Pancreatic Injury Secondary to Renal Ischemia/Reperfusion (I/R) Injury: Possible Role of Oxidative Stress

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Summary

Recent studies demonstrated remote effects of renal ischemia/reperfusion (I/R) injury on some organs such as brain, liver, and lungs. We investigated the effects of renal I-R injury on function, histology and oxidative stress state of pancreas. Twenty-four male adult Sprague-Dawley rats were divided equally into 2 groups; sham group: rats underwent midline laparotomy and dissection of renal pedicles without renal ischemia, and ischemic group: rats underwent bilateral renal ischemia for 45 min. Renal functions (serum creatinine and BUN), pancreatic functions (serum amylase, lipase and insulin) and fasting blood glucose were measured at 2 h, 1 day, 3 days and 7 days after ischemia. Also, pancreatic histology and malondialdehyde (MDA), catalase and reduced glutathione (GSH) were examined at 2 h and 7 days after ischemia. The ischemic rats showed significant increase in serum creatinine and BUN with significant increase in serum amylase and lipase at 2 h, 1 day and 3 days after ischemia. Blood glucose and fasting insulin showed no significant change apart from significant increase in insulin in sham group at 1 day after ischemia. Pancreas isolated from ischemic rats showed significant increase in histopathological damage score and significant increase in MDA and catalase enzyme with decrease in GSH. In conclusion, bilateral renal ischemia for 45 min caused significant impairment of pancreatic functions and histology. This might be due to deficiency of antioxidant and increased lipid peroxidations in pancreatic tissues.

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Introduction

Renal ischemia/reperfusion injury (I/R) is an inevitable consequence of kidney transplantation and after local renal hypoperfusion following partial nephrectomy, renal vascular surgery and aortic cross-clamping (Weight *et al.* 2001). Ischemia/reperfusion injury is a complex phenomenon that induces cell damage through a bi-phasic process. Ischemia initiates the injury by deprivation of the energy needed to maintain ionic gradients and homeostasis, which may ultimately lead to cellular dysfunction and death. Reperfusion exacerbates this damage triggering an inflammatory reaction in which participate free radicals, endothelial factors and leukocytes (Devarajan 2006). Reactive oxygen species (ROS) play an important role in mediating cell damage during I/R injury (Noiri *et al.* 2001) and I/R induced-tissue injury is caused by an imbalance between production of ROS and antioxidant capacity (Erdogan *et al.* 2006).

Renal ischemia/reperfusion (I/R) elicits tissue damage in a number of organs: heart, lung and liver (Serteser *et al.* 2002, Kelly 2003, Yildirim *et al.* 2003, Gulec *et al.* 2008). In the liver, it has been demonstrated that renal I/R injury might cause liver oxidative stress and increase lipid peroxidation in liver tissue (Yildirim *et al.*

2003, Vaghasiya *et al.* 2010). Moreover, Kadkhodaee *et al.* (2009), recently, demonstrated significant decrease in liver GSH, as well as a significant increase in proinflammatory cytokines e.g. TNF-α and anti-inflammatory cytokines e.g. IL-10 concentrations in liver after renal I/R injury. In lungs, the role of proinflammatory and proapoptotic factors such as leukocyte trafficking, cytokines/chemokines, activation of caspases, oxidative stress and uremic toxins in lung injury secondary to renal I/R injury have been demonstrated (White *et al.* 2011). In the heart, cardiac myocyte apoptosis and neutrophil infiltration are two of the most important contributors to the pathophysiology of myocardial infarction during renal I/R injury (White *et al.* 2011). In the pancreas, although, a recent *in vitro* study demonstrated impairment of kidney function by pancreatic I/R injury through xanthine oxidase enzyme (Khoury *et al.* 2010), there is no study, up to the best of our knowledge, investigated the impact of renal I/R injury on pancreatic function and histology. So, the aim of this study was to investigate the impact of renal I/R injury on the markers of pancreatic function and histology of pancreas as well as on the oxidative stress state in pancreas.

Materials and Methods

Experimental animals

This study was conducted on 24 adult male healthy Sprague Dawley rats weighing between 200-250 g aging 4-6 months. Animals were bred and housed in the animal house of the Medical Experimental Research center (MERC), Mansoura University, at a temperature of 20 °C, fed a standard laboratory chow and had a free access to tap water. All experimental protocols were approved by our local ethics committee.

Experimental design

Rats were allocated into 2 groups (12 rats each): Group I (sham); rats were subjected to midline laparotomy and dissection of renal pedicles without any renal ischemia. Group II (ischemic); rats were subjected to all experimental procedure with renal ischemia.

Renal I/R injury model

The anesthesia was induced by placing rats for 30 s in a glass container containing a piece of cotton soaked with 10 ml of halothane. Then animals were maintained on sodium thiopental at a dose of 12 mg/100 g body weight injected intraperitoneally. Under anesthesia, the animal was fixed in the supine position on a thermoregulated heating board to maintain body temperature at 37 °C. A midline laparotomy was done, then both renal pedicles were exposed and clamped for 45 min using a vascular Bulldog clamp. Blood samples were collected at 2 h and at day 1, day 3 and day 7 after ischemia. Pancreatic tissues were collected 2 h after reperfusion from half of animals (6 rats), and at day 7 from the other half (6 rats) and prepared for histopathological examinations and measurement of markers of oxidative stress.

Biochemical assay

Serum creatinine and BUN, serum amylase, lipase, fasting insulin and fasting blood glucose were measured at 2 h, day 1, 3 and 7 after ischemia. All of these measurements were done by commercially available kits (Fortress diagnostics, UK).

Histological procedures

After formalin fixation (10 % phosphate-buffered) and dehydration, paraffin-embedded pancreatic sections (4 μm) were stained by hematoxylin and eosin. Histopathology for all tissues was evaluated per section in at least 10 randomly selected non-overlapping fields at × 400 magnifications of the sections. Pancreatic sections were evaluated for the presence of congestion, cellular degenerative changes, cytoplasmic vacuolization and leukocyte infiltration. The histological grading of edema was made using a scale ranging from 0 to 3 (0 = no edema, 1 = interlobular edema, 2 = interlobular and moderate intralobular edema, and 3 = interlobular edema and severe intralobular edema). Leukocytic infiltration was also graded from 0 to 3 (0 = absent, 1 = scarce perivascular infiltration, 2 = moderate perivascular and scarce diffuse infiltration, 3 = abundant diffuse infiltration). Grading of vacuolization was based on the appropriate percentage of acinar cells involved: 0 = absent, 1 = less than 25 %, 2 = 25-50 % and 3 = more than 50 % of acinar cells. Hemorrhage was graded: 0 = no hemorrhage, 1 = 1-2 hemorrhagic foci per specimen (slide), 2 = 3-5 hemorrhagic foci per slide, 3 = more than 5 hemorrhagic foci per slide. Necrosis was graded: 0 = no necrosis, 1 = less than 15 % of pancreatic cells involved, 2 = 15-35 % of cells involved, 3 = more than 35 % of cells involved (Warzecha *et al.* 2004).

Table 1. Effect of 45 min bilateral renal ischemia on renal functions, pancreatic functions and blood glucose.

	Group	2 h	1 day	3 days	7 days
Renal functions					
Serum creatinine	Sham group	36.24 ± 4.42	68.07 ± 10.61	44.20 ± 4.20	38.89 ± 0.88
(µmol/l)	Ischemic group	72.49 ± 6.19*	93.70 ± 13.26*	138.79 ± 10.61*	58.34 ± 13.26
Serum BUN (mmol/l)	Sham group	7.09 ± 0.68	12.48 ± 2.39	16.65 ± 0.59	8.6 ± 1.15
	Ischemic group	17.42 ± 1.53*	48.39 ± 6.59*	37.41 ± 3.18*	10.59 ± 1.54
Pancreatic functions					
Serum amylase (U/l)	Sham group	264.0 ± 44.43	255.0 ± 22.53	249.17 ± 33.06	383.67 ± 40.82
	Ischemic group	287.67 ± 44.68	315.83 ± 62.26*	498.33 ± 103.84*	459.5 ± 54.38
Serum lipase (U/l)	Sham group	32.00 ± 5.51	18.67 ± 6.47	24.0 ± 9.18	29.83 ± 10.05
	Ischemic group	36.00 ± 8.03	54.67 ± 12.25*	74.83 ± 5.56*	42.33 ± 15.38
Fasting insulin	Sham group	66.74 ± 9.79	93.76 ± 19.79	60.60 ± 4.03	68.62 ± 8.26
(pmol/l)	Ischemic group	46.39 ± 11.67	57.29 ± 9.45*	58.38 ± 12.29	55.21 ± 8.75
Fasting blood	Sham group	5.84 ± 0.75	6.97 ± 0.44	6.09 ± 0.44	6.05 ± 0.25
glucose (mmol/l)	Ischemic group	6.45 ± 1.57	6.86 ± 1.13	7.18 ± 1.43	5.51 ± 0.49

All data expressed as Mean ± SD. Independent T-test. * significant vs. sham group ($p < 0.05$). BUN = blood urea nitrogen

Estimation of lipid peroxidation and antioxidant enzymes in pancreatic tissues

Pancreas tissues, harvested at 2 h and 7 days after ischemia, were kept in cold conditions (precooled in Petri dish inverted on ice). It was cross-chopped with surgical scalpel into fine slices in chilled 0.25 M sucrose and quickly blotted on a filter paper. The tissue was minced and homogenized in 10 mM Tris-HCl buffer, pH 7.4 (10 % w/v), with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for assays of lipid peroxidation (MDA content) and endogenous antioxidant enzyme catalase (CAT), and reduced glutathione (GSH) by using colorimetric kit (Bio-Diagnostics, Dokki, Giza, Egypt) according to manufacturer's instructions commercially available kits.

Statistical analysis

The results are given as mean ± SD. Independent T test was used to find statistical difference between sham and ischemic group. The null hypothesis was rejected at the 0.05 level of significance. SPSS 14.0 software was used for data analysis.

Results

Effects of 45 min bilateral renal ischemia on renal functions (serum creatinine and BUN)

Table 1 shows the results of serum creatinine

and BUN in sham and ischemic groups at 2 h, day 1, day 3 and day 7 after ischemia. Compared to sham group, serum creatinine and BUN were significantly high in ischemic group at 2 h, day 1, and day 3 only after ischemia ($p < 0.05$). At day 7 there was no significant difference between sham and ischemic group.

Effects of 45 min bilateral renal ischemia on pancreatic functions (serum amylase, lipase, fasting insulin) and fasting blood glucose

Compared to sham group, serum amylase showed significant increase in ischemic group at 2 h, day 1 and day 3 after ischemia ($p < 0.05$), while serum lipase showed significant increase in control group only at days 1 and 3 (Table 1). Compared to sham group, fasting insulin showed significant decrease in ischemic group only at day 1 ($p < 0.001$). Fasting blood glucose showed no statistically significant difference between sham and ischemic groups at different periods of follow up (Table 1).

Effect of 45 min bilateral renal ischemia on oxidative stress markers and antioxidants in pancreatic tissues

The results of the MDA, catalase and GSH analyses are shown in Figure 1. Compared to sham group, the level of MDA was significantly high in the pancreatic tissue of the ischemic group at 2 h, but not at 7 days (Fig. 1A). Also, pancreatic catalase level was
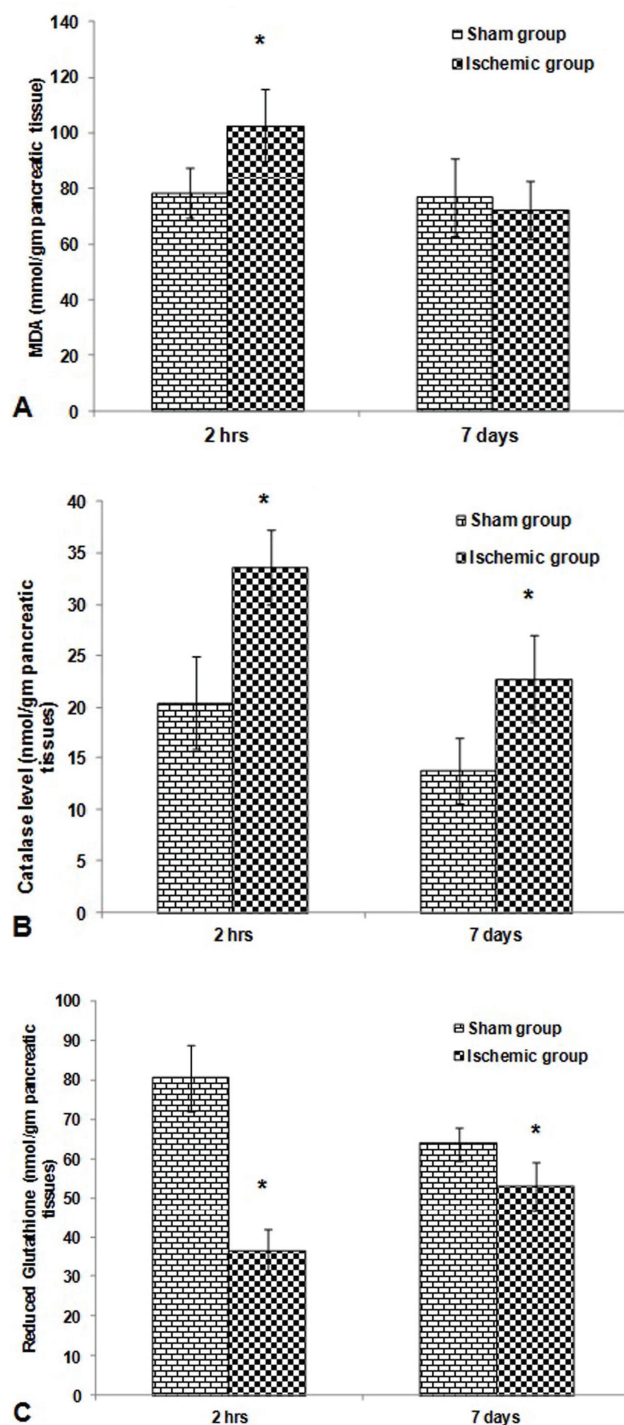
Fig. 1. Effect of 45 min bilateral renal ischemia on pancreatic oxidative stress MDA (**A**), Catalase (**B**), and reduced glutathione (GSH) (**C**). All data expressed as Mean ± SD. Independent T-test. * significant vs. sham group (p≤0.05)

significantly high in ischemic group when compared with that of sham group at 2 h and 7 days after ischemia (Fig. 1B). Lastly, compared to sham group, GSH concentration in pancreatic tissues was significantly low in ischemic group at 2 h but not at 7 days (Fig. 1C).

Effect of renal ischemia on the weight and histopathology of pancreas

Table 2 shows the results of pancreatic weight and damage score. Compared to sham group, the weight of pancreas was significantly increased in ischemic group at 2 h after ischemia and at the end of experiment. Also, compared to sham group, pancreatic edema, leucocytic infiltration, and vacuolization were significantly high in ischemic group at 2 h after ischemia and at the end of the experiment. However, pancreatic necrosis was present only in control ischemic group at 2 h after ischemia, as well as both groups showed no pancreatic hemorrhage. Figures 2, 3, 4, and 5 are samples of ischemic group.

Discussion

The main findings of the present study include that, renal I/R injury resulted in a) significant elevation in markers of pancreatic function such as serum amylase and lipase, b) significant elevation of pancreatic damage score, c) significant elevation of pancreatic oxidative stress state. To the best of our knowledge, this is the first study that addressed the effect of renal I/R injury on pancreatic function and morphology. Most of research in renal I/R injury has focused on the local renal response to this injury (Kadkhodaee *et al.* 1996, Hussein *et al.* 2011). However, recent studies emerged to investigate the remote effects of renal I/R injury on distant organs such as liver, brain and lungs (Serteser *et al.* 2002, Kelly 2003, Yildirim *et al.* 2003, Gulec *et al.* 2008). Moreover, Johnson and Nabseth (1970) reported occurrence of pancreatitis in patients with renal transplantation by 2 % and half of these cases were fatal. They attributed this pancreatitis to immunosuppressive drugs, hyperparathyroidism, surgical traumatic pancreatitis and immunologic considerations. So, in the present study, we examined the effect of renal I/R injury on pancreatic functions and histology, as well as on pancreatic oxidative stress status.

In the present study, we showed that 45 min bilateral renal ischemia caused significant elevation of serum amylase and lipase after the different time intervals. These findings indicate renal I/R injury may result in impairment of pancreatic exocrine functions. In agreement with our study, Warzecha *et al.* (2008) demonstrated, in a rat model of pancreatic I/R injury, that renal not direct ischemic preconditioning caused more elevation in serum amylase and lipase at 12-24 h after reperfusion. This effect seems to be related to the mechanism of blood flow regulation of the gut. Ischemic

Table 2. Effects of 45 min bilateral renal ischemia on pancreatic weight and histopathological damage score.

	Group	Weight (mg)	Oedema	Leucocytic infiltration	Vacuolization	Necrosis	Hemorrhage
2 h	*Sham group*	98.80 ± 29.40	0-1	0-1	0	0	0
	Ischemic group	$120.50 \pm 30.31^*$	1-2*	1-2*	0-1*	0-1*	0
7 days	*Sham group*	120.17 ± 81.55	0-1	0-1	0	0	0
	Ischemic group	$174.00 \pm 56.39^*$	1-2*	1-2*	1-2*	0	0

Data of pancreatic weight are expressed as Mean ± SD. Independent T-test. * significant vs. sham group ($p < 0.05$). Numbers representing the predominant grading of pancreatic damage
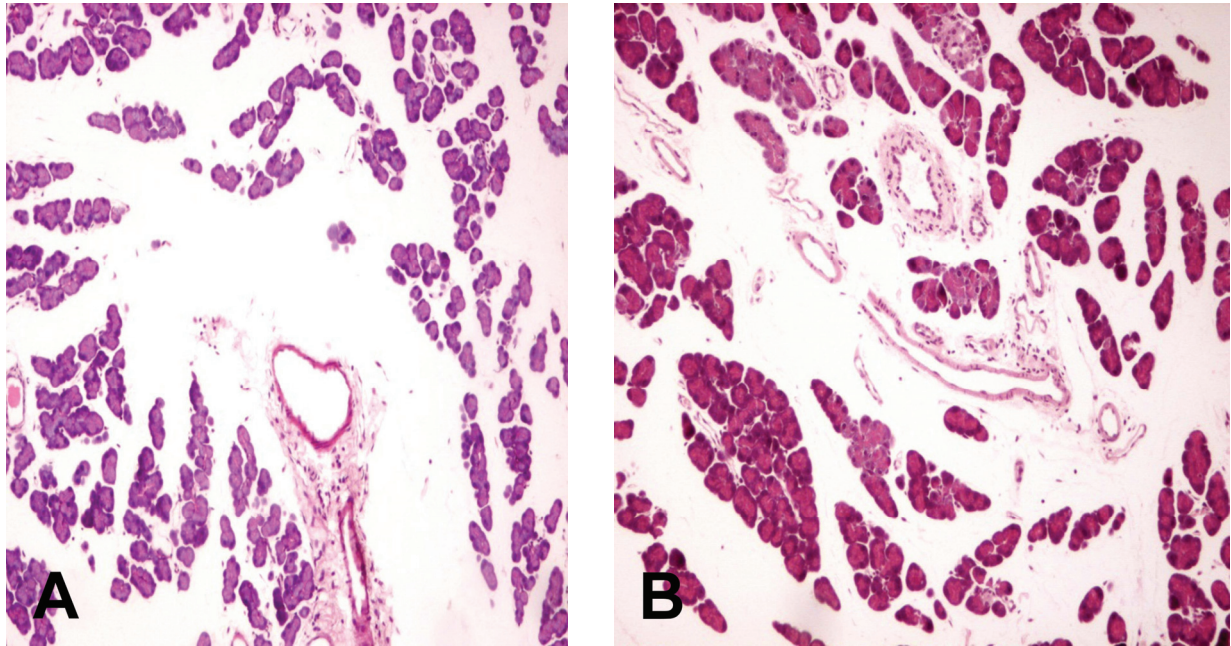

Fig. 2. Edema expanding the interstitial tissue separating pancreatic acini and lobules in ischemic group (**A** and **B**) (H & E, X400).
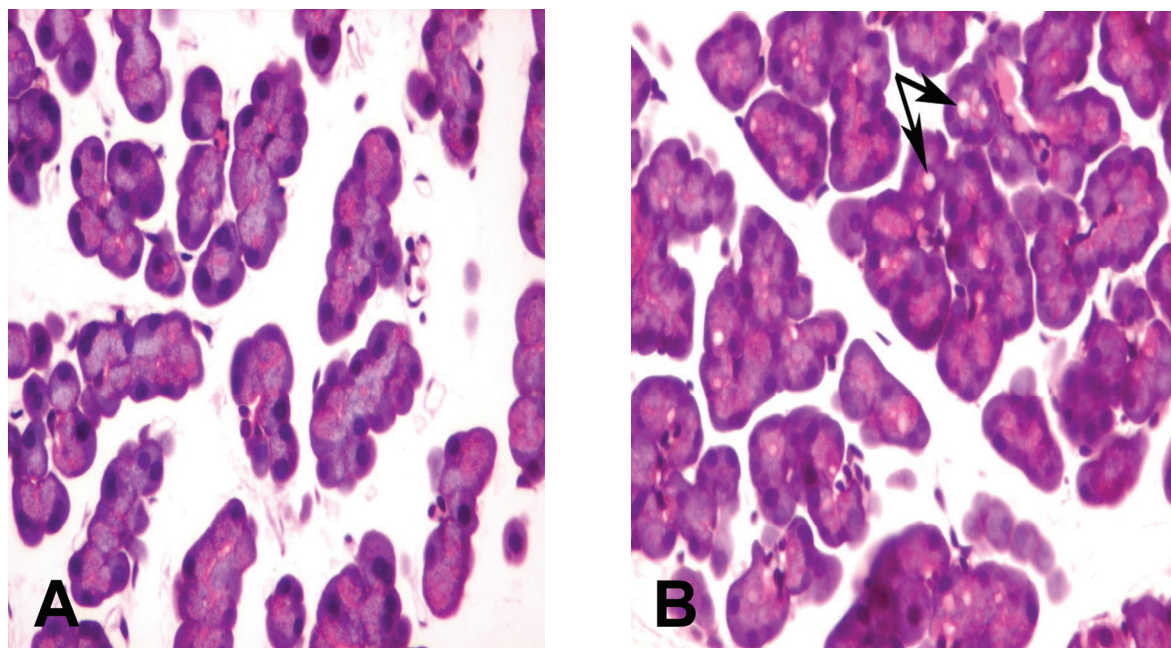
Fig. 3. Pancreatic acinar cell vacuolization (**A**), vacuoles appear as unstained holes within cytoplasm (arrows) (**B**) (ischemic group) (H & E, X400).
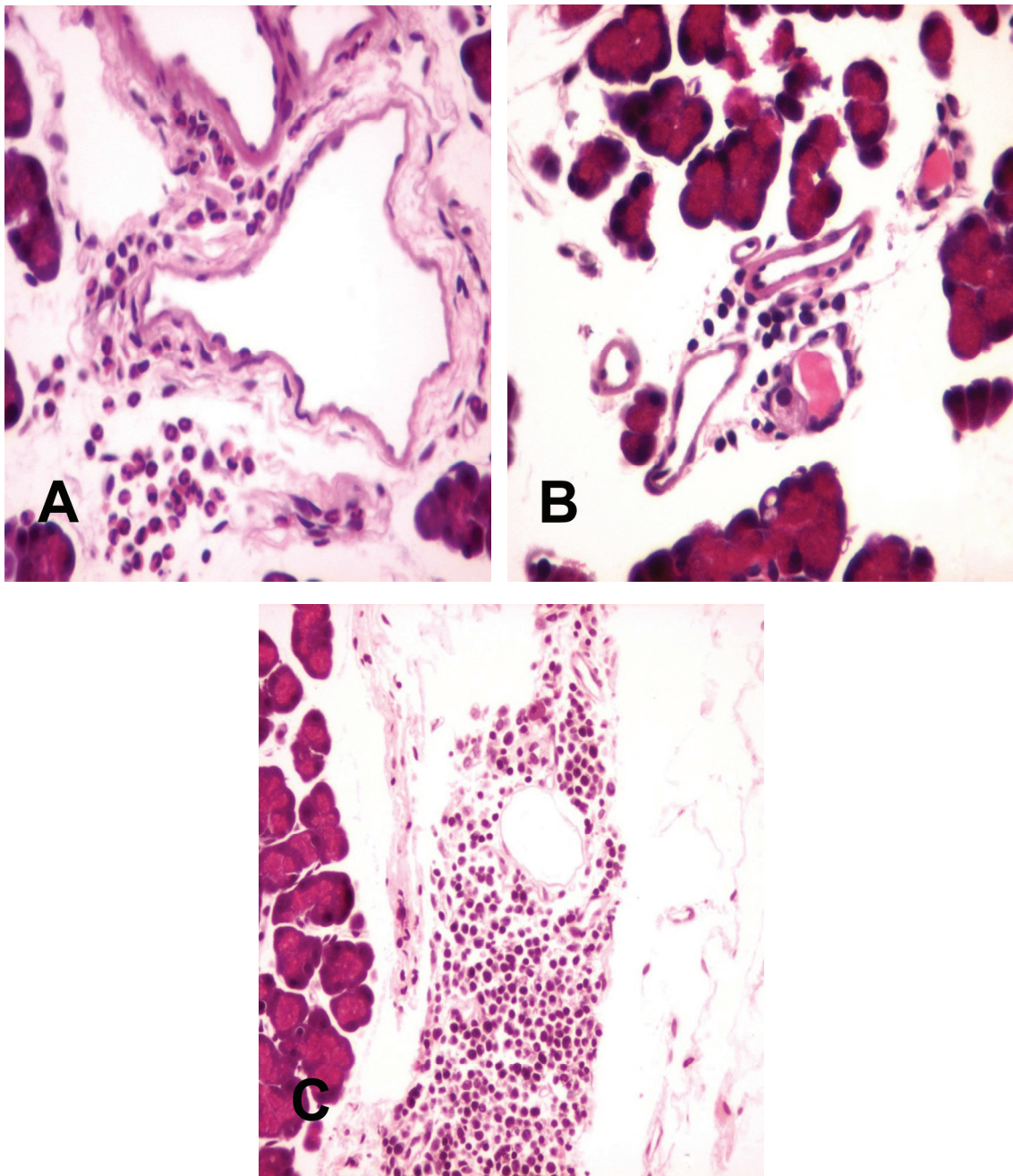
Fig. 4. Mild perivascular inflammatory infiltrate formed mainly of neutrophils (H&E) (**A**), mild dense perivascular inflammatory infiltrate (**B**) (H & E, X400), and moderately dense perivascular infiltrate (**C**) (ischemic group), (H & E, X400).

preconditioning of the kidney is a kind of trauma that results in centrally integrated autonomic mediated cardiovascular response that are designated to increase heart rate, systemic vascular resistance and maintain the arterial blood pressure to vital organs at the expense of gut and skeletal muscle blood flow (Muir 2006). This effect is probably intensified by activation of renin angiotensin system by renal ischemia. This speculation was supported the data reported by Warzecha et al. (2008) who demonstrated reduction of pancreatic blood flow between 6 h to 24 h by renal ischemic

preconditioning. However, this point was not investigated in our study. On the other hand, fasting insulin showed no significant change in ischemic rats when compared to sham group apart from significant decrease in day 1. Moreover, fasting blood glucose showed no the endocrine functions of pancreas. These findings suggested that the pancreatic injury induced by renal ischemia was not sufficient to affect the endocrine functions of pancreas or to affect glucose homeostasis. Further studies are needed to assess the functions of β cells of islets of Langerhans and its histology.
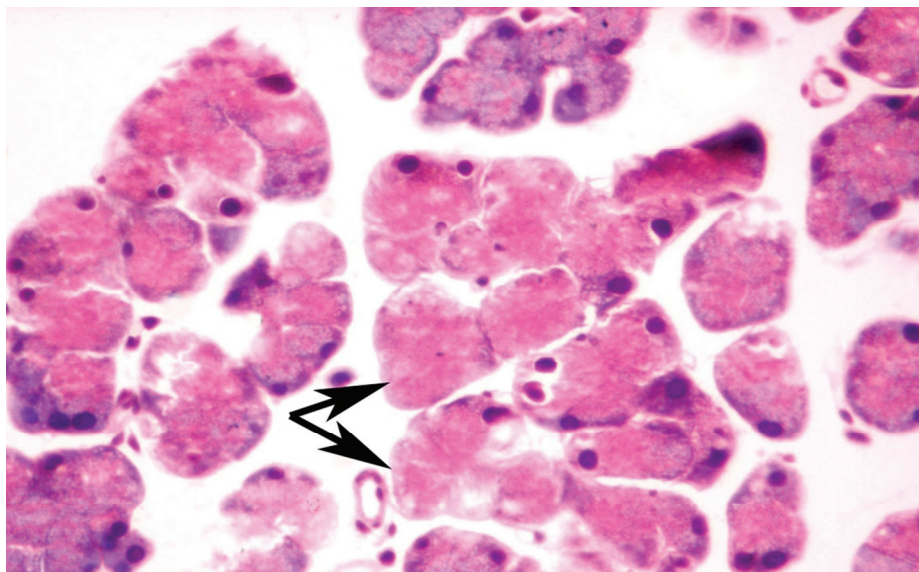
Fig. 5. Groups of necrotic acinar cells (arrows) (ischemic group), (H & E, X400).

Oxidative stress injury of the kidney may play a major role in inducing remote organ injury. The involvement of ROS in I/R injury to the kidney and other organs is widely accepted. We examined oxidative stress state in pancreas during renal I/R injury. We found significant increase in concentration of MDA (marker of lipid peroxidation), and catalase enzyme and significant decrease in GSH in pancreatic tissues after renal I/R injury. These findings suggest increased oxidative stress in pancreas during renal I/R injury. In consistence with these findings Kadkhodaee *et al.* (2009) demonstrated significant reduction of hepatic level of GSH with different periods of renal ischemia. Moreover, Khoury *et al.* (2010) demonstrated that the ROS, generated in the ischemic pancreas, could be associated with edema in the vasculatures of both ischemic-nontreated and ischemic-treated pancreases and the exposed kidneys (Weinbroum *et al.* 2001). The hyperamylasemia and hyperlipasemia could be explained by pancreatic injury caused by ROS. This is evidenced in the present study by significant increase in MDA (marker of lipid peroxidation) and GSH (marker of endogenous antioxidant) in ischemic rats. Tamura *et al.* (1993) suggested that the active oxygen species that are generated by short-term pancreatic ischemia and reperfusion injure the endothelium and cause hyperamylasemia. Also, Junior *et al.* (2010) demonstrated that pancreatic I/R injury produced hyperamylasemia probably due to ROS damage.

The histological examination of pancreas revealed edema, leucocytic infiltration, and vacuolization in ischemic group at 2 h after ischemia and at the end of the experiment and pancreatic necrosis in ischemic group at 2 h after ischemia, but, there was no pancreatic hemorrhage. So, these pathological findings indicate that bilateral renal ischemia for 45 min cause acute inflammatory reaction in pancreas. This inflammatory reaction may be similar to that observed in liver, heart and lung in previous studies (Serteser *et al.* 2002, Jeyarajah *et al.* 2003, Kadkhodaee *et al.* 2009). Some studies demonstrated significant increase in proinflammatory cytokines TNF-α and IL-10 concentrations in liver after renal I/R injury (Serteser *et al.* 2002, Jeyarajah *et al.* 2003, Kadkhodaee *et al.* 2009). Serteser *et al.* (2002) have suggested that 30 min ischemia and 60 min reperfusion is sufficient to elicit remote effects of I/R injury. In the present study 2 h reperfusion is sufficient to elicit the remote effects of renal I/R injury on pancreas. Also, Warzecha *et al.* (2008) demonstrated significant elevation in serum IL1β in a model of pancreatic I/R injury by renal ischemic preconditioning with maximal rise 6 h after reperfusion. Moreover, White *et al.* (2011) demonstrated the involvement of proinflammatory and proapoptotic factors such as leukocyte trafficking, cytokines/chemokines, and activation of caspases in pulmonary injury induced by renal I/R injury.

Conclusion

We conclude that 45 min bilateral renal ischemia causes detrimental changes in pancreatic exocrine functions and morphology as well as it enhances oxidative stress and inflammatory states in pancreas. We recommend, care should be taken to protect other organs such as pancreas remote from I/R sites, especially during renal surgery.

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