EXPERIMENTALLY- INDUCED PANCREATIC DAMAGE CAUSED BY RENAL ISCHEMIA REPERFUSION INJURY (LIGHT MICROSCOPIC STUDY)

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ABSTRACT

Background Renal ischemia reperfusion injury is the major cause of acute renal failure. Acute pancreatitis occurs as remote effect of renal ischemic insult.

Aim of the work: This study was conducted to demonstrate the pancreatic histological alterations secondary to induced renal ischemia-reperfusion.

Material and Method: Eighteen healthy male adult albino rats were classified into two groups; Control group: 12 rats subdivided into two equal subgroups, ischemia reperfusion group: 6 rats subjected to bilateral renal pedicles clamping for 45 min. The pancreas was dissected and processed for histopathological examination by light microscope.

Results: Our results revealed thick connective tissue septa, thick walled congested blood vessels. Distorted vacuolated pancreatic acini which were characterized by dark stained nuclei. The cells of islets of Langerhans appeared with dark nuclei. Dilated congested islets' blood capillaries were seen.

Conclusion: Renal ischemia reperfusion had deleterious effect the pancreas.

KEYWORDS: Renal ischemia- reperfusion; pancreas.

I. INTRODUCTION

Renal ischemia reperfusion is a complex phenomenon which induces cellular damage in a bi-phasic manner. Ischemic phase initiated by deprivation of the energy needed to maintain ionic gradients and homeostasis leading to dysfunction and death of the cell [1]. This damage was exacerbated by reperfusion which triggers an inflammatory reaction in which participate free radicals, endothelial factors and leukocytes [2]. Reactive oxygen species (ROS) play an important role in mediating cell damage during I/R injury and this damage is caused by an imbalance between production of ROS and antioxidant capacity [3].

Switching from aerobic to anaerobic metabolism caused by reduced blood supply result in the ischemic injury. The initial ischemic insult followed by the sudden reoxygenation upon the reestablishment of vascular flow causes reperfusion injury which to a large extent is attributed to the production of ROS and associated cellular injury [4].

Renal ischemia reperfusion injury causes renal epithelial cell death and contributes to the delayed recovery of kidney function. Chronic renal hypoxia is an important mechanism in the development of tubulointerstitial fibrosis and progression of chronic renal disease [5].

Pancreatic injuries are associated with high morbidity and mortality. Massive production of ROS during renal ischemia reperfusion leads to tissue injury, causing acute pancreatitis and apoptosis. The formation of ROS is the frequently blamed mechanism that augments local tissue damage and affects organs remote from the site of IR [6&7].
II. MATERIALS AND METHODS

Animals:
Eighteen healthy adult male albino rats weighing 180–200gm, aged 6-7 weeks were utilized. They were received and kept according to the guidelines of animal house, Faculty of Medicine, Zagazig University. All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee and accepted by the Faculty of Medicine; Zagazig University.

Experimental design:
The rats were divided into two main groups:

- **Group (I):** (Control group): 12 rats equally subdivided into two subgroups:
  - **Subgroup Ia (negative control group):** were kept without treatment.
  - **Subgroup Ib (sham operated group):** underwent identical surgical procedures as an Ischemia-reperfusion group without bilateral renal clamping.

- **Group (II):** (Ischemia - Reperfusion group): Included six animals. Anesthesia was induced with ketamine 50 mg/kg intra peritoneal. The animals were placed on a heating mat, to keep the body temperature at 36 ±1°C. A midline laparotomy was performed, both kidneys were located, and the renal pedicles, containing the artery, vein, and nerve supplying each kidney, were carefully isolated. Rats were subjected to bilateral renal pedicles clamping for 45 min [8]. Once reperfusion commenced the artery clips were removed. The occlusion was verified visually by a change in the color of the kidneys to a paler shade and reperfusion by a blush [7].

Animals were sacrificed after one week of the beginning of reperfusion.

At the end of the experiment, rats were anaesthetized with 50mg/kg body weight of sodium phenobarbital through intra-peritoneal injection [9]. Intra-cardiac perfusion was carried out through the heart apex with 2.5% glutaraldehyde in 0.1 mol/ liter cacodylate buffer (pH 7.3) for 5 min for partial fixation of pancreas. A midline upper abdominal incision was performed and dissection of pancreas was carried out. The specimens were then processed for light microscopic study.

Light microscopic study:
The pancreas of each rat was carefully dissected for light microscopic preparations.

1) Haematoxylin and Eosin stain: [10].
2) Mallory’s trichrome stain: [10].

III. RESULTS

- **H and E results :**
The sub groups (a, b) of control group showed nearly similar results; so, only histological results of the sub group (a) were presented.

Examination of H& E stained sections of the control group rats' pancreas showed that the lobules were formed of serous acini. The serous acini were lined by pyramidal secretory acinar cells; these cells were characterized by basal rounded nuclei, apical acidophilic and basal basophilic cytoplasm (Fig. A&B).Islets of Langerhans appeared as pale stained areas in between the serous acini. Their cells were characterized by pale nuclei and pale cytoplasm. Blood capillaries were observed in between the islets' cells (Fig. B).
Fig. (A): A photomicrograph of a control rat's pancreas showing its general architecture. Serous acini (a) are lined by pyramidal cells with basal rounded nuclei (N) and apical acidophilic (star) cytoplasm. Thin connective tissue septa (thin arrows), duct (d) and blood vessels (bv) are seen. (H&EX200)

Fig. (B): A photomicrograph of a control rat's pancreas showing its general architecture. Serous acini (a) are lined by pyramidal cells with basal rounded nuclei (N) and apical acidophilic (star) cytoplasm. An islet of Langerhans appears as a group of cells with small pale nuclei (thick arrows). Blood capillaries are observed (zigzag arrows). (H&EX200)

Examination of H & E stained sections of the pancreas of ischemic group showed thickening of the connective tissue septa, thick walled congested blood vessels (Fig. C). In addition, the acini appeared distorted, vacuolated and characterized by dark basal nuclei (Fig. D). The cells of islets of Langerhans appeared with dark nuclei. Dilated congested blood capillaries were seen in-between the islets' cells (Fig.E).
Fig. (C): A photomicrograph of an ischemic rat's pancreas showing acini (a), branches of intralobular ducts (d), congested blood vessels (bv) and thickened connective tissue septa in between (arrows). (H&E X200)

Fig. (D): A photomicrograph of an ischemic rat's pancreas showing: some cells with pyknotic nuclei (arrow heads) and vacuolated cytoplasm (curved arrows). Dilated blood vessel (bv) and wide septa (arrow) are seen (H&E X400).
**Fig. (E):** A photomicrograph of an ischemic rat’s pancreas showing cells of islet of Langerhans appears with dark nuclei (arrow heads). Blood capillaries are congested and dilated (zigzag arrows). (H&E X400)

- **Mallory's trichrome stain results:**

Examination of **Mallory's trichrome** stained sections of **control** rats’ pancreas of the control group showed scanty collagen fibers around the blood vessel (**Fig. F**).

**Fig. (F):** A photomicrograph of control rat's pancreas showing scanty o collagen fibers (arrow) around blood vessel (Mallory trichrome x400)

Examination of **Mallory's trichrome** stained sections of **ischemic group** rats’ pancreas of the showed extensive collagen fibers (arrows) around blood vessel(bv) (**Fig. G**).
Fig. (G): A photomicrograph of an ischemic rat's pancreas showing marked deposition of collagen fibers (arrows) around blood vessel (bv) (Mallory trichrome x400).

IV. DISCUSSION

Distant organ effects of acute kidney injury (AKI) are evident. Progression to multiple organ failure following AKI is the leading cause of morbidity and mortality; it is the end point of remote effects of IRI. Several studies have demonstrated that injured kidneys affect different remote organs. However, remote effects of renal ischemia reperfusion injury on the pancreas remain unclear [8&11].

In the current work, examination of H&E stained sections of ischemic rats’ pancreas showed vacuolated cytoplasm of both acinar and islets cells with darkly stained nuclei which was in agreement with Soliman et al., (2014) [12]. Hypoxia occurring secondary to tissue ischemia leads to electron transport chain dysfunction and decrease in ATP production resulting in failure of sodium and calcium pumps on the cell surface. Finally sodium retention inside the cells leads to hyperosmolarity and subsequent water influx to the cell causing their distention appearance of vacuolations [13&14].

The H&E stained sections of ischemic rats’ pancreas showed dilated congested blood vessels and inflammatory cells infiltration, similar findings were described previously by Zhou et al., (2002) [15]. Mast cells secrete newly synthesized lipid mediators derived from metabolism of arachidonic acid. Mast cell degranulation and mediator release contribute to the inflammatory response, eliciting vascular fluid leakage and resulting edema and recruitment of leukocytes [16&17]. They also reported that numerous factors associated with ischemia reperfusion including superoxide, complement components, calcitonin gene-related peptide, platelet activating factor, leukotriene B4, and bacterial toxins have been shown to activate mast cells. Upon activation, mast cells degranulate, releasing mainly pro-inflammatory mediators, including monoamines such as histamine and serotonin, cytokines such as TNFα, and proteases [16&17].

Also excess nitric oxide production due to increased Nitric oxide synthase activity resulted in high tissue levels of nitric oxide that had a direct pancreatic acinar cells toxic effect as observed in our work in the form of vacuolated cytoplasm and dark nuclei and vascular congestion [18].

Examination of Mallory's trichrome stained sections of ischemia reperfusion group showed increased amount of collagen around blood vessels. Similar observations were reported [12]. Ischemia reperfusion induced cytokines activation which has the ability to activate pancreatic stellate cells transforming them into myofibroblast like cells [19]. Activating factors of PSCs such as platelet derived growth factor, Interleukin-1 and angiotensin II induce fibrosis by induction of pancreatic stellate cells to synthesize and secrete extracellular matrix proteins [20&21].
our result revealed the histological alteration occurs in the pancreas caused by renal ischemia reperfusion injury.

Conflict of Interest statement: The authors declare that there are no conflicts of interest.

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REFERENCES: