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Study of Histopathological Changes in Kidney of Male Wistar Rats after Contrast Induction

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Abstract

Use of iodinated contrasts in day to day clinical practice is increased in diagnosis and treatment. Its impact on the kidney has not studied extensively. As per the previous literature reviews, use of iodinated contrast has a impact on kidney leading to AKI (acute Kidney Injury). Current research is under take to fill the lacunae of histopathological changes after inducing the contrast. 36 male wister rats used in the study to evaluate the impact of contrast on histopathological changes. Animals were grouped in to 6 groups including control group. Animals were euthanized at 3 hours, 6 hours, 12 hours, 24 hours, 48 hours of contrast induction and kidney were dissected out for histopathological studies. The light microscopic finding in the kidney of male wistar rats has shown normal appearance of the kidney in control animals. But contrast exposed kidneys of group 1 (3 hours), group 2 (6 hours) and group 3 (12 hours) has showed remarkable changes such as decreased bowmen's capsular space, infiltration of lymphocytes, mild to moderate inflammation with infiltration of inflammatory cells particularly lymphocytes noticed in renal pelvis region of the kidney, mild epithelial cell hyperplasia noticed in renal pelvis region. Tissue necrosis and acute tubular necrosis was also observed. The present histopathological study confirmed significant changes in tubular vacuolar transformation, interstitial edema and tubular degeneration, infiltration of lymphocytes, hyperplasia at real pelvis region when Contrast media (CM) are used frequently in various diagnostic procedures.

Key words: Acute Kidney Injury (AKI), Contrast Media (CM), Contrast induced nephropathy (CIN).

Introduction

Contrast induced acute kidney injury (AKI) is defined as an abrupt or rapid decline in renal filtration function of the kidney on contrast exposure. Glomerular pressure depends primarily on renal blood flow and combined resistances of renal afferent and efferent arterioles control the glomerular pressure. Reduction in RBF represents a common pathologic pathway for decreasing glomerular filtration rate (GFR) regardless of the cause of AKI. The etiology of AKI consists of 3 main mechanisms namely prerenal, intrinsic (renal) and obstructive. In prerenal failure GFR is decreased but tubular and glomerular function remains normal. Intrinsic renal failure includes diseases of the kidney itself which predominantly affects the glomerulus and renal tubules which are associated with the release of renal afferent vasoconstrictors.

Ischemic renal injury is the most common cause of intrinsic renal failure. Renal vasoconstriction figures prominently in the proposed pathogenesis of CIN based on animal experiments. Depressed RBF eventually leads to ischemia and cell death. This may happen before frank systemic hypotension is present and is referred to as normotensive ischemic AKI. The initial ischemic insult triggers a cascade of events, including production of oxygen free radicals, cytokines and enzymes, endothelial activation and leukocyte adhesion, activation of coagulation and initiation of apoptosis. These events continue to cause cell injury even after restoration of RBF. Tubular cellular damage results in disruption of tight junctions between cells, allowing back leak of glomerular filtrate and further depressing effective GFR. In addition, dying cells slough off into the tubules, forming obstructing casts, which further decrease GFR and lead to oliguria. During this period of decreased renal blood flow (RBF), the kidneys are particularly vulnerable to further insults; this is when iatrogenic renal injury is most common. Substance such as radio contrasts, aminoglycosides, ACE inhibitors and NSAID causes renal insult. The mechanisms underlying contrast media nephrotoxicity has not been fully studied, may be due to several factors, including renal ischemia, particularly in the renal medulla, the formation of reactive oxygen species (ROS), reduction of nitric oxide (NO) production and tubular epithelial and vascular endothelial injury. The current study is focused on histopathological changes of the kidney when exposed to contrast media in male wistar rats.

Materials and methods

Experimental protocol

The experimental protocol was approved by Institutional Ethical Committee and met the guidelines of CPCSEA, Ministry of Social Justice and Empowerment, Government of India. (Approval No SU/BRULAC/ RD/001/2014). 36 male adult Wistar rats' were acquired and maintained at Department of Research and Development, Saveetha University, Chennai, throughout the duration of experimental protocol. A fixed dose of 0.6 mL of contrast (IOHEXOL) was given intraperitoneally to 30 animals and grouped them into 3 hours, 6 hours, 12 hours, 24 hours and 48 hours and grouped in ascending order from 1 to 5 and a control group which has not received any contrast. Animals were anesthetised under Isoflurane USP (inhalation anesthesia) and were euthanized to collect the kidney after the contrast exposure at different time intervals as per the research protocol. Harvested tissue was stored in 10 % formalin solution for further investigations.

Method of preparation of histology slides of kidney

The collected soft tissue kidney was stored in 10 % formalin (containing 0.03 M NaH_2PO_4 and 0.045 M $Na_2H_2PO_4$). Tissue was dehydrated by using different grades of alcohol, cleared with xylene and embedded in paraffin wax. The processed tissue was sectioned by rotary microtome section was dewaxed, hydrated and stained with hematoxylin (2.5 gm hematoxylin, 50 gm potash alum, 0.5 gm sodium iodate and 20 mL of absolute alcohol in 500 mL of water) for 10 minutes. Stained tissue washed with water differentiated acid alcohol (10 % HCL in 70 % alcohol), stained in 1 % eosin and washed dehydrated and mounted.

Results

After harvesting the renal tissue, the kidney were fixed immediately in 10 % formalin, embedded in paraffin, sectioned at 3 µm thickness and the sections were stained with hematoxylin and eosin. The extents of tubular injury, dilatation, vacuolation, and necrosis were evaluated quantitatively with the method (Raij et al, 1984). Briefly, the extent of tissue damage was graded from 0–4 according to the severity of tubular necrosis, tubular vacuolation and tubular dilatation.

The scoring system followed was 0 = no change in the tubules, 1 to tubular injury (mild), 2 to 50 % of tubular involvement (moderate), 3 = 50 % to 75 % of tubules showing characteristic change (severe), and 4 for tubular damage (very severe). Fifty fields were counted from each slide. All the assessments were done in a blinded fashion.

The light microscopic finding in the kidney of male wistar rats has shown normal appearance of the kidney. Glomerulus and tubular region appear to be normal. There is no evidence of cast, vacuolation, necrosis and acute tubular injury was observed in control group of rats which were not induced with the contrast (Figure 1). But in group 1 (3 hours), group 2 (6 hours) and group 3 (12 hours) of contrast exposed kidney has showed remarkable changes, decreased bowmen's capsular space, infiltration of lymphocytes, mild to moderate inflammation with infiltration of inflammatory cells particularly lymphocytes noticed in renal pelvis region of the kidney, mild epithelial cell hyperplasia noticed in renal pelvis region. Tissue necrosis and acute tubular necrosis was also observed (Figure 1). Group 4 (24 hours) and group 5 (48 hours) has not shown any histological changes, kidney appears to be normal even after the contrast exposure.

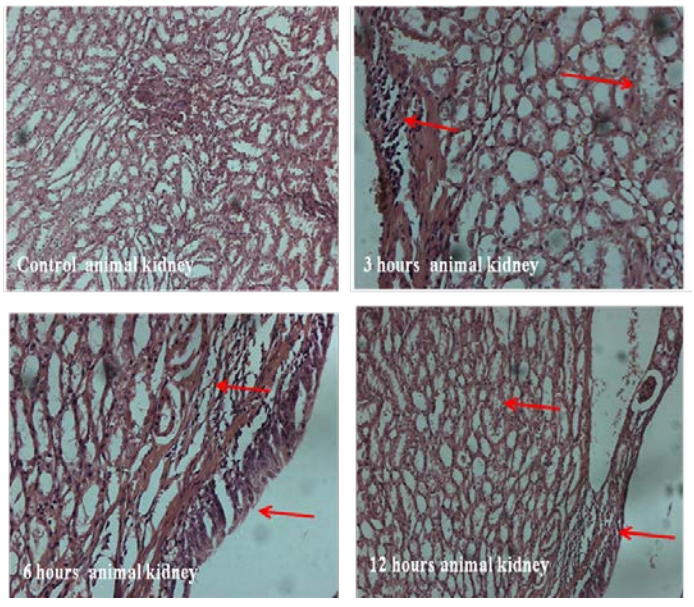

Fig 1- Red arrows indicate the site of lesion. Hours indicate after the induction of contrast

Discussion

The present histopathological study confirmed significant changes in tubular vacuolar transformation, interstitial edema and tubular degeneration, infiltration of lymphocytes, hyperplasia at real pelvis region. Contrast media (CM) are frequently used for various diagnostic procedures including X-rays, computed tomography and magnetic resonance imaging and angiographic procedures. CM induced nephropathy (CIN) or AKI is a leading cause of acute kidney injury and is associated with significant mortality and morbidity [1]. Histological alterations including proximal tubular vacuolar transformation, interstitial edema, and tubular degeneration following contrast administration have also been reported earlier [2]. The mechanism of CM induced renal impairment at cellular level is not fully clear, recent reports suggested both direct and indirect effects on renal tubules including biochemical and hemodynamic disturbance due to CM induced prerenal dehydration, hypotension and medullary ischemia are the probable causes [3,4]. Contrast media caused impairment of renal perfusion leading to hypoxic conditions resulting in acute tubular necrosis [5]. It has been reported that patients with diabetes and preexisting renal insufficiency may be at a higher risk of CM induced nephrotoxicity compared to patients with normal renal function [6]. The pathogenesis of CM induced nephropathy is poorly understood, however, numerous pathways have been suggested to participate in the process of renal tissue injury. While some studies support the direct action of CM on tubular toxicity and other investigators suggested that the final pathway for contrast induced renal injury might be due to medullary hypoxia, conditioned by medullary ischemia affecting metabolically active and hyperfiltering nephrons. CM infusion induces renal vasoconstriction [1]. An imbalance between endothelium derived vasoconstrictive and vasodilatory

factors [8,9]. Depending on the severity of CM induced vasoconstriction, ischemic tubular injury may result in cell detachment, apoptosis or necrosis, the ischemic insult is often accompanied by reperfusion injury involving excessive generation of potentially toxic free radicals and inflammatory mediators [9]. In another study a significant increase in TBARS and decrease in GSH in kidneys of rats exposed to CM, indicated a state of oxidative stress [10]. Present study has found similar histopathological changes in contrast exposed male wistar rats which supports the earlier studies.

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