

Association of Antioxidant Enzymes and MDA level in Diabetic Nephropathy Patients in Indore Region of Madhya Pradesh

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(Received: 18 February 2014; accepted: 21 April 2014)

Diabetic Nephropathy is a microvascular complication of diabetes, representing the leading cause of end stage renal disease in the world, and a major cause of morbidity and mortality in type 2 diabetic subjects. The aim of the study was to evaluate the effect of antioxidant enzymes on Diabetic Nephropathy patients. Superoxide dismutase (SOD), Catalase, Malondialdehyde (MDA), fasting blood sugar, serum urea, serum creatinine and serum uric acid were assayed in 160 subjects In which 71 Diabetic Nephropathy patients, 36 Nephropathy patients without diabetes and 53 healthy control. In our study, we found statistical significantly decrease SOD, Catalase level and increase production of MDA in DM-CKD and also found deranged renal function. Reduced activity of serum antioxidant enzymes and increased level of MDA were observed in Diabetic Nephropathy patients compare to healthy control.

Key words: Diabetes mellitus (DM), Chronic kidney disease (CKD), Oxidative stress, Urea, Creatinine, Uric acid.

Diabetic nephropathy is the most common cause of microvascular chronic complication of type 2 diabetes mellitus which is associated with considerable morbidity and mortality, finally leading to end-stage renal disease¹ Diabetic nephropathy is a progressive disease that takes several years to develop. Glomerular hyperfiltration and increased excretion of urinary albumin (microalbuminuria) are early manifestations of diabetic nephropathy. It also involves various functional clinical abnormalities of the kidney such as elevated creatinine, urea, albuminuria, decline glomerular filtration rate, elevated arterial blood pressure, and fluid retention^{2,3}. The pathogenesis of diabetic nephropathy is likely to be

multifactorial: it strongly dependent on the duration of diabetes; other risk factors include oxidative stress induced poor glycemic control, hypertension, hypertriglyceridemia, with production of cytokines IL-6 and Tnf- α causing inflammation responsible for endothelial dysfunction⁴.

According to the World Health Organization (WHO), the prevalence of diabetes for all age-groups Worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030⁵. Estimation of the prevalence of earlier stages of chronic kidney disease (CKD) in the US population and ascertainment of trends over time is central to disease management and prevention planning, particularly given the increased prevalence of obesity and diabetes⁶. Oxidative stress has been defined as a loss of balance between reactive oxygen species (ROS) and protective antioxidant defense system⁷. Increased oxidative stress induced by hyperglycemia may be due to multiple

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mechanisms (eg, the activation of polyol pathway, inhibition of pentose phosphate pathway, mitochondria dysfunction, activation of NAD(P)H oxidase, and uncoupling of endothelial NO synthase [eNOS], as well as impairment of antioxidant defense system^{8,9}. The oxidative stress generated by hyperglycemia increases reactive oxygen species (ROS), which leads to the activation of various redox-sensitive cell signalling molecules and the production of cytotoxic materials. This is followed by cellular dysfunction and damage, and ultimately results in diabetic micro and macrovascular complications^{10,11}.

This study was therefore designed to assess the effect of some antioxidant enzymes as well as lipid peroxides and relate diabetic nephropathy to nephropathy without diabetes and diabetes subjects.

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 71 diabetic nephropathy patients who were consecutively recruited from the nephrology clinic of the hospital between 1 September 2013 to 30 May 2014.

The study was conducted in 160 human subjects with and without diabetic nephropathy patients. The diabetic nephropathy patients 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 diabetes, 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. Diabetic patients suffering from any other medical problems were excluded from the study.

5 ml of blood sample was withdrawn from the antecubital 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 °C for biochemical investigations. Fasting blood sugar level was estimated by GOD-POD method. Urea, Creatinine and uric acid were estimated by enzymatic method. All biochemical investigation done by fully automated analyzer Hitachi 902.

Serum super oxide dismutase (SOD) activity was estimated by the method of Marklund and Marklund (1988)¹². Serum catalase activity was assayed by the method of Aebi (1984) (13). Plasma Malondialdehyde (MDA) was estimated by Jean CD¹⁴. Correlation analysis was done by using SPSS version 16. Results were expressed as mean \pm SD and were analyzed by unpaired student's t-test. Values of $p < 0.01$ were considered significantly.

RESULTS

Table 1 shows serum urea, serum creatinine and serum uric acid concentrations. In group 2nd, mean serum urea levels were 91.8 ± 21.4 , serum creatinine levels were 3.7 ± 1.4 and serum uric acid level 8.5 ± 2.6 . All these were significantly raised ($p < 0.01$) as compared to control. Group 3rd CKD patients without DM, mean serum urea levels were 97.1 ± 24.9 , serum creatinine levels were 5.7 ± 2.4 and serum uric acid level were 7.3 ± 2.2 . All

Table 1. Increased level of serum urea, serum creatinine and serum uric acid of Diabetic nephropathy patients. (mean \pm SD)

Subjects	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)	Serum Uric Acid (mg/dl)
1.Control(n = 53)	26.6 \pm 5.87	0.8 \pm 0.2	4.6 \pm 1.2
2.DM-CKD (n = 71)	91.8 \pm 21.4*	3.7 \pm 1.4*	8.5 \pm 2.6*
3.Non DM-CKD (n = 36)	97.1 \pm 24.9*	5.7 \pm 2.4*	7.3 \pm 2.2*

* $p < 0.01$ significantly raised activity. DM: diabetes malitus: CKD: chronic kidney disease

Table 2. Demographic data and fasting blood glucose level of Diabetic nephropathy patients (with and without Diabetes mellitus dysfunction) and controls. (mean± SD)

Subjects	Age	Male : Female	BMI (kg/m ²)	Blood Pressure (mm/hg)	Glucose (mg/dl)
1. Control(n = 53)	46±4	28 : 25	27.2 ± 1.7	<140/90	85.2± 6.2
2. DM-CKD (n = 71)	57±8	42 : 29	22.3 ± 2.1 *	>140/90	190± 8.2 *
3. Non DM-CKD (n = 36)	44±4	15 : 21	22.4 ± 2.4 *	>140/90	87.7 ± 8.9

* p<0.01 significantly raised activity. DM: diabetes mellitus; CKD: chronic kidney disease

Table 3. Increase oxidative stress and renal dysfunction in patients suffering from diabetic nephropathy. (mean± SD)

Parameter	SOD activity (Units/gmHb)	Catalase activity (Units/gmHb)	Serum MDA (nmol/ml)
1. Control(n = 53)	6.1±1	7.1±0.9	1.5±0.2
2. DM-CKD (n = 71)	3.1±0.6*	4.1± 0.5*	5.1±0.4*
2. Non DM-CKD (n = 36)	3.5±0.4*	5.2±0.6*	3.8±0.2*

* p<0.01 significantly raised activity. DM: diabetes mellitus; CKD: chronic kidney disease.

these these levels of group 3rd significantly raised ($p < 0.01$) as compared to control but compare to group 2nd serum urea and serum creatinine level were higher and serum uric acid level was lower. The data clearly indicates the increased risk of kidney dysfunction in patients suffering from diabetes mellitus.

Table 2 shows demographic data and fasting blood glucose level of diabetic nephropathy patients. Body mass index (BMI) of Chronic Kidney Disease (CKD) patients with or without diabetes were found significantly increased from control. Majority of the patients group 2nd and group 3rd were on antihypertensive treatment and their blood group were higher to control. Blood glucose level of group 2nd was significantly higher to control ($p < 0.01$).

Table 3 showed antioxidant profile of CKD with and without diabetes mellitus. Group 2nd and group 3rd showed significantly low level ($p < 0.01$) of SOD and Catalase activity and significantly high ($p < 0.01$) level of MDA in comparison to normal subjects. CKD without diabetes mellitus group showed higher activity of SOD, Catalase and lower level of MDA compare from group 2nd.

DISCUSSION

In our study oxidative stress and impairment of renal function was studied by assessing kidney function tests and the results suggested that renal functions were profoundly deranged in patients of non DM-CKD as compared to DM-CKD. However serum uric acid was higher in patients of DM-CKD than non DM-CKD despite higher levels of serum urea and serum creatinine in non DM-CKD.

In this study, Non DM-CKD patients were relatively younger than DM-CKD patients. This slight disparity is due to the fact that patients of DM were enrolled only if he/she did not show any evidence of micro-albuminuria which is an early marker of renal injury in DN, despite having DM for at least 10 years, so the patients of DM were relatively older. Sex matched Healthy Control and patients were recruited in the present study and there was no significant difference between study groups with respect to sex distribution pattern. Blood pressure was higher in patients of both group 2nd and 3rd as compared to control. It is well known that hypertension is the major cause of non diabetic CKD¹⁵. Our study shows that there may be poor blood glucose control as reflected by higher fasting

plasma glucose in patients with DM-CKD in compared to patients of non DM-CKD.

ROS produced in hyperglycemia increases peroxidation of cellular membrane lipids as well as increasing the oxidation of proteins that yield protein carbonyl derivatives, producing high level of MDA in the diabetic nephropathy subjects which is a suggestive feature of oxidative stress in long standing type-2 diabetes. Our results are also consistent with the study reported by Cvetkovic *et al*, 2009^{16, 17, 18}.

Renin angiotensin system activation results in increased, all which stimulates nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases¹⁹. Thresulting NADPH/NADH suppresses superoxide dismutase and increases reactive oxygen species. In the present study too, a significant decrease in serum SOD and catalase activity was observed in CKD patients irrespective of whether they were having renal dysfunction or not. Compromised antioxidant functions result in the well known cascade of hypoxic ischemic injury, inflammation, apoptosis and cell death¹⁹.

Since it was an observational study and not a comparative one, hence the robustness of blood urea, serum creatinine and uric acid over other new biomarkers cannot be questioned. The results obtained from the present study is only a small representation of the population actually suffering from CKD, hence more and more data is required to evaluate the role of oxidative stress in diagnosis and management of renal disorder. A follow-up study aiming at investigating the Cystatin C levels in a healthy population would be beneficial to have a better idea of its variations during a course of acquired CKD.

ACKNOWLEDGEMENTS

Authors are thankful to colleague and the patients for their valuable support and cooperation.

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