

Manganese Superoxide Dismutase (SOD2) Gene Val16Ala polymorphism in type 2 diabetic patients

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Abstract: *Background:* Reactive oxygen species generated by hyperglycemia modify structure and function of lipids, proteins and other molecules taking part in chronic vascular changes in diabetes mellitus (DM). In patients with DM low activity of scavenger enzymes has been observed. Within mitochondria, the main defence against oxidative stress is provided by Manganese superoxide dismutase (Mn SOD). Val16Ala may be a single nucleotide polymorphism (SNP) within the Mn SOD gene, predicted to affect intra-mitochondrial transport of the enzyme. Functional polymorphisms of these antioxidant enzyme are reported to be involved in pathogenesis of T2DM individuals. Low activity of scavenger enzymes may be deteriorated by oxidative stress. This study was undertaken to investigate the association between SOD2 gene polymorphisms and Total Antioxidant capacity of T2DM. *Objective:* To find the V16A polymorphism of Manganese superoxide dismutase (SOD2) and its association with Total antioxidant capacity in Type 2 Diabetic patients. *Methods:* We assessed SOD2 gene V16A polymorphism, Total Antioxidant Capacity (TAC) and Lipid Profile in 100 patients of type 2 diabetes Mellitus and 100 healthy controls. This study was conducted in Rama Medical College, Hospital & Research centre, Kanpur. Statistical analysis was done by chi-square test and student t test. *Result:* Significant differences in allele and genotype distribution among T2DM and control persons were found in SOD2 genes. Serum TAC levels was significantly decreased in T2DM subjects compared to the control group. TAC was higher in CC than in TT and CT genotype of SOD2 gene. *Conclusion:* the results of our study demonstrate that oxidative stress in DM can be accelerated not only due to increased production of ROS caused by hyperglycemia but also by reduced ability of antioxidant defense system caused at least partly by SNPs of SOD2.

Keywords: Oxidative stress, SOD2 gene V16A polymorphism, Total antioxidant capacity, Type 2 Diabetes mellitus.

Introduction

Superoxide dismutase (SOD) is that the first acting antioxidant enzyme and plays a critical role in protecting cells against ROS-induced damage [1]. The SOD family dismutates superoxide radicals ($O_2^{\bullet-}$) derived from extracellular sources or produced within the mitochondrial matrix as a by-product of O_2 metabolism through the ETC [2].

SOD family of antioxidant enzymes includes intracellular (Cu Zn-SOD), mitochondrial (MnSOD), and extracellular (EC-SOD) enzymes also mentioned as SOD types 1, 2 and 3, respectively. The MnSOD isoform becomes a key antioxidant enzyme in the protection of cells

from $O_2^{\bullet-}$ ions due to its unique genetic organization and mitochondria matrix localization [3-4]. Human MnSOD is encoded by MnSOD nuclear gene located in chromosome 6q25 and it is a homotetrameric molecule [5-6]. The MnSOD enzyme is synthesized with a mitochondrial targeting sequence (MTS) and is translated within the cytoplasm, transported into the mitochondria, processed, and assembled into a lively homotetramer. MnSOD is that the only known antioxidant enzyme present within the mitochondria and it's been considered a singular tumor suppressor protein presenting a pivotal role in regulating events of cell death [4].

The most common genetic mutation studied in humans is the single nucleotide polymorphism (SNP), which occurs when gene single bases are changed or deleted, resulting in amino acids modification at specific positions [7]. A few SNPs are silent, while others may produce to altered phenotypes across protein modulation or function, and possibly affect homeostasis. In humans a minimum of 111 SNPs are identified for Cu/ZnSOD, 190 for MnSOD, and 100 for ECSOD. Considering the relevance of MnSOD because the first line defense to reactive oxygen species (ROS) production, structural and/or functional SNPs of the MnSOD encoding gene are of high importance within the maintenance of ROS cell levels [5].

The most commonly studied MnSOD SNP is the Val16Ala, characterized by a structural mutation substituting a thiamine (T) for a cytosine (C) in the exon2. The substitution affects the codon 16, translating the alanine (GCT) into valine amino acid (GTT) [8]. During MnSOD processing the single peptide is removed these modification plays a key role in targeting the enzyme into mitochondria. The alanine to valine substitution produces a β -sheet secondary structure instead of α -helix structure, which may decrease the transport efficiency of the enzyme into the mitochondria, modifying the antioxidant defense against ROS [8].

The Ala variant can cross both outer and inner mitochondrial membranes to reach the matrix, while most of the Val variant is embedded within the inner membrane [9]. This may be due to the α -helical structure of the Ala-containing precursor which results in an enhanced MnSOD transport [10]. As compared with the Val-MnSOD precursor the Ala-MnSOD generates 30 - 40% more of the active MnSOD homotetramer [11].

Excessive oxidative stress has been considered a major factor in the onset of diabetes, and mitochondrial $O_2^{\bullet -}$ overproduction plays an important role in the development of diabetic complications [12-13]. Val/Val carriers presented higher risk for diabetes development in comparison to Ala carriers after adjustment for age, gender, systolic blood pressure, total cholesterol, and body mass index and insufficient ROS scavenging related to the MnSOD gene genotype could also be associated with

susceptibility to glucose intolerance [14]. Diabetes mellitus is a chronic metabolic disorder characterized by impaired glucose and lipids metabolism due to defect in insulin secretion (beta cell dysfunction) or action (insulin resistance) or both [15]. Which may affect all age groups and sex [16].

Increase in the incidence of Diabetes worldwide is due to Changes in human lifestyle and behaviour over the last century [17]. Long term complications of diabetes are diabetic nephropathy, neuropathy, retinopathy and cardiovascular diseases, which bring high cost for both individual and society due to the development of complications affecting the vascular system, kidney, retina and peripheral nerve in diabetic patients [18-20].

Worldwide 415 million people are diabetic and it is expected to rise to >642 million by 2040. The Indian statistics showed prevalence is 8.7 and 69.2 million Diabetic cases in adult population [21]. This is according to IDF diabetes atlas 7th edition-2015 update. Oxidative stress is Oxidative damage of cell, tissues or organs by free radicals [22-23].

Oxidative stress plays a important part in diabetic progression [24]. In pathogenesis of diabetes and relationship between oxidative stress and complications, free radicals play major role [25-26]. Non enzymatic protein glycation, glucose auto oxidation and activation of polyol path way with increased oxidative stress is the possible cause of long term and high blood glucose levels in uncontrolled diabetes mellitus [27-28]. A way of assessing the antioxidant levels of plasma recently proposed as TAC.

The total radical trapping antioxidant parameter (TRAP) also used as TAC or TAS [29]. The antioxidant capacity of all the antioxidants present in serum/plasma, foods (dietary total antioxidant capacity) and other body fluids considered as TAC [30-32]. TAC gives cumulative parameter instead of simple sum of measurable antioxidants. Total antioxidant capacity measurement is useful indicator of risk associated with activity of free radicals in type 2 diabetics [33].

Material and Methods

A total of 100 patients with type 2 diabetes and 100 non diabetic healthy controls were included in this study. The study was conducted in the Department of Biochemistry with the collaboration of Department of General Medicine in Rama Medical College, Hospital and Research Centre, Kanpur. The control group consists of age, sex matched non- diabetic healthy subjects and Age group between 30-60 years from both sex.

The study group consists of Type 2 Diabetes patients with age group of 30-60years from both sex and duration of Diabetes Mellitus is 5 years or more. Patients with type I Diabetes Mellitus, Pregnant and lactating females, Patients taking diuretics and lipid lowering drugs, patients with smoking, liver disease, thyroid disease, tuberculosis, cancer patients and acute or chronic inflammatory diseases are excluded from the study.

Blood sampling and biochemical analysis: Venous blood collected in plain, fluoride and heparin coated tubes was centrifuged. The separated plasma and serum were stored until further analysis at -50°C. Glucose and Lipid profile were estimated on fully automated analyzer and Total antioxidant capacity (TAC) was analyzed by ferric reducing ability of plasma (FRAP).

DNA analysis: Genomic DNA was extracted by using a DNA extraction kit from peripheral blood cells. Genotyping of Val(16)Ala of Mn-SOD was done by PCR- restriction fragment length polymorphism methods. Briefly, the primers for V16A polymorphism were 5'-GCTGTGCTTTCTCGTCTTCAG-3' (forward primer) and 5'-TGTACTTCTCCTCGGTGACG-3' (reverse primer).

The PCR involved 38 cycles of 94°C for 30s, 60°C for 30s and 72°C for 30s. Then the PCR products were digested overnight at 60°C with BsaI, electrophoresed on 2.5% agarose gel, and stained with ethidium bromide.

Statistical analysis: Results are reported as the mean ± SD. The statistical significance of differences in mean values was analyzed by the student's t-test and the χ^2 test, respectively.

Results

Data analysis was done using SPSS programme. p value was used to compare the groups. p value < 0.05 was considered significant. Age and HbA1C were significantly higher in the diabetic patients than in the healthy subjects (P < 0.0001; age, 52±3.24 vs 46±4.85 years; HbA1c: 8.52±0.61% vs 6.24±1.1%), whereas both the allele frequency and the genotype distribution were different between the two groups {allele frequency [V/A], 0.72/0.28 vs 0.35/0.65; genotype [VV/VA/AA (%)], 55/34/11 vs 09/52/39; diabetic patients vs healthy subjects}. The frequency of genotype in each group was consistent with the Hardy-Weinberg equilibrium.

Table-1: Characteristics of the diabetic patients and control group

Variables	Controls	DM	p
n	100	100	-
Sex (male/female)	69 / 31	42 / 58	<0.001
Age (years)	46 ± 4.85	52 ± 3.24	<0.001
BMI (kg/m ²)	22.6 ± 0.4	24.0 ± 0.5	<0.001
HbA1C	6.24 ± 1.1%	8.52 ± 0.61%	<0.001
HDL-C	53.29 ± 9.37	41.80 ± 14.32	<0.001
LDL-C	113.71 ± 24.82	149.62 ± 54.13	<0.001
TG	115.03 ± 25.63	135.81 ± 56.05	<0.001
TC	152.91 ± 31.38	174.62 ± 37.18	<0.001
TAC	1.44 ± 0.52	0.62 ± 0.39	<0.001
FBS	91.48 ± 4.61	162 ± 12.49	<0.001
PPBS	113.21 ± 5.29	242.43 ± 39.68	<0.001
FBS-fasting plasma glucose, PPBS- postprandial plasma glucose, HbA _{1c} -glycosylated hemoglobin, TC-total cholesterol, TG-triglycerides, HDL-C – high density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, TAC-total antioxidant capacity.			

Clinical characteristics of the diabetic patients are shown in Table-1. Plasma high-density lipoprotein cholesterol (HDL-C) was lower in the DN2 group compared with the control group (P < 0.0001). In the DN2 group, body mass index (BMI), HbA1c, plasma total cholesterol(T-CHO), Plasma triglycerides and Plasma low density

lipoprotein cholesterol were higher than in the control group (BMI, P <0.0001; HbA1c, P < 0.0001; T-CHO, P< 0.0001; TG, P< 0.0001; LDL-C, P<0.0001). TAC levels were significantly decreased in diabetics than in control group (0.62±0.39 vs 1.44±0.52).

SNP	Sequence of used primers	Restriction endonuclease
SOD2 V16A	5'-GCTGTGCTTTCTCGTCTTCAG-3' 5'-TGGTACTTCTCCTCGGTGACG-3'	Bsaw I

		TT (val/Val)	CT (Ala/Val)	CC (Ala)
Controls	n(%)	09 (9)	52 (52)	39(39)
	TAC	1.42±0.27	1.43±0.21	1.45±0.13
T2DM	n(%)	55(55)	34(34)	11 (11)
	TAC	0.59±0.09	0.61±0.17	0.64±0.25

The occurrence of genotypes in studied polymorphisms among Type 2 diabetic patients and healthy subjects (controls). Total antioxidant capacity (TAC) levels separated according to the genotypes in compared groups

Discussion

In the present study we analyzed the Val(16)Ala polymorphism of Mn-SOD and then evaluated the association of this polymorphism with TAC in diabetic patients and non diabetic subjects. The TT genotype (Val/Val) was most common in T2DM, CT genotype (Ala/Val) is most common in both control and T2DM and CC genotype (Ala/Ala) was common in control group. In T2DM TAC activity was highest in the CC genotype (Ala/Ala) and the lowest in the TT genotype (Val/Val). The presence of TT genotype (Val/Val) in SOD2 gene was related to poorer diabetic control as compared with CT (Ala/Val) and CC (Ala/Ala) genotypes. M.G petrovic have found that the VV genotype of the V16A polymorphism of the Mn-SOD gene might be a risk factor for diabetic retinopathy in the Slovene population (Caucasians) with type 2 diabetes [34]. Chistyakov et al. have reported an association between diabetic neuropathy and VV genotype of the V16A polymorphism [35].

Amanda Crawford was shown that the Val/Val genotype was associated with an increased risk of diabetic nephropathy (DN) in both T1DM and

T2DM [36]. The present study confirms the previous findings that the V allele is associated with increased risk of oxidative stress. Glucose induces several pathways which will contribute to the event of diabetic complications, including the activation of protein kinase C and nuclear factor κB (NFκB) and therefore the formation of advanced glycation end-products.

Nishikawa et al. demonstrated that when the quantity of mitochondrial reactive oxygen species were normalised, these glucose-induced changes were prevented, which shows the importance of keeping reactive oxygen species at low levels, particularly for diabetic patients[37], one among the essential enzymes for this is often the MnSOD and therefore the V allele results in lower levels of MnSOD and decreased degradation of reactive oxygen species, patients carrying this allele are likely to possess a better burden of oxidative stress [38]. TAC is significantly decreased in diabetic patients compared with control group. TAC is also known as Total radical trapping antioxidant parameter (TRAP). Existence of low levels of circulating

antioxidants in diabetic patients suggestive of decreased TAC. O.M. Akinosun have found a marked decrease in TAC levels in diabetic patients with compared to control group [33]. In this study TAC levels were decreased may be due to: Auto oxidation of glucose and non enzymatic glycation in diabetes mellitus most likely increased sources of free radicals [39], As a result of Diabetes Mellitus excess free radicals are produced which are neutralized by antioxidants [40].

Conclusion

The presented findings show that the genotype distribution of the SOD2 in patients with DM can

differed from non diabetic individuals. We are aware of the limitation of this study with relatively small sample size as compared with wide epidemiological studies, especially by providing subgroup analysis within the group with DM. Nevertheless the results of small studies with similar conclusions may set off sequent research. Genetic background could even be a minimum of partly associated with disease control of diabetes and consequently enzyme activities protecting against oxidative stress. Combined genetic and metabolic changes may leads to vascular disorders like atherosclerosis.

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