

Effect of complete and partial capsulotomy on the renal function tests and oxidative stress markers in rats undergoing ischemia-reperfusion injury



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ABSTRACT

Objective: To compare the effect of complete and partial renal capsulotomy on the renal function tests and oxidative stress markers in rats undergoing ischemia-reperfusion injury.

Design: Randomized controlled experimental study.

Animals: A 60 Sprague-dawely rats weighing 180 ± 50 g.

Procedures: Rats were divided into 3 groups in triplicate (6 each). In addition, 6 rats were subjected to blood and renal tissues sampling for estimation of normal parameters. Group 1 (Positive control): ischemia reperfusion (IR) injury; Group 2: Complete capsulotomy + I R; Group 3: Partial capsulotomy + IR. Six rats from each group were sacrificed at 2, 7 and 14 days post- surgery.

Results: The complete capsulotomy induced a significant decrease in the serum creatinine at 2 and 7 days post- capsulotomy in comparison with partial capsulotomy ($P < 0.05$), whereas at 14 days, the partial capsulotomy induced the significant decrease ($P < 0.05$). Complete capsulotomy showed a significant improvement in creatinine clearance in comparison with partial capsulotomy at 2, 7 and 14 days post- surgery ($p < 0.05$). At 2 and 7 days, BUN of IR+ Capsulotomy group showed a significant decrease ($P < 0.05$) compared to the other groups, while at 14 days partial capsulotomy, the serum BUN reached to the normal value. Serum sodium level showed a significant decrease ($P < 0.05$) at 2 days after partial capsulotomy, and at 14 days after complete capsulotomy ($P < 0.05$). Nitric oxide level in IR + partial capsulotomy group showed a significant decrease at 7 and 14 days ($P < 0.05$). Results of MDA of IR+ partial capsulotomy groups showed a significant decrease ($P < 0.05$) compared to the IR+ complete capsulotomy groups at 2, 7 and 14 days.

Conclusion and clinical relevance: The partial capsulotomy ameliorates could improve serum creatinine, BUN and could lower the oxidative stress at 14 days. Partial capsulotomy could also improve the renal tissues at both short and long-term. So this study indicates the importance of the presence of intact renal capsule for ischemic acute kidney injury.

Keywords: Capsulotomy, Kidney, IRI, Laparotomy, Creatinine, Rats.

1. INTRODUCTION

An acute kidney injury (AKI) is a sudden, possible reversible, decrease in renal function over a period of hours to days [1]. AKI is accompanied with rapid fall in glomerular filtration rate (GFR), retention of nitrogenous waste products and non-nitrogenous metabolic waste products that are normally excreted in urine [2, 3]. It increases the mortality in the patient of intensive care units (ICU), prolongs hospital stays and accelerates chronic kidney disease [1, 4]. Despite the reversibility of the loss of renal function in most patients who survive, the mortality of AKI remains alarmingly high (over 50%) [5].

When the kidney is exposed to sudden temporary restriction of the blood supply as in case of kidney transplantation or coronary bypass, the renal blood flow may be stopped completely [6], with subsequent tissue

damage [7]. During the restoration of blood supply and re-oxygenation, the increased formation of free radicals and inflammatory response intensify the tissue damage [8]. Necrosis and apoptosis in the renal tubules, as well as more release of ROS have been recorded [9]. Superoxide dismutase, catalase and glutathione peroxidase, a potent antioxidant enzymes [10], are responsible for elimination of ROS under normal condition, but are insufficient during the IR [11].

Renal IRI is mainly induced either by systemic hypoperfusion as surgery requiring clamping of the aorta, renovascular surgery, shock and trauma or by local hypoperfusion such as renal transplantation [12, 13]. An IRI activates different programs of cell death, such as necrosis, apoptosis, or autophagy associated cell-death, multiorgan dysfunction and increased vascular permeability [14]. The pathophysiological changes are very complex and

overlapping, but some of these pathways are activation of neutrophils, platelets, cytokines, reactive nitrogen species, ROS, the coagulation system, the endothelium, and the xanthineoxidoreductase enzyme system [15, 16].

The renal tissues in compartment syndrome should be decompressed to regain adequate tissue perfusion and to avoid the detrimental effects [17]. Therefore, capsular incision of ischemically damaged and edematous kidneys may be a logical technique to improve renal perfusion [18].

Capsulotomy is performed either via induction of a longitudinal incision in the greater curvature of the renal capsule (complete capsulotomy) or by small incision in the renal capsule (partial capsulotomy). Capsulotomy may be useful during renal grafts with extensive IRI, edema and high intrarenal pressures. It has to be determined at which renal flow capsulotomy improves renal perfusion and outweighs the potential risk of parenchymal damage [19].

Thus, our hypothesis was that decompressing the swollen and edematous kidney during renal graft via complete or partial renal capsulotomy may alleviate the harmful effect of IRP during the course of ischemia and may improve the renal function as well as oxidant/antioxidant balance.

2. MATERIAL AND METHODS

2.1. Animals

A total of 60 female Sprague -Dawley rats at 2-3 month of age and weighing 180-230 gm were used. Rats were bred at temperature 20-25°C in the animal house, Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University. They were conditioned in standard metallic cages (6 rats per cage) with an alternating 12 h light – dark cycle. They were acclimatized to the laboratory conditions, fed standard rat chow and water was available ad libitum. The experimental protocol of this work was approved by Mansoura Local Ethical Committee (MMREC).

2.2. Experimental design

Sixty rats were allocated randomly into the following three experimental groups (18 each) in triplicate (6 each). Additionally, 6 rats were subjected to blood and renal tissues sampling for estimation of normal parameters. Group 1 (Positive control): ischemia reperfusion injury, Rats were subjected to IRI to the left kidney for 1 h combined with unilateral nephrectomy to the contralateral one. Group 2: Capsulotomy + IRI (IR+ CAP) complete capsulotomy of the renal capsule was performed where the capsule was opened along the entire greater curvature of the kidney, then IRI for 1hr to the left kidney and nephrectomy to the right kidney was performed. Group 3: Partial capsulotomy+ IRI (IR+PC), the rats were subjected to partial capsulotomy where a small part of the posterior pole of the left kidney was incised then IRI to left kidney for 1 hour and nephrectomy to the right one were performed. The rats in the 3 groups were divided into 3 subgroups according to the time of their scarification. Rats were sacrificed after 2, 7 and 14 days from ischemia.

2.3. Surgical procedures

All rats were anaesthetized using 7.5mg/kg ketamine Hcl (Ketamax 50, Troikaa Pharmaceuticals Ltd, Gujarat, India) and 0.1mg/kg xylazine Hcl (Xylajet, ADWIA, Egypt). Amixture of them was injected IP in one syringe according to [20].

Aseptic preparation to the surgical site was performed. A midline incision was performed in the ventral abdomen including meso-gastric and hypogastric region. The intestine is rewarded to right side for good exposure of the left kidney. IRI technique was conducted according to [21]. Briefly, the renal pedicle (renal artery, vein and nerve) was dissected carefully from adjacent peri renal fat and adventitia (Figure 1A). The renal pedicle was clamped by a non-trumatic vascular clamp for 1 hour (Figure 1B). The intestine redirected to the abdomen, the edges of the abdominal incision adjusted to each other and covered with a piece of gauze soaked with warm isotonic saline (37 °C) and the rats were putted under heating lamp to avoid hypothermia until performing the ischemia. After 1 hour the gross signs of ischemia were appeared on the kidney. The clamp was removed and the signs of reperfusion began to appear gradually.

Capsulotomy technique was performed according to [19]. Complete capsulotomy; a longitudinal incision was performed from anterior pole to the posterior pole of the kidney along the greater curvature of the left kidney by using microsurgical scissor before induction of ischemia (Figure1C). Partial capsulotomy; a small incision was created in the posterior pole of the kidney toward the middle of the kidney befor induction of ischemia (Figure1D).

For nephrectomy, a right kidney was exposed before the removal of vascular clamp, completely dissected from adherent perirenal fat and liver (Figure 2A). The renal pedicle and ureter were double ligated using 2/0 non absorbable suture (Sofisilk company; Figure 2B) then cutting in between them and remove the kidney (Figure 2C, D) after removal of the bulldog clamp on the left renal pedicle. The internal organ was readjusted to its sites and irrigated by isotonic warm saline. Then the wound was closed by usual manner. Dressing the wound was performed post operatively and the heating lamp was adjusted on the operated rats until recovery from anesthesia. All rats were kept on a soft diet in the cages, monitoring of the general health and care of the wound were evaluated till the time of scarification. Rats were euthanized by single over dose of thiopental sodium injected IP 7.5 mg /100g BW [22].

2.4. Biochemical analysis in blood and urine

Determination of serum and urine creatinine was performed (Diamond Diagnostics, Egypt) according to [23] for calculation of creatinine clearance; determination of BUN (Stanbio Lab., Texas, USA); determination of serum sodium (Spectrum Diagnostics, Egypt) according to [24].

2.5. Biochemical and histopathological analysis of the renal tissues

Determination of MDA (SIGMA) according to [25]; determination of NO (SIGMA) according to [26] these parameters were measured using an automated spectrophotometer (Slim Plus, Italy).

In histopathological examination, the kidneys were fixed in 10% neutral buffered formalin. The samples were dehydrated in graded ethanol and embedded in paraffin wax. Four μm -thick paraffin embedded sections were cut and routinely stained with hematoxyline and eosin [27]. Each section was examined by light microscopy. The tissue sections were stained by Masson trichrome stain to investigate the collagen fibers [28]. Also, investigation was carried out by staining histological section in periodic acid Schiff reaction (PAS) for observing basal lamina and cell membranes of renal structure [29] as well as with methanamine silver stain (MSS) for observing cell membranes, nuclei and reticular fibers and demonstrating the degree of thickening of glomerular and tubular basement membrane [3].

2.6. Statistical analysis

Statistical analyses were carried out using a commercial software program (SPSS 20, USA). As an initial step, homogeneity of the groups was tested by Kruskal Wallis test, and then descriptive statistics was done. General linear model was conducted to assess the effect of time and treatment. When the results were significant, one-way ANOVA was performed at each time point. Results were considered significant at $p < 0.05$.

3. RESULTS

3.1. Serum creatinine

Based on time and treatment interaction, there was a significant variation of serum creatinine after treatments. Accordingly, the complete capsulotomy showed a significant decrease ($P < 0.05$) in the serum creatinine at 2 and 7 days post-surgery in comparison with partial capsulotomy. While at 14 days, partial capsulotomy showed a significant decrease ($P < 0.05$) in comparison with complete capsulotomy (Table 1).

3.2. Creatinine clearance

Effect of complete capsulotomy on creatinine clearance at all three times (2, 7 and 14 days) showed a significant improvement compared with partial capsulotomy (Table 2).

3.3. Serum BUN level

At 2 and 7 days BUN of IR+CAP group showed a significant decrease ($P < 0.05$) compared to IR+PC while at 14 days the opposite occurs (Table 3).

3.4. Serum sodium

Serum sodium level after partial capsulotomy showed a significant decrease ($P < 0.05$) when compared with complete capsulotomy at 2 days. While at 14 days complete capsulotomy showed a significant decrease ($P < 0.05$) than partial capsulotomy (Table 4).

3.5. NO and MDA level in renal tissue extract

NO level of IR+PC groups at 7 and 14 days showed a significant decrease ($P < 0.05$) compared to IR+CAP groups (Table 5).

Results of MDA of IR+PC groups showed a significant decrease ($P < 0.05$) compared to the IR+CAP groups at 2, 7 and 14 days (Table 6).

3.7. Histopathological examination

The renal tubular epithelium showed coagulative necrosis and eosinophilic hyaline cast in the lumen of the renal tubule of the kidney at 2 days of IRI group (Figure 3B, F). IR + CAP group at 2 days; kidney shows normal renal glomeruli and degenerative changes in the renal tubular epithelium lining renal tubules. (Figure 3C, G). Kidney of IR + PC group at 2 days shows mild degenerative changes in the renal tubular epithelium lining renal tubules. (Figure 3D, H).

Kidney is showing interstitial nephritis expressed by lymphocytic infiltration in interstitial tissue at 7 days from ischemia reperfusion injury (Figure 4B, F). IR+CAP group at 7 days, Kidney is showing interstitial nephritis represented by mild lymphocytic exudate in interstitial tissue. (Figure 4C, G). Kidney of IR + PC group at 7 days is showing normal renal glomeruli and degenerative changes in renal tubular epithelium lining renal tubular epithelium. Kidney is showing bluish stained intraglomerular fibrous tissue proliferation. Kidney is showing normal renal glomeruli and normal renal tubules. (Figure 4D, F).

IRI group at 14 days showed normal renal glomeruli and normal renal tubules. Kidney showed bluish stained fibrous tissue septa in interstitial tissue. (Figure 5B, F). IR + CAP group at 14 days; kidney shows severe interstitial nephritis represented by intense lymphocytic exudate in interstitial tissue. (Figure 5C, G). Kidney of IR + PC group, at 14 days showed normal renal glomeruli and normal renal tubules with normal renal tubular epithelium. Kidney shows normal parenchyma without any fibrous proliferation (Figure 5D, H).

Table 1. Effects of capsulotomy on serum creatinine (mg/dl; mean \pm SD) in rats undergoing ischemia-reperfusion injury.

Group	Time post treatments (days)			
	0	2	7	14
Control	0.54 \pm .018	.53 \pm .018 ^a	.53 \pm .018 ^a	.53 \pm .018 ^a
IR	0.54 \pm .018	.97 \pm .114 ^b	.92 \pm .175 ^b	.66 \pm .102 ^a
IR + CAP	0.54 \pm .018	.71 \pm .091 ^a	.61 \pm .083 ^a	1.24 \pm .199 ^c
IR + PC	0.54 \pm .017	1.35 \pm .243 ^c	1.09 \pm .155 ^c	.95 \pm .279 ^b

In each column, the means with different superscript letters are significantly different at $P < 0.05$. IR refers to Ischemia reperfusion injury group, CAP refers to complete capsulotomy and PC refers to Partial capsulotomy.

Table 2. Effects of capsulotomy on serum Creatinine clearance (ml/min; mean \pm SD) in rats undergoing ischemia-reperfusion injury.

Group	Time post treatments (days)			
	0	2	7	14
Control	0.53 \pm .008	0.53 \pm .013 ^b	.53 \pm .008 ^a	.54 \pm .013 ^b
IR	0.53 \pm .008	0.29 \pm .079 ^a	.39 \pm .145 ^a	.69 \pm .408 ^b
IR + CAP	0.54 \pm .013	.026 \pm .224 ^a	.38 \pm .326 ^a	.14 \pm .118 ^a
IR + PC	0.53 \pm .008	0.09 \pm .036 ^c	.062 \pm .015 ^b	.057 \pm .035 ^a

In each column, the means with different superscript letters are significantly different at $P < 0.05$. IR refers to Ischemia reperfusion injury group, CAP refers to Complete capsulotomy and PC refers to Partial capsulotomy.

Table 3. Effects of capsulotomy on serum BUN (mg/dl; mean \pm SD) in rats undergoing ischemia-reperfusion injury.

Group	Time post treatments (days)			
	0	2	7	14
Control	31.26 \pm 7.7	31.26 \pm 7.7 ^c	30.43 \pm 7.06 ^c	30.46 \pm 6.99 ^a
IR	30.43 \pm 7.1	106.03 \pm 11.3 ^a	117.73 \pm 36.6 ^b	57.95 \pm 20.64 ^b
IR + CAP	30.93 \pm 7.4	82.25 \pm 17.6 ^b	71.88 \pm 13.38 ^a	84.70 \pm 25.97 ^c
IR + PC	34.16 \pm 6.3	118.13 \pm 15.3 ^a	81.80 \pm 9.98 ^a	46.75 \pm 27.31 ^{ab}

In each column, the means with different superscript letters are significantly different at $P < 0.05$. IR refers to Ischemia reperfusion injury group, CAP refers to Complete capsulotomy and PC refers to Partial capsulotomy.

Table 4. Effects of capsulotomy on serum Na (mEq/L; mean \pm SD) in rats undergoing ischemia-reperfusion injury.

Group	Time post-treatments (days)			
	0	2	7	14
Control	132.83 \pm 1.72	132.83 \pm 2.31 ^a	134.33 \pm 2.80 ^b	134.50 \pm 2.66 ^a
IR	132.83 \pm 2.32	197.41 \pm 36.92 ^b	216.65 \pm 24.37 ^a	174.85 \pm 28.89 ^a
IR + CAP	134.33 \pm 2.80	203.53 \pm 17.70 ^c	198.73 \pm 29.47 ^a	193.76 \pm 15.56 ^a
IR + PC	134.83 \pm 2.56	134.98 \pm 31.88 ^a	196.65 \pm 12.89 ^a	265.66 \pm 104.98 ^b

In each column, the means with different superscript letters are significantly different at $P < 0.05$. IR refers to Ischemia reperfusion injury group, CAP refers to Complete capsulotomy and PC refers to Partial capsulotomy.

Table 5. The effect of capsulotomy on nitric oxide in renal tissue of rats undergoing ischemia-reperfusion injury (mmol /g tissue; mean \pm SD).

Group	Time post treatments (days)			
	0	2	7	14
Control	9.64 \pm .302	9.64 \pm .302 ^b	9.53 \pm .24 ^c	9.64 \pm .30 ^a
IR	9.64 \pm .302	19.16 \pm 3.07 ^a	13.13 \pm 5.91 ^a	13.26 \pm 3.08 ^{ab}
IR + CAP	9.64 \pm .302	16.05 \pm 5.36 ^a	22.25 \pm 5.41 ^b	19.78 \pm 6.62 ^c
IR + PC	9.64 \pm .302	17.68 \pm 5.97 ^a	16.01 \pm 2.69 ^a	15.06 \pm 4.25 ^{bc}

In each column, the means with different superscript letters are significantly different at $P < 0.05$. IR refers to Ischemia reperfusion injury group, CAP refers to Complete capsulotomy and PC refers to Partial capsulotomy.

Table 6. The effects of capsulotomy on serum malodialdehyde in renal tissues of rats undergoing ischemia-reperfusion injury (mmol/g tissue; mean \pm SD).

Group	Time post-treatments (days)			
	0	2	7	14
Control	53.19 \pm .79	53.19 \pm .79 ^a	53.19 \pm .79 ^a	53.19 \pm .79
IR	53.19 \pm .79	86.58 \pm 7.9 ^b	93.7 \pm 22.1 ^c	69.6 \pm 10.34
IR + CAP	53.19 \pm .79	69.33 \pm 38.3 ^a	79 \pm 17.193 ^b	70 \pm 24.99
IR + C	53.19 \pm .79	61.83 \pm 16.6 ^a	60.16 \pm 7.85 ^a	57 \pm 13.61

In each column, the means with different superscript letters are significantly different at $P < 0.05$. IR refers to Ischemia reperfusion injury group, CAP refers to Complete capsulotomy and PC refers to Partial capsulotomy.

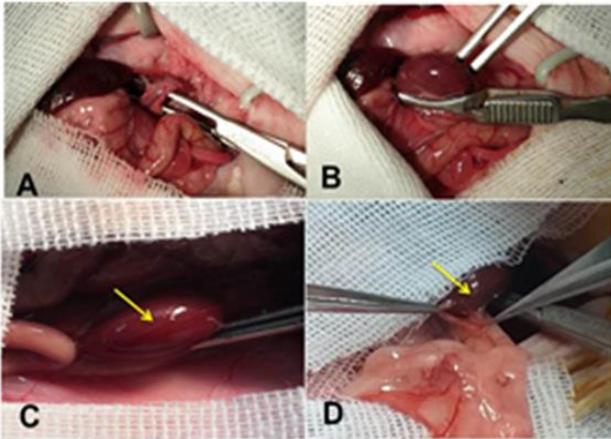


Figure 1. Dissection of the left renal pedicle (A), clamping of the left renal pedicle (B), complete capsulotomy of the left kidney (C) and partial capsulotomy of the left kidney (D).

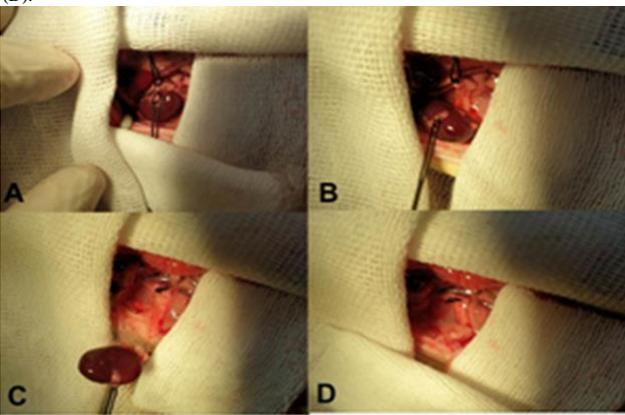


Figure 2. Two ligations in the right renal pedicle (A), cutting in between the two ligations (B), and removal of the right kidney (nephrectomy) (C&D).

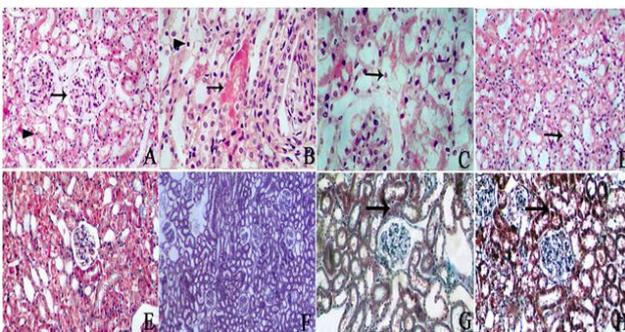


Figure 3. A- Control group, kidney is showing normal renal glomeruli (arrow) and normal renal tubules lined by normal renal tubular epithelium (arrow head) (HE, 400x), E- control group, Kidney is showing normal parenchyma without any fibrous proliferation (Masson trichrome, 400x), B- IR 2 days, kidney is showing coagulative necrosis of renal tubular epithelium (arrow head) and eosinophilic hyaline cast in the lumen of the renal tubule (arrow) (HE, 400x), F-IR 2 days, Kidney is showing normal parenchyma without any fibrous proliferation. (Masson trichrome, 100x), C- IR+CAP 2 days, kidney is showing normal renal glomeruli and degenerative changes in the renal tubular epithelium lining renal tubules (arrow) (HE, 400x), G- Kidney is showing normal parenchyma without any fibrous proliferation. (Masson trichrome, 400x), D- IR+PC 2 days, kidney is showing mild degenerative changes in the renal tubular epithelium lining renal tubules (arrow). (HE,100x). H- Kidney is showing normal parenchyma without any fibrous proliferation. (Masson trichrome, 100x).

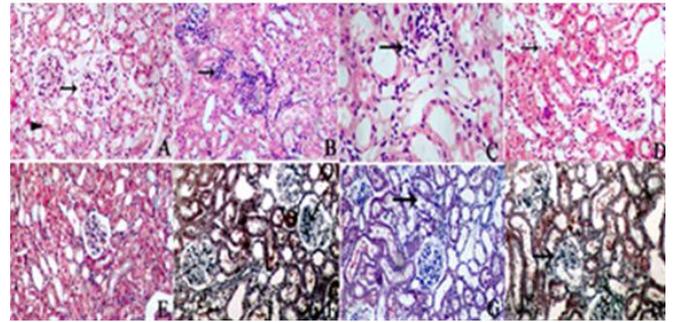


Figure 4. A- Control group, kidney is showing normal renal glomeruli (arrow) and normal renal tubules lined by normal renal tubular epithelium (arrow head) (HE, 400x), E- control group, Kidney is showing normal parenchyma without any fibrous proliferation (Masson trichrome, 400x), B-IR 7 days, Kidney is showing interstitial nephritis expressed by lymphocytic infiltration in interstitial tissue (arrow). (HE, 100x). F-IR 7 days, kidney displays dense bluish stained of glomerular basement membrane (arrow). (Masson trichrome, 400x), C- IR+CAP 7 days, Kidney is showing interstitial nephritis represented by mild lymphocytic exudate in interstitial tissue (arrow). (HE, 400x), G- IR+CAP kidney is showing normal parenchyma without any fibrous proliferation (arrow). (Masson trichrome, 400x), D- IR+PC 7 days, Kidney is showing normal renal glomeruli (arrow head) and degenerative changes in renal tubular epithelium lining renal tubular epithelium (arrow). (HE, 100x), H-IR+PC kidney is showing bluish stained intraglomerular fibrous tissue proliferation (arrow). (Masson trichrome, 100x).

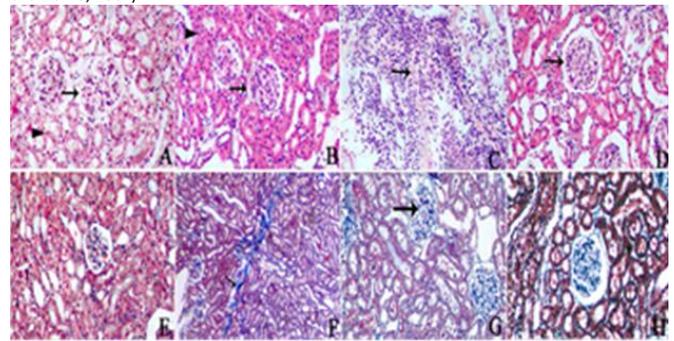


Figure 5. A- Control group, kidney shows normal renal glomeruli (arrow) and normal renal tubules lined by normal renal tubular epithelium (arrow head) (HE, 400x); E- control group, Kidney shows normal parenchyma without any fibrous proliferation (Masson trichrome, 400x); B- IR 14 days, kidney shows normal renal glomeruli (arrow) and normal renal tubules lined by normal renal tubular epithelium (arrow head) (HE, 100x); F- IR14days, Kidney shows bluish stained fibrous tissue septa in interstitial tissue (Masson trichrome, 100x); C- IR+CAP 14 days, kidney shows severe interstitial nephritis represented by intense lymphocytic exudate in interstitial tissue (arrow) (HE, 100x),G- IR+CAP 14 days, Kidney shows normal parenchyma without any fibrous proliferation (arrow, Masson trichrome, 100x); D- IR+PC14 days, kidney is showing normal renal glomeruli (arrow) and normal renal tubules lined by normal renal tubular epithelium (arrow head, HE, 400x). H- IR+PC14 days, Kidney shows normal parenchyma without any fibrous proliferation. (Masson trichrome, 400x).

4. DISCUSSION

Acute kidney injury is the deterioration of renal function over a period of hours to days, which decreases the kidney ability to excrete metabolic waste products and the control of fluid and electrolyte homeostasis [30]. It is still responsible for high percentage of the mortality rate of many patients, although of new advanced therapies protocols [3]. There is still some question marks about the pathophysiology of AKI because of its complication and incomplete information till now [31]. The main causes of AKI in intensive care unit patients are renal ischemia in the setting of low cardiac output and hypotension and sepsis [32].

The rat is one of the most common models in AKI studies, because it develops marked blood pressure

elevation and glomerular injury [33]. The severity of damage occurs in renal epithelial cells depends on initiation of inflammatory reactions, onset of hypertension and continuous nephrotoxic medication [31]. The renal tissue has ability to regenerate after the reperfusion of blood is occurred but not all of injured tissue, because of the more damaged cells subjected to necrosis or apoptosis [34].

This study pointed to relieve the severity AKI, especially excessive edema, swelling of the kidney and hyporenovascular perfusion which occur as a result of renal transplantation by induction of complete and partial renal capsulotomy. The effects of such intervention on kidney function tests and oxidative stress markers were investigated.

In the present study, IR group induced asignificant increase in serum ceatinine and BUN with significant decrease in Cr. Clearance at 2 days and 7 days of examination, suggesting the deterioration of glomerular function as a result of renal vasoconstriction [38, 39]. In addition, there was a significant increase in serum Na at the both times, suggesting impairment of tubular function. These results are in agreement with that of previous reports [4, 40]. At 14 days post-surgery, all parameters declined to their normal values, proposing the beneficial effect of our intervention [41].

The renal tissue affected by IRI produces large amount of ROS which causes oxidative stress [10, 42]. Oxygen free radicals is produced during reperfusion phase of IRI from the blood flow which cause lipid peroxidation that considered as main pathway of free radical tissue injuries [10]. Nitric oxide, oxidative stress marker is produced by inducible nitric oxide synthase in the renal proximal tubules in response to ischemic injury is a toxic agent [41]. Moreover,MDA which is one of the end products of lipid peroxidation, is also used as a marker for free radicals detection in the damaged cell [43, 44]. The oxidative stress injury is evaluated by measuring MDA and NO concentration, which are used as an indirect indicators of ROS,because they are stable end product of lipid peroxidation produced by ROS [45]. MDA was increased significantly after 2,7 and 14 days, these results suggest the role of ROS during IRI and exposed the harmful effects on renal tissues [4, 40, 46]. Also, NO was increased significantly after 2 days of IRI, but with non-significant increase at 7and 14 days. Such findings are in agreement with that reported in a previous study [21] where IRI activates nitric oxide synthase (NOS) and increases the expression of NOS proteins [47].

In this study, the renal tissues IRI group at 2 days reflected the signs of acute tubular necrosis, coagulative necrosis, hyper cellularity of the mesengial cells and apoptosis in the renal tubular epithelium. Easinophilic hyaline cast in the lumen of the renal tubule with histeocyte, lymphocyte and plasma cell infiltration were also recorded. Neutrophilic infiltration and dissolution in the renal glomeruli were observed. However, the kidney at 14 days of IRI showed normal glomeruli and normal renal tubules lined by normal tubular epithelium which ensure the theory of the

regenerative ability of the renal tissues that agreed with [48]. This regeneration was registered as intertubular proliferation, presence of festooned appearance of lining epithelium, regard solid sheet and mitotic activity. These repair appeared as dedifferentiated epithelial cells had multiplied mitotic appearance. Also there repair may be attributed to the stem cells, which has the ability to differentiate in vitro into endothelium and renal epithelium [49].

The results of serum creatinine and creatinine clearance showed good improvement in cases subjected to IR+ Cap then sacrificed after 2 and 7days where these parameters become near to normal levels. This improvement in this parameter may be attributed to that; the capsulotomy may improve the renovascular perfusion that decrease the deterioration of renal cell and the function of the kidney was improved.

IR+ Cap group sacrificed at 14days did not show this improvement in serum creatinine and creatinine clearance. On the other hand, BUN in all groups of IR+ Cap showed significant decrease than IRI groups. Similar findings were recorded in a previous report [50] who reported that renal decapsulation in monkeys could increase the creatinine and urea level in blood triple times. It has been mentioned that the renal decapsulation in rats lowered the renal function [51]. It has been also reported that a significant increase in creatinine was observed in capsulotomy treatment in patients with compartment syndrome. [52]

At 2 days, IR+CAP treated animals showed an improvement in renal glomeruli with mild degenerative changes in the renal tubular epithelium lining of renal tubule which may be attributed to the improvement of the renal function parameters at 2 days, comparing with that in IRI group. In spite of this result of complete capsulotomy at 2 days it causes severe interstitial nephritis at 14 days which also may be accompanied with the increase of serum creatinine that occurred at this time.

Conclusion

The partial capsulotomy could decrease serum creatinine, BUN and diminish the oxidative stress at 14 days post-surgery. It could also improve the renal tissue at short time (2 days) or at the acute stage of injury and at a long term time (14 days).

Conclusion

Finally, this study indicates the importance of the presence of intact renal capsule for ischemically acute kidney injury. Where the normal intact elastic renal capsule is capable for absorbing the variability of the intrarenal hydrostatic pressure under different states of renal pathology and also able to re-maintain the renal function tests and return the oxidative stress factors to the normal values.

Conflict of interest statement:

The authors declare that there is no any conflict of interest in the current research work.

Research ethics committee permission

The current research work is permitted to be executed according to standards of Research Ethics committee, Faculty of Veterinary Medicine, Mansoura University.

Authors' contribution

Y. K. and A. R. conducted the experiment, analytical procedures, research writing, M. A. conducted the experiment design, and revised the manuscript, A. Z. revised the manuscript

5. REFERENCES

- [1] Zager RA. Marked protection against acute renal and hepatic injury after nitrated myoglobin + tin protoporphyrin administration. *Transl Res* 2015;166:485-501.
- [2] Lameire N/Ajon, transplantation. The definitions and staging systems of acute kidney injury and their limitations in practice. *Arab J Nephrol Transplant* 2013;6:145-52.
- [3] Mohammad BI, Raheem AK, Hadi NR, Jamil DA, Al-Aubaidy HA. Reno-protective effects of TAK-242 on acute kidney injury in a rat model. *Biochem bioph res commun* 2018;503:304-8.
- [4] Korkmaz A, Kolankaya D. Protective Effect of Rutin on the Ischemia/Reperfusion Induced Damage in Rat Kidney. *J Rurg Res* 2010;164:309-15.
- [5] Liaño F, Pascual J, The Madrid Acute Renal Failure Study G. Epidemiology of acute renal failure: A prospective, multicenter, community-based study. *Kidney Int* 1996;50:811-8.
- [6] Bucuvic EM, Ponce D, Balbi AL. Risk factors for mortality in acute kidney injury. *Rev Assoc Med Bras* 2011;57:156-61.
- [7] Hsu Cy, McCulloch CE, Fan D, Ordoñez JD, Chertow GM, Go AS. Community-based incidence of acute renal failure. *Kidney Int* 2007;72:208-12.
- [8] Ozer Şehirli A, Şener G, Ercan F. Protective Effects of Pycnogenol against Ischemia Reperfusion-Induced Oxidative Renal Injury in Rats. *Ren Fail* 2009;31:690-7.
- [9] Wang Z-s, Liu X-h, Wang M, Jiang G-j, Qiu T, Chen Z-y, et al. Metformin attenuated the inflammation after renal ischemia/reperfusion and suppressed apoptosis of renal tubular epithelial cell in rats. *Acta Cir Bras* 2015;30:617-23.
- [10] Paller MS, Hoidal JR, Ferris TF. Oxygen free radicals in ischemic acute renal failure in the rat. *J Clin Invest* 1984;74:1156-64.
- [11] Cakir M, Polat A, Tekin S, Vardi N, Taslidere E, Rumeysa Duran Z, et al. The effect of dexmedetomidine against oxidative and tubular damage induced by renal ischemia reperfusion in rats. *Ren Fail* 2015;37:704-8.
- [12] Sharfuddin AA, Molitoris BA. Pathophysiology of ischemic acute kidney injury. *Nat Rev Nephrol* 2011;7:189-200.
- [13] Kellum JA, Lameire N. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit Care* 2013;17:204.
- [14] Salvadori M, Rosso G, Bertoni E. Update on ischemia-reperfusion injury in kidney transplantation: Pathogenesis and treatment. *World J Transplant* 2015;5:52.
- [15] Fan L-h, He L, Cao Z-q, Xiang J, Liu L. Effect of ischemia preconditioning on renal ischemia/reperfusion injury in rats. *Int Braz J Urol* 2012;38:842-54.
- [16] Sedaghat Z, Kadkhodae M, Seifi B, Salehi E, Najafi A, Dargahi L. Remote per-conditioning reduces oxidative stress, downregulates cyclo-oxygenase-2 expression and attenuates ischaemia-reperfusion-induced acute kidney injury. *Clin Exp Pharmacol Physiol* 2013;40:97-103.
- [17] Koliás AG, Kirkpatrick PJ, Hutchinson PJ. Decompressive craniectomy: past, present and future. *Nat Rev Neurol* 2013;9:405-15.
- [18] Herrler T, Wang H, Tischer A, Schupp N, Lehner S, Meyer A, et al. Decompression of Inflammatory Edema along with Endothelial Cell Therapy Expedites Regeneration after Renal Ischemia-Reperfusion Injury. *Cell Transplant* 2013;22:2091-103.
- [19] van Smaalen TC, Mestrom MGAM, Kox JHFM, Winkens B, van Heurn LWE. Capsulotomy of Ischemically Damaged Donor Kidneys: A Pig Study. *Eur Surg Res* 2016;57:89-99.
- [20] Flecknell P, Lofgren JLS, Dyson MC, Marini RR, Michael Swindle M, Wilson RP. Preanesthesia, Anesthesia, Analgesia, and Euthanasia. *Lab Anim Med*: Elsevier; 2015. p. 1135-200.
- [21] Shoskes DA, Xie Y, Gonzalez-Cadavid NF. Nitric oxide synthase activity in renal ischemia-reperfusion injury in the rat. *Transplant* 1997;63:495-500.
- [22] Close B, Banister K, Baumans V, Bernoth E-M, Bromage N, Bunyan J, et al. Recommendations for euthanasia of experimental animals: Part 1. *Lab Anim* 1996;30:293-316.
- [23] Murray RL. Creatinine In: A KL, Pesce AJ, editors. *Clinical Chemistry; Theory, Analysis and Correlation*: CV Mosby Co., St. Louis; 1984. p. 1247-53.
- [24] Tietz NW, Ash Koj CC. *Clinical Guide to Lab Tests*, 3rd ed. Philadelphia. WB. Saunders, 1995;41:1548-.
- [25] Tokmak M, Sehitoglu MH, Yuksel Y, Guven M, Akman T, Aras AB, et al. The axon protective effects of syringic acid on ischemia/reperfusion injury in a rat sciatic nerve model. *Turk Neurosurg* 2017;27:124-32.
- [26] Grisham MB, Johnson GG, Lancaster JR. Quantitation of nitrate and nitrite in extracellular fluids. *Methods Enzymol* 1996. p. 237-46.
- [27] Bancroft JD, Stevens A, Turner DR. *Theory and Practice of Histological Techniques*: Churchill Livingstone. Churchill Livingstone, New York, London, Tokyo. 1996:100-50.
- [28] Čunderlikova M, Balazova V. Selected chapters from histological techniques. *Osveta Martin, Slovakia* 1990:167.
- [29] Sheehand D, Hrapchak B. *Theory and Practice of Histotechnology*, Columbus, OH. Battelle Press; 1987.
- [30] Schrier RW, Wang W, Poole B, Mitra A. Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *J Clin Invest* 2004;114:598-.
- [31] Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. *J Clin Invest* 2011;121:4210-21.
- [32] Mehta RL, Pascual MT, Soroko S, Savage BR, Himmelfarb J, Ikizler TA, et al. Spectrum of acute renal failure in the intensive care unit: The PICARD experience. *Kidney Int* 2004;66:1613-21.
- [33] Leelahavanichkul A, Yan Q, Hu X, Eisner C, Huang Y, Chen R, et al. Angiotensin II overcomes strain-dependent resistance of rapid CKD progression in a new remnant kidney mouse model. *Kidney Int* 2010;78:1136-53.
- [34] Chawla LS, Eggers PW, Star RA, Kimmel PL. Acute Kidney Injury and Chronic Kidney Disease as Interconnected Syndromes. *New Engl J Med* 2014;371:58-66.
- [35] Malek M, Nematbakhsh M. Renal ischemia/reperfusion injury; from pathophysiology to treatment. *J Renal Inj Prev* 2015;4:20.
- [36] Jang HR, Rabb H. The innate immune response in ischemic acute kidney injury. *Clin Immunol* 2009;130:41-50.
- [37] Tirapelli LF, Barione DF, Trazzi BFM, Tirapelli DPC, Novas PC, Silva CS, et al. Comparison of Two Models for Evaluation Histopathology of Experimental Renal Ischemia. *Transplant Proceed* 2009;41:4083-7.
- [38] Singh D, Chander V, Chopra K. Carvedilol attenuates ischemia-reperfusion-induced oxidative renal injury in rats. *Fund Clin Pharmacol* 2004;18:627-34.
- [39] Kusaka J, Hagiwara S, Hasegawa A, Kudo K, Koga H, Noguchi T. Cepharanthine Improves Renal Ischemia-Reperfusion Injury in Rats. *J Surg Res* 2011;171:212-7.
- [40] Chander V, Chopra K. Protective Effect of Nitric Oxide Pathway in Resveratrol Renal Ischemia-Reperfusion Injury in Rats. *Arch Med Res* 2006;37:19-26.
- [41] Peresleni T, Noiri E, Bahou WF, Goligorsky MS. Antisense oligodeoxynucleotides to inducible NO synthase rescue epithelial cells from oxidative stress injury. *Am J Physiol* 1996;270:F971-F7.
- [42] Johnson KJ, Weinberg JM. Postischemic renal injury due to oxygen radicals. *Curr Opin Nephrol Hypertens* 1993;2:625-35.
- [43] Li C, Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am J Physiol* 2002;282:C227-C41.
- [44] Erdogan H, Fadillioglu E, Yagmurca M, Uçar M, Irmak MK. Protein oxidation and lipid peroxidation after renal ischemia-reperfusion injury: protective effects of erdosteine and N-acetylcysteine. *Urol Res* 2006;34:41-6.
- [45] Munshi R, Hsu C, Himmelfarb J. Advances in understanding ischemic acute kidney injury. *BMC Med* 2011;9.
- [46] Rodriguez F, Bonacasa B, J. Fenoy F, G. Salom M. Reactive Oxygen and Nitrogen Species in the Renal Ischemia/Reperfusion Injury. *Curr Pharm Des* 2013;19:2776-94.

- [47] Viñas JL, Sola A, Genescà M, Alfaro V, Pí F, Hotter G. NO and NOS isoforms in the development of apoptosis in renal ischemia/reperfusion. *Free Radical Bio Med* 2006;40:992-1003.
- [48] Kulkarni OP, Hartter I, Mulay SR, Hagemann J, Darisipudi MN, Kumar Vr S, et al. Toll-Like Receptor 4-Induced IL-22 Accelerates Kidney Regeneration. *J Am Soc Nephrol* 2014;25:978-89.
- [49] Humphreys BD, Valerius MT, Kobayashi A, Mugford JW, Soeung S, Duffield JS, et al. Intrinsic Epithelial Cells Repair the Kidney after Injury. *Stem Cell* 2008;2:284-91.
- [50] Stone HH, Fulenwider JT. Renal Decapsulation in the Prevention of Post-ischemic Oliguria. *Ann Surg* 1977;186:343-55.
- [51] Khraibi AA, Haas JA, Knox FG. Effect of renal perfusion pressure on renal interstitial hydrostatic pressure in rats. *Am J Renal Physiol* 1989;256:F165-F70.
- [52] Al Ghazal A, Schnoeller TJ, Baechele C, Steinestel J, Jentzmik F, Steffens S, et al. Capsulotomy for treatment of compartment syndrome in patients with post extracorporeal shock wave lithotripsy renal hematomas: safe and effective, but also advisable? *Urol J* 2014;11:1569-74.