ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

https://ijrsms.com

International Journal of Recent Surgical and Medical Sciences

Article in Press

Original Article

Erythrocyte Superoxide Dismutase as a Marker for Nephropathy in Type 2 Diabetes Mellitus: A Pilot Study

Shilpa Bhardwaj[1], Kamna Singh[2], Ritu Singh[3]

[1]Department of Neurochemistry, Institute of Human Behaviour and Allied Sciences, Delhi, [2]Department of Biochemistry, Sarojini Naidu Medical College, Agra, UP, [3]Department of Biochemistry, Lady Hardinge Medical College, Delhi, India.

***Corresponding author:**
Dr. Shilpa Bhardwaj,
Department of Neurochemistry,
Institute of Human Behaviour
and Allied Sciences, Delhi,
India.

drshilpa2001@gmail.com

Received: 15 April 2024

Accepted: 03 July 2024

EPub Ahead of Print:
20 December 2024

Published: *****

DOI
10.25259/IJRSMS_23_2024

Quick Response Code:

ABSTRACT

Objectives: Hyperglycemia, characterized by elevated blood glucose levels, is a consequent upregulation in the generation of reactive oxygen species (ROS). These ROS possess the capacity to induce deleterious modifications in endothelial function. Superoxide dismutase (SOD), an enzymatic antioxidant, stands as the primary scavenger of superoxide, a specific type of ROS, within the extracellular space. This study aims to elucidate the association between the levels of erythrocyte SOD and routinely measured renal function parameters (serum urea and serum creatinine) in type 2 diabetes mellitus (T2DM) patients with nephropathy.

Material and Methods: Diagnosed patients of T2DM were enrolled in the study conducted at a tertiary care hospital in Delhi, India (n = 120). Erythrocyte SOD concentrations (expressed as units/g of hemoglobin) were determined in 60 T2DM patients with nephropathy (cases) and 60 type 2 diabetes patients without any complications (controls) using a commercially available colorimetric assay kit on an automated analyzer. Serum urea and creatinine levels were measured spectrophotometrically on a clinical chemistry automated analyzer. Data were analyzed using SPSS Statistics (v.20) for statistical inference.

Results: We observed significantly lower activity of erythrocyte SOD with mean levels of 183 ± 0.78 units/g hemoglobin in subjects with DM nephropathy compared with mean levels of 186 ± 0.95 units/g hemoglobin in the control subjects ($P = 0.03$). Our analysis revealed a statistically significant negative association (p-value = 0.02) between erythrocyte SOD activity and serum creatinine levels in DM patients diagnosed with nephropathy.

Conclusion: Our findings suggest that lower levels of erythrocyte SOD are associated with nephropathy in T2DM.

Keywords: Diabetes mellitus, Nephropathy, Superoxide dismutase

INTRODUCTION

Diabetes mellitus (DM) represents a chronic, multifactorial metabolic disorder characterized by persistent elevations in blood glucose (hyperglycemia). The globally rising epidemic of DM has not spared the developing countries, with approximately 70 million people expected to be affected by 2025.[1] Oxidative stress is a well-known precursor of progressive vascular damage and the eventual complications seen in DM. Endothelial dysfunction, characterized by an imbalance between the markers of oxidative stress and various antioxidants, plays a crucial role in initiating vascular injury and inflammation in diabetes. This chronic vascular insult results in progressive damage to kidneys, and once nephropathy ensues, measures can only be taken to halt its progression. Currently, DM nephropathy represents the predominant aetiology contributing

to both morbidity and mortality in the context of chronic diabetic complications. Several prospective studies have established a link between high blood glucose levels and cardiovascular morbidity in diabetes.[2] Several mechanisms have been postulated for hyperglycemia-mediated damage, such as cytotoxicity, enhanced extracellular matrix production, vascular dysfunction, and oxidative stress.[3] Persistent hyperglycemia due to defective insulin secretion or insulin insensitivity leads to the generation of ROS species from glucose autoxidation and protein glycosylation.[4] Free radicals generated during normal metabolism are scavenged by endogenous enzymatic antioxidants like superoxide dismutase (SOD). In DM, an excess of free radicals is generated, or their destruction by radical scavenging systems is reduced. This imbalance results in vascular injury and predisposes to various complications like DM nephropathy. Recent studies have suggested that elevated blood glucose levels trigger the increased production of superoxide anions within the mitochondrial electron transport chain (ETC). It is the prime step in the pathogenesis of hyperglycemia-induced complications in DM.[5] Superoxide mediates further damage via activation of other pathways, resulting in the production of peroxynitrites, which are even stronger oxidants.[6] These free radicals induce DNA damage to modify the expression of glucose transporter type 4 (GLUT4) in adipocytes and muscle, impair the production and secretion of insulin from β cells in the pancreas, and induce endothelial dysfunction.[7]

SOD is a ubiquitous enzymatic class with the function of catalyzing the dismutation of superoxide anions. In eukaryotic cells, there is a spatial distribution of SOD isoforms: CuZnSOD is predominantly localized within the cytosol, while MnSOD is found primarily within the mitochondrial matrix. The enzymatic action of SOD degrades superoxide ions into peroxides, which are rapidly broken down into water and oxygen by other enzymes. A functional polymorphism in the SOD gene has been linked with type 2 DM in Japanese and Korean populations.[8,9] This investigation aimed to elucidate a potential association of erythrocyte SOD with type 2 DM nephropathy in the North Indian population.

MATERIAL AND METHODS

Selection of subjects

This hospital-based case-control study was conducted at a tertiary care hospital in Delhi by recruiting subjects from among the patients attending the diabetes clinic at a tertiary care hospital in Delhi, India. The case group was formed of 60 patients (age group, 40–70 years old) diagnosed with type 2 diabetes mellitus with nephropathy (based on American Diabetes Association, 2011 criteria). In the context of type 2 DM, nephropathy is diagnosed by either persistent albuminuria exceeding 300 mg/day or 200 μg/min measured on at least two separate occasions three to six months apart or a progressive reduction in glomerular filtration rate (GFR). The control group was comprised of type 2 DM patients without any microvascular or macrovascular complications (n = 60). None of the patients were on insulin treatment.

Exclusion criteria

Pregnant and nursing women and subjects with dehydration; muscular dystrophy; rhabdomyolysis; glomerulonephritis; pyelonephritis; urinary tract infections; severe neurological, endocrinological, dermatological, acute or chronic debilitating illness; end-stage renal disease, or on dialysis were screened out.

Informed written consent was obtained from all participants, and data was obtained using a standard questionnaire prepared with information on age, sex, duration and family history of DM. Anthropometric indicators like blood pressure and body mass index (kg/mt^2) were measured in the morning after taking fasting venous samples. All biochemical analyses were carried out in the clinical biochemistry laboratory of a tertiary care hospital on automated analyzers using standard kits and protocols. All analyses were run in duplicates, and average values were used.

Sample collection

A 5-mL fasting venous sample was collected under sterile conditions from the median cubital vein and immediately processed for separation of plasma (NaF and EDTA vacutainer) for the estimation of glucose and serum (no anticoagulant vacutainer) for urea and creatinine on an automated analyzer (CX series; Beckman Coulter, Inc., Brea, California, USA). Whole blood lysate was employed for the quantification of erythrocytic SOD activity on an automated analyzer platform.

Biochemical assays

Fasting plasma glucose

The concentration of glucose in plasma was determined using a commercial enzymatic assay kit employing the glucose oxidase-peroxidase (GOD-POD) method. 5μL of samples and standard solution (100 mg/dL) were incubated with a reaction mixture for 5 minutes at 37 °C. The reaction mixture comprised 100 mM phosphate buffer at pH 7.5, supplemented with 5 mM phenol, 10 U/mL glucose oxidase, 1 U/mL peroxidase and 0.4 mM 4-aminoantipyrine. Following incubation, a spectrophotometric analysis was performed on the solution at 505 nm using an automated instrument to quantify absorption.

Serum urea estimation

Urease-GLDH, a kinetic UV-based spectroscopic technique was employed for measuring serum urea levels. A working reagent of urea was prepared by dissolving 1 tablet of reagent R.2 (urease 3750 U/L, glutamate dehydrogenase 6000 U/L, NADH 0.32 mmol/L) in 20 mL of R.1 (Tris pH 7.8, α- ketoglutarate). Ten microliters of sample and standard (50 mg/dL) were incubated at 37°C with a working reagent. Absorbance was read after 30 seconds (A1) and 90 seconds (A2) at 340 nm.

Serum creatinine determination

The alkaline picrate kinetic method was used for measuring levels of serum creatinine. A working solution of creatinine reagent solution was prepared by combining equal volumes of solution R1a (picric acid, 35 mmol/L) and R1b (sodium hydroxide, 0.32 mol/L). Ten microlitres of samples and standards were incubated with 100 µL of working reagent, and absorbance was recorded at 490 nm.

SOD; E.C.1.15.1.1 activity estimation

After carefully sampling erythrocytes from the bottom of the tubes, washing was done thrice with isotonic saline solution. Double-distilled water containing 5 mL/L Triton X-100 was added to lyse the erythrocytes and stored on ice for 10 minutes following vortex mixing. SOD activity in erythrocytes was quantified using commercially available RANSOD kits (cat. No. SD 125; Randox Labs., Crumlin, Northern Ireland). This assay utilizes xanthine oxidase, which generates superoxide radicals by acting on its substrate, xanthine. The superoxide radicals then interact with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT), leading to the formation of a red-coloured formazan dye. SOD activity is quantified by its capacity to inhibit the rate of a specific reaction. Absorbance was measured at 505 nm on an automated analyzer. SOD activity is quantified in whole blood (initial state) as units per milliliter. One unit signifies the enzyme quantity necessary to achieve a 50% reduction in formazan formation rate. This was then converted to units per gram of hemoglobin (specific activity). A standard curve was plotted using standards by serial dilutions of a commercially available SOD calibrator along with the kit.

Statistical analyses

This study utilized SPSS Statistics version 20 (SPSS, Inc., Chicago, IL) for data analysis. Data are presented as mean ± SEM. Independent t-tests assessed intergroup differences in quantitative measures. Pearson correlation coefficients assessed the strength and direction of the linear relationship between study variables. Regression analysis was conducted to identify the variable with the most significant predictive power for nephropathy in type 2 DM. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Based on the presence of diabetic nephropathy, the study population was categorized into two distinct groups for further analysis. The case group comprised individuals diagnosed with T2DM and concomitant nephropathy (n = 60). The control group consisted of T2DM patients without any complications (n = 60). Demographic and disease-related characteristics of the participants at baseline are presented in Table 1 to facilitate group comparisons. Statistical analysis revealed no significant discrepancies between the groups' mean age and diabetes duration. Biochemical analysis results have been depicted in Table 1. Fasting plasma glucose levels were not markedly different between the two groups, even though the values were on the higher side in cases compared to controls (P = 0.07). Serum urea (P = 0.02) and serum creatinine (P = 0.002) levels were significantly different with mean levels higher in cases compared to controls. Erythrocyte SOD activity, measured in units per gram of hemoglobin, was significantly lower in subjects with type 2 DM and nephropathy compared to those with type 2 DM alone (p-value = 0.03). Correlation analysis revealed a statistically significant negative association between serum creatinine concentration and SOD (SOD) activity. The coefficient of –0.337 suggests a moderate negative relationship, indicating that higher levels of creatinine are associated with lower SOD activity. This finding was statistically significant at a p-value less than ($p < 0.05$), as detailed in Table 2. Multivariate regression analysis using SOD as a dependent variable showed its significant predictability for DM nephropathy with variations in serum creatinine (P = 0.01), as shown in Table 3.

Table 1: Baseline and biochemical characteristics of study groups.

Characteristics	Controls mean ± SEM	Cases mean ± SEM	*P* value
Age (years)	58.42 ± 1.12	58.96 ± 1.02	0.14
Duration of diabetes (years)	5.82 ± 0.64	5.94 ± 0.56	0.12
Fasting plasma glucose (mg/dL)	126 ± 2.64	132 ± 3.2	0.07
Serum urea (mg/dL)	26 ± 1.0	31.2 ± 1.8	0.02*
Serum creatinine (mg/dL)	0.9 ± 0.02	1.1 ± 0.05	0.002*
Erythrocyte SOD activity (units/g hemoglobin)	186 ± 0.78	183 ± 0.95	0.03*

SOD: Superoxide dismutase, SEM: Standard error of mean
* *P* value significant at <0.05

Table 2: Correlation analysis between superoxide dismutase and renal function parameters.

Parameter	Pearson coefficient "r"	*P* value
Serum urea	−0.07	0.64
Serum creatinine	−0.337	0.02*

**P* value significant at <0.05

Table 3: Regression analysis with superoxide dismutase as dependent variable.

Parameter	Beta value (standardized coefficient)	*P* value	95% Confidence Interval
Serum urea	0.174	0.28	−0.08 to 0.259
Serum creatinine	−0.433	0.01*	−13.5 to −1.9

**P* value significant at <0.05

DISCUSSION

Nephropathy is a common and debilitating complication of type 2 DM, which drastically impairs quality of life. Once diagnosed, its progress is difficult to control, and a complete cure is hard to achieve, but in developing stages, its progression can be controlled. We conducted this study to establish the association of erythrocyte SOD with type 2 DM nephropathy. We tried to evaluate the role of erythrocytic SOD as an early biomarker in a scenario where urinary markers are presently considered superior to serum markers for diagnosing and monitoring the progression of the disease, keeping in mind that the feasibility of procuring an ideal urine sample for analysis has more limitations, including refrigeration, contamination, and cumbersome collection, as compared to a blood sample.[10]

This investigation focused on T2DM patients with nephropathy who had significantly lower levels of erythrocyte SOD activity as compared to their respective controls ($P = 0.03$). Our results were comparable to the previous studies, which also reported decreased SOD activity in DM nephropathy.[11] SOD scavenges the superoxide reactive oxygen species (ROS), which are formed in the mitochondria during prolonged hyperglycemia.[12] A long-term prospective study over 20 years showed that nephropathy with the fast track is associated with enhanced mitochondrial ROS production in DM patients as compared to slow-track nephropathy in DM.[13] Shen *et al.*[13] suggested a protective role of increased expression of SOD in reducing DM cardiomyopathy.[14] A recent study showed decreased activity of renal SOD in the pathogenesis of nephropathy in mouse models with diabetes.[15]

We also observed significantly higher levels of biochemical indicators of renal function (i.e., serum urea [$P = 0.02$] and creatinine [$P = 0.002$]) in DM patients with nephropathy as compared to controls. Idonije *et al.* reported similar findings in type 2 DM patients with nephropathy.[15] These two serum metabolites have been used as predictors of nephropathy in diabetic patients as they are excreted through the kidneys. Serum creatinine is considered a better marker as it is largely excreted and not reabsorbed by the kidneys and it is not affected by diet, age and other factors that affect serum urea levels. Creatinine clearance is used as an estimate of the glomerular filtration rate, which declines as renal function is impaired in chronic kidney disease. We observed that serum creatinine levels were more significantly associated with nephropathy as compared to serum urea. Siddiqui K reported the use of serum creatinine as a marker for progression of DM nephropathy.[16]

The levels of plasma glucose in the two groups were not significantly different, but the DM patients with nephropathy had higher levels of plasma glucose as compared to DM patients with no complications ($P = 0.07$). Chronic or uncontrolled hyperglycemia contributes to complications in DM through five major pathways in which the overproduction of reactive oxygen species forms the primary event.[17-19]

In our study, Pearson's correlation analysis demonstrated a statistically significant negative association between serum creatinine levels and SOD activity within the case group ($p = 0.02$). This rise in serum creatinine suggests progressive renal damage and decreased glomerular mass, which correlates with the decreased activity of SOD. Studies have suggested that downregulation of SOD in DM patients with nephropathy can be mediated by TNF-α (Tumour necrosis factor- alpha) and other cytokines, thus mitigating the protective effect of SOD in oxidative stress in complicated DM.[20] We also found a negative correlation between serum urea and SOD activity in DM patients with nephropathy, even though it did not reach statistically significant levels.

Multiple regression analysis showed that serum SOD activity (dependent variable) changed significantly with slight changes in serum creatinine levels (independent variable) with β value/standardized coefficient value of −0.433 significant at $P = 0.01$, keeping the other independent variable, serum urea, as a constant. No significant change was observed in SOD activity variations with serum urea when creatinine was kept as a constant variable ($P > 0.05$). The rate of change of the conditional mean of serum SOD activity with respect to serum creatinine was expected to be between 1.9 and 13.5 (95% CI) in the negative direction, suggesting a higher predictability of SOD for DM nephropathy.

The present study indicates that the levels of SOD decrease significantly in type 2 DM patients with nephropathy, suggestive of oxidative stress-mediated damage. Thus, the lower

erythrocyte SOD levels in patients with type 2 DM may contribute to the progression of renal complications.

Limitations of the study

In this study, we investigated the relationship of the superoxide scavenger, superoxide dismutase, with the most common cause of chronic renal disease, DM nephropathy. The limitations of the study were the confounding factors or other mechanisms which may play a role in the pathogenesis of complications in DM. Moreover, in this study, we could not correlate the levels of erythrocyte SOD with urinary markers (GFR and microalbumin or proteinuria), which are presently considered to be the earliest markers of nephropathy in DM and for which further studies are anticipated. Nevertheless, we could establish a role for SOD as a better predictor than routinely utilized serum indicators (urea and creatinine) in the pathogenesis of DM nephropathy, but for a cause-and-effect relationship, large-scale studies would be required.

CONCLUSION

This case-control study suggested that nephropathy is associated with reduced activity of erythrocyte SOD in type 2 diabetes mellitus due to hyperglycemia-mediated toxic effects, and therefore, the increased oxidative stress may lead to progression of the disease and its associated complications. Since erythrocytic SOD levels can be estimated on a routine basis in clinical labs using automated analyzers, it is an easy alternative to cumbersome urine-based investigations for early diagnosis of DM nephropathy. The role of free radicals cannot be underestimated in other causes of renal failure. As our study focused on the role of erythrocytic SOD in type 2 DM, similar work can be carried out in type 1 DM as hyperglycemia is a common feature. Moreover, inflammation has now been well recognized as one of the mechanisms for CAD and CVD which opens the platform for research of this biomarker to look at their predictability. Large-scale studies would be needed to establish more specific guidelines for this parameter. At present, the role of antioxidants in diabetic patients cannot be denied, and supplementation in the early stages may be beneficial in delaying the development of complications in type 2 diabetes mellitus.

Acknowledgement

The authors would like to thank the patients and the hospital staff, especially technicians, for their cooperation during the study.

Ethical approval

The Institutional Review Board has waived the ethical approval for this study.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation:

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of Type 2 Diabetes: Indian Scenario. Indian J Med Res 2007;125:217–30.
2. Zhang Y, Pan XF, Chen J, Xia L, Cao A, Zhang Y, *et al*. Combined Lifestyle Factors and Risk of Incident Type 2 Diabetes and Prognosis Among Individuals with Type 2 Diabetes: A Systematic Review and Meta-analysis of Prospective Cohort Studies. Diabetologia 2020;63:21–33.
3. Li H, Wang D, Chen Y, Yang M. β-Caryophyllene Inhibits High Glucose-induced Oxidative Stress, Inflammation and Extracellular Matrix Accumulation in Mesangial Cells. Int Immunopharmacol 2020;84:106556.
4. Kang GG, Francis N, Hill R, Waters D, Blanchard C, Santhakumar AB. Dietary Polyphenols and Gene Expression in Molecular Pathways Associated with Type 2 Diabetes Mellitus: A Review. Int J Mol Sci 2020;21:140.
5. Kumar V, Agrawal R, Pandey A, Kopf S, Hoeffgen M, Kaymak S, *et al*. Compromised DNA Repair is Responsible for Diabetes-Associated Fibrosis. EMBO J 2020;39:e103477.
6. Palma FR, He C, Danes JM, Paviani V, Coelho DR, Gantner BN, *et al*. Mitochondrial Superoxide Dismutase: What the Established, the Intriguing, and the Novel Reveal about a Key Cellular Redox Switch. Antioxid Redox Signal 2020;32:701–14.
7. Hussain T, Tan B, Murtaza G, Liu G, Rahu N, Kalhoro MS, *et al*. Flavonoids and Type 2 Diabetes: Evidence of Efficacy in Clinical and Animal Studies and Delivery Strategies to Enhance their Therapeutic Efficacy. Pharmacol Res 2020;152:104629.
8. Nomiyama T, Tanaka Y, Piao L, Nagasaka K, Sakai K, Ogihara T, *et al*. The Polymorphism of Manganese Superoxide Dismutase is Associated with Diabetic Nephropathy in Japanese Type 2 Diabetic Patients. J Hum Genet 2003;48:138–41.
9. Mistry KN, Dabhi BK, Joshi BB. Evaluation of Oxidative Stress Biomarkers and Inflammation in the Pathogenesis of Diabetes and Diabetic Nephropathy. *Indian J Biochem Biophys* 2020;57:45–50.

10. Lane C, Brown M, Dunsmuir W, Kelly J, Mangos G. Can Spot Urine Protein/Creatinine Ratio Replace 24 h Urine Protein in Usual Clinical Nephrology? Nephrol (Carlton) 2006;11: 245–49.
11. Kasai S, Shimizu S, Tatara Y, Mimura J, Itoh K. Regulation of Nrf2 by Mitochondrial Reactive Oxygen Species in Physiology and Pathology. Biomolecules 2020;10:320.
12. Huang C, Kim Y, Caramori ML, Moore JH, Rich SS, Mychaleckyj JC, *et al*. Diabetic Nephropathy is Associated with Gene Expression Levels of Oxidative Phosphorylation and Related Pathways. Diabetes 2006;55:1826–31.
13. Shen X, Zheng S, Metreveli NS, Epstein PN. Protection of Cardiac Mitochondria by Overexpression of MnSOD Reduces Diabetic Cardiomyopathy. Diabetes 2006;55:798–805.
14. Fujita H, Fujishima H, Chida S, Takahashi K, Qi Z, Kanetsuna Y, *et al*. Reduction of Renal Superoxide Dismutase in Progressive Diabetic Nephropathy. J Am Soc Nephrol 2009;20:1303–13.
15. Idonije BO, Festus O, Oluba OM. Plasma Glucose, Creatinine and Urea Levels in Type 2 Diabetic Patients Attending a Nigerian Teaching Hospital. Res J Med Sci 2011;5:1–3.
16. Siddiqui K, Joy SS, Nawaz SS, Alnaqeb D, Mujammami M, Al-Rubeaan K. Association of Urinary Non-albumin Protein with the Different Urinary Marker for Glomerular and Tubular Damage in Patients with Type 2 Diabetes. BMC Nephrology 2020;21:1–9.
17. Moussa, SA. Oxidative Stress in Diabetes Mellitus. Romanian J Biophys 2008;18:225–36.
18. Irazabal MV, Torres VE. Reactive oxygen species and redox Signalling in Chronic Kidney Disease. Cells 2020;9:1342.
19. Nasri M, Mahdavifard S, Babaeenezhad E, Adibhesami G, Nouryazdan N, Veiskarami S, *et al*. Ameliorative Effects of Histidine on Oxidative Stress, Tumor Necrosis Factor-alpha (TNF-α), and Renal Histological Alterations in Streptozotocin/ Nicotinamide-induced Type 2 Diabetic Rats. Iran J Basic Med Sc 2020;23:714–23.
20. Ebaid H, Bashandy SA, Abdel-Mageed AM, Al-Tamimi J, Hassan I, Alhazza IM. Folic Acid and Melatonin Mitigate Diabetic Nephropathy in Rats via Inhibition of Oxidative Stress. Nutr Metab (Lond) 2020;17:6.

How to cite this article: Bhardwaj S, Singh K, Singh R. Erythrocyte Superoxide Dismutase as a Marker for Nephropathy in Type 2 Diabetes Mellitus: A Pilot Study. Int J Recent Surg Med DOI: 10.25259/IJRS MS_23_2024