The Diagnostic Value of HIF-2 alpha to Determine The Development and Efficacy of Treatment for Contrast Induced Nephropathy: An Experimental Study

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Background and objectives:

Contrast-induced nephropathy (CIN), is an acute renal damage due to contrast agents. This study is conducted to determine the potential diagnostic value of hypoxia-inducible factor 2-alpha (HIF2-α) and to evaluate the renal protective effects of N-acetyl cysteine (NAC) and sildenafil in a rat CIN model.

Material/Methods:

This randomized, controlled, interventional animal study was conducted on Wistar rats. Totally, rats (n=36) were randomly assigned to four groups: control (n=9), CIN group (n=9), CIN+NAC group (n=9), and sildenafil (n=9). The rat model was used to form iohexol-originated CIN. During the modelling, prophylactic treatment was performed at 24th and 48th hours. After 48 hours of the modelling; blood, urine, tissue samples were obtained for biochemical analyses. HIF-2-α levels were measured in renal tissue, serum and urine samples. Renal sections were performed in order for histopathologic and immunohistochemical evaluations.

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Results:

In the CIN model, HIF-2α levels and other biochemical parameters were significantly increased ($p<0.01$). Both sildenafil and NAC, efficiently decreased the renal damage due to contrast agents ($p<0.05$). Similarly, after treatment with sildenafil and NAC, HIF-2α levels were significantly decreased ($p<0.05$).

Conclusions:

The current study constructs an experimental base for the use of HIF-2α for clinical prevention and treatment of CIN. Several mechanisms may be postulated for the changes in HIF-2α levels. Besides, the increased HIF-2α levels with CIN and decreased HIF-2α levels after treatment may be used for the treatment and follow-up of patients with CIN.

Keywords : Contrast-induced nephropathy, Hypoxia-inducible factor 2α, N-acetyl-cysteine, Sildenafil

INTRODUCTION

Contrast-induced nephropathy (CIN) is an acute renal damage due to the use of contrast agents. The diagnosis is made with the 25% increment or 5 mg/dL increase of serum creatinine (Scr) versus basal levels within the 48 hours of contrast agent use[1]. In recent years, along with the fast development in medical imaging techniques, the examinations and treatments with intravenous contrast agents in emergency services or other services may induce CIN. CIN is one the most important causes of acute renal damage/injury in patients followed at emergency services and inpatients[2]–[4]. In the current literature, the rates for CIN was reported between 0.2-2.0%, after tomography undertaken with contrast agents [1].

Pathophysiologically, CIN is closely related with renal hemodynamic changes, medullar ischemic injury, oxidative stress injury formed with reactive oxygen species (ROS), secondary damage to tubules and tubular obstruction[5]. In many experimental

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studies, chronic hypoxic damage is pointed out as an eventual common way to cause the progression of chronic renal disease (CRD) to end-stage renal failure [2]. Thus, the therapeutic intervention to hypoxia may be a valid tool to cease the CRD.

Heterodimeric nuclear transcription factor, HIF, is a crucial intermediate form for the protection mechanisms against hypoxia. HIF forms reactions to preserve the renal hypoxic tissues and to decrease the damage after the decrease in hypoxia [6]. In chronic and acute renal failure, HIF is being activated [7], [8]. There are studies emphasizing HIF activation in chronic renal fibrosis in CRD [6]. In situations like CIN, acute hypoxic renal damage occurs[2], [5]. The decrement in intramedullar blood flow secondary to hypoxia and direct tubular damage induce CIN[2], [9].

There are two well-known forms of HIFα: HIF1α and HIF2α [6]. In studies, HIF2α is detected higher in renal cells and as responsible for eritropoetin production [10], [11]. In the literature, HIF2α levels were demonstrated to be specific to renal cells [7]. The increment in HIF2α levels under hypoxic conditions is a key mediator for cellular oxygen homeostasis [12]. In a number of experimental studies conducted with unstable metals like cobalt and nickel, hypoxia induced an increment in HIFs and had a renal protective effect [13]. Accordingly, HIF plays and important role in acute renal injury and is the most important factor for the development of hypoxia, inflammation and angiogenesis [7]. Pinelopi et al. [14], detected HIF2α levels to prevent the ischemic renal injury.

In a vast of studies, risk factors and prophylaxis strategies for CIN is determined. Except volume therapies, there is no consensus or an exact protocol for the use in emergency services. N-acetyl cysteine (NAC) is commonly used for the treatment of CIN [1]. Besides, in recent years sildenafil is determined to be effective in experimental CIN models [15].

Nephropathy scoring is used in order to test the efficacy of contrast nephropathy treatments [1]. There is no current biomarker to be used in diagnosis and monitoring

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for CIN. Normal blood urea nitrogen (BUN) and creatinine levels do not point out the absence of CIN. In the current literature, there is no biomarker to demonstrate CIN injury. Therefore, in the current study, we aimed to evaluate the potential diagnostic value of HIF2α and renal protective effects of NAC and sidenofil in rat CIN model.

MATERIAL AND METHODS

Experimental materials

All the procedures with animals in this study were approved by the Ethical Committee of Erciyes University Experimental Research and Application Center (Approval date and number:14.06.2017 17/063). Forty, sixteen weeks-old Wistar albino, female rats weighing 200-250 g in the same condition were selected (Erciyes University Experimental Research Center). They were provided with adequate commercial feed (Produced by Purina, Düzce,Turkey) and tap water. The rats were arranged into four groups and each group were arranged in four cages (25x40x20). Each cage contained two or three rats and provided coarse sawdust bedding (Kayseri, Turkey). Rats were accommodated under conventional experimental animal housing conditions with controlled temperature (23±2°C), humidity (50±5%), air change (12 air change per hour), 12 h of light and darkness and ad libitum feed. General health status of the rats was monitored prior, during and at the end of the study.

NAC was purchased from Basel Pharmaceutical Co. Ltd. (Turkey); Sildenafil was purchased from Actavis Pharmaceutical Co. Ltd. (Turkey), The low-osmolar, non-ionic contrast media agent (Iohexol) was obtained from Opakim Pharmaceutical Co. Ltd (Turkey).

Model and grouping

The rats were randomly assigned to four groups: control group, CIN group, CIN+NAC group, and sildenafil group, with nine rats in each group (Figure 1). CIN rats were subjected to CIN protocol as follows: [16], [17]. Rats in the CIN model, NAC, and

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sildenafil group were anesthetized with 60 mg/kg pentobarbital. Pentobarbital sodium anesthesia was followed by CIN induction, which was performed with drug administration into a tail vein. Drugs administered were consisted of low-osmolar, non-ionic contrast medium agent (Iohexol) at a dose of 1600 mg iodine/kg. This is the standard contrast medium dose for clinical purposes and other related experiments in rat studies [5], [17], [18]. For each time, control group rats were provided an equivalent amounts of saline, in terms of volume. Rats in the NAC group received intragastric administration of NAC (150 mg/kg) 48 h prior to the CIN-inducing injections. Rats in the sildenafil group also received intragastric administration of sildenafil (50mg/kg) 48 h prior to the CIN-inducing injections. The control group and the CIN group were given an equal volume of saline by intragastric administration.

After the protocol, rats in all groups were put into their routine nutritional environment. According to the KM providing hours, earliest at 48th hours under anesthetic conditions, blood and tissue samples were obtained from rats and blood and serum markers were measured. 5ml intracardiac blood samples were taken from rats under ketamin/xylasine anesthetics. Control groups and other groups were sacrified concurrently. After taken into dry tubes, blood samples were centrifuged at 3000 rpm for 10 minutes. The obtained serum samples were stored at -80◦C until analyses time.

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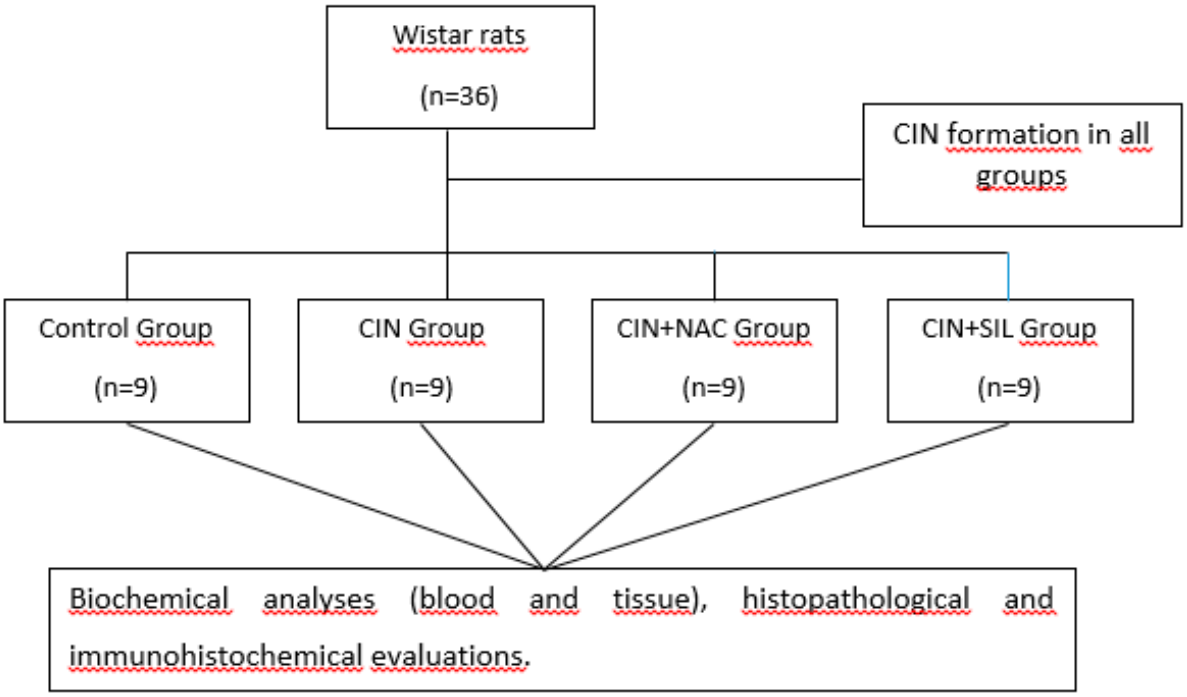

Figure 1. Flow chart of the study

Biochemical analyses

Serum, urine and tissue HIF-2a levels were detected by a commercial kit relied on the quantitative sandwich enzyme immunoassay technique (Human [HIF2a] ELISA kit; SunRed Biotechnology Company, Shangai, PRC). Serum creatinine was measured with modified Jaffe's reaction and urea was measured by coupled enzymatic method by an Autoanalyzer (Beckman Coulter AU 5800, USA)

Biochemical evaluation of tissue samples

Renal tissue samples of rats were cut on middle and weights are adjusted to 0.25 g. Then, frozen tissues and 1 ml of phosphate buffered saline (pH 7.4) was put on a screw cap 2.0 ml tube with 0.4 g of sterile zirconium beads (0.3 g of 0.1 mm and 0.1 g of 0.5 mm). Tubes were placed in the BeadBug™ (D2400 BeadBlaster 24 Microtube Homogenizer, USA) and processed for 1 minute and 6 cycles with 30 seconds intervals, at speed of 6.5 m/s. Tubes were incubated in cold nitrogen tank for 3 minutes and the

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same process was repeated on homogenizer. Tubes were centrifuged at 4ºC, 16000xg for 10 minutes. Supernatants were transferred to a fresh 2.0 ml tube for further analysis.

Histopathological evaluation

For histological examination, routine paraffin wax embedding procedures were used. The kidneys were taken out, divided into sections, fixed in 10% formalin and processed by routine histological methods. After embedding in paraffin, 5µm thick paraffin sections were removed from each sample and placed on poly-L-lysine slides. In order to evaluate the morphological characteristics of the tissue and structure before assesment by light microscopy, all sections were coloured with hematoxylin-eosin (H&E) (Olympus BX51, Tokyo, Japan). Renal injury was graded as follows: At least 10 random, non-overlapping fields (200×magnification) were observed for each slice and afterwards, the mean percentage of the injured renal tubules was calculated. The following grading system was implemented for the histopathological evaluation of tissues under light microscopy; no damage was marked as 0; <25% damage was marked as 1; 25–50% damage was marked as 2; 50–75% damage was marked as 3 and>75% damage was marked as 4 [19].

Immunohistochemistry

The renal tissues were fixed in 10% buffered formalin solution, and, after routine laboratory methods, embedded in paraffin. 5µm paraffin tissue sections were placed on poly-L-lysine slides. The slides were air-dried and the tissue was deparaffinized. 5-µm tissue sections were rinsed in de-ionized water and antigen retrieval was performed by incubation in 10% citrate buffer (pH 6.0) at 300 W for ten minutes, afterwards cooled to room temperature for 20 minutes. The sections were incubated in 3% H_2O_2 for ten minutes, then rinsed in phosphate-buffered saline (PBS). Anti-Polyvalent HRP kit (Thermo Scientific, *USA*) was used for the following steps. To reduce non-specific staining, sections were pretreated with normal block serum for 20

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minutes. Primary antibodies used were raised against HIF2α (HIF-2 alpha Polyclonal Antibody, cat no PA1-16510). The slides were incubated overnight at 4°C in a humidified chamber. After washing three times for five minutes in PBS, sections were incubated with the biotinylated secondary antibodies was applied for 15 min. After washing in PBS was applied 3,3 P-diaminobenzidine tetrahydrochloride (DAB) as a chromogen, and the sections were counterstained with hematoxylin. The stained sections were examined for HIF2α immunoreactivity under an Olympus BX-51 light microscope (Olympus BX-51, Tokyo, Japan). Two histologists continuously observed at least 10 high-power fields (×200) for each slice, and calculated the immunoreactivity intensity to reflect the intensity by using Image J software.

Quantitative immunohistochemistry

Quantitative immunohistochemistry and histomorphometry were performed using Image J software. The TUNEL-positive cells were counted in the kidney tissue sections without distinguishing cortex and medulla. Immunoreactivity intensity values for HIF2α were calculated for sections in which HIF2α staining was applied.

Statistical analyses

Statistical analyses were performed by SPSS 22.0 (Chicago, USA). One way Anova test (Post hoc test was used to compare the BUN, SCr, Urine BUN , Urine Cre , HIF-2α-tissue, HIF-2α-plasma, HIF-2α-urine and QIRIAR results. Kruskal –Wallis (posthoc Dunn's and Benforini) test was used to compare tubular damage score in groups. Statistical significancy was set at $p<0.05$ level.

3. RESULTS

There was no death among the rats in this study. There were no significant anomalies in nutrition or activity of rats in groups. CIN model was formed and the parameters were measured in CIN model. Among groups (Control, CIN, CIN+NAC, CIN+HIF) were compared renal functions, HIF-2α levels and QIRIAR and demonstrated in Table 1.

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Comparison of renal function among four groups

When renal function variables were compared between groups, BUN and SCr were detected as significant ($p<0.001$), while urine BUN and urine Cre variables were non-significant ($p=0.678$ and $p=0.788$, respectively). According to multiple comparison test (post-hoc test: Tukey), BUN was significantly different in CIN+SIL and CIN groups ($p<0.05$) versus the control group. According to the same test, SCre was not significant between the second and third groups ($p>0.05$). Other possible pairwise comparisons were statistically significant ($p<0.05$) (Table 1).

Comparison of HIF-2α levels among four groups

HIF-2α-plasma, HIF-2α-tissue, HIF-2α-urine values were measured in the control group and effects of SIL and NAC on CIN rats were shown in Table 1. When SIL and NAC were given to rats in the CIN group compared versus CIN group, plasma HIF-2α levels and kidney tissue HIF-2α levels were both decreased. As the HIF-2α levels were compared according to groups, kidney tissue (ng/gr) and plasma levels were significant ($p<0.001$), however urine levels were non-significant ($p=0.382$).

According to multiple comparison tests(post-hoc test: Tukey); tissue HIF-2α levels were significant for CIN group and control group, CIN+SIL and CIN+NAC groups. Additionally, the difference between control and CIN+NAC groups were also significant ($p<0.05$) (Table 1).

The difference between groups in terms of QIRIAR numbers were significant ($p<0.001$). According to multiple comparison test(post-hoc test: Tukey); all possible dual comparisons were significant ($p<0.05$) (Table 1).

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Table 1. Comparison of four groups according to laboratory variables

Variable	Groups				*p*
	Control	CIN	CIN+SIL	CIN+NAC	
BUN (mg/dL)	21.440±2.45	17.610±1.61	22.889±1.965	20.111±1.90	<0.001
SCr (mg/dL)	0.380±0.34	0.344±0.03	0.294±0.022	0.283±0.025	<0.001
Urine BUN (mg/dL)	63.250±122.94	170.000±245.85	164.111±202.093	135.333±224.997	0.678
Urine Cr (mg/dL)	1.220±2.43	2.000±4.09	2.333±3.000	1.000±3.000	0.788
HIF-2α-tissue (ng/gr)	49.110±15.74	71.082±13.086	44.811±9.735	31.638±6.448	<0.001
HIF-2α-plasma (ng/ml)	5.770±2.01	8.430±1.330	5.252±1.206	3.627±0.839	<0.001
HIF-2α-urine (ng/ml)	0.024±0.006	0.044±0.453	0.025±0.007	0.0441±0.453	0.382
QIRIAR	82.159±0.437	91.864±0.634	76.076±0.378	79.423±0.366	<0.001

Results are expressed as mean ± SEM. SIL: Sildenafil; CIN: contrast-induced nephropathy, NAC: N-acetyl cysteine, SCr: serum creatinine, mg: miligram. According to multiple comparison test (post-hoc test: Tukey), BUN was significantly different in CIN+SIL and CIN groups ($p<0.05$) versus the control group. According to the same test, SCre was not significant between the second and third groups ($p>0.05$). Other possible pairwise comparisons were statistically significant ($p<0.05$).

In Figure 2; plasma and tissue HIF-2α levels were given for all groups in comparison and with box-plot graphics.

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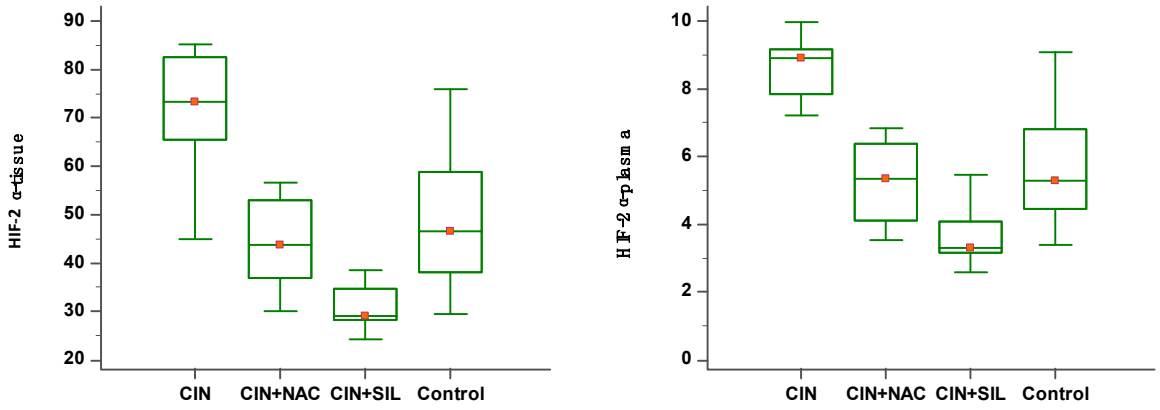

(a) (b)

Figure 2 (a,b). Multiple comparison test of HIF-2α-tissue (Figure 2a) and HIF-2α-plasma (figure 2b) for all groups. According to multiple comparison tests(post-hoc test: Tukey); tissue HIF-2α levels were significant for CIN group and control group, CIN+SIL and CIN+NAC groups. Additionally, the difference between control and CIN+NAC groups were also significant ($p<0.05$).

Effects of Sildenafil on Kidney Histopathological Alterations, Histopathologic findings in CIN Rats and treatment groups.

H&E staining of kidney tissues showed that the renal tubular epithelial cells of the control group presented a normal morphology and structure as shown in Figure 3. However, CIN markedly increased hemorrhage, shedding of the brush border, tubular vacuolization and degeneration, infiltration of mononuclear cells and intratubular obstruction by granular casts were detected in rat kidney compared versus the control. Specifically, the most severe alterations were observed in the renal cortico-medullary boundary zone. Moreover, renal injury in Sil-treated CIN group had fewer histological changes than NAC-treated CIN group.

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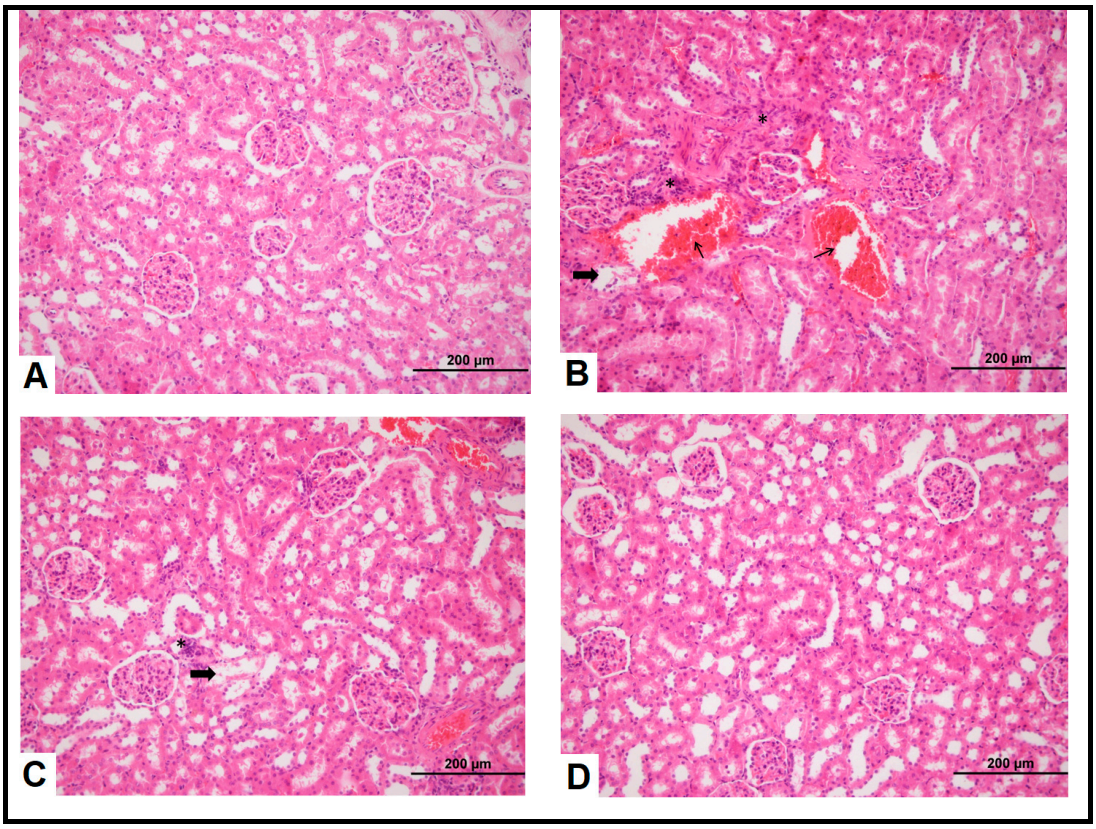

Figure 3. Pathological observations of kidney tissue in rats after modelling for 24 h (H&E staining, ×200). (A) Control group; (B) Model group; (C) NAC group; (D) Sildenafil group (arrow; hemorrhage,*; mononuclear cell infiltration, thick arrow; tubular damage, for B and C thick arrow; tubular damage)

In Figure 4; tubular damage scores were given for all groups in comparison and with box-plot graphics.

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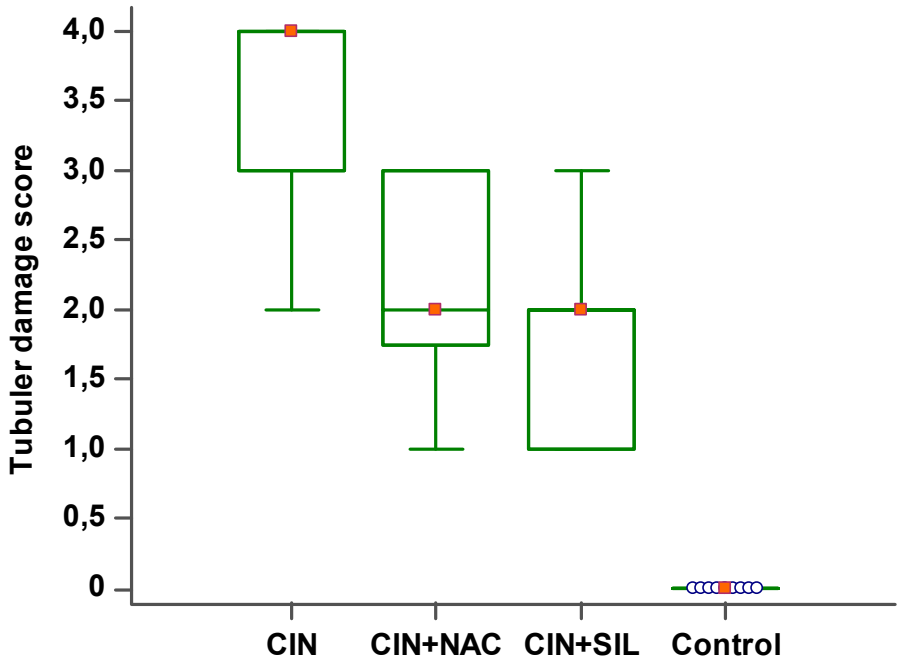

Figure 4. Tubular damage scores of rats after modelling for 48 h. According to multiple comparison tests(post-hoc test: Tukey); the difference between control group and other groups and CIN and CIN+NAC groups were significant ($p<0.05$) (Table 2).

Table 2: Multiple comparison of tubular damage scores of rats after modelling for 48 h.

Variable	Groups				*p*
	Control	CIN	CIN+SIL	CIN+NAC	
Tubuler damage score	0 (0-0)	4 (2-4)	2 (1-3)	2 (1-3)	<0.001

Results are expressed as median (min-max).

According to groups, tubular damage scores were significant ($p<0.001$). According to multiple comparison test; all possible dual comparisons were significant ($p<0.05$)

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(Table 1). According to the same test, the difference between control group and other groups and CIN and CIN+NAC groups were significant ($p<0.05$) (Table 2).

Observation of renal immunohistochemistry for four groups

The conventional immunohistochemistry method was used to perform HIF-2α immunohistochemical staining on paraffin sections. As observed under a light microscope, the tubules in the control group presented a very low immunoreactivity intensity of HIF-2α positive tubules, and the staining was light than CIN group as shown in Figure 5.

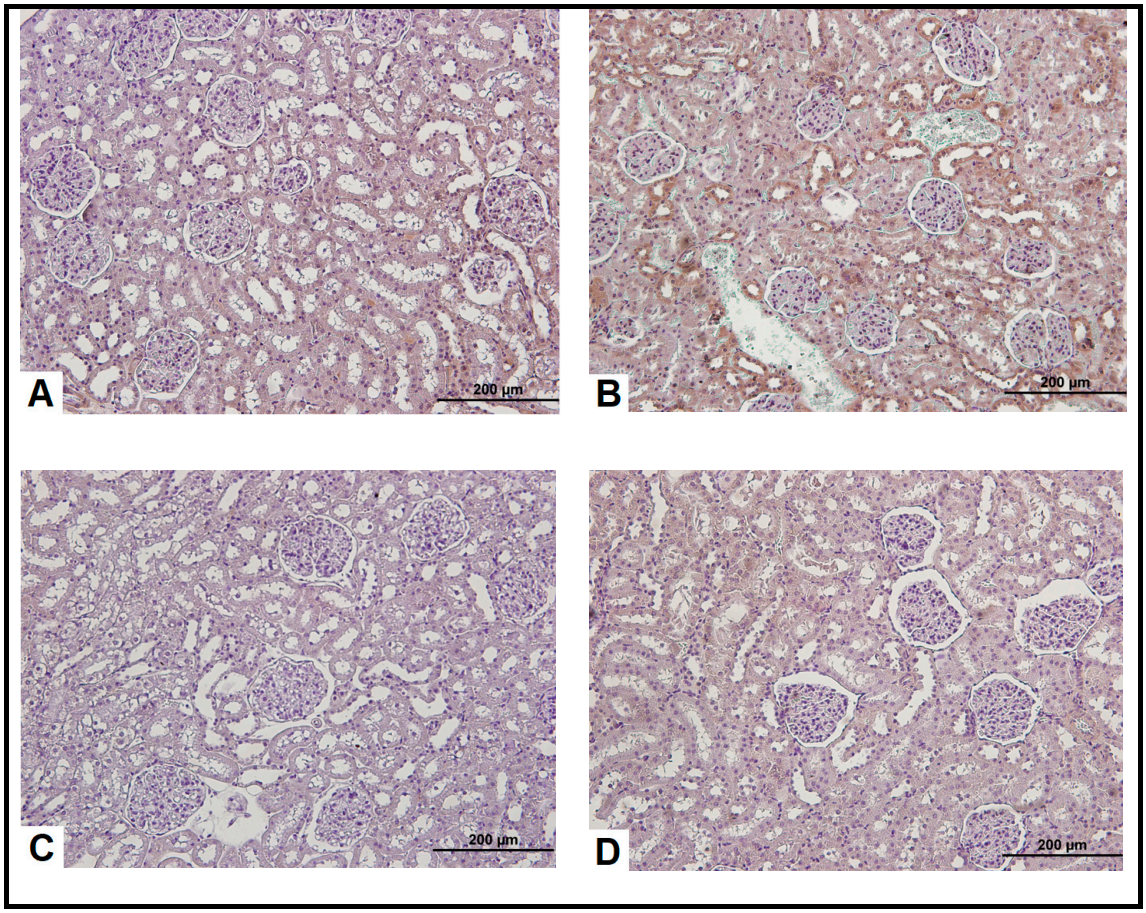

Figure 5. Immunohistochemistry of HIF2α in rat kidney section after modelling 24 h (×200). (**A**) Control group; (**B**) Model group; (**C**) NAC group; (**D**) Sildenafil group.

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In Figure 6; QIRIAR counts were given for all groups in comparison and with box-plot graphics.

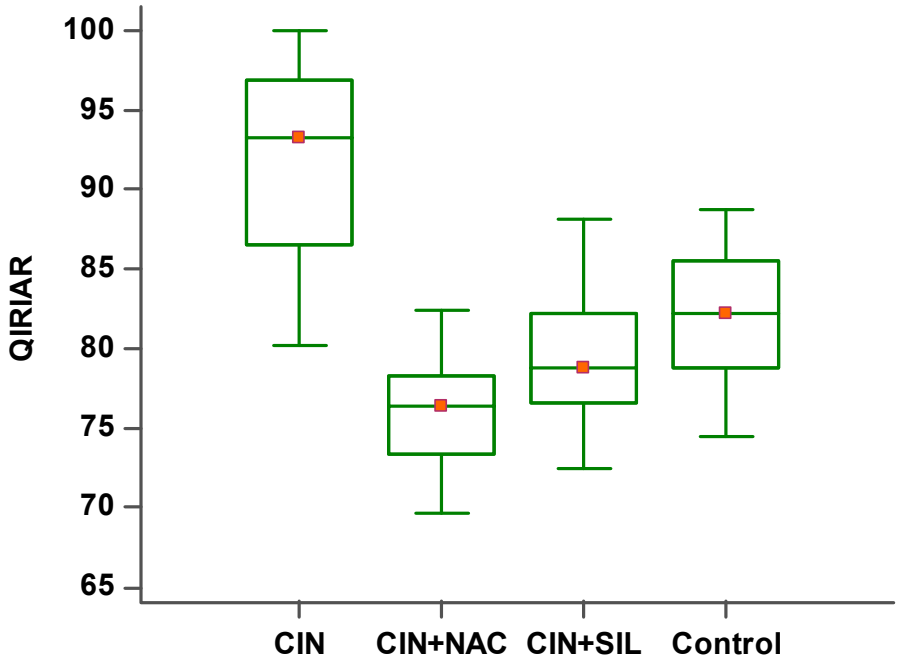

Figure 6. HIF-2α immunostaining in CIN model kidney of control, CIN and treated groups. Ratio of HIF-2α positive tubular with immunolocalization staining in rats after modelling. According to multiple comparison test(post-hoc test: Tukey); all possible dual comparisons were significant ($p<0.05$).

DISCUSSION

Owing to the increased use of iodine contrast agents worldwide, CIN is increasing day-by-day. Prevalent in all age and patient groups, CIN risk is increased among patients with diabetes and hypertension [1], [20]. In the current literature, the rates of CIN was reported between 0.2-2.0% after contrast tomography taken in emergency services [1]. Generally, along with increased creatinine levels for 3-5 days, it may be

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deleterious in long-term and dialysis requirement may occur. In the current study, in order to form the CIN model, the non-ionic low-osmolar contrast medium agent was used as in the study of Sun et al. [17]. In all rats in the experiment model, CIN was developed significantly versus the control group. The effects of HIF-2α in CIN and treatment groups were demonstrated by using the modified CIN protocol of Sun et al. [17]; with biochemical parameters, histopathological analyses, immunohistochemical tests.

Of the biochemical parameters; serum BUN and Cre, urine BUN and Cre levels were significantly increased in CIN group, however, solely serum Cre and urine BUN levels were significantly decreased in treatment groups. Similarly with our results, Wang et al. [16], detected a decrement in increased serum Cre levels in CIN group after treatment with statins.

In the entire world, the main aim of the studies conducted to reveal the pathogenesis of CIN is related to diagnosis and treatment. In recent years, all clinicians, especially the ones working at emergency services, conduct studies in order to increase the awareness on CIN. Along with a number of difficulties in CIN diagnosis and treatment, BUN and Cre are the mostly preferred biochemical markers for diagnosis. These markers are necessary for the diagnosis, however they are not sufficient to demonstrate the efficacy of the treatment and the ischemic injury.

In the current study, both as a diagnostic agent and also to evaluate the treatment efficacy, HIF-2α is studied on rats modelled with CIN. Our study is among the preliminary studies to determine HIF-2α levels in rats modelled with CIN. A number of studies are performed on a number of biomarkers related with CIN [1].

As the underlying mechanism of contrast agents to induce CIN is complex, diagnosis and treatment are also complex situations. In the clinical treatment, mainly two different mechanisms are used. The first is the sufficient hydration of the patient, and the second is the antioxidant treatment [1], [21]. The mostly known antioxidant

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treatment is NAC, which scavenges the ROS and increases the vasodilatative effect of nitric oxide [2]. Despite the exact mechanism against renal damage induced with contrast agents is unknown, NAC is the widely used agent in the world in CIN due to its renal protective effect and antioxidant property [2]. Especially, NAC may decrease the oxidative stress formed with contrast agents efficiently [2]. In the current study, in CIN formed rats HIF-2α is significantly increased in both tissue and plasma. After treatment with NAC for 48 hours, a significant decrease was detected in HIF-2α levels. Besides, NAC may protect kidneys with more than one mechanism; as deletion of ROS, inducing glutathion (GSH) synthesis and stabilizing nitric oxide [1]. Apart from NAC; many other antioxidants like sildenafil and vitamin C are used for the treatment of CIN [20], [22]. Thus, we used NAC to compare with sildenafil as a positive control drug. As performing comparisons, we measured both the treatment efficacy of NAC and also the treatment efficacy of sildenafil.

Sildenafil is a vasoactive agent used for erectile dysfuncion, pulmonery artery hypertension in humans, besides being used in pig model during cardiac by-pass and in rat model for gentamisin-induced nephrotoxicity [22], [23]. de Almeida et al. [15], regarded sildefanil as successful to prevent nephropathy in CIN-formed rats. In the current study on CIN-modelled rats, sildenafil treatment efficiently protected renal functions, decreased both plasma and tissue HIF2 α levels, and also SCr levels; however not effected the serum BUN, urine BUN and urine Cre levels. In the histopathological evaluation, we detected significant differences in rats modelled with CIN.

Important evidences obtained from experimental studies point out to the chronic hypoxic damage of tubulointerstitium as a common eventual pathway to induce the progression of chronic kidney disease to end-stage renal disease [2]. Thus, therapeutic intervention to prevent hypoxia may be a valid way to terminate the progression of CIN. HIF, heterodimeric nuclear transcription factor, is an essential intermediate for defense mechanisms against hypoxia [24]. HIF-α cumulates in the

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cell, moves to the nucleus and by binding to β-subunit, undertakes functions in erythropoesis, angiogenesis, cell metabolism, cell growth and apoptosis [6]. In chronic and acute renal failure, HIF is activated. There are studies referring to HIF activation to be responsible for renal fibrosis in chronic renal failure [6]. HIF is efficient in preserving the hypoxic tissues, decreasing the hypoxia, decrementing the injury [9]. In hypoxic states, there is no oxygen available for molecular hydroxylation. In states like CIN; there is an acute renal damage secondary to hypoxia, a decrease in intramedullar blood flow and a direct tubular damage [9], [17].

In general, oxidative stress was revealed as an important factor [2]. Thus, several antioxidant agents were used as being important factors for the mechanism of oxidative stress [6]. Although an exact consensus does not exist, in practice, NAC becomes the widely preferred agent [1]. After their injection into the body, the contrast agents produce oxygen radicals through pathophysiologic effects. Contrast agent primarily cause vasoconstriction that plays a directly role on production of oxygen radicals, adenosil residues and calcium ions. Afterwards, glomerular basal membrane and mesengial cells are damaged and oxygen radicals are formed by the increment in leukocyte chemotaxis and xantine oxidase activity. Oxygen radicals are claimed as the causative factor for CIN due to contrast agents and these molecules may lead to toxic ischemic reaction or tissue damage related to immune system [1,22,23]. In the current study, for the model group, we detected significantly increased HIF-2α levels after modelling and the levels were significant in renal tissue.

Our study revealed the HIF activation in CIN model and CIN treatment had histopathological and immunohistochemical effects. Several studies demonstrated HIF-2α activation in renal ischemic models[7], [11], [25]. In the current study, in rat models with CIN and in rats treated with NAC and sildenafil, HIF-2α activation is measured and differences were detected. In CIN model, HIF-2α activation is determined and in CIN+NAC and CIN+SIL models, this activation was decreased versus CIN model. Kong et al. [26], demonstrated late phase renal tubular HIF-2α

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activation to be protective on renal fibrosis and renal dysfunction, and also its use as a therapeutic agent in the late phase of chronic kidney disease.

In the current study, HIF-2α levels measured in tissues were as follows: 49.11±15.74 ng/mL in control group, 71.082±13.086 ng/mL in CIN group, 44.881±9.735 ng/mL in CIN+SIL group and 31.638±6.448 ng/mL in CIN+NAC group, respectively. Accordingly, the increase for HIF-2α levels in CIN group versus control group was significant ($p<0.001$). Zheng et al [27], in ischemia/reperfusion injury mice model, detected increased HIF-2α levels in kidney. Again in the same study, treatment with sevoflurance induced a significant decrease in HIF-2α levels.

In our study, the decrease in HIF-2α levels in treatment (CIN+SIL, CIN+NAC) groups versus CIN group was significant ($p<0.01$). HIF-2α levels measured in plasma and also in tissue were significant between groups. Urine HIF-α levels were non-significant in treatment groups versus control group. All these measured values may be used to evaluate the efficacy of treatment with HIF-2α levels in CIN treatment. Similarly, BUN levels were non-significant for CIN and treatment groups. Oppositely, SCr levels were significant in treatment groups versus CIN group. Urine BUN levels were significant for CIN+NAC and CIN groups ($p<0.05$), however, non-significant for CIN+SIL group. There were no significant differences for urine Cr in treatment groups versus CIN group. Our current results related to BUN and SCr were in consistent with the current literature[1], [18], [22].

CONCLUSION

In the current study, HIF-2α levels were significantly increased in CIN model. After CIN treatment with NAC and sildenafil, HIF-2α levels were significantly decreased. NAC and sildenafil efficiently reduced the renal injury due to contrast agent implementation. Increased HIF-2α levels in CIN formation and decreased HIF-2α levels after treatment may be beneficial in monitoring and treatment of patients with CIN. The underlying mechanism for the change in HIF-2α levels states, where CIN or acute renal damage is presumed, may be associated with a decrement in

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411 regional reactive oxidative stress and renal pathological changes. Thus, these
412 conclusions may construct an experimental base for the use of HIF-2α levels in clinical
413 prevention and treatment of CIN. Despite the use of NAC and sildenafil in CIN
414 treatment, we determined NAC treatment as more significant.

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