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Investigation of sod2 gene polymorphism in the patients associated with type-2 diabetes mellitus patients

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Abstract---Introduction: Type-2 Diabetes mellitus (type-2DM) is a multifactorial metabolic disorder, characterized by hyperglycemia. Oxidative stress plays a crucial role in the progression of diabetes and its complications. The polymorphism in the SOD2 gene (superoxide dismutase) results in minimal production of SOD enzymes, which may affect the scavenging of free radicals. Aim: to see the level of SOD enzyme in type-2DM patients associated with SOD2 gene polymorphism. Material& Methods: It is a comparative study conducted in the department of anatomy in collaboration with the Department of General Medicine, RKDF medical college Hospital& research center, Bhopal. 5 ml of venous blood from type-2 DM patients (n=30) and control subject (n=30) was collected, followed by estimation of blood glucose (HbA1c, FBS& PPBS) and the genetic test was done to detect the SOD2 gene polymorphism. Results: Blood glucose levels (HbA1c, FBS &PPBS) were high and SOD levels were low in type-2 DM patients when compared with control subjects (p<0.05). When a comparison was done among three genotypes, the levels of SOD enzyme were high in the CC genotype followed by CT and TT genotypes. Discussion & conclusion: SOD enzyme detoxifies the free radicals, and low levels of SOD enzyme indicate the elevation of oxidative stress. The 'C' allele plays a protective role whereas the 'T' allele plays a pathogenic role. The severity of diabetes mellitus is high in TT genotype than in CT& CC in terms of glycemia.

Keywords---Superoxide dismutase 2, Diabetes mellitus, SOD2 gene.

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, it occurs due to a lack of insulin/ action or both. Persistent hyperglycemia may result in damage/ dysfunction or failure of different organs including kidneys, heart, eyes, blood vessels and kidneys, it may leads increase mortality and morbidity¹. Polyuria, polydipsia, polyphagia, fatigue and weight loss are the typical symptoms of type DM². In the 21st century, T2DM has become one of the major public health challenges. The prevalence of T2DM has been rising worldwide; nearly 180 million individuals are suffering currently. Worldwide diabetic burden generally comes from India and China where over 75% of diabetic subjects will reside in 2025³. India has been considered as “diabetes capital” of the world. According to current statistics, the prevalence of type-2 diabetes mellitus in India is about 8.7⁴.

Excess formation and incomplete removal of highly reactive substances such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are called oxidative stress. ROS and RNS play a crucial role in the pathogenesis and progression of type-2DM^{5, 6}. Superoxide dismutase 2 is an important enzyme from the SOD family involved in the scavenging of free radicals, it is also known as manganese superoxide dismutase (Mn-SOD). There is evidence that hereditary variations are an essential factor in the pathogenesis and advancement of DM and its complications^{7, 8}. The gene of SOD2 is present on chromosome 6q25. Ala16Val polymorphism has been found in exon 2 of the human SOD2 gene, where normal GCT is mutated to GTT. Ala 16 Val (rs 4880) is the most frequently studied polymorphism in the SOD2 gene and it has functional significance. Due to this SNP, at the 16th position, the amino acid Alanine (Ala) is changed to Valine (Val). The presence of Valine ('T' allele) prompts the development of unstable mRNA and decreases the transport of enzyme into the mitochondrial matrix and then its antioxidant function, therefore, rise in oxidative stress^{9, 10}. Hence the present study is designed to see the level of Mn-SOD enzyme and blood glucose in patients of T2DM associated with SOD 2 gene polymorphism.

Materials and Methods

It is a comparative study conducted in the department of anatomy in collaboration with the department of General Medicine in RKDF medical college Hospital& research center. The study was conducted after getting approval from the institutional ethical committee and informed concerns from each participant. Study participants were recruited as per the following inclusion/exclusion criteria.

Inclusion criteria

- Normal subjects.
- T2 DM patients having HbA1c: >6.5% or FBS: 126 mg/dl to 200mg /dl or PPBS: 200 mg/dl to 300mg/dl.
- Patients with the age group of 30 to 60 years from both sexes.
- Patients are willing to give informed concerns.

Exclusion criteria

- Type-1 Diabetes mellitus patients.
- Type-2 diabetes mellitus patients without SOD2 gene polymorphism.
- Patients with other medical problems.
- Pregnant & lactating women.
- Unwilling to participate and give informed concern or mental incapacity to take the drugs.

30 healthy subjects and 30 T2DM patients (associated with SOD gene polymorphism) were recruited, followed by 5 ml of venous blood collected from a peripheral vein using Di sodium EDTA vacutainers to evaluate the SOD2 gene polymorphism by simple PCR technique. All the collected samples were aliquoted and stored at -20 C until tested.

Genomic DNA was extracted from peripheral blood cells by salting out method using a Qiagen Kit (spin protocol procedure and standard protocol followed). The targeted sites of DNA were amplified using specific primers: The forward sequence was 5- GCTGTGCTTTCTCGTCTTCAG -3, and the reverse sequence was 5-TGGTACTTCTCCTCGGTGACG -3. Genotyping of Ala 16 Val of Mn-SOD was done by PCR restriction fragment length polymorphism methods. The PCR involved 38 cycles of 94°C for the 30s, 60°C for 30s, and 72°C for the 30s. Then the PCR products were digested overnight at 60°C with BsaW I, electrophoresed on 2.5% agarose gel and stained with ethidium bromide. Followed by 5 ml of blood was collected from both control subjects and T2DM patients associated with SOD2 gene polymorphism for estimating HbA1c (Ion Exchange Resin Method), FBS& PPBS (GOD&POD method) and superoxide dismutase enzyme (Xanthine oxidase enzymatic method) level.

Statistical Analysis

Data was entered in MS excel and analyzed by the unpaired t-test. Results are expressed as the mean ± SD.

Results

Among 30 type-2 diabetes mellitus patients, 18 were males and 12 were females. The majority of patients were belonging to the age group 51 to 60 years (72%) and the remaining 28 % were from the age range between 41 to 50 years. CC, CT and TT genotypes were detected in control (CC=15; CT=9; TT=5) and diabetes mellitus patients groups (CC=4; CT=10; TT=16). Table-1 Blood glucose level of control subjects was normal i.e. HbA1c: 5.071(%), FBS: 108 mg/dl and PPBS: 180.9 mg/dl whereas type-2 diabetes mellitus patients were shown elevated blood glucose levels (HbA1c: 8.25± 0.16; FBS: 149.9± 8.90; PPBS: 239.5±15.91), which was statistically significant when compared with the control subjects (p<0.05). Table-2 There was a significant difference found in the mean SOD2 level of when diabetic group compared with the control group (p<0.05). When a comparison was done among three genotypes in diabetic groups, the HbA1c, FBS and SOD2 levels were significantly different (p<0.05).Table-3

Table-1: Distribution of genotypes

Genotypes	Control subjects(n=30)	Type-2DM patients(n=30)
CC	15	4
CT	9	10
TT	6	16

Table- 2: Blood glucose& SOD level in control subjects& type-2DM patients

Variables	Control subjects Mean \pm SD	T2DM Mean \pm SD	Mean difference	p-value
FBS (Mg/dl)	108 \pm 6.52	173.6 \pm 10.61	65.64 \pm 2.92	0.001**
PPBS (Mg/dl)	180.9 \pm 12.68	273.7 \pm 12.36	92.82 \pm 3.74	0.001**
HbA1c (%)	5.071 \pm 0.29	8.52 \pm 0.33	3.452 \pm 0.10	0.001**
SOD(U/l)	162 \pm 5.67	120.2 \pm 6.85	41.78 \pm 2.96	0.001**

Significant*; highly significant **

Table-3: Blood glucose and SOD levels in CC, CT and TT genotype of type-2 DM patients

Variables	Genotype	Mean \pm SD	p-value
HbA1c (%)	CC	8.22 \pm 0.19	0.001**
	CT	8.53 \pm 0.29	
	TT	8.81 \pm 0.19	
FBS(Mg/dl)	CC	170.1 \pm 7.41	0.025*
	CT	171.1 \pm 9.57	
	TT	179.8 \pm 12.12	
PPBS(Mg/dl)	CC	271.8 \pm 9.21	0.712 ^{ns}
	CT	273.6 \pm 16.8	
	TT	275.7 \pm 10.31	
SOD(U/l)	CC	131.5 \pm 4.34	0.001**
	CT	115.9 \pm 5.82	
	TT	97.18 \pm 5.38	

Significant*; highly significant **

Discussion

The present study aims to find out the level of blood glucose and superoxide dismutase enzyme (Mn-SOD) in patients of type-2 diabetes mellitus in association with Ala 16 Val polymorphism. Among 30 type-2 diabetes mellitus patients, 18 were males and 12 were females, which indicates the high prevalence of diabetes mellitus in males, similar results were found in another study¹¹. The majority of patients were belonging to the age group 51 to 60 years (72%) and the remaining 28 % were from the age range between 41 to 50 years. The distribution of CC genotype (n=15) was more in control group i.e 50%, followed by CT 30% (n=9) and TT genotype 20% (n=6). Whereas in diabetes group 13.33% was CC genotype (n=4), 33.33% CT genotype (n=10) and 53.33% was TT genotype (n=16). In the present study, the frequency of TT genotype distribution among the diabetic

group was more, which is supported by a similar previous study¹². Blood glucose (HbA1c, FBS& PPBS) levels were significantly high in the diabetes group as compared to control subjects ($p < 0.05$), which is in line with the previous study¹³. The present study revealed that Mn-SOD levels were low in the diabetes group than in control subjects which are in line with another study^{14, 15& 16} but it has been opposed by another study¹⁷. There is a significant variation in mean Blood glucose i.e HbA1c and FBS level and SOD level in between the CC (HbA1c: 8.22 ± 0.19 ; FBS: 170.1 ± 7.4 ; SOD: 131.5 ± 4.34), CT (HbA1c: 8.53 ± 0.29 ; FBS: 171.1 ± 9.57 ; SOD: 115.9 ± 5.82) and TT (HbA1c: 8.81 ± 0.19 ; FBS: 179.8 ± 12.12 ; SOD: 97.18 ± 5.38) ($p < 0.05$) genotypes in diabetic group these findings were supported by another study¹². There are three enzymes present in the SOD family including SOD1 (Cu Zn-SOD), SOD2 (Mn-SOD), and SOD3 (EC-SOD), which are present in intracellular, mitochondrial and extracellular sites respectively^{18, 19& 15}. The level of SOD2 declined in type 2 diabetes mellitus patients, which leads to the progression of diabetes mellitus and its complications²⁰.

Conclusion

The present study concludes that the blood glucose and serum SOD levels are high in diabetes patients. The frequency distribution of TT genotype in diabetes patients was high, whereas in normal subjects it was CC genotype. The “T” allele is associated with low SOD levels, it may be responsible for the high prevalence and severity of diabetes mellitus, which need to be confirmed by further investigations on large scale.

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