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Research Article

Oxidative stress and Antioxidant Enzyme levels In Hypertensive Chronic Kidney Disease patients

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Abstract

Background: Hypertension is one of the most important factor associated with the progression of both diabetic and non diabetic CKD (chronic kidney disease). In the general population showed that hypertension is a strong independent risk factor for ESRD (end stage renal disease).

Methods: Total Antioxidant Capacity (TAC), Superoxide dismutase (SOD), Catalase, Malondialdehyde (MDA), serum urea, serum creatinine and serum uric acid were assayed in 241 subjects In which 78 CKD patients with hypertension, 72 CKD patients without hypertension and 91 healthy controls.

Results: In our study, we found statistical significantly significant decreased level ($p<0.001$) of Total Antioxidant Capacity (TAC), Superoxide dismutase (SOD), Catalase and significant increase level ($p<0.001$) of malondialdehyde (MDA) in CKD with and without hypertension and also found deranged renal functions.

Conclusion: The reduced activities of antioxidant enzymes status and increased production of malondialdehyde in the hypertensive patients confirms the presence of oxidative stress The data suggest that alteration in antioxidant status and MDA in hypertensive CKD patients that binds support to role of oxidative stress in hypertensive patient.

Keywords: Chronic kidney disease (CKD), Reactive Oxygen Species (ROS), oxidative stress, Hypertension (HTN)

1. Introduction

Chronic kidney disease (CKD) is defined as persistent kidney damage accompanied by a reduction in the glomerular filtration rate (GFR) and the presence of albuminuria. CKD is a worldwide public health problem in both developed and developing country. There is increasing incidence and prevalence of renal failure with poor outcome and high cost and an even higher prevalence of earlier stage of CKD. The rise in incidence of CKD is attributed to an aging population and increases in hypertension (HTN), diabetes, and obesity within the U.S. population. HTN has been reported to occur in 85% to 95% of patients with CKD (stages 3-5).[1] The relationship between HTN and CKD is cyclic in nature. Uncontrolled HTN is a risk factor for developing CKD, is associated with a more rapid progression of CKD, and is the second leading cause of ESRD in the U.S.[2] Meanwhile, progressive renal disease can exacerbate uncontrolled HTN due to volume expansion and increased systemic vascular resistance. Multiple guidelines discuss the importance of lowering blood pressure (BP) to slow the progression of renal disease and reduce cardiovascular morbidity and mortality.[3] However, in order to achieve and maintain adequate BP control, most patients with CKD require combinations of antihypertensive agents; often up to three or four medication classes may need to be employed.[4]

There is accumulating evidence that oxidative stress plays a role in the progression of hypertension.[5] Oxidative stress occurs when there is an imbalance between the generation of reactive species (ROS) and the antioxidant defence system so that the latter become overwhelmed.[6] Hypertension effects of oxidative stress are most due to endothelial dysfunction resulting from disturbances of vasodilator systems, particularly degradation of nitric oxide (NO) by oxygen-free radicals.[7] Oxidative stress raises blood pressure by promoting functional NO deficiency (through NO inactivation and tetrahydrobiopterin depletion) and by augmenting arachidonic acid oxidation and formation of vasoconstrictive prostaglandin $F_{2\alpha}$. ROS producing enzymes involved in increased oxidative stress within vascular tissue include NADPH oxidase, xanthine oxidase, and mitrochondrial superoxide producing enzymes. Superoxide produced by the NADPH oxidase may react with NO, thereby stimulating the production of the NO/superoxide reaction product peroxynitrite.

This study was therefore designed to assess the effect of some antioxidant enzymes as well as degree of oxidative stress in Hypertensive CKD patients.

2. Material and Methods

The study was conducted in Department of Biochemistry at SAIMS medical college and hospital, Indore MP. Study was approved by the Ethical committee of the institute. Informed consent was obtained from all patients. The study population comprised 91 control (Group 1), 78 CKD with hypertension (group 2nd) and 72 CKD without hypertension (group 3rd), who were consecutively recruited from the nephrology clinic of the hospital between 01 September 2013 to 11 July 2014.

The study was conducted in 241 human subjects. The CKD patients with and without diagnosed by department of nephrology in SAIMS, hospitals were included in this research work by their consent. A structured questionnaire regarding the demographic data such as age, sex, duration of Hypertension, height and body weight were measured while wearing light weight clothing, but not shoes. Blood pressure, smoking habit, family history of diabetes, renal disease and hypertension was recorded for each patient. Hypertensive patients suffering from any other medical problems were excluded from the study.

5 ml of blood sample was withdrawn from the anticubital vein following overnight fasting. The blood sample was collected in plain, fluoride and EDTA vacutainers. The blood sample was centrifuged for 15 min. at 3000 rpm at room temp. The serum was stored at 4 ºC for

biochemical investigations. Urea, Creatinine and uric acid were estimated by enzymatic method. All biochemical investigation done by fully automated analyzer Hitachi 902.

Serum total antioxidant capacity (TAC) was estimated by the method of D Koracevic and G Koracevic (2001).[8] Serum super oxide dismutase (SOD) activity was estimated by the method of Marklund and Marklund (1988).[9] Serum catalase activity was assayed by the method of Aebi (1984).[10] Plasma Malondialdehyde (MDA) was estimated by Jean CD.[11] Correlation analysis was done by using SPSS version 17. Results were expressed as mean ± SD and were analyzed by unpaired student's t-test. The level of significant was set as $p < 0.001$: significant and $p > 0.05$: non-significant.

3. Result

Table 1 shows serum urea, serum creatinine and serum uric acid concentrations. All these levels of Group 2nd (CKD with hypertension) were significantly raised ($p < 0.001$) as compared to control. All these levels of group 3rd (CKD without hypertension) significantly raised ($p < 0.001$) as compared to control but compare to group 2nd serum urea and serum creatinine and serum uric acid level were lower. The data clearly indicates the increased risk of kidney dysfunction in patients suffering from CKD with hypertension.

Table 2 shows demographic data hypertensive CKD patients. Body mass indexes (BMI) of Chronic Kidney Disease (CKD) patients without hypertension were found significantly increased from control. BMI of group 2nd were similar to control and no significant change found. Group 2nd were on antihypertensive treatment and their blood pressure was significantly higher compare to control and group 3rd was showed higher blood pressure compare to control.

Table 3 showed antioxidant profile of CKD with and without hypertension. Group 2nd and group 3rd showed significantly low level ($p < 0.001$) of total antioxidant capacity, SOD and Catalase activity and significantly high ($p < 0.001$) level of MDA in comparison to control subjects. CKD without hypertension (group 3rd) showed higher activity of SOD, Catalase, MDA and lower level of TAC compare to group 2nd.

Table 1: Renal profile of hypertensive patients of CKD (Mean ± SD)

Parameter	Control (n = 91)	CKD with HTN (n = 78)	*p* value	CKD without HTN (n = 72)	*p* value
Serum Urea (mg/dl)	24.6±6	111.7±35.9	<0.001	98.9±32.6	<0.001
Serum Creatinine (mg/dl)	0.8±o.2	5.9±2.1	<0.001	5.7±2.3	<0.001
Serum Uric Acid (mg/dl)	5.1±2.1	7.1±1.9	<0.001	6.6±1.8	<0.001

p<0.001 significantly raised activity. CKD: chronic kidney disease. Hypertension: HTN.

Table 2: Demographic data of hypertensive CKD patients and controls (Mean± SD)

Parameter	Control (n = 91)	CKD with HTN (n = 78)	*p* value	CKD without HTN (n = 72)	*p* value
Age	40±9	48±8	NS	44±5	NS
Male : Female	51:40	43:35	NS	30:42	NS
BMI (kg/m^2)	21±1.7	21.5±2.7	NS	22.4±1.9**	<0.001
SBP (mm/hg)	123.2±5.2	156.1±4.2**	<0.001	134±3.1	NS
DBP (mm/hg)	79.2±4.4	95.7±2.0**	<0.001	84.7±2.4	NS

p<0.001 significantly raised activity. NS: Non significant. CKD: chronic kidney disease. Hypertension: HTN.

Table 3: Increase oxidative stress and renal dysfunction in hypertensive chronic kidney disease patients (Mean± SD)

Parameter	Control (n = 91)	CKD with HTN (n = 78)	*p* value	CKD without HTN (n = 72)	*p* value
TAC (mmol/L)	2.4±0.3	1.7±0.2	<0.001	1.5±0.3	<0.001
SOD activity (Units/gmHb)	6.0±1.1	3.3±0.4	<0.001	3.5±0.4	<0.001
Catalase activity (Units/gmHb)	7.2±0.9	4.1±0.5	<0.001	5.1±0.6	<0.001
Serum MDA (nmol/mL)	1.5±0.2	3.7±0.3	<0.001	3.9±0.4	<0.001

p<0.001 significantly raised activity. CKD: chronic kidney disease, Hypertension: HTN.

4. Discussion

In the present study we observed a strong association between blood pressure and some oxidative stress-related parameters. The present findings demonstrate a strong significant decrease ($p<0.001$) in Total antioxidant capacity (TAC), superoxide dismutase (SOD) and Catalase as compared to the control subjects. However malondialdehyde (MDA) levels were found significantly increased ($p<0.001$) as compared to controls (Table 3). The increased oxidative stress levels that we observed in hypertensive CKD patient are consistent with the finding of several previous studies.[12] In the our study, hypertensive subjects showed an impairment of the antioxidant defense system as assessed by a diminution of plasma and erythrocyte antioxidant status and this is in agreement with previous data.[13]

It is well documented that exposure to ROS increases the expression of antioxidant enzymes.[14] It may be that the compensatory mechanism is triggered in most hypertensive CKD patients in response to their ROS levels, which were shown to be elevated in the hypertension in the present study compared to normal subjects without hypertension. Increased ROS production results in reduction in the endothelium dependent vasodilation of the vascular smooth muscle cells of hypertensive patients.[15] It is also likely that elevations of blood pressure could also contribute to the increase of ROS, thereby enhancing the mechanism of ROS-mediated hypertension.

In the present study, there is significant decrease in superoxide dismutase and significant increase of malondialdehyde levels ($p<0.001$) (Table-3) in hypertensive CKD subjects. There was significant decrease in the activity of superoxide dismutase in the study group, indicating that either the scavenging system has been consumed during CKD or is suppressed. The major reason for decreased superoxide dismutase activity is the glycosylation of superoxide dismutase which has been shown to lead to enzyme inactivation[11,16]. Compromised antioxidant functions result in the well known cascade of hypoxic ischemic injury, inflammation, apoptosis and cell death.[17]

The significant decrease activity of Catalase in present study agreement with previous studies found a low antioxidant enzyme activity[11,18] and a negative correlation of Catalase activity with both day time SBP and DBP in hypertensive CKD patients.[19] Some other study found elevated level of Catalase and possible explanation for this is that rise in Catalase activity in these groups could be a compensatory mechanism by the body to prevent tissue damage by the increased free radicals as it was not supported by a corresponding increase in the activities of other antioxidant enzymes.[20] It is believed that modification of low density lipoprotein (LDL) increases its atherogenicity.[21] Amirkhizi *et al* reported that activities of erythrocyte antioxidant enzymes decrease in pre-hypertensive and hypertensive women, which may eventually lead to atherosclerosis and other high blood pressure related health problem.[22]

An explanation for the observed reduction in TAC among the hypertensive CKD patients could be due to the presence of high amount of free radicals and other oxygen derived (superoxide) species. Short lived free superoxide and nitric oxide have been shown to react chemically to form highly reactive free radicals such as peroxynitrite that triggers the depletion of plasma antioxidants and increases lipid peroxidation.[11] The reduced activities of antioxidant enzymes status and increased production of malondialdehyde in the hypertensive patients confirms the presence of oxidative stress. Possible explanation production of ROS in hypertension increases peroxidation of cellular membrane lipid as well as increasing the yield protein carbonyl derivatives, producing high level of MDA in the hypertensive CKD subjects which is a suggestive feature of oxidative stress in hypertension. Our results are also consistent with the previous study.[11]

This preliminary investigation shows that antioxidant status is modulated through changes in antioxidant enzymatic activity in hypertensive CKD patients and data provide evidence of blood pressure modulation by measurable oxidative stress–related parameters. Accordingly, antioxidant might one day be considered as a novel therapeutic target for the therapy of hypertensive CKD patients.

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