ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 7, Issue 5, 2014

ISSN - 0974-2441

Research Article

SERUM NO_X AND RED BLOOD CELLS LYSATE SOD LEVELS IN DIABETIC PATIENTS AND THEIR RELATION WITH DURATION OF DIABETES MELLITUS

RENUKA P*, NAVEEN CR

Department of Biochemistry, SRM Medical College Hospital & Research Centre, Kattankulathur, Kancheepuram - 603 203, Tamil Nadu, India. Email: rpangaluri@yahoo.com

Received: 16 August 2014, Revised and Accepted: 13 September 2014

ABSTRACT

Objective: Oxidative stress is one of the major causes for complications in diabetic patients. Oxidative stress might be due to an imbalance in elimination and production of free radicals. Nitric oxide (NO) is a reactive oxygen species (RNS) and a potent vasodilator. When NO reacts with superoxide anion, it forms RNS. Superoxide dismutase (SOD) is an antioxidant enzyme which eliminates O_2^-.

Methods: In the present study, 53 diabetic patients have been selected randomly and grouped into two groups based on the diabetic duration to evaluate the effect of duration on oxidative stress.

Results: Red blood cells lysate SOD (4.745±1.047) and serum NO (52.2±25.8) are increased in diabetic patients compared with healthy controls. However, when compared between groups of different diabetic duration, SOD is decreased whereas NO is increased.

Conclusion: Decrease in SOD activity might be due to the loss of its activity by glycation, whereas increase in NO might be due to the induction of endothelial NO synthase by glucose.

Keywords: Diabetes mellitus, Nitric oxide, Oxidative stress, Superoxide dismutase.

INTRODUCTION

Oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications [1-3]. High glucose levels produce reactive oxygen species (ROS), as a result of glucose auto-oxidation, metabolism and the development of advanced glycosylation end products. General scavenging mechanism includes an enzymatic elimination of free radicals by the activity of enzymes like glutathione reductase, catalase, superoxide dismutase (SOD) and glutathione peroxidase (POD). SOD is considered a primary enzyme since, it is involved in the direct elimination of ROS [4,5]. SOD is an important defense enzyme which catalyzes the dismutation of superoxide radicals. Nitric oxide (NO) is a highly reactive molecule generated enzymatically from L-arginine [6] and participates in many chemical reactions in the body. Even though, NO has important biological functions, its high activity leads to reactive nitrogen species, which cause damage to the body like ROS. NO is reported to be generated at a higher rate in oxidative stress conditions [7,8]. Similarly, SOD is decreased, which helps in the removal of free radicals. Hence, this study is undertaken to evaluate SOD and NO simultaneously in diabetic patients and their relation with duration of diabetes.

METHODS

Subjects

Fifty-three random patients have attended Diabetology Department, SRM Hospital and Research Center, and 26 age and sex matched healthy subjects were selected for the study. Patients who were taking statins and antioxidant drugs in any form were excluded from the study.

Consent was obtained from all the subjects before collecting the blood sample. The ethical clearance for the study was obtained from SRM University according to the norms of the university.

Methods

Fasting blood sample was collected. NO was estimated by Griess method after deproteinizing the sample with 96% ethanol. Fasting blood glucose was estimated by GOP-POD method and HbA1c by immunoturbidometry method using Beckhman Coulter AU 400 auto-analyzer.

Ethylenediamine tetraacetic acid blood sample was used for estimating SOD. SOD was estimated using RanDox kit following the procedure given in the kit.

Student's t-test is used to compare the groups. All statistical analysis was performed using IBM SPSS statistics for Windows, Version 20.0 (IBM Corp. Released 2011).

RESULTS

Serum NO_x and red blood cells (RBC) SOD are significantly elevated in the diabetic patients compared to controls (Table 1). When the patients were grouped based on duration, NO was found to be significantly increased in patients with >7 years duration of diabetes but SOD levels decreased in patients with diabetes for more than 7 years (Table 2).

DISCUSSION

In our study, diabetic patients were shown to have significantly increased SOD activity. With increasing duration of disease, the antioxidant enzyme activity in RBC is found to be decreased minimizing the scavenging mechanism (Chart 1). Similar results were demonstrated in a study, stating that the highest SOD activity in RBC at the onset of diabetes and subsequent decrease in its activity [9]. Increased SOD levels observed in our study might be due to increased synthesis in response to oxidative stress. Supporting our results, a study reported increase in extra cellular-SOD (EC-SOD) irrelevant of their EC-SOD phenotype [10]. Kawamura et al., showed an increase in RBC glycated SOD, which would cause a decrease in activity in diabetic patients [11]. It is known that SOD enzyme is upregulated in response to an increase in oxidative stress [12]. In our study with prolongation of disease duration, SOD activity is decreased, which might be explained by long-term exposure of enzyme to high concentration of glucose

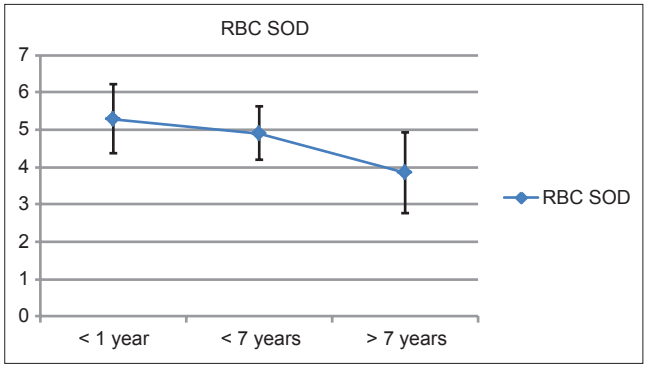

Chart 1: Changes in RBC SOD (Units) with duration of diabetes mellitus expressed as mean±SD

Table 1: Biochemical parameters of diabetic patients and controls expressed as mean±SD

	FBS (mg/dL)	HbA1c (%)	Serum NO_x (µM)	RBC lysate SOD (units)
Controls	124.96±17.02	5.86±0.51	28.01±6.97	0.65±0.29
Patients	200.3±76.67**	8.75±1.73**	52.2±25.8**	4.745±1.047*

**p<0.01, *p<0.05, P<0.05 are considered significant, FBS: Fasting blood glucose, SOD: Superoxide dismutase, RBC: Red blood cells

Table 2: Serum NO and RBC lysate SOD of patients with diabetic duration <7 years and >7 years (mean±SD)

Patients with diabetic duration	Serum NO (µM)	RBC lysate SOD (units)
Duration <7 years	45.87±27.11	4.9±0.72
Duration >7 years	64.9±25.91**	3.84±1.08*

**p<0.01, *p<0.05, p<0.05 are considered significant, NO: Nitric oxide, SOD: Superoxide dismutase, RBC: Red blood cells

leading to formation of increased glycated of SOD from which is less active compared to normal enzyme [13].

NO levels were elevated in diabetic patients and is proportional to duration of diabetes, which might be explained by high glucose induction of NO production and decrease in SOD leading to O_2^- increase, which reacts with NO and forming NO metabolites (Table 2). Similar results were noted in a study conducted by Cosentino et al., summarizing that elevated concentrations of glucose increase endothelial nitric oxide synthase (eNOS) gene and protein expression as well as NO release. However, upregulation of eNOS and increased NO release are associated with a marked concomitant increase of O_2^- production. Increased NO might also be explained by stimulation of inducible NO by high circulating lipopolysaccharides in diabetic patients [14].

CONCLUSION

Oxidative stress was seen in patients with diabetes mellitus with an increase in NO levels and SOD levels. Even though SOD levels are initially increased in diabetic patients in response to free radicals, prolonged duration of diabetes causes a decrease in SOD levels, which might be the reason for increased NO levels with duration as there is less scavenging mechanism of SOD in eliminating O_2^-. Further research is warranted to understand the relationship between SOD and NO levels and the mechanism of regulation of their synthesis.

ACKNOWLEDGMENT

We thank Dr. J. S. Kumar, Consultant Diabetologist, SRM MCH and RC for his extensive support in helping us with patient selection.

REFERENCES

1. Ceriello A. Oxidative stress and glycemic regulation. Metabolism 2000;49 2 Suppl 1:27-9.
2. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes 1999;48(1):1-9.
3. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40(4):405-12.
4. Halliwell B, Gutteridge JM. Free radicals and toxicology. In: Free Radicals in Biology and Medicine. Oxford: Clarendon Press; 1997. p. 1-27.
5. Mehran MJ, Zendehbad H, Malla S. Free radical scavenging and antioxidant potential activity of cassava plants. Asian J Pharm Clin Res 2014;7 Suppl 1:66-70.
6. Arya A, Aggarwal S, Yadav HN. Pathogenesis of diabetic nephropathy. Int J Pharm Pharm Sci 2010;2 Suppl 2:24-9.
7. Kar S, Kavdia M. Local oxidative and nitrosative stress increases in the microcirculation during leukocytes-endothelial cell interactions. PLoS One 2012;7(6):e38912.
8. Naveen CR, Renuka P. Nitric oxide in diabetic patients and its relation with HbA1c. Int J Pharm Clin Res 2014;6(2):146-8.
9. Satoh M, Fujimoto S, Haruna Y, Arakawa S, Horike H, Komai N, et al. NAD(P) H oxidase and uncoupled nitric oxide synthase are major sources of glomerular superoxide in rats with experimental diabetic nephropathy. Am J Physiol Renal Physiol 2005;288(6):F1144-52.
10. Kimura F, Hasegawa G, Obayashi H, Adachi T, Hara H, Ohta M, et al. Serum extracellular superoxide dismutase in patients with type 2 diabetes: Relationship to the development of micro-and macrovascular complications. Diabetes Care 2003;26(4):1246-50.
11. Kawamura N, Ookawara T, Suzuki K, Konishi K, Mino M, Taniguchi N. Increased glycated Cu, Zn-superoxide dismutase levels in erythrocytes of patients with insulin-dependent diabetis mellitus. J Clin Endocrinol Metab 1992;74(6):1352-4.
12. Huie RE, Padmaja S. The reaction of no with superoxide. Free Radic Res Commun 1993;18(4):195-9.
13. Li W, Yanoff M, Jian B, He Z. Altered mRNA levels of antioxidant enzymes in pre-apoptotic pericytes from human diabetic retinas. Cell Mol Biol (Noisy-le-grand)1999;45(1):59-66.
14. Lee HY, Noh HJ, Gang JG, Xu ZG, Jeong HJ, Kang SW, et al. Inducible nitric oxide synthase (iNOS) expression is increased in lipopolysaccharide (LPS)-stimulated diabetic rat glomeruli: Effect of ACE inhibitor and angiotensin II receptor blocker. Yonsei Med J 2002;43(2):183-92.