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Research Article

Correlation of renal ischemia with oxidative stress and generation of reactive nitrogen species

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Abstract
Reactive oxygen species (ROS) and nitric oxide (NO) are important mediators of tissue injury and renal ischemia (RI). The study was aimed to investigate the correlation of oxidative stress with RI. Blood samples were collected from 25 RI patients and 25 normal individuals. Plasma urea, uric acid and creatinine levels were monitored as markers of renal injury. Glutathione oxidase, Superoxide dismutase (SOD), catalase and malondialdehyde (MDA) were used as markers of oxidant stress. NO was used as a biomarker of reactive nitrogen species (RNS) formation. SOD, MDA, give complete name of this first, catalase and NO were assessed by spectrophotometric assay while renal parameters were estimated by enzymatic kits. Significant increase in plasma creatinine, urea and uric acid were found showing renal injury. Significant decrease in catalase, SOD and GSH with compare able increases in MDA and NO were observed. The data was evaluated statistically by using t-test according to which overall significant difference was observed in oxidative stress indices, reactive nitrogen species and renal indices in case group of renal ischemia as compared to control group as $p<0.05$. Negative correlation was observed in oxidative stress parameters with NO and renal parameters except for MDA with positive correlation. Oxidative stress and RNS generation occur in the kidney during ischemia.
Keywords: Renal ischemia (RI) Oxidative stress, Reactive oxygen species, Nitric Oxide.

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Introduction

Renal ischemia/reperfusion (I/R) injury is a syndrome that develops following a sudden transient drop in blood flow to the kidney [1]. Renal I/R injury are a relatively common cause of acute renal failure (ARF) [2]. The pathogenesis of renal I/R injury involves the release of proinflammatory cytokines such as tumour necrosis factor, transforming growth factor, nuclear factor and reactive oxygen species [3]. Nitric oxide (NO) plays an important role in mediating cell damage during I/R injury [4]. Acute renal failure (ARF) or acute kidney injury (AKI), as it is now referred to in the literature, is defined as an abrupt or rapid decline in renal filtration function. This condition is usually marked by a rise in serum creatinine concentration or by azotemia (a rise in blood urea nitrogen [BUN] concentration) [5]. Ischemia is a feature of organ transplantation. In mammalian kidneys, ischemia results in a wide range of physiologic changes, including the generation of reactive species in a process known as oxidative stress [6, 7]. The reactive species important in this setting appear to include oxygen free radicals, such as superoxide, hydroxyl and reactive oxygen species, of hydrogen peroxide [7, 8]. These molecules can induce complex cascades of pathologic changes involving cellular lipids, proteins, nucleic acids, and other molecules [9, 10]. Collectively, these events are known as oxidative stress [10].

Superoxide dismutase (SOD) and catalase are the most important enzymatic antioxidant systems in the body. SOD, as the first and most important line of defense against reactive oxygen metabolites (ROM), transforms superoxide ion to H2O2 which is a less reactive molecule [11]. The ability of superoxide dismutase (SOD), allopurinol, deferoxamine and other antioxidants to attenuate renal I-R injury suggests that reactive oxygen species contribute to the development of renal injury [12-14]. However, these compounds also scavenge or inhibit the formation of peroxynitrite (ONOO–), a highly reactive species derived from nitric oxide (NO) and superoxide [15, 16, 17, 18]. Indeed, several lines of evidence now implicate reactive nitrogen species (RNS) as contributors to renal I-R injury [19-22]. There are a number of RNS derived from NO [23]. Of these, peroxynitrite (ONOO–) is the best characterized and appears having the most biological activity [24]. Although NO, generated from L-arginine by nitric oxide synthase (NOS), participates in numerous physiological processes in kidney [25], NO also appears to contribute to renal I-R injury [20-22]. Oxidative stress in organisms leads to the peroxidation of all major biomolecules, such as DNA, proteins and lipids. The most widely used method to find oxidative stress is to determine lipid

peroxidation with the thiobarbituric acid reactive substances (TBARS) method. Among these targets, the peroxidation of lipids is basically damaging because the formation of lipid peroxidation product leads to spread of free radical reactions. The general process of lipid peroxidation consists of three stages: initiation, propagation and termination [26]. Owing to the correlation and effect of oxidative stress with renal ischemia the present study was carried out to determine the relationship of generation of reactive oxygen species and nitrogen reactive species in oxidative stress with the occurrence of renal ischemia.

Materials and Method

In this study, a total of 50 samples were collected from 25 patients with renal ischemia and 25 control subjects. Patients with renal ischemia were chosen from the outpatient clinic of kidney in Mayo Hospital. Blood taken was subjected to centrifugation in order to separate serum within one hour after collection of blood. The sample was processed and analyzed for the estimation of renal parameters (urea, creatinine and uric acid), lipid peroxidation (LPO), antioxidant enzymes activity (SOD, Catalase and Glutathione) and nitric oxide (NO). Renal parameters (urea, creatinine and uric acid) were measured by the enzymatic kit method. GSH count was assessed by the method of Tietze [27]. Catalase activity was measured by the method of Aebi [28]. Superoxide dismutase (SOD) activity was determined by the method of Kakkar [29]. Malondialdehyde (MDA) in tissue was estimated by the method of Ohkawa [30]. Nitrite concentration (NO) was typically measured by a well-known method of colorimetric Griess assay [31]. Measurements were made spectrophotometrically. Oxidative stress parameters (catalase, SOD, GSH, MDA and NO) and renal parameters (urea, creatinine and uric acid) in the control group were compared with the same parameters of blood samples of renal ischemic patients. In statistical analysis, SPSS test was used (Independent T-test).

Results and Discussion

In this study effects of ischemia on renal catalase and SOD activities as well as renal GSH, MDA and NO levels were evaluated. Data showed that in spite of a decrease in renal GSH, SOD and catalase activity in ischemic condition, MDA activity was increased. NO levels in renal ischemic patients group were significantly higher than that of control group. According to descriptive statistics, the mean values of catalase in control and renal ischemic patients were 164.30±10.86 and 151.47±0.58 respectively. The observed mean value of SOD in control was 9.55±2.21 while in renal ischemic patients it was 4.35±1.73. The mean value of MDA in control and renal ischemic patients were 3.68±1.22 and 5.97±1.73 respectively. In control, the mean value of GSH was 9.96±2.47 while the observed mean value in renal ischemic patients was 3.44±1.16. NO in control was observed as 28.72±3.92 while in renal ischemic patients, the observed mean value of NO was 47.78±9.66 (**Table 1**).

Significant increase in both BUN and creatinine showed that ischemia resulted in serious renal injury. According to descriptive statistics, the mean values of urea in control and renal ischemic patients were 32.75±4.84 and 175.75±60.60 respectively. Acute renal failure (ARF) is characterized by the abrupt failure of the kidneys to regulate water and electrolyte homeostasis leading to high levels of urea and BUN in individuals with renal failure. The maximum level of urea could be more than 300 IU [9].

Table 1: Mean ± SD values of oxidative stress parameters

Groups	Parameters analysed				
	Catalase	SOD	MDA	GSH	NO
Control	164.3±10.9	9.5±2.2	3.6±1.2	9.96±2.5	28.7±3.9
RIP	151.4±0.6	4.3±1.7	5.97±1.7	3.4±1.2	47.8±9.7

RIP = Renal Ischemic Patients

The observed mean value of creatinine in control group was 0.91±0.18 while in renal ischemic patients it was 7.33±2.65. Uric acid in control was observed as 5.08±0.74 while in renal ischemic patients, the observed mean value of uric acid was 33.46±11.06 (**Table 2**).

Table 2: Mean ± SD values of renal parameters

Groups	Parameters analysed		
	Urea	Creatinine	Uric acid
Control	32.75±4.84	0.91±0.18	5.08±0.74
RIP	175.75±60.6	7.33±2.65	33.46±11.06

RIP = Renal Ischemic Patients

Correlation showed significant difference ($p<0.05$) in all the parameters (catalase, SOD, MDA and GSH) of oxidative stress as compared to reactive nitrogen species (NO) and renal parameters and as compared to each other. Oxidative stress parameters (SOD, GSH and catalase) showed negative correlation with MDA, NO and renal parameters (urea, creatinine and uric acid) as SOD, GSH and catalase decreases while other increases. NO showed positive correlation with lipid peroxidation， MDA and renal parameters (urea, creatinine and uric acid) all these increase in renal ischemic patients. However, all the parameters were highly correlated (**Table 3**).

Table 3: Correlation of oxidative stress and renal parameters

		Catalase	GSH	Creatinine	MDA	NO	SOD	Urea	Uric Acid
CATALASE	Pearson Correlation	1	0.509**	-0.583**	-0.558**	-0.604**	0.382*	-0.574**	-0.581**
	Sig. (2-tailed)		0.001	0.000	0.000	0.000	0.015	0.000	0.000
	N	40	40	40	40	40	40	40	40
GSH	Pearson Correlation	0.509**	1	-0.724**	-0.433**	-0.612**	0.693**	-0.725**	-0.771**
	Sig. (2-tailed)	0.001		0.000	0.005	0.000	0.000	0.000	0.000
	N	40	40	40	40	40	40	40	40
CREATININE	Pearson Correlation	-0.583**	-0.724**	1	0.520**	0.737**	-0.673**	0.923**	0.525**
	Sig. (2-tailed)	0.000	0.000		0.001	0.000	.000	0.000	0.000
	N	40	40	40	40	40	40	40	40
MDA	Pearson Correlation	-0.558**	-0.433**	0.520**	1	0.435**	-.284	0.459**	0.450**
	Sig. (2-tailed)	0.000	0.005	0.001		0.005	0.076	0.003	0.004
	N	40	40	40	40	40	40	40	40
NO	Pearson Correlation	-0.604**	-0.612**	0.737**	0.435**	1	-0.681**	0.727**	0.566**
	Sig. (2-tailed)	0.000	0.000	0.000	0.005		0.000	0.000	0.000
	N	40	40	40	40	40	40	40	40
SOD	Pearson Correlation	0.382*	0.693**	-0.673**	-0.284	-0.681**	1	-0.673**	-0.637**
	Sig. (2-tailed)	0.015	0.000	0.000	0.076	0.000		0.000	0.000
	N	40	40	40	40	40	40	40	40
UREA	Pearson Correlation	-0.574**	-0.725**	0.923**	0.459**	0.727**	-0.673**	1	0.586**
	Sig. (2-tailed)	0.000	0.000	0.000	0.003	0.000	0.000		0.000
	N	40	40	40	40	40	40	40	40
URIC ACID	Pearson Correlation	-0.581**	-0.771**	0.525**	0.450**	0.566**	-0.637**	0.586**	1
	Sig. (2-tailed)	0.000	0.000	0.000	0.004	0.000	0.000	0.000	
	N	40	40	40	40	40	40	40	40

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

There was a decrease in renal GSH after renal ischemia, as had been shown in other studies [32, 33, 34]. This decrease in GSH level could be explained by its consumption in scavenging free radicals and maintaining the redox state of the cell during ischemic injury [33,35]. There are many evidences of involvement in renal ischemic injury [36, 37] and both tubular and vascular endothelial cells are involved in Reactive oxygen species (ROS) production [36]. Superoxide (O_2^-) radicals are among the most important free radicals responsible for ischemic injury. O_2^- is converted to hydrogen peroxide (H_2O_2) by SOD and resulted H_2O_2 is inactivated by glutathione peroxidase (GPX) and catalase [14]. Decrease in renal catalase activity after ischemia in present study is similar to other published data, which remarked decrease in catalase activity as well as a reduction of its gene expression after ischemia [32]. Increased MDA level after ischemia was seen which is same as seen in other studies in various models of kidney ischemia injury [34].

Collectively, data revealed that in the kidney, ischemia alone causes both oxidant stress and RNS formation. GSH depletion and altered GSH/GSSGfirst use complete name ratios can signal the development of oxidant-mediated tissue injury [12, 38] and have been observed in several models of oxidant-mediated acute renal failure [39,40]. GSH peroxidase can scavenge a number of oxidants and RNS such as $ONOO^-$ [23, 41, 42] directly or through the actions of GSH. The increases in GSSG content and percent oxidized GSH equivalents show that GSH oxidation occurs during ischemia and indicates the development of oxidative stress prior to reperfusion. Lipid peroxidation is an autocatalytic pathway that causes oxidative damage to cell membranes and results in the release of reactive lipid aldehydes. These cytotoxic metabolites such as 4-HNE [43, 44] diffuse from the site of production and react with cellular macromolecules. The appearance of 4-HNE-protein adducts during ischemia is additional evidence that oxidative stress occurs during ischemia. However, we cannot rule out the possibility that the increase in oxidant stress products during ischemia may be, at least in part, a result of accumulation due to reduced clearance. Lipid peroxidation is frequently used as an indicator of oxidative damage in the kidney [14, 45, 46] and provides additional support for oxidant-mediated

injury in rat I-R model. 4-HNE-protein adducts have also been detected in the kidney following treatment with iron [47] and during ischemia [48] using immunohistochemistry.

We recently showed immunohistochemical detection of 3-NT-protein adducts 6 h following reperfusion [49], and others have shown the appearance of immunoreactive 3-NT containing protein 24 h following reperfusion [19]. In the present study, a highly specific and quantifiable method was used to detect 3-NT. These data provide the first evidence that the generation of $ONOO^-$ precedes the development of renal injury and failure. $ONOO^-$ is a potent and versatile oxidant that can react with cellular lipids, proteins, and DNA [50]. Nevertheless, 3-NT-modified proteins can affect protein function [51-53] and should be considered as potential contributors to renal cell injury. In this study, $ONOO^-$, which is a metabolite of NO increases, showed that reactive nitrogen species (NO) generated during renal ischemia contribute to the renal injury.

Conclusion

In conclusion, Oxidative stress is a deleterious process that can be an important mediator of damage to cell structures and consequently various disease states and ageing. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism. ROS/RNS are known to act as secondary messengers controlling various normal physiological functions of the organism and therefore the production of NO• by NOS and superoxide by NAD(P)H is tightly regulated by hormones, cytokines, and other mechanisms. It was concluded from the outcomes of present study that oxidative stress increases in renal ischemia along with the generation of reactive nitrogen species (NO) contributing to the renal injury.

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