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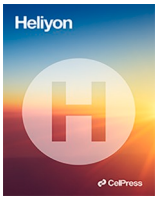

Research article

Does cardiac impairment develop in ischemic renal surgery in rats depending on the reperfusion time?

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ABSTRACT

Background: Renal dysfunction is known to cause heart failure. However, renal dysfunction associated with kidney surgeries (mediated by reperfusion injury) that affects the cardiac physiological function, especially during the recovery and repair phase of renal surgery is unknown.
Method: Male Wistar rats (238 ± 18 g) were subjected to renal sham and ischemia-reperfusion (IR-bilateral clamping for 15 min/45 min and reperfusion for 24 h/48 h/7 days) surgeries. At the end of the experiment, the heart was isolated from the animal (to exclude neurohormonal influence) and perfused for 60 min with Krebs-Hanseleit buffer to study the physiological changes.
Result: Renal artery bilateral occlusion for 45 min that creates ischemia, followed by 24 h of reperfusion did not impart any significant cardiac physiological functional decline but 48 h of reperfusion exhibited a significant decline in cardiac hemodynamic indices (Rate pressure product in $x10^4$ mmHg*beats/min: Sham- 3.53 ± 0.19, I45_R48–2.82 ± 0.21) with mild tissue injury. However, 7 days of reperfusion inflict significant physiological decline (Rate pressure product in $x10^4$ mmHg*beats/min - 2.5 ± 0.14) and tissue injury (Injury score- 4 ± 1.5) in isolated rat hearts. Interestingly, when the renal artery bilateral occlusion time was reduced to 15 min the changes in the hearts were negligible after 7 days. Cellular level exploration reveals a positive relation between functional deterioration of mitochondria and elevated mitochondrial oxidative stress and inflammation with cardiac physiological decline and injury linked with renal ischemia-reperfusion surgery.
Conclusion: Cardiac functional decline associated with renal surgery is manifested during renal repair or recovery. This decline depends on cardiac mitochondrial health, which is negatively influenced by the renal IR mediators and kidney function.

1. Introduction

Each year, more than 300 million surgical procedures are performed worldwide in which approximately 1 % of these surgeries are in the kidney [1]. Acute kidney injury (AKI) is a predominant renal problem often treated by surgical and non-surgical methods where the outcome of surgical procedures negatively influences the other organs including the brain, lung, heart and liver [2,3]. Many studies have reported the complex interaction between the heart and kidney in various pathological conditions, which often leads to

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cardiorenal syndrome (CRS), which can manifest in dual organ dysfunction [4–6] [4–6] [4–6]. Incidentally, cardiac events such as heart attacks, heart failure and heart rhythm disorders were reported in association with different renal surgery [6,7].

Renal ischemia-reperfusion injury (IRI) is a common unavoidable clinical complication that develops during surgical procedures such as kidney transplantation or aortic surgery and is associated with a high incidence of AKI and chronic kidney disease (CKD) [8,9]. It is characterized by transient obstruction of blood flow to the kidneys, with inadequate local blood supply [8,10]. Even though the focus of such surgical interventions is primarily aimed to restore renal function, recent evidence suggests that these procedures may have broader implications on distant organs, particularly the heart (CRS type 3) [4,6]. However, the limited information available in this direction indicates that cardiac patients who experienced AKI are two times more likely to get admitted to the hospital for heart failure within 1.4 years. According to Kuhar et al., 48 h after renal IRI, functional changes in the heart occur including left ventricular dilation, increased ventricular diameters, prolonged relaxation time and decreased fractional shortening. Notably, these cardiac modifications are specific to IRI and are not observed in bilateral nephrectomy [11]. Echocardiography conducted on rats with renal failure indicates impaired cardiac function 48 h after renal ischemia, even in the absence of differences in systolic blood pressure, hematocrit, or body weight between the sham surgery and renal ischemia groups [12]. Additionally, a prior study by Caio-Silva et al. on mice induced with renal IRI revealed oxidative stress in the heart eight days after kidney injury induction [13]. Hence, understanding the temporal pattern of distinct cardiac effects due to renal surgery is important and it can aid early identification of complications, recovery or repair process of renal tissues after surgery.

The mechanisms underlying the distant cardiac effects of renal IRI remain to be fully elucidated, but they are likely to involve a complex interplay of systemic inflammation, neurohormonal activation, mitochondrial dysfunction, oxidative stress, and endothelial dysfunction [14–16] [14–16] [14–16]. Among these pathological mediators, mitochondrial dysfunction is generally overlooked despite its potential to be involved in the crosstalk between the pathologies and coordinate them all due to the difficulty in mitochondrial health assessment as compared to other mentioned parameters [17,18]. A recent proteomic study in CRS rats provides evidence for the altered cardiomyocyte metabolisms like myocardial pyruvate metabolism, glyoxylate and dicarboxylic acid metabolism, starch and sucrose metabolism, and amino acid biosynthesis and dysregulation of cardiac signalling pathways and mitochondrial bioenergetic function [18]. Cardiac pathological trigger from renal surgery executed by cardiac mitochondrial dysfunction is believed to be the consequential effect of systemic inflammation, neurohormonal activation and endothelial dysfunction [17,18].

Understanding the temporal patterns of distant cardiac effects of renal IRI is of utmost clinical importance, to managing post-renal surgical patients from postoperative complications, rehospitalization and effective rehabilitation procedures [19]. Monitoring the heart of renal surgical patients enables to implementation of various strategies to reduce cardiac-associated mortality and morbidity. Thus, in the present study, we explored renal ischemic impact on the heart in various reperfusion time durations. Moreover, the present study uncovers the relationship of renal IR physiological recovery on cardiac physiology, injury and cellular mediators.

2. Material methods

2.1. Animals

Male Wistar rats weighing 238 ± 18 g were obtained from the central animal facility of SASTRA University. Prior to the experiments, animals were housed in the animal unit for at least one week under standard conditions of temperature (22 ± 1 °C), humidity (55 ± 5 %), and 12 h/12 h light/dark cycles with free access to food and water. All animal experiments were conducted with prior approval from the institutional animal ethical committee (IAEC) and following the Committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines (546/SASTRA/IAEC/RPP).

2.2. In vivo renal ischemia-reperfusion

All animal experiments adhere to the ARRIVE (Animal research: reporting of in vivo experiments) guidelines. Prior to the surgical procedure, rats were placed in an anaesthesia induction chamber and induced with 3–4% isoflurane (Matrx, VIP 3000®, United States) in 100 % oxygen at a rate of 1.5 L/min until reflex loss. Once induction of anaesthesia was complete, the animal was transferred to a heating pad (Vet Tech, United Kingdom) thermostatically regulated at 37.5 ± 1 °C, and anaesthesia was maintained at 2 % through the nasal cone. The renal tissue and vascular pedicles were exposed via a dorsal approach. Using non-traumatic mini-bulldogs, both renal pedicles were occluded in animals undergoing clamping. After the ischemic period, the kidney is perfused and the restraints are removed to achieve reperfusion. After surgery, the animals were recuperated and given postoperative care. Prior to suturing, saline was infused into the peritoneal cavity to counteract fluid loss. To prevent sepsis, povidone-iodine and neosporin were administered to the sutured area, and meloxicam (1 mg/kg) was injected subcutaneously to alleviate pain [20].

Monitoring Anaesthesia: We confirmed the animal is adequately anaesthetized before beginning surgery by observing the pedal withdrawal reflex (pinching the foot pads on both hind feet). If the foot pad pinch causes a response, we supplied additional anaesthesia and re-tested before starting the procedure, to make sure the induction of anaesthesia. There should also be a lack of eye blink reflex. During anaesthesia, we monitored the respiratory rate and depth, by visualizing the slow regular rhythm of up and down movement of the chest and adequate oxygenation by observing the pink mucous membrane colour. During the entire duration of the surgical procedure we regularly rechecked the anaesthetic depth. If the animal exhibits an increase in respiration rate, is responsive to painful stimuli, or, on the other hand, has a lack of colour of the ears, tail, gums, or foot pads, we adjusted the anaesthesia accordingly [21].

In this study, a total of 36 rats were used rats were randomized into 3 major groups:

I) Sham-operated (S): Surgical procedures were adopted except for the occlusion of renal arteries. After the sham procedure, the animals were sacrificed after 7 days (n = 6).
II) Ischemic group (I_45): Bilateral renal clamping was performed for 45 min followed by removal of tissues (n = 6) without any reperfusion.
III) Ischemia-reperfusion (IR): Bilateral renal clamping was done to begin ischemia (15 min/45 min) and maintained for a particular reperfusion time (24 h/48 h/7 days), followed by removal of clamps and recovery of the animal. Based on the time of ischemia and reperfusion there were 4 subgroups
- I15_R7- After the renal ischemic induction for 15 min, the kidneys were reperfused for 7 days (n = 6)
- I45_R24- After the renal ischemic induction for 45 min, the kidneys were reperfused for 24 h (n = 6)
- I45_R48- After the renal ischemic induction for 45 min, the kidneys were reperfused for 48 h (n = 6)
- I45_R7- After the renal ischemic induction for 45 min, the kidneys were reperfused for 7 days (n = 6)

The blood samples were collected in tripotassium ethylenediaminetetraacetic acid (K3EDTA) tubes from all rats before the surgery and on the day of the necropsy. Plasma was separated by centrifuging the tubes at 3000 rotations per minute (rpm) for 10 min and stored at −80 °C for further analysis. Plasma levels of creatinine and blood urea nitrogen (BUN) were estimated at the end of reperfusion using the kit method according to the manufacturer's instructions (Agape, India). Kidneys from each group were preserved in formalin (10 % v/v), embedded in paraffin, and sectioned at 5 μm for histopathological examination with hematoxylin/eosin. The following pathologies were used to evaluate the pathomorphological lesions under a microscope based on the EGT (Endothelium, Glomeruli, Tubular) scoring system: glomerular congestion, tubular dilatation, tubular degeneration, tubular necrosis, and the presence of eosinophilic casts. Based on the severity, the changes were rated on a 4-point scale (0–nil/absent, 1-mild, 2-moderate, 3-marked, 4-severe), and the average score is provided [22].

2.3. Estimated glomerular filtration rate (eGFR)

Animals used for urine collection were housed in metabolic cages. The animals had free access to water during the entire experiment, which could last up to 24 h/48 h/7 days post-surgery. The urine was collected every 24 h after the surgery to determine the urine volume. Creatinine and blood urea nitrogen were measured in both plasma and urine.

eGFR calculation done using the equation by Pestel et al. (2007) [23]:

- Creatinine clearance = (1000 * urine volume * concentration of creatinine in urine)/concentration of creatinine in serum
- BUN clearance = (urine volume * concentration of BUN in urine)/concentration of BUN in serum
- eGFR = mean of (creatinine clearance, BUN clearance)

2.4. Ex vivo isolated rat heart perfusion

At the end of renal procedures in groups I, II and III, the rat hearts from all the groups were excised after anaesthetization of the animal with sodium thiopentone (60 mg/kg of body weight). The excised hearts were then mounted onto Langendorff apparatus maintained at 37 °C and perfused continuously with Krebs-Hensleit (KH) buffer (118.5 mM NaCl, 5.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 11 mM glucose, 25 mM $NaHCO_3$, pH-7.4) equilibrated with carbogen (95 % O2 + 5 % CO2) for 120 min. Throughout the experiment, heart rate (HR), left ventricular developed pressure (LVDP), rate pressure product (RPP) and the force of contraction (dp/dt) were recorded and calculated using LabChart physiological data analysis software (AD Instruments Inc, Sydney, Australia). Perfusates were collected during the end of the reperfusion and used for further biochemical analysis. At the end of the experiment, the hearts were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis [24].

2.5. Cardiac injury parameters

Utilizing the enzymatic activity of the injury marker enzymes lactate dehydrogenase (LDH) and creatine kinase (CK), the extent of cardiac injury was determined. The enzymatic activity of these enzymes was measured in perfusate and tissue samples from the different experimental groups, as described previously. By measuring the conversion of lactate to pyruvate and observing the absorbance of nicotinamide adenine dinucleotide (NADH) at 340 nm, the LDH activity was determined. The concentration of inorganic phosphate, which is liberated during ATP conversion, was measured with a reagent and calculated at 660 nm in order to ascertain CK activity [24].

2.6. Histopathology

The isolated hearts were cleansed of extraneous tissue, weighed, and rinsed with ice-cold normal saline solution. A heart section was fixed with 10 % formalin (v/v), embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for histopathological examination under a light microscope. The following pathologies were used to evaluate the pathomorphological lesions under a microscope-congestion, haemorrhage, mononuclear infiltration, wavy fibres, myocardial oedema and disarray of cardiomyocytes. Based on the severity, the changes were rated on a 4-point scale (0–nil/absent, 1-mild, 2-moderate, 3-marked, 4-severe), and the average score is provided [24].

2.7. Isolation of mitochondria from peripheral blood mononuclear cells (PBMC)

Blood mitochondria were isolated from PBMC using a modified version of the previous method [25]. Blood was diluted with PBS (1:4) and layered on density gradient separation media (HiSepTM-LSM-1077, Himedia). To isolate mononuclear cells from RBC and plasma, the tubes were centrifuged at 2000 rpm for 20 min at 10 °C without brakes. Plasma was promptly utilized for RNA isolation and ATP measurement. PBS was used to collect and cleanse the PBMC fraction. The solution was centrifuged at 5000 rpm for 5 min at 4 °C to pellet the PBMC, and the pellet was stored at −80 °C after the supernatant was discarded. Frozen 200 μL of isolation buffer [IB-0.25 M sucrose and 10 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH = 7.5] was added to the leukocyte particle. After the addition of IB, five cycles of repeated freeze-thaw were performed to separate the mitochondria from the cells, and the mixture was centrifuged at 1000 g for 10 min at 4 °C to separate shattered cells. A mitochondrial particle was obtained by separating and centrifuging the supernatant containing mitochondria at 20,000 g.

In the isolated blood mitochondria The analysis of mitochondrial complex-I was done as per the previously described procedure [26]. Briefly, the mitochondrial pellet was lysed by giving an osmotic shock in a hypotonic medium (25 mM K_2PO_4, 5 mM $MgCl_2$ pH 7.2). To 25 μg of mitochondrial protein, 200 μL of reaction buffer (50 mM Tris, 0.8 mM NADH, 240 μM KCN, and 4 μM antimycin A pH = 8.0) was added. The reaction was initiated with the addition of 50 μM decylubiquinone. For 5 min the reaction was monitored at 340 nm using a spectrophotometer with the temperature maintained at 37 °C (Synergy H1, BioTek, USA). The rotenone-insensitive activity was estimated after the addition of 4 μM rotenone and the monitoring was continued for an additional 3 min. The enzyme activity was expressed as nM/min/mg protein.

Mitochondrial NADH-ubiquinone oxidoreductase chain-1 (ND1) expression by quantitative polymerase chain reaction (qPCR).

Plasma total RNA was isolated using TRIzol™ reagent according to the kit's instructions (15596026, Thermo Scientific, USA). Using the Verso cDNA synthesis reagent (#AB1453AThermo Fischer Scientific, USA), the cDNA conversion was carried out. The mitochondrial ND1 primers were designed to amplify the gene of interest using SYBR green chemistry. The polymerase chain reaction (PCR) analysis was done in real-time using the Applied Biosystems™ 7500 with 6-carboxyl-X-Rhodamine (ROX) reference dye. The expression was normalized with β actin as a control. The gene expression was calculated by the method of Livak and Schmittgen [27].

2.8. ATP measurement in blood plasma

The separated plasma was immediately treated with a lysis buffer containing an inhibitor of endogenous ATPase and shaken for 10 min. Substrate buffer 100 μL was then added, and incubated in the dark for 10 min and the luminescence intensity was recorded as per the kit instructions (ATP lite, PerkinElmer, USA) using Synergy H1 multimode reader (BioTek, USA). The standard curve for ATP was drawn from 10 μM to below for an estimation of the ATP level in the sample [28].

Table 1
Primer details.

PRIMER	SEQUENCE (5′ to 3′)
TNF- α –F	CGCTCTTCTGTCTACTGAAC
TNF- α –R	TTCTCCAGCTGGAAGACTCC
IL-6 –F	CACTTCACAAGTCGGAGGCT
IL-6 –R	AGCACACTAGGTTTGCCGAG
Pgc 1α –F	GAGGGACGAATACCGCAGAG
Pgc 1α –R	CTCTCAGTTCTGTCCGCGTT
Dnm1-F	TTGCCCTCTTCAACACTGAGC
Dnm1-R	ATGAAGCTGTCAGAGCCGTT
Parkin-F	AGTTTGTCCACGACGCTCAA
Parkin-R	CAGAAAACGAACCCACAGCC
Mfn1-F	TGACTTGGACTACTCGTGCG
Mfn1-R	GGCACAGTCGAGCAAAAGTG
Mfn2-F	CTCTGTGCTGGTTGACGAGT
Mfn2-R	TCGAGGGACCAGCATGTCTA
Mff-F	GAAAACACCTCCACGTGTGC
Mff-R	CTGCTCGGATCTCTTCGCTT
Fis 1-F	CCAGAGATGAAGCTGCAAGGA
Fis 1-R	TTCCTTGAGCCGGTAGTTGC
Pink-F	TGTATGAAGCCACCATGCCC
Pink-R	TCTGCTCCCTTTGAGACGAC
Optn-F	GGGTTTCCCAGAACCGACTT
Optn-R	AAGGTCCGCTTTCTCAAGCC
Tfam-F	GTTGCTGTCGCTTGTGAGTG
Tfam-R	GTCTTTGAGTCCCCCATCCC
Polg –F	CTTTGGGCTCCAGCTTGACT
Polg –R	TGGAGAAAATGCTTGGCACG
β-actin-F	GTGTGGTCAGCCCTGTAGTT
β-actin-R	CCTAGAAGCATTTGCGGTGC
ND 1-F	CTCCCTATTCGGAGCCCTAC
ND 1-R	GGAGCTCGATTTGTTTCTGC

2.9. Isolation of mitochondria

Heart tissue mitochondria were isolated using the differential centrifugation technique as described previously [29]. The following analysis was performed on isolated mitochondrial function.

i) Activity of electron transport chain enzymes, namely rotenone-sensitive NADH-oxidoreductase (NQR), succinate decylubiquinone DCPIP reductase (SQR), ubiquinol cytochrome-c reductase (QCR), and cytochrome *c* oxidase (COX), analyzed spectrophotometrically according to the previously described method [29].
ii) ATP level and ATP producing capacity: The ATPlite (Perkin Elmer) apparatus was used to measure ATP levels in the isolated mitochondria of all groups using the luminescence produced by the reaction of ATP with the substrates luciferase and α-luciferin under non-energized conditions [29].

2.10. Inflammation

i) Estimation of the tissue level of tumour necrosis factor alpha (TNF- α) and interleukin-6 (IL-6) were performed in blood using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instruction (Krishgen biosystems, Mumbai).
ii) Gene expression: The expression for nuclear-encoded β actin, TNF- α and IL-6 were analyzed using qPCR (ABI7500, Thermo Scientific, USA) in rat heart samples. The DNA isolation is done using the phenol-chloroform-isoamyl alcohol method according to the manufacturer's instructions (Himedia, Mumbai). The mRNA extraction was carried out using TRIzol reagent (15596026, Thermo Scientific, USA) as per the instructions, and gene expression was quantified using Sybr green chemistry (F415, Thermo Scientific, USA). Gene expressions were estimated in cDNA and normalized with β actin in respective samples. The expression of genes was calculated as per the procedure of Livak and Schmittgen [27]. The primer sequence of the genes is presented in Table 1.

2.11. Reactive oxygen species (ROS) level measurement using dihydrodichlorofluorescein diacetate (DCFH/DA)

ROS levels in the heart were conducted as described before [30]. H_2 DCFH-DA, a non-polar compound that can rapidly react with ROS to produce the highly fluorescent dichlorofluorescein. Briefly, a concentration of 5 mg tissue/ml was achieved by diluting the tissue homogenate with ice-cold Locke's buffer. The reaction mixture (1 ml) comprising Locke's buffer (pH 7.4), 0.2 ml homogenate or mitochondria (0.5 mg protein), and 10 ml DCFH-DA (5 mM) was incubated at room temperature for 15 min. Using a spectrophotometer set with excitation at 484 nm and emission at 530 nm, the conversion of DCFH-DA to the fluorescent product DCF was determined 30 min after initial incubation [30].

2.12. Gene expression

The expression for the mitochondrial encoded Nd 1, and nuclear-encoded β actin, Tfam, Polg, Pgc 1α, Fis 1, Mff, Dnm 1, Mfn 1, Mfn 2, Opa 1, Pink 1, Parkin, and Optn was analyzed using qPCR (ABI7500, Thermo Scientific, USA) in rat heart samples. The primer details are presented in Table 1. Nd 1 expression was analyzed in both cellular DNA and cDNA whereas other gene expressions were estimated in cDNA and normalized with β actin in respective samples. The DNA isolation is done using the phenol-chloroform-isoamyl alcohol method according to the manufacturer's instructions (Himedia, Mumbai). The mRNA extraction was carried out using TRIzol reagent (15596026, Thermo Scientific, USA) as per the instructions, and gene expression was quantified using Sybr green chemistry (F415, Thermo Scientific, USA). The expression of genes was calculated as per the procedure of Livak and Schmittgen [27].

2.13. Statistical analysis

The data presented in this study are reported as the mean ± standard deviation of the mean (SD). Intergroup comparisons were carried out using a one-way analysis of variance (ANOVA), followed by post hoc Dunnet's test to assess the distinctions across the groups. A significance level of $p < 0.05$ was applied to define statistical significance. For the histopathological score, the intergroup comparisons were done based on Kruskal-Wallis analysis followed by post hoc Dunnet's test.

3. Results

3.1. Renal recovery assessment after 45 min of bilateral renal artery ligation

Renal physiological recovery after 45 min of bilateral renal ligation was studied in 24 h, 48 h and 7 days after post-surgical intervention and the results are given in Fig. 1. The calculated eGFR in rats after 24 h, 48 h and 7 days were 0.22 ± 0.01, 0.38 ± 0.05 and 0.41 ± 0.04 ml/min respectively and found to be significantly lower from the sham control animal (16.81 ± 2.1 ml/min). The sign of renal functional improvement was noted in 7 days after surgery. These findings were well supported by elevated BUN to creatinine levels in the plasma (S: 27.24 ± 2.44, I45_R24: 32.76 ± 4.12, I45_R48: 53.64 ± 3.15, I45_R7: 49.10 ± 2.98) for 24 h, 48 h and 7 days respectively.

Further analysis in the histology section of renal tissue demonstrated a higher injury score for 48 h tissue sample (14 ± 2) as compared to 24hr (11 ± 1.5) and 7 days (12 ± 1) samples obtained from the animal subjected to 45 min of bilateral renal artery

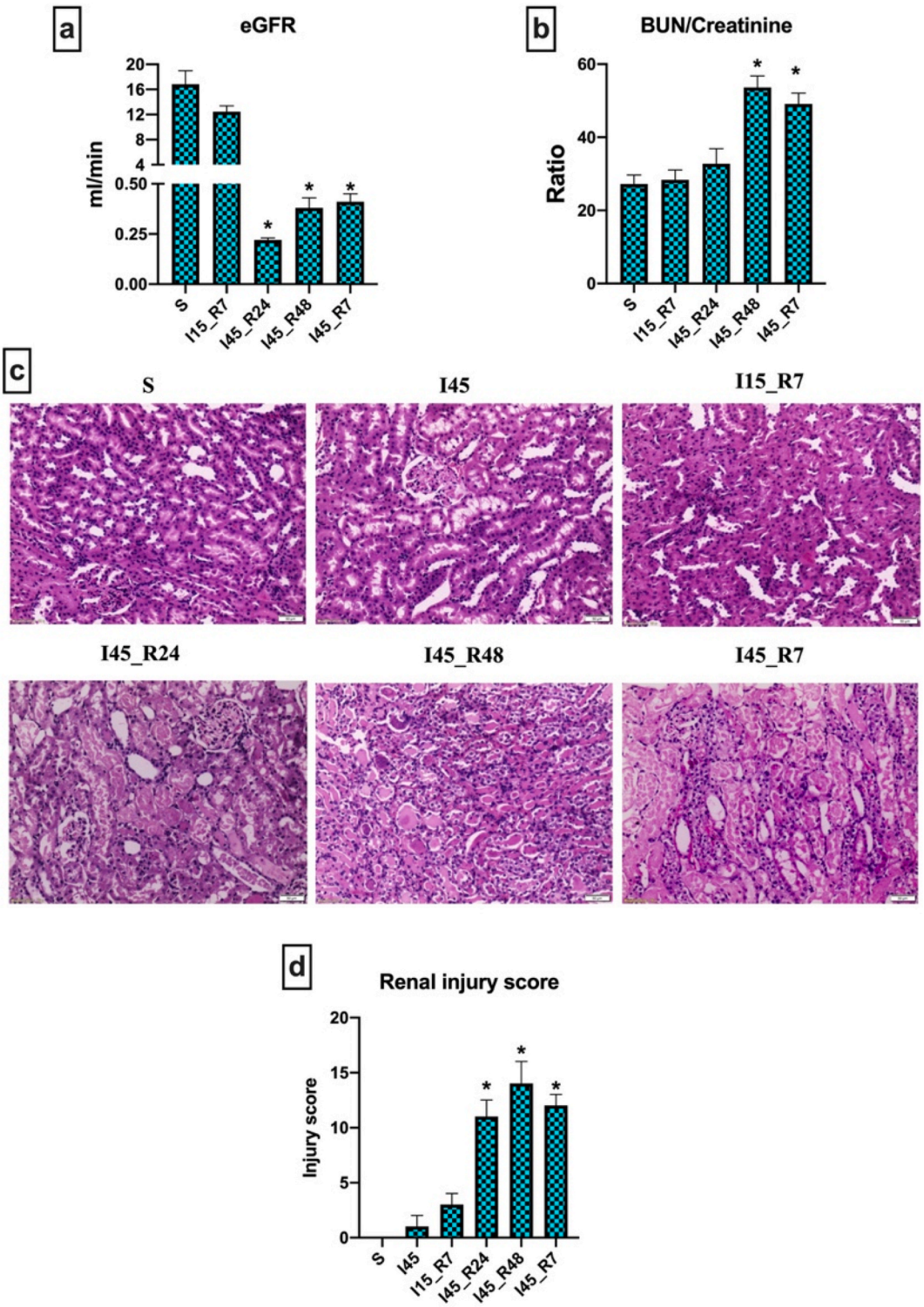

Fig. 1. Renal function and injury assessment in rats subjected to IR-a) eGFR, b) BUN/Creatinine ratio *p < 0.05 vs S. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. c) Representative H and E stained images (The images are presented at 10× magnification). d) Renal injury score. Intergroup comparison made by Kruskal-Wallis test. *p < 0.05 vs S. S- Sham, I-ischemia, R- Reperfusion.

ligation.

However, the animal with 15 min of bilateral renal artery ligation recovered the eGFR and corresponding plasma BUN/creatinine to near normal level by 7 days after surgery (eGFR- 12.46 ± 0.93 BUN/Cr- 28.37 ± 2.71).

3.2. Alterations in cardiac hemodynamic indices in response to renal ischemia and subsequent varying time of reperfusion

The hemodynamic parameters of isolated rat hearts from 24 h, 48 h and 7-day renal reperfused rats showed a substantial decrease in hemodynamic indices by I45_R7 and the results are given in Table 2. Decreased cardiac contractility (dp/dt), LVDP and RPP on the 7th day of rat heart after 45 min of renal artery ligation suggested 10 %, 10 %, and 16 % variation respectively from 48 h post renal IR heart and 21 %, 16 %, 33 % changes from 24 h post IR rat heart. On the other hand, 15 min of renal artery ligation did not alter any significant change in the hemodynamic parameters in 7 days post renal IR tissue from the sham control (Table 2).

3.3. Cardiac injury assessment following renal ischemia-reperfusion

Fig. 2 shows the injury score measured from the histopathological studies of the heart and the results suggested prominent injury only in post 7-day surgery sample of 45-min renal ischemia. The corresponding injury scores 2 ± 1, 3 ± 1 and 4 ± 1.5 were measured in groups I45_R24, I45_R48 and I45_R7 respectively. The cardiac injury marker measured in the coronary perfusate like CK and LDH showed a 30 % and 18 % increase in the I45_R7 sample from the sham where its corresponding tissue level activity declined.

3.4. Changes in blood biochemistry after renal ischemia-reperfusion injury

Fig. 3 shows the level of TNF-α, IL-6, ATP and mitochondrial copy number in the blood that showed a significant increase in 24 h, 48 h and 7 days after 45 min of ischemia.

TNF-α showed a 57 %, 60 %, and 50 % hike in its level from sham whereas IL-6 levels were increased by 57 %, 61 %, and 54 % in 24 h, 48 h and 7 days respectively after an ischemic period of 45 min (Fig. 3a and b). Mitochondrial copy number remained at a similar elevation in I45_R24, I45_R48 and I45_R7 from the sham (Fig. 3c). Similarly, ATP levels exhibited 22 %, 29 % and 33 % increase from the sham control in the respective groups (Fig. 3d). However, the corresponding activity of mitochondrial complex 1 in peripheral blood mononuclear cells (PBMCs) exhibited a significant decline in activity across all three groups compared to the sham group, indicating that the observed elevation in mitochondrial DNA (mtDNA) and ATP in the blood may be originated from an external source (Fig. 3e).

3.5. Cardiac tissue inflammation, oxidative stress and mitochondrial bioenergetic function following renal ischemia-reperfusion

Fig. 4 shows the results of TNF-α and IL-6, DCFH/DA level and Fig. 5 shows the activities of ETC enzymes in the cardiac tissue samples. Unlike ischemic tissue, reperfusion inflict increased DCFH/DA that measure ROS level (S: 1 ± 0.02, I45_R24: 1.65 ± 0.01, I45_R48: 1.79 ± 0.18, I45_R7: 2.18 ± 0.17), TNF-α (S: 1 ± 0.03, I45_R24: 2.26 ± 0.18, I45_R48: 2.67 ± 0.2, I45_R7: 2.65 ± 0.14) and IL-6 (S: 1 ± 0.02, I45_R24: 1.53 ± 0.14, I45_R48: 2.11 ± 0.02, I45_R7: 2.29 ± 0.04) gene expression in the myocardium, that persist even in post 7 days tissues (Fig. 4). Mitochondrial bioenergetic enzymes like NQR, SQR, QCR and COX along with ATP levels declined significantly by 7 days after 45 min of ischemia (23 %, 13 %, 13 %, 29 % and 28 % respectively from control) (Fig. 5a–e).

3.6. Mitochondrial quality control gene expression in myocardial tissue of rats subjected to renal ischemia-reperfusion

Mitochondrial quality was assessed by measuring the expressions of key genes involved in mitochondrial biogenesis, mitochondrial fission, mitochondrial fusion and mitophagy, and the results are given in Figs. 6–7. Mitochondrial copy number showed a significant decline of 13 % after 48 h and exhibited a 19 % decline after 7 days of reperfusion from the sham (Fig. 6a). The downregulation of the mitochondrial master regulatory gene, peroxisome proliferator-activated receptor-gamma coactivator (Pgc 1α) along with upregulation of mitochondrial translation regulator, transcription regulation factor A (Tfam) and replication regulator, mitochondrial DNA polymerase γ (Polg) was evident and the changes were significant in the tissue after 48 h and 7 days of reperfusion except Polg (Fig. 6b–d). A similar pattern of upregulation in dynamin-1 (Dnm 1) (1.5, 1.67 and 1.75-fold), mitochondrial fission protein 1 (Fis 1)

Table 2
Hemodynamic changes in the heart of rats subjected to renal IR.

	S	I45	I15_R7	I45_R24	I45_R48	I45_R7
LVDP (×10 mmHg)	12.54 ± 2.0	12.01 ± 1.6	12.18 ± 1.4	11.1 ± 1.7	10.28 ± 2.8	9.24 ± 1.3[a]
HR (beats/min)	281 ± 34	293 ± 35	282 ± 27	281 ± 25	248 ± 21[a]	236 ± 25[a]
RPP ($x10^4$ mmHg*beats/min)	3.51 ± 0.19	3.52 ± 0.23	3.39 ± 0.22	3.14 ± 0.12	2.52 ± 0.23[a]	2.1 ± 0.10[a]
(-dp/dt) (×10 mmHg)	95.32 ± 10.1	98.24 ± 7.3	90.24 ± 16	87.69 ± 7.9	76.21 ± 11.5[a]	70.5 ± 6.8[a]
(+dp/dt) (×10 mmHg)	132.9 ± 17.2	139.2 ± 12.5	121.81 ± 8.3	114.25 ± 10.4	99.6 ± 14.1[a]	103.66 ± 6.2[a]

[a] $p < 0.05$ vs S. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. S- Sham, I- ischemia, R- Reperfusion.

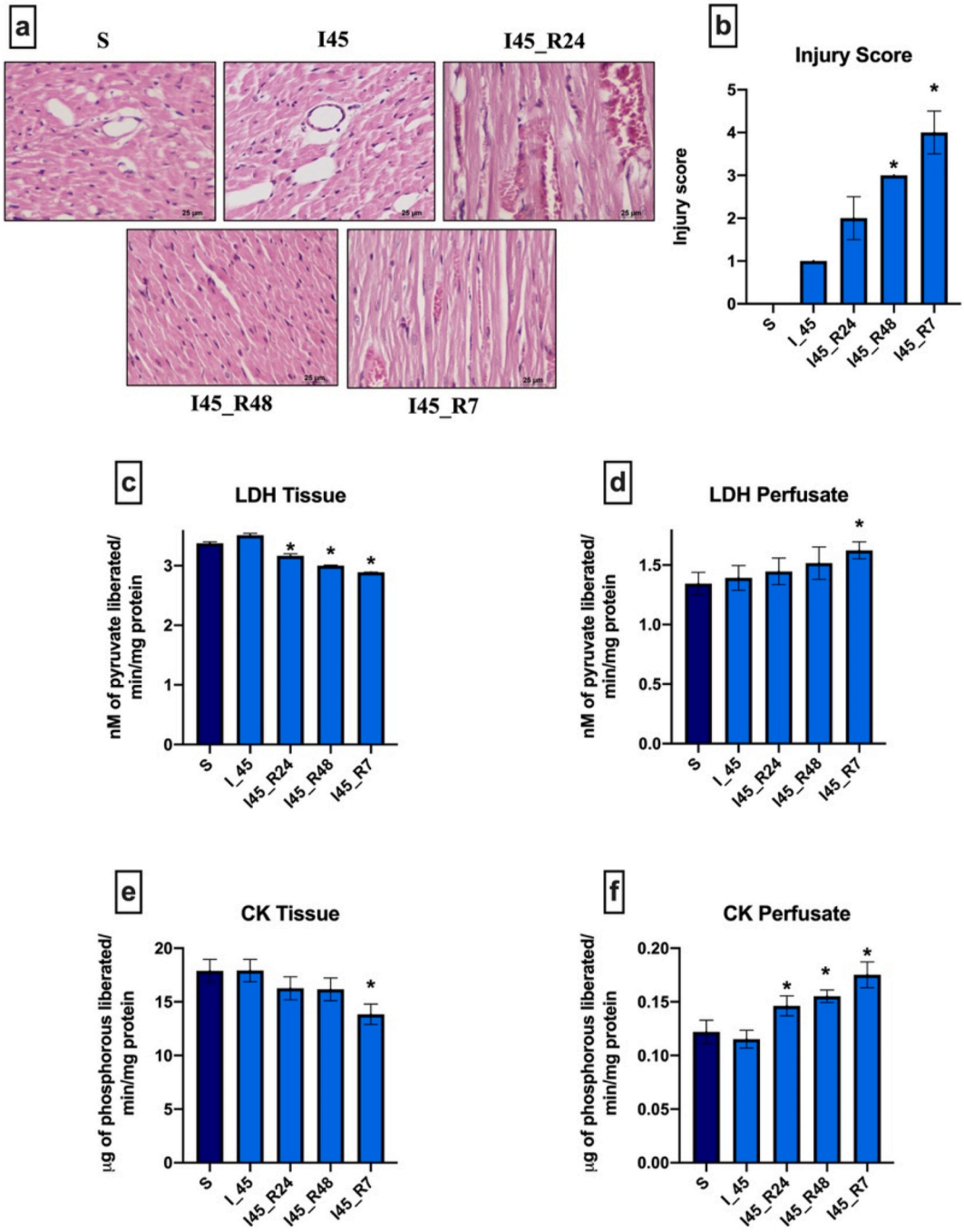

Fig. 2. Cardiac injury assessment in rats subjected to IR a) Representative H and E images of different experimental groups (The images are presented at 10× magnification) b) Cardiac injury score. Intergroup comparison made by Kruskal-Wallis test. *$p < 0.05$ vs S. Biochemical analysis of c) LDH activity in the tissue d) LDH activity in the perfusate e) CK activity in the tissue and f) CK activity in the perfusate. *$p < 0.05$ vs S. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. S- Sham, I- ischemia, R-Reperfusion.

(1.5, 2 and 2.13-fold) and mitochondrial fission factor (Mff) (1.33, 1.49 and 1.45 fold) genes in 24 h, 48 h and 7 days respectively was recorded (Fig. 7a–c). On the other hand, in all cardiac tissue isolated from reperfused rats, mitochondrial fission protein 1 (Mfn1) and mitochondrial fission protein 2 (Mfn 2), the key genes for mitochondrial fusion were significantly downregulated (Fig. 7d–e). Mitophagy genes like pink and parkin showed a similar pattern as that of ischemic tissue and were all upregulated (Fig. 7f–g). But optineurin (Optn) gene expression significantly down regulated in 48 h and 7-day tissue samples compared with the sham control

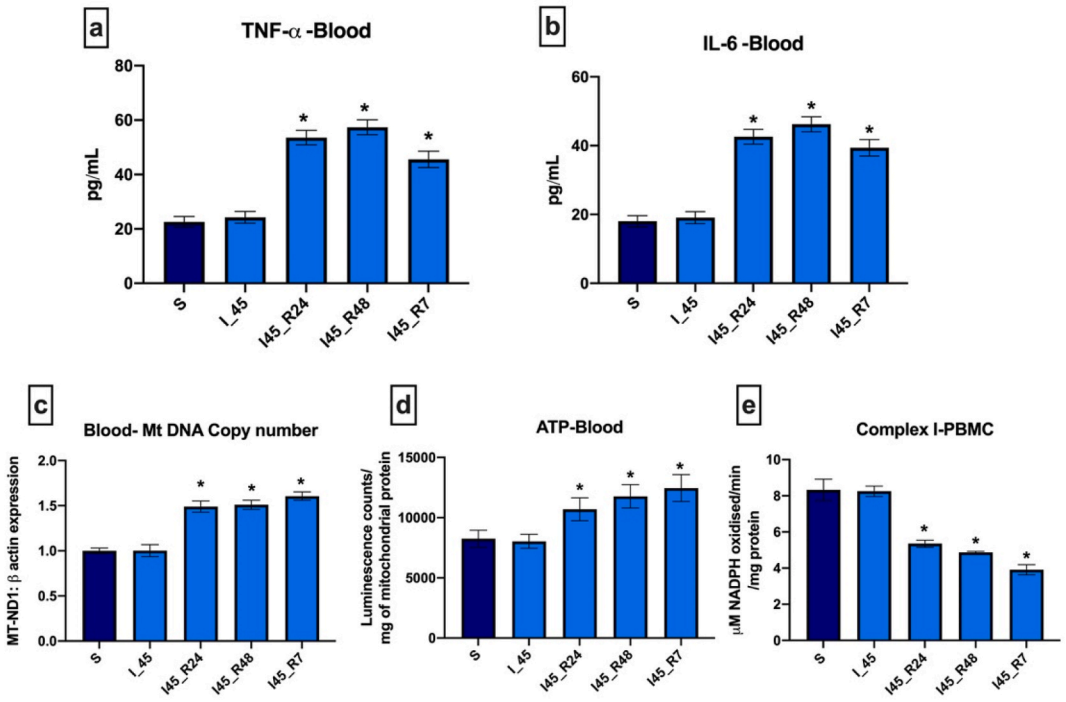

Fig. 3. Changes in the blood chemistry after renal IR a) TNF- α level in blood, b) IL-6 level in blood, c) Mitochondrial copy number in blood, d) ATP level in the plasma, and e) Complex I activity in peripheral blood mononuclear cells (PBMCs). *p < 0.05 vs S. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. S- Sham, I- ischemia, R- Reperfusion.

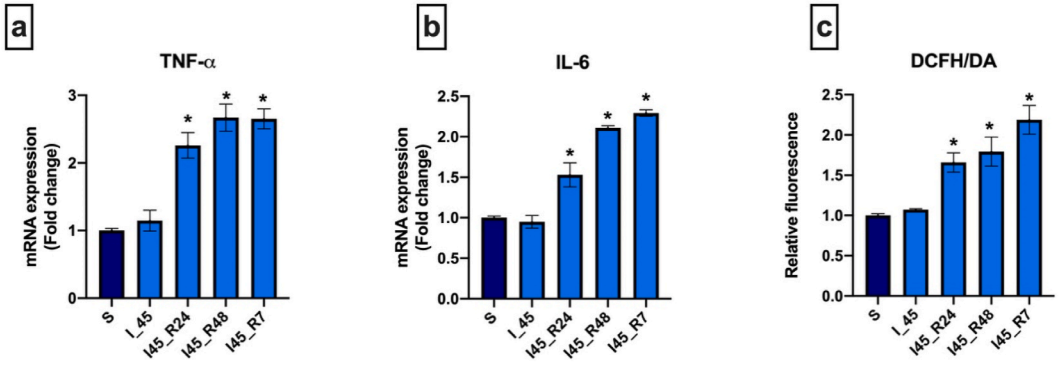

Fig. 4. Cardiac tissue inflammation and oxidative stress after renal IR. Gene expression analysis of a) TNF-α and b) IL-6 in the cardiac tissue, c) DCFH/DA analysis in cardiac tissue. *p < 0.05 vs S. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. S- Sham, I- ischemia, R- Reperfusion.

(Fig. 7h).

4. Discussion

Effective post-renal surgical repair and recovery is one of the unexplored areas of research that often create concerns for clinicians in the management of their patients [31,32]. In fact, inefficient post-renal repair can lead to long-lasting morbidity and even mortality due to renal and non-renal causes [33]. Many studies have shown that patients after kidney transplantation surgery develop ischemic heart disease, congestive heart failure and left ventricular hypertrophy [6,15]. In the present study, we explored the connectivity between renal IR surgery repair and recovery with cardiac function. The major findings of the present study are as follows.

i) Cardiac dysfunction and associated injury in rats subjected to renal IR showed a direct relationship with duration of reperfusion, where cardiac physiological impairment developed after 48 h and cardiac injury was noted after 7 days of 45 min of renal

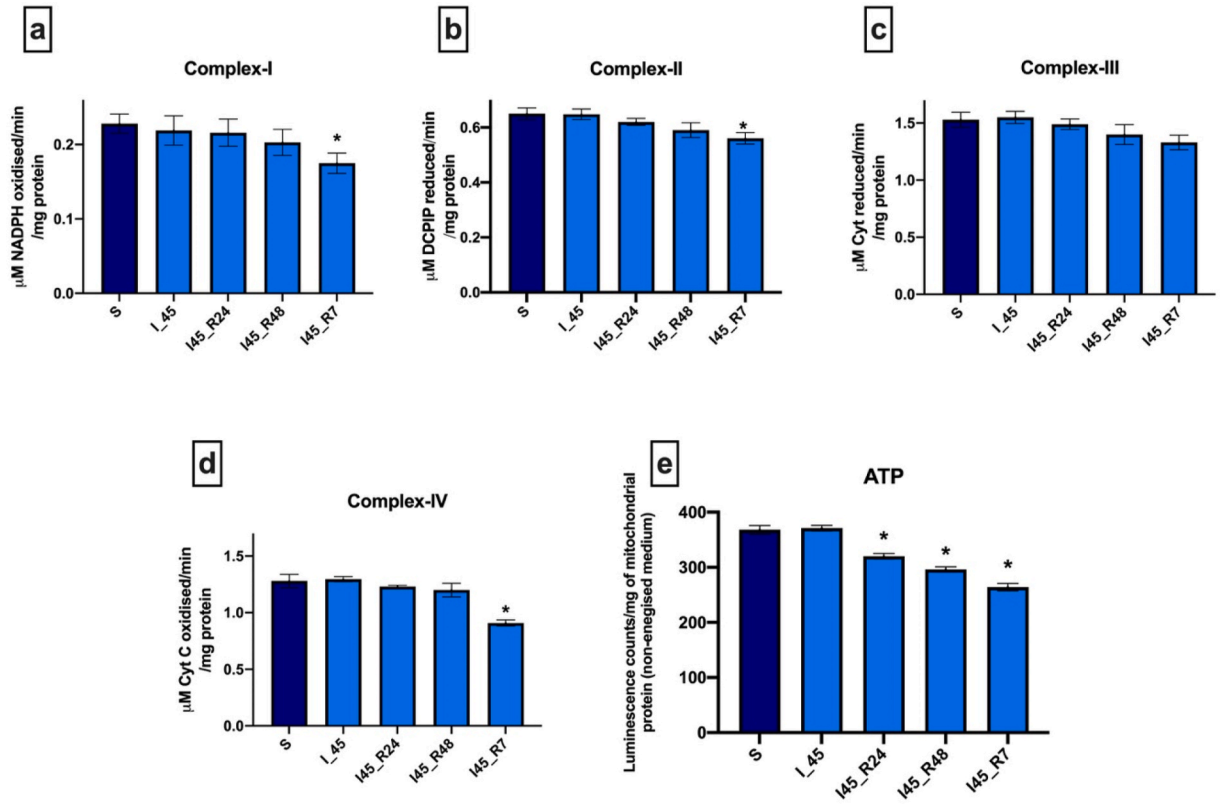

Fig. 5. Mitochondrial electron transport chain enzyme activities in cardiac mitochondria using spectrophotometry-based analysis a) NQR (Complex I), b) SQR (Complex II), c) QCR (Complex III), d) COX (Complex IV) activities and e) ATP level in the mitochondrial samples isolated from heart tissues. *$p < 0.05$ vs S. The data are presented as mean ± SD ($n = 6$/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. S- Sham, I- ischemia, R- Reperfusion.

ischemia. Incidentally, tissue injury repair was observable in renal tissue seven days following renal ischemia. Interestingly, during the early phases after renal ischemia (up to 24 h), both cardiac hemodynamics and tissue injury were minimal.

ii) The cardiac hemodynamics and tissue injury are also influenced by the duration of renal ischemia. Following 15 min of renal ischemia, cardiac hemodynamics and tissue injury remained negligible even after 7 days

iii) Interestingly, the blood-borne inflammation and mitochondrial copy number and ATP levels were high in systemic circulation, during the reperfusion period and perhaps triggered the cardiac oxidative stress, inflammation, emphasising stressful environment persist even after 7 days of ischemia.

iv) Cardiac mitochondrial bioenergetic decline along with deterioration in mitochondrial quality maintenance gene expression, and decreased mitochondrial copy number was observed after 7 days of 45 min of renal ischemia, indicating that the negative effect of renal IR on the heart was a delayed impact.

Literature suggests that following an episode of AKI, progressive or persistent impairment in renal function may exist and the early recovery lasts up to 7 days and late recovery lasts for 7–90 days post-injury in normal rats [32,34]. However, this timeframe may vary in diseased kidneys. A study conducted in our lab revealed histological changes in the kidney following ischemia, leading to AKI, characterized by loss of brush border in proximal convoluted tubule (PCT), inflammatory cell infiltration, and sloughing of epithelial cells, particularly notable in rats fed with a high-fat diet [35]. We also noted that rats fed a high-fat diet for 16 weeks showed increased sensitivity of the heart to renal ischemia-reperfusion-induced injury [36]. It is well established that the repopulation of damaged cells in nephrons relies on surviving tubular cells with stem cell characteristics, which may themselves be affected by ischemic insult [37]. Consequently, in the current study, we observed that 45 min of renal ischemia led to a significantly higher degree of AKI with a considerable decline in estimated glomerular filtration rate (eGFR) even after 24 h post-surgery, a phenomenon absent in kidneys subjected to 15 min of renal ischemia. Incidentally, the response of surviving cells to the regenerative mechanisms in AKI nephrons following renal ischemia, if unsuccessful, may result in partial epithelial-mesenchymal transition, cell senescence, G2/M cell cycle arrest, ultimately perpetuating pro-inflammatory mediators in tissue and blood [38].

An inseparable relationship exists between cardiac and renal physiology where cardiac dysfunction can result in kidney injury and vice versa [39]. Similarly, renal insufficiency can ultimately lead to uremic cardiomyopathy or myocardial fibrosis [40]. The fibroblast growth factor (FGF-23) released by CKD is reported to cause myocardial hypertrophy [41]. All this evidence and many other findings

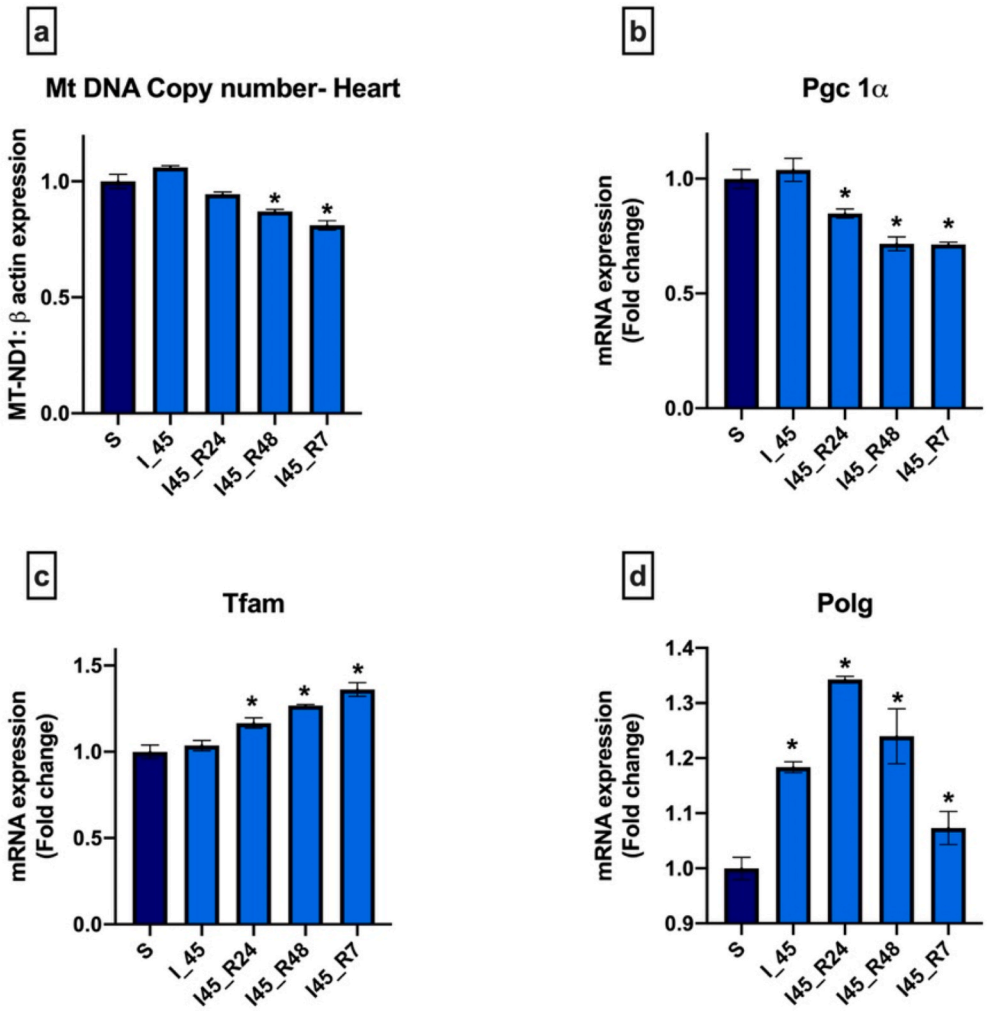

Fig. 6. Gene expression analysis in heart tissue isolated from rats subjected to renal IR injury. The graph represents (a) mitochondrial copy number and (b) Pgc 1α expression (c) Tfam expression and (d) Polg in renal tissues. *$p < 0.05$ vs S. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. S- Sham, I- ischemia, R- Reperfusion.

in the literature indicate that kidney disease can accelerate heart disease even before it ravages the kidneys [42]. Post-renal transplant patients may exhibit left ventricular hypertrophy and reduced coronary perfusion that promote cardiac failure in the long run [43]. The recent disease guidelines reported in the European society of cardiology in 2021 emphasize the significance of cardio-renal medicine for long-term therapy, that provides insight to explore the cardiac changes in renal surgery [44]. Accordingly, in the present study, we found a negative impact of renal surgery on cardiac physiology that eventually leads to tissue injury. In fact, in the present study, we found significant cardiac physiological alterations in the early days of reperfusion that resulted in tissue injury by 7 days after 45 min of bilateral renal artery ligation.

The induction of cardiac failure following renal surgery is a complex phenomenon influenced by various factors [45]. Hemodynamic changes associated with renal surgery, including alterations in blood volume and fluid balance, can impact cardiac preload, afterload, and contractility, potentially leading to cardiac dysfunction [45]. Electrolyte imbalances, particularly hyperkalemia, arising from surgical interventions can disrupt normal cardiac electrical conduction and contractility, contributing to arrhythmias and cardiac failure [46,47].

Hemodynamic variation in the heart was noted after 48 h of 45 min of bilateral renal artery ligation, which was worsened by 7 days of perfusion. During this period, AKI induced by ligation triggered the repair and recovery phase in the kidneys, which simultaneously elevated systemic inflammation. Since corresponding increase in cardiac inflammation and subsequent elevation in oxidative stress suggest the presence of negative stimulus on the heart, that comes from systemic circulation during the renal repair process. Accordingly, we noted elevated mitochondrial DNA (mtDNA) in the circulation, which is known to promote inflammation. Sterile inflammation involved in the development of heart failure can triggered by mtDNA [48]. According to previous studies, this sterile inflammatory response contributes to cardiac dysfunction by promoting leukocyte infiltration, cytokine release, and oxidative stress

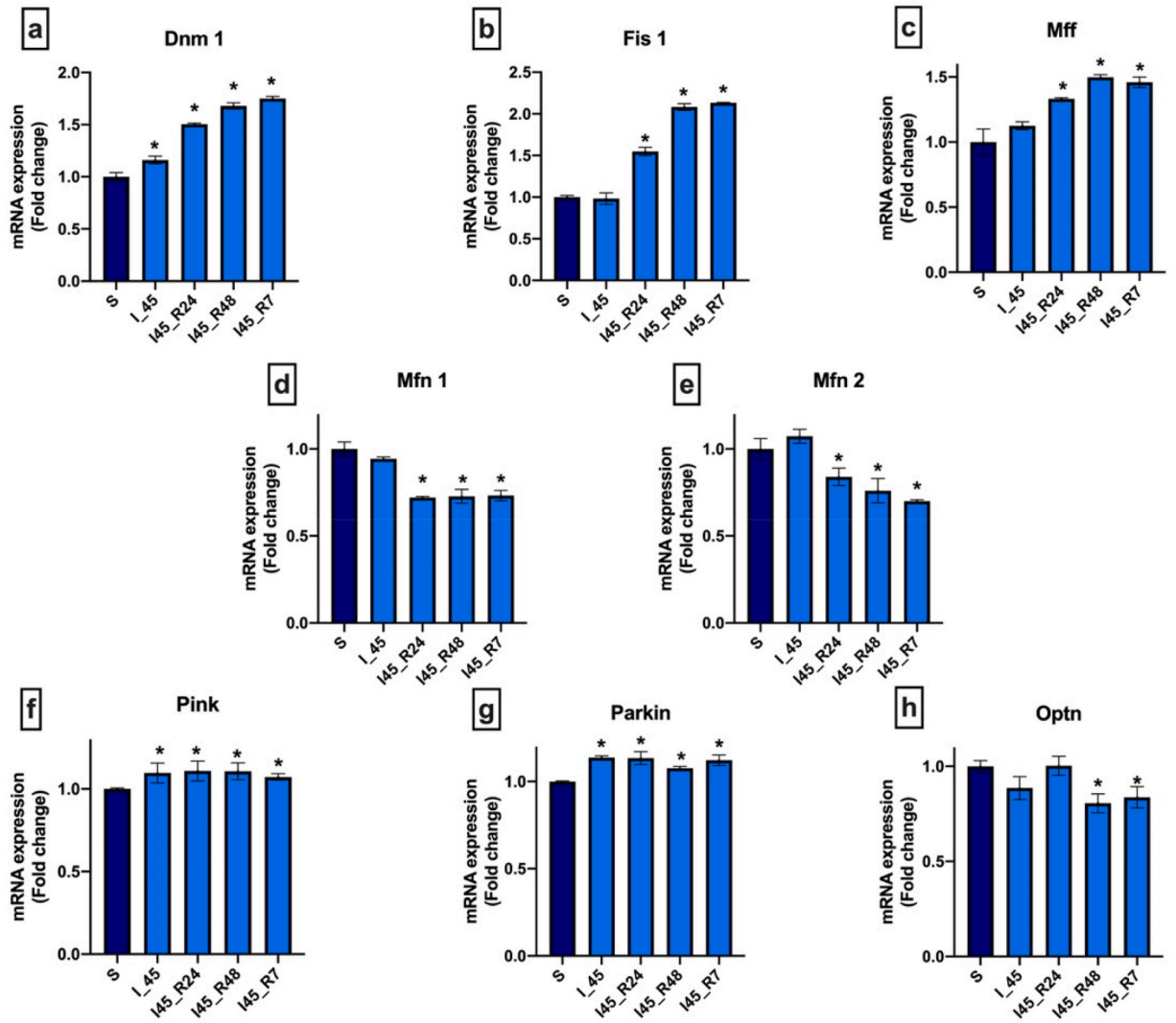

Fig. 7. Expression analysis of genes encoding mitochondrial fission, fusion and mitophagy in cardiac tissues isolated from rats subjected to renal IR injury. The graph represents the mitochondrial fission genes (a) Drp 1, (b) Fis 1, and (c) Mff; Mitochondrial fusion genes (d) Mfn 1 and (e) Mfn 2; Mitophagic genes (f) Pink, (g) Parkin, and (h) Optn. *$p < 0.05$ vs S. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. S- Sham, I- ischemia, R- Reperfusion. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

[49,50]. Furthermore, mitochondrial ROS production during renal IR injury can directly damage cardiomyocytes by inducing oxidative stress and impairing mitochondrial function [51]. Excessive ROS generation leads to lipid peroxidation, protein oxidation, and DNA damage, all of which contribute to cardiomyocyte injury and apoptosis [52]. In the current study, the tissue injury observed in the myocardium was owing to the increased inflammation in the circulation and the cardiac tissue. This in turn triggered the cell death mechanisms by disrupting the mitochondrial quality and its function.

Growing evidence showed a close relationship between inflammation and oxidative stress [53]. Thus, elevated inflammation triggers cardiac oxidative stress that in turn initiates mitochondrial dysfunction [54,55]. In the present study, we noted progressive damage to cardiac mitochondrial overall quality by increasing reperfusion time. 24-hour post-ischemic cardiac tissue showed insignificant change in the cardiac bioenergetics and copy number but changes identified in the mitochondrial fusion, fission and mitophagy gene expressions were significant. But 48-h post-ischemic tissue showed a reduction in the mitochondrial number and changes in mitophagy, fission and fusion gene expressions. By day 7 a significant reduction in mitochondrial number, bioenergetics and quality mechanisms were observed. This indicates that strategies that focus on mitochondrial quality, maintenance or targeting mitochondrial preservation can be promising approaches in the prevention of cardiac failure associated with renal IR.

5. Conclusion

The distant organ injury and the consequential impact of altered systemic effect following renal ischemia-reperfusion is considered to be one of the prime reasons for the high mortality in AKI patients, despite improved technology. The majority of the studies focused on the release of cytokines, extravasation of leukocytes and oxidative stress as the reason for remote organ damage. However, the extent to which the repair process in response to AKI can affect distant organs is not well explored. In the present study, we demonstrated that at the late phase of the early repair process (7th day) of renal ischemic injury of 45 min, the perpetuated biochemical alterations in the heart eventually manifested in injury and overall physiological decline. However, the physiological decline measured in the present manuscript was in an isolated rat heart model where the neurohormonal influences were missing. Thus, intact animal physiology via echocardiogram can provide better insight towards the temporal relationship of cardiac physiological changes in response to renal IR. The loss of cardiac resistance or adaptation by the 7th day of post-IR renal surgery may due to the significant deterioration of mitochondrial quality, quantity and function in cardiac tissue.

Ethics statement

The animal experiments were conducted by the guidelines of the committee for the purpose of conduct and supervision of experiments on animals, in India, with prior approval from the institutional animal ethical committee (546/SASTRA/IAEC/RPP).

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Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Priyanka N. Prem: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis. **Gino A. Kurian:** Writing – original draft, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Priyanka N Prem reports financial support was provided by Council of Scientific & Industrial Research. The corresponding author Dr. Gino A Kurian is a member of the Heliyon editorial team by acting as an section editor and was not involved in the editorial review or the decision to publish this article.

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