Original Article

Effects of renal ischemia-reperfusion on biochemical factors and histopathological alterations in the liver of male rats

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Abstract

Introduction: One of the main reasons for acute renal failure is the renal ischemia-reperfusion. It seems that renal ischemia-reperfusion-induced oxidative stress not only lead to alterations in renal function but also causes tissue alterations in distant organs such as the liver. The purposes of this study were to investigate the effects of renal ischemia-reperfusion on biochemical factors and histopathological changes in the liver of male rats.

Methods: Forty male rats were randomly divided into 4 groups: control, the sham group (only laparotomy), right nephrectomy and ischemic-reperfusion (right nephrectomy + left ischemic- reperfusion). In the end, following anesthesia, blood and liver samples were taken for the measurement of biochemical factors (malondialdehyde (MDA), superoxide dismutase (SOD), aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) levels) and histopathological changes.

Results: The result of this study indicated that renal ischemia-reperfusion significantly decreased SOD and increased MDA, AST, ALP and ALT compared to sham-control group ($P<0.05$). In addition, histopathological findings show that ischemia-reperfusion significantly increased apoptotic cells ($P<0.05$) and causes the disorganized arrangement of the hepatic plate, severe hepatocellular cytoplasmic vacuolation and extensive nuclear pyknosis.

Conclusion: Our findings suggest that renal ischemia-reperfusion-induced liver damage, accompanied by decreasing of antioxidant capacity and increasing of oxidative stress and apoptosis in the liver.

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Introduction

Ischemia is a restriction in blood supply to tissues, causing a shortage of glucose and oxygen needed for cellular metabolism. Blood flow's restoration to the ischemic tissue is called reperfusion. In spite of the indisputable advantages of reperfusion of blood to an

ischemic tissue, reperfusion itself can elicit a cascade of harmful reactions that injure tissue (Bonventre, 1993).

Renal ischemia-reperfusion is the main reason for acute kidney injury, perhaps progress to chronic kidney disease that may lead to death in patients (Bonventre and Yang, 2011; Sharfuddin and Molitoris, 2011). Several studies have discovered that renal ischemia is related to lipid peroxidation in cellular membranes of many organs. It was proposed that lipid peroxidation, as a free radical generating system, closely associated with ischemia-reperfusion-induced tissue damage (Eschwege et al., 1999).

Previous studies have shown that renal ischemia-reperfusion in addition to kidney tissue, also affects distant organs such as the brain (Liu et al., 2008), lung (Yousefi et al., 2014), liver (Park et al., 2011) and heart (Eltzschig and Eckle, 2011; Alihemmati et al., 2017). Nitric oxide and reactive oxygen species (ROS) perform essential roles in mediating cell destruction during renal ischemia-reperfusion (Basireddy et al., 2006; Noiri et al., 2018). Renal ischemia-reperfusion results in tissue injury and apoptosis through oxidative stress production by an imbalance between the formation of ROS and the antioxidant capacity (Erdogan et al., 2006). In addition, myeloperoxidase is available in neutrophils and catalyzes the development of hypochlorous acid, a toxic agent to cellular components and initiates apoptosis and oxidative damage (Altunoluk et al., 2006).

The liver is one of the distant organs that are damaged during renal ischemia-reperfusion. It is exhibited that renal ischemia-reperfusion injury might cause liver oxidative stress and enhance lipid peroxidation in the liver tissue (Yildirim et al., 2003). Reduction of antioxidant enzyme activities in the liver of rat is well reported after bilateral renal ischemia-reperfusion (Sural et al., 2000). Because studies on renal ischemia-reperfusion on the liver are limited and mostly bilateral ischemia is done, this study examined the effects of unilateral renal ischemia-reperfusion, similar to kidney transplantation model (Yousefi et al., 2014; Alihemmati et al., 2017), on oxidative stress factors, apoptosis and tissue injury in the liver to further identify the mechanisms involved in injury caused by renal ischemia-reperfusion.

Materials and methods

Animals

In this experimental study, forty adult male Wistar rats weighing 200-250g were used. Rats were prepared of the experimental animal research center of Tabriz University of Medical Sciences and were housed in the controlled room (temperature of 22-24°C with the cycle of light/dark for 12 hours). The study was approved by the Tabriz University of Medical Sciences Ethics Committee.

Experimental protocol and surgery

Animals were randomly divided into 4 groups (n=10 for each group): control, the sham group, right nephrectomy and ischemic-reperfusion (right nephrectomy + left ischemic- reperfusion). The control group was without any procedure and the sham group is also under anesthesia that was performed an only laparotomy. In the nephrectomy group, the skin on the back was disinfected with the povidone-iodine solution after shaving. Then the animals were placed in the right flank position (under anesthesia) and a small operation was made under the last ribs and the right kidney was removed and then, the operation was sutured.

In the ischemic-reperfusion group, after right nephrectomy, the rats were kept for recovery for 15 days and then under anesthesia and in the left flank position left renal pedicle (artery and vein) was exposed. In order to perform ischemia renal, pedicle was occluded by placing an atraumatic microvascular clamp for 45min and then subjected to reperfusion for 24h. At the end of 45min ischemia, the clamp was removed slightly and the left kidney was observed for 5min to make sure the reflow process. Then, sterile saline (1ml, 37°C) was injected intraperitoneally and the incision was closed (Yousefi et al., 2014). In the end, following anesthesia, blood and liver samples were taken for the measurement of biochemical factors (malondialdehyde (MDA), superoxide dismutase (SOD), aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) levels) and histopathological changes.

Liver enzymes analysis

After reperfusion period, the blood was taken and put into heparinized tubes. Plasma was separated by centrifugation (3000 rpm for 10min at room temperature) for biochemical studies. The serum

samples were kept in -20°C until measurement of the serum for measurement of hepatic enzymes. ALT, AST and ALP were measured by appropriate commercial kits (Pars Azmoon, Tehran, Iran) photometrically.

Lipid peroxidation and superoxide dismutase measurement

First, the tissue sample was homogenized in lysis buffer (pH 7.4) in the presence of protease inhibitor cocktail (Sigma-Aldrich, St Louis, MO) with the homogenizer. The levels of MDA evaluated by thiobarbituric acid reactive substances (TBARS) in homogenates. In brief, 250µl of the samples were mixed with thiobarbituric acid (1ml of 0.67%) and trichloroacetic acid (1ml 10%). Then samples were placed in a boiling water bath (15min) and next supplemented by n-butyl alcohol (2:1 V:V) solution. After cooling, the samples were centrifuged (3500g, 5min) at room temperature and then read the absorbance at 535nm, by a spectrophotometer (Pharmacia Biotech; England). Tissue SOD activity was evaluated by a spectrophotometric method based on the inhibition of a superoxide-induced reduced nicotinamide adenine dinucleotide (NADH) oxidation. Also, protein concentration of supernatant was assessed using the Bradford technique (Kruger, 2002).

Histopathological examination

At the end of the treatment period, liver was removed and then fixed in buffered-formalin solution (10%) at the room temperature. After fixation, the liver specimens were dehydrated with an ascending ethanol sequence, cleared with xylene and embedded in paraffin. Sections of 5µm were obtained, stained with hematoxylin-eosin (H&E) and observed with a light microscope (Olympus BH-2,

Tokyo, Japan) for assessment of histological alterations. The morphological changes were scored on an ordinal scale as follows: (0: no evidence of injury; 1: minimal; 2: mild; 3: moderate and 4: severe) in order to perform the comparison between the groups.

Apoptotic cell detection

Hepatocytes apoptosis were evaluated by TUNEL proxidase assay kit (In situ cell death detection Kit-POD, Roch, Germany). Sections of 5µm were obtained and placed on poly-L-lysine coated slides for TUNEL technique. All procedures were performed according to the manufacturer's kit (Hazrati et al., 2018). The apoptosis quantification was calculated by counting the number of TUNEL-positive cells per high power field.

Statistical analysis

All biochemical data were expressed as the mean ± standard deviation (M ± SD). The statistical analyses of the data were performed using the one-way ANOVA test and Tukey's post hoc test. *P*<0.05 was considered statistically significant. For histological variables, a minimum of 10 fields in each liver slide was obtained for TUNEL staining and H&E evaluation (n = 7 for each group). Histological variables have been analyzed with Kruskal-Wallis nonparametric test using SPSS 13 (SPSS® Inc; Illinois, USA).

Results

Liver enzymes levels

Serum level of AST, ALT and ALP showed a significant (*P*<0.05) increase at 24h of reperfusion after 45min of ischemia in compared with control and sham groups. In addition, the levels of AST, ALT and ALP increased significantly in nephrectomy group

Table 1: Serum level of liver enzymes (ALT, AST and ALP; n= 10; mean±SD)			
Group	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	75.80±6.39	73.63±6.96	1101.10±54.29
Sham	77.81±5.72	75.79±7.71	1128.06±81.43
Nephrectomy	139.10±5.68*	111.63±9.07*	1380.75±67.12*
Ischemia/ Reperfusion	189.86±6.53*°	143.74±7.38*°	1533.11±59.72*°

*P<0.05 compared to control and sham groups. °P<0.05 compared to nephrectomy group. AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase.

Table 2: Liver SOD and MDA levels (n= 10, mean±SD)

	MDA (nmol/ml)	SOD (U/mg protein)
Control	2.47±0.38	5.12±0.43
Sham	3.06±0.78	4.93±0.55
Nephrectomy	4.97±0.53*	3.08±0.27*
Ischemia-reperfusion	7.12±0.65*°	1.71±0.42*°

*$P<0.05$ as compared with control and sham groups. °$P<0.05$ compared with nephrectomy group. MDA: malondialdehyde, SOD: superoxide dismutase.
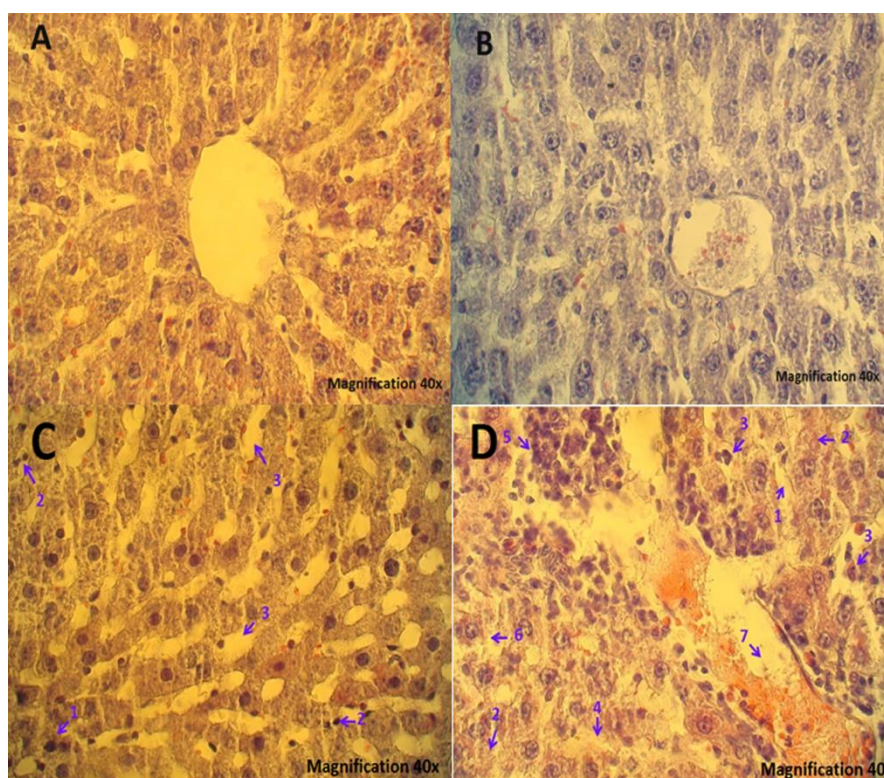

Fig.1. Histopathologic examination in control (A) and sham (B) groups showed liver sections with normal hepatic cells and prominent nucleus, cytoplasm and central vein. Histopathology examination in nephrectomy group (C) showed liver injury with little nuclear pyknosis (1), few inflammatory cells infiltration (2) and dilated sinusoids (3). Evaluation of the liver sections in ischemia reperfusion group (D) showed injury with disorganized of arrangement of hepatic plate (1). In these sections showed injury with severe hepatocellular cytoplasmic vacuolation (2), extensive nuclear pyknosis (3), dilated sinusoids (4), severe inflammatory cells infiltration (5) and loss of intercellular borders (6) and vascular congestion (7).

than the control and sham groups ($P<0.05$). Also, the levels of these enzymes significantly ($P<0.05$) increase in the ischemic-reperfusion group than to the nephrectomy groups (Table 1).

Tissue SOD and MDA levels
As shown in Table 2, the level of MDA in the liver was significantly higher in ischemic-reperfusion and nephrectomy groups compared to the control group ($P<0.05$). On the other hand, the activity of SOD was significantly diminished in ischemic-reperfusion and

nephrectomy groups compared to the control group ($P<0.05$). Also, in the group of ischemic-reperfusion significantly ($P<0.05$) the level of MDA increased and the level of SOD decreased compared to the nephrectomy group.

Histological changes
The liver sections of control and sham groups were shown to have normal morphological structure. This section showed regular hepatic cords with the normal hepatic cells and few spaced sinusoids arranged

Table 3: Median score and inter quartile range (IQR) of histological changes in control, Sham, nephrectomy and ischemia/reperfusion groups.

Histological criteria	Control		Sham		Nephrectomy		Ischemia/ Reperfusion	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
PMN.Infiltration	1	1	1	1	2*	2	4*°	1
Nuclear.pyknosis	0	0	0	1	2*	2	3*°	1
Cytoplasm.vacuolization	0	1	1	0	2*	1	4*°	1
Disorganized.Hepatic.cords	0	1	0	1	3*	1	4*	1

A minimum of 10 fields in each liver slide were obtained and stained with H&E to evaluation of severity of tissue injury (n=10 for each group). *$P<0.05$ compared to control and sham groups. °$P<0.05$ compared to nephrectomy group. PMN: polymorphonuclear neutrophils.

Table 4: Apoptotic positive hepatic cells

Group	TUNEL-positive cells
Control	5.00±1.29
Sham	5.57±1.39
Nephrectomy	9.57±1.51*
Ischemia/ Reperfusion	19.56±1.27*°

*$P<0.05$ compared to control and sham groups. °$P<0.05$ compared to nephrectomy group.

between the hepatic cords and central vein. Also, hepatic cells showed well-preserved and eosinophilic cytoplasm with prominent round nuclei (Figs. 1A and B). Histopathology evaluation in the nephrectomy group showed injury with little nuclear pyknosis, sporadic inflammatory cells infiltration and dilated sinusoids (Fig. 1C). The histopathological finding in the ischemia-reperfusion group showed injury with disorganized of the arrangement of the hepatic plate. In these sections showed injury with severe hepatocellular cytoplasmic vacuolation extensive nuclear pyknosis, severe inflammatory cells infiltration and loss of intercellular borders and vascular congestion (Fig. 1D). The degrees of structural change in the liver of rats with the nephrectomy and ischemia-reperfusion were increased markedly as compared with control and sham groups ($P<0.05$). Furthermore, structural changes in the group of ischemia-reperfusion were significantly ($P<0.05$) increased compared to the nephrectomy group (Table 3).

Apoptotic cell detection

More TUNEL positive cells observed in the liver sections obtained 24h after renal ischemia-reperfusion (Table 4). Furthermore, TUNEL positive cells were rarely present following the nephrectomy group. But there were not any TUNEL positive cells in sham and control groups.

Discussion

The results of this research exhibited that renal ischemia-reperfusion exerts adverse effects on liver enzymes, MDA and SOD levels and causes histopathological alternating in the liver of adult rats. Renal ischemia-reperfusion is confronted in many clinical conditions such as partial nephrectomy, transplantation, sepsis or hydronephrosis (Bonventre and Zuk, 2004). In the present study, we assessed hepatic enzymes activities including AST, ALT and ALP to evaluate the liver injury. During renal ischemia-reperfusion, liver functional indexes such as blood ALT and AST were raised and spermine, spermidine acetyltransferase, an enzyme up-regulated in initial phases of liver injury, was increased (Khastar et al., 2011a). Similar results in our study showed that increases in ALT and AST cause liver injury in the renal ischemia-reperfusion group. After 45min of ischemia followed by 24h of

reperfusion, the levels of ALT, AST and ALP in liver were significantly increased in the renal ischemia-reperfusion rats compared to the control group. Our result showed significant increases in ALT level in the renal ischemia-reperfusion group. ALT level, AST level and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for liver health (Kumar and Boon-Bee Goh, 2016).

Renal ischemia-reperfusion results in endothelial and leukocyte activation and oxidative stress in the kidney (Ahmadiasl et al., 2014). Oxidative stress can destruct biomembranes and enzyme proteins, cause apoptosis, and promote leukocyte-endothelial cell adherence (Granger and Korthuis, 1995). Our findings showed 45min unilateral renal ischemia followed by 24h reperfusion significantly increased MDA level in the liver. MDA is utilized as an indicator of lipid peroxidation (Salimnejad et al., 2018). Increase in lipid peroxidation level has been investigated in many liver diseases, including exposure to hepatotoxic agents. It was shown that lipid peroxidation and protein oxidation products were markedly increased, whereas GSH levels were significantly decreased (Sundari et al., 1997). These findings indicate impairment of protein redox and thiol status in hepatic tissue. The fact that neutrophil depletion decreases lipid peroxidation suggests that neutrophilic oxidants contribute to ischemia-reperfusion injury by oxidation, modifying the biomolecules in tissues, resulting in lipid peroxidation, protein destruction or DNA abnormalities (Serteser et al., 2002).

In addition, the results of this study indicated that renal ischemia-reperfusion decreased SOD activities compared to sham-control rats. Decrease SOD activity in the liver is suggested to be the outcome of inactivation by lipid peroxyl radicals and their breakdown products. The lowest SOD activities were previously detected after 60min renal ischemia–1h reperfusion. The increasing in TBARS levels and the declining in SOD activities can support this hypothesis (Serteser et al., 2002; Stadtman and Berlett, 2002). Related to reduced SOD activity, CAT activity was similarly found to be decreased as a result of the declined production of hydrogen peroxide by SOD and increased usage of hydrogen peroxide as fuel in the MPO-catalyzed reaction (Serteser et al., 2002). In addition, it was showed significant decreases in liver SOD and CAT activities

after 60min of ischemia followed by 3h of reperfusion. These results confirmed that bilateral renal ischemia-reperfusion induces induction of liver oxidative stress (Khastar et al., 2011b).

SOD and CAT are the first lines of hepatic tissue protection against oxidant substances that release from cells (Granger and Korthuis, 1995, Khastar et al., 2011a). Other investigations have shown SOD level was decreased in conditions for example oxidative stress (Asaga et al., 2008). It has been showed that leukocytes have a role in the local tissue ischemia-reperfusion injury after induction of reperfusion injury (Khastar et al., 2011b).

The histopathological evaluation of the liver sections in the ischemia-reperfusion group, showed many apoptotic positive cells and injury with severe hepatocellular cytoplasmic vacuolation, extensive nuclear pyknosis, vascular congestion and other changes in the liver as mentioned above. Similar to our results, the previous study presented the significant progress in histopathological damage score, apoptosis, necrosis and MDA and catalase enzyme activity in pancreatic isolated tissue from ischemic rats. Bilateral kidney ischemia for 45min induced impairment of islets functions and histopathology. This can be because of deficiency of antioxidant activity and induced lipid peroxidations in pancreatic tissues (Hussein et al., 2014).

Conclusion

In conclusion, unilateral renal ischemia-reperfusion after right nephrectomy increased tissue injury in the liver of rats. It is exhibited that renal ischemia-reperfusion injury might cause liver oxidative stress and enhance lipid peroxidation in the liver tissue by decreasing tissue SOD activities and increasing MDA, AST, ALT and ALP levels. Also by the destruction the liver morphological changes which occurred after 45min of ischemia followed by 24h reperfusion in the kidney.

Acknowledgments

Conflict of interest

the author declared that there are no conflict interests.

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