickness in fellow eye of the sham group (166.7±9.7 µm) without any apoptotic changes (p<0.01). Figure 2 demonstrates the TUNEL stained sections of the study and control group eyes.

## DISCUSSION

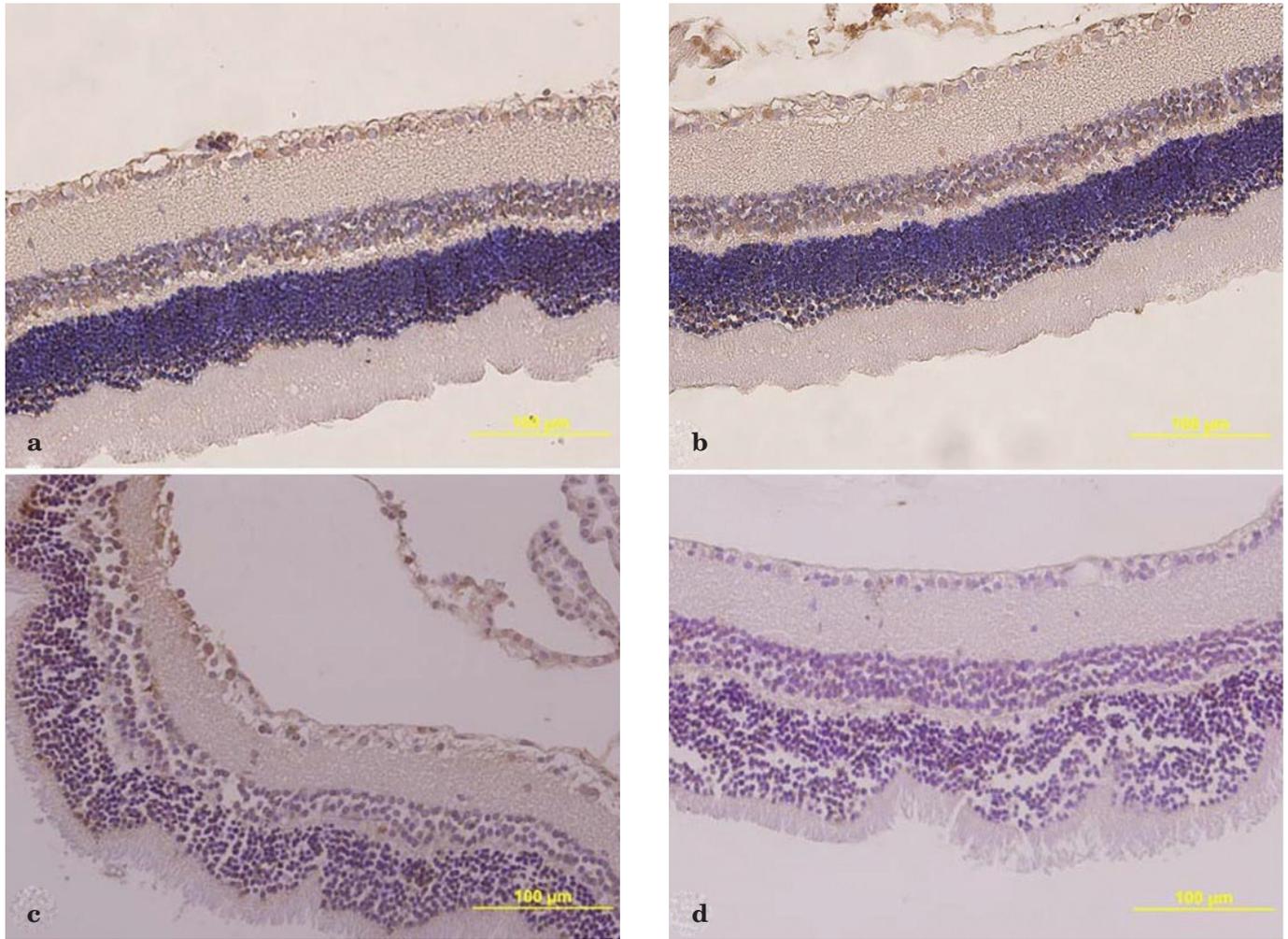
Restoration of blood flow to organs that are deprived of blood supply for a period of time is critical to prevent the irreversible injury.



**Figure 1a-d:** Hematoxyline and eosin retinal staining. Control group right eye (a), control group left eye (b), IR eye of the study group (c), fellow non-ischemic eye of the study group (d).

However, reperfusion may cause more damage in the tissue due to the ischemia produced pro-inflammatory state, mostly to free radicals.<sup>9</sup> The resultant injury is termed as ischemia reperfusion injury (IR) that mediates its damage by different mechanisms including oxidative stress. Tissues in physiologic conditions are capable of eliminating oxidative stress with antioxidant mechanisms. IR injury increases the oxidative stress (reactive oxygen and nitrogen species) and depletes the antioxidative reserve in the tissues and this new oxidant rich environment causes inflammation, protein and lipid peroxidation, DNA adducts and eventually apoptosis of the cells.<sup>5</sup> Retina is a highly functioning neurosensory organ that is highly dependent on blood supply and thus so sensitive to IR injuries. Example to ocular diseases that is associated with IR based retinal injuries are premature retinopathy, retina artery and vein occlusions, diabetic retinopathy and acute angle closure glaucoma.<sup>10</sup> Therefore, understanding the pathophysiology of IR based injuries is crucial and study designs for this purpose should be reliable. Some of the retinal IR studies performed in animals set the control eye as the non-ischemic fellow eye of the IR induced eye.<sup>7,8</sup> It is known that IR injuries may extend beyond the ischemia site and affect remote non-ischemic organs.<sup>6</sup>

From this point of view, we tried to express the status of the fellow eye in IR injury and compared it to the control eye. Retinal thickness accurately reflects the toxic state of the retina and is used in some of the IR injury studies.<sup>9, 11</sup> In a recent study, Kim et al.,<sup>12</sup> demonstrated retinal thickening indicative of edema in mice 3 days following IR, which was followed by continuous retinal layer thinning for as long as 4 weeks after IR. Our study design is based on the 24<sup>th</sup> hour findings so the expected finding was the increment of retinal thickness. IR injury resulted in increment of retinal thickness to  $228.7 \pm 13.1 \mu\text{m}$  in IR eyes compared to  $143.9 \pm 4.2 \mu\text{m}$  in the control group. The fellow non-ischemic eyes retinal thickness values were  $166.72 \pm 9.7 \mu\text{m}$  in sham group and  $143.3 \pm 3.5 \mu\text{m}$  in control group fellow eye. The results revealed a statistically higher retinal thickness in sham fellow eye compared to the control. The TUNEL staining is one of the commonly used immunohistological method to evaluate apoptosis. IR increased the number of apoptotic cells in the retina of the IR eyes. Although the fellow eye retina of study group seemed inflamed and edematous in H&E stained sections, TUNEL staining revealed no apoptotic changes. Therefore, we can speculate that inflammatory response in the fellow non-ischemic retinas was not severe enough to result in apoptosis.



**Figure 2a-d:** TUNEL staining. Control group right eye (a), control group left eye (b), IR eye of the study group (c), fellow non-ischemic eye of the study group (d).

The major limitation of our study is the absence of the biochemical changes for supporting the fellow eye involvement that we demonstrated in histological sections. Our results revealed that the fellow non-ischemic eye is also injured in retinal IR injury with increased retinal inflammation, edema and retinal thickness and thus should not be set as the control eye in retinal IR injuries.

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