Original Article

Effectiveness Of Glimepiride And Glimepiride + Vitamin-C In Type-2 Diabetes Mellitus Patients Associated With SOD2 Gene Polymorphism

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Abstract

Introduction: Diabetes mellitus (DM) is characterized by hyperglycemia, usually associated with the rise in oxidative stress. Oxidative stress plays a major role in diabetes and its complications. Methods: 40 newly diagnosed type-II DM patients were recruited after measuring glycolated hemoglobin (HbA1c), fasting blood sugar (FBS) & postprandial blood sugar (PPBS) & malondialdehyde (MDA) & total antioxidant capacity (TAC). Genetic test was done for detecting SOD2gene polymorphism (Superoxide dismutase). Patients were divided into two groups, group-A (n=20) &B (n=20). Group–A patients were given Glimepiride (1mg) and group-B patients were given Glimepiride (1mg) + vitamin C (1000 mg) OD for 3 months. After the commencement of the study glycemic level and oxidative stress were measured and compared with basal values. Results: 3 genotypes were identified, such as CC, CT and TT. After 3 months of study, there was a significant reduction in mean HbA1c, FBS and PPBS ($p<0.05$) in all genotypes of both group-A& B, but overall the mean reduction was more in CC genotype followed by CT and then TT genotype. Oxidative stress was reduced significantly in the CC and CT genotype ($p<0.05$) but not in the TT genotype of the group–B, whereas group-A (all 3 genotypes) had shown elevation of oxidative stress. Conclusion: Vitamin C supplementation have better control over the glycemic level and oxidative stress.

Keywords: Oxidative stress, Diabetes mellitus, vitamin C.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by a rise in blood glucose levels resulting from a lack of insulin secretion, action, or both. Hyperglycemia is associated with disturbances in lipid and protein metabolism. Polyuria, polyphagia and polydipsia are the three hallmarks of diabetes. Uncontrolled hyperglycemia is associated with micro and macrovascular complications such as diabetic neuropathy, diabetic nephropathy and retinopathy and atherosclerosis, coronary artery disease, arrhythmias and cerebrovascular disease. Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease.1, 2 The diabetic population in India is predicted to be 109 million by 2035.

Many factors responsible for diabetes include environmental factors, obesity, age, cigarette smoking, alcohol, sedentary lifestyle, Sleep Disturbances and genetic factors3. Among genetic factors, SOD2 gene polymorphism is associated with diabetes and its complications. Superoxide dismutase 2 is the key enzymatic anti-oxidant in the SOD family and acts against free radicals. It is also called Manganese superoxide dismutase (Mn-SOD), it is encoded by the nuclear SOD2 gene. Human chromosome 6q25 is the site where the SOD2 gene is located. Ala 16 Val is a more frequently studied single nucleotide polymorphism (NSP), which has been identified in axon 2 of SOD 2gene. Instead of α-helix structure, the β-sheet secondary structure is produced due to Ala 16 Val polymorphism resulting in a fall of Mn-SOD activity leading to a rise in oxidative stress.4 Maximal

SOD activity is found in the CC genotype followed by CT and finally TT genotype. Lack of Ala allele affects the removal of ROS which also may lead to glucose intolerance.5Ala allele can reduce the risk of both forms of diabetes (Type-1 and 2) and its microvascular complications like diabetic neuropathy, nephropathy and retinopathy.6

Currently, many antidiabetic drugs are available but unfortunately, none of them control oxidative stress, hence present study is designed to see the additional benefits of supplementation of vitamin C along with oral hypoglycemic agents on blood glucose and oxidative stress control.

METHODS

The present study is a prospective, open-label, randomized controlled clinical trial, conducted in the department of pharmacology collaborated with the department of general medicine at Rama medical college hospital& research center, Kanpur. Before conducting the present study permission was taken from the institutional ethical committee and written informed concerns were taken from each study participant. 40 newly diagnosed type-2 diabetes mellitus patients were recruited as per inclusion and exclusion criteria as given below.

Inclusion criteria:

- Newly Diagnosed Type-2 Diabetes Mellitus Patients
- Glycosylated Haemoglobin (HbA1c) >6.5%.
- Subjects with the age group of 30-60years from both sexes.
- Patients willing to give informed concern

Exclusion criteria:

- Type-1 Diabetes mellitus patients.
- Smokers/ alcoholics
- Pregnant & lactating women.
- Patients on steroid therapy, history of chronic infections.
- Hypersensitivity to vitamin C and any of the study drugs.
- Unwilling to participate or mental incapacity to take the drugs.

The medical history of all the patients was taken at beginning of the study followed by 3 ml of venous blood drawn from the peripheral vein to measure blood glucose (HbA1c, FBS and PPBS). Followed by 5 ml of venous blood from patients whose glycosylated haemoglobin is (HbA1c) >6.5% was collected for measuring oxidative stress (MDA& MDA) and to detect SOD2 (ALA 16 VAL) gene polymorphism.

Estimation of HbA1c was by Ion exchange resin method and FBS &PPBS were by GOD POD enzymatic method. TAC was estimated by the ferric reducing ability of plasma (FRAP) and MDA was by (Malondialdehyde) TBARA Assay.

Patients have their SOD2 gene was analyzed for the SNP rs4880 sequence (V16A) located on exon 2 of chromosome 6q25. Qiagen Kit for whole blood used for extraction of DNA by spin protocol procedure and standard protocol followed as given in the Qiagen kit.

Following primers were used to detect gene presence and followed by polymorphism.

Forward Primer: 5'- GCTGTGCTTTCTCGTCTTCAG-3' Tm=54oC

Reverse Primer: 5'TGGTACTTCTCCTCGGTGACG3' Tm=58.3oC

Restriction Endonuclease: BsaWI

A total of 40 newly diagnosed type-2 DM patients were recruited and divided into 2 groups, group-A& group B. Group-A patients were given Glimepiride 1mg/day, and group-B patients were given Glimepiride 1mg+ vitamin C1000 mg/day for 3 months. The study had 7 visits, each visit scheduled after every 15 days except 1st visit, which was held after 1 week. At every visit, each patient was examined for general medical conditions and investigations for glycemic control (FBS and PPBS) were done. All the biochemical investigations for blood glucose (HbA1c, FBS, and PPBS), and oxidative stress (TAC& MDA) were repeated after the commencement of the study and the values were compared with baseline.

STATISTICAL ANALYSISI

Data were statistically analyzed using Statistical Package for the Social Sciences (SPSS) version23.0. The results were expressed as mean± SD (Standard deviation).

RESULTS

Among 40 patients of newly diagnosed type-2DM, 18(45%) patients were females and 22(55%) were males. Maximum patients belonged to the age group 45-55 years (n=23) followed by 56- 65(n=14) years and the remaining 3 patients were belongs to the age group 35-44 years. 3 types of genotypes were observed in SOD2 gene polymorphism associated type-2 DM patients such as CC homozygous (n=27.5%), CT heterozygous (32.5%) and TT homozygous (40%) at 260bp, 180bp and 84bp respectively.

After 3 months of treatment, there was a significant reduction in mean HbA1c, FBS and PPBS observed in all three genotypes but the mean reduction was more in the CC genotype followed by CT and then TT ($p<0.05$).Table-I After 3 months of treatment there was a significant reduction in mean TAC and elevation in mean MDA values found in the TT genotype($p<0.05$). Whereas CC& CT genotype also showed a reduction in mean TAC and elevation in mean MDA values, which is not statistically significant ($p>0.05$).Table-II After 3 months of treatment there was a significant reduction in mean HbA1c, FBS and PPBS levels observed in all three genotypes but the mean reduction was more in the CC genotype followed by CT and then TT($p<0.05$). Table-III After 3 months of treatment there was a significant elevation in mean TAC and reduction in mean MDA values found in CC and CT genotypes ($p<0.05$). Whereas TT genotype also showed elevation in mean TAC and reduction in mean MDA values, which is not statistically significant ($p>0.05$).Table-IV

Table-I: Glimepiride effect in blood glucose levels of SOD2 gene positive type-2 DM patients

Variables	Genotype	Baseline± (Mean± SD)	3 months (Mean± SD)	Mean difference	p-value
HbA1c (%)	CC	7.16±0.25	6.58 ± 0.14	0.58± 0.10	0.005**
	CT	7.33±0.15	6.8 ±0.26	0.53± 0.11	0.015**
	TT	7.48± 0.04	7.05 ±0.29	0.43±0.25	0.018**
FBS (mg/dl)	CC	161.3±6.34	141.5±7.86	19.83 ±1.52	0.005**
	CT	162.7±10.5	145 ± 4	17.67 ±6.5	0.042**
	TT	168.3±11.62	151.3 ± 8.80	17 ±2.81	0.043**
PPBS (mg/dl)	CC	249 ± 6.13	225.5 ± 8.38	23.5± 2.25	0.001**
	CT	257± 6.55	237.3 ±6.80	19.67 ±0.25	0.041**
	TT	261± 8.718	244.3 ±4.16	16.67±4.55	0.050**

$p<0.05$**Significance

Table-II: Glimepiride effect in oxidative stress of SOD2 gene positive type-2 DM patients

Variables	Genotype	Baseline (Mean± SD)	3 months (Mean± SD)	Mean difference	p-value
TAC (mmol/l)	CC	0.83± 0.10	0.8± 0.10	0.03±0.006	0.695
	CT	0.7 ± 0.08	0.62 ± 0.09	0.07± 0.01	0.444
	TT	0.7 ±0.1	0.56 ± 0.12	0.13±0.03	0.050**
MDA (mmol/l)	CC	4.41± 0.48	4.53 ± 0.47	-0.11±0.01	0.608
	CT	5.05 ± 0.19	5.15 ±0.20	-0.1±0.01	0.514
	TT	5.56± 0.37	5.76± 0.38	-0.2 ±0.01	0.008**

p<0.05**Significance

Table-III: Glimepiride+ vitamin-C effect in blood glucose levels of SOD2 gene positive type-2 DM patients

Variables	Genotype	Baseline (Mean± SD)	3 months (Mean± SD)	Mean difference	p-value
HbA1c (%)	CC	7.18 ± 0.18	6.46 ± 0.19	0.71 ±0.01	0.001**
	CT	7.2 ± 0.25	6.52± 0.15	0.67 ± 0.10	0.004**
	TT	7.43±0.11	6.8 ±0.2	0.63± 0.08	0.011**
FBS (mg/dl)	CC	155.3±10.42	132.8 ± 8.93	22.5± 1.48	0.001**
	CT	164.2 ±10.72	144 ± 9.89	20.25±0.82	0.001**
	TT	168± 6.55	149± 3.464	19±3.09	0.012**
PPBS (mg/dl)	CC	254.8±19.79	230±16.1	24.83±3.69	0.001**
	CT	255.8 ± 16.7	233.2±8.77	22.5 ± 7.93	0.011**
	TT	263.3 ±7.50	244 ±7.81	19.33±0.30	0.015**

p<0.05**Significance

Table-IV: Glimepiride +Vitamin C effect in oxidative stress of SOD2 gene positive type-2 DM patients

Variables	Genotype	Baseline (Mean± SD)	3 months (Mean± SD)	Mean difference	p-value
TAC (mmol/l)	CC	0.78±0.09	0.95 ± 0.12	-0.16±0.02	0.012**
	CT	0.6 ±0.08	0.72± 0.12	-0.12±0.04	0.015**
	TT	0.6 ±0.1	0.68 ± 0.08	-0.08±0.01	0.374
MDA (mmol/l)	CC	4.61±0.50	4.03± 0.49	0.58±0.01	0.018**
	CT	5.3± 0.23	4.97± 0.20	0.32±0.02	0.032**
	TT	5.92 ± 0.43	5.74 ± 0.48	0.18±0.05	0.221

p<0.05**Significance

DISCUSSION

Oxidative stress is a major leading factor that deteriorates diabetes and is also responsible for diabetic complications. Currently, many antidiabetic drugs are available but unfortunately, none of them have a negligible role on oxidative stress, hence in the present study patients were supplemented with 1000 mg of vitamin C along with Glimepiride to see the additional benefits on glycemic and oxidative stress control.

The majority of the study population i.e.55% were males(n =22) which is in line with findings of another study7 but a similar study found a high prevalence in females.8 57.5% of study subjects belonged to the age group 45-55 years, findings are similar to another study7 but another study found the majority of patients in the age above 61 years.9 Among 40 SOD2 gene-positive patients 15 belonged to the TT genotype, 14 patients were CT genotype and the remaining 11 patients were belongs to the CC genotype which is not similar to the findings of another study.10 Among 3 genotypes TT had shown high HbA1c, FBS, PPBS and oxidative stress followed by CT and CC genotypes which are in line with another study11, which indicates the pathogenic role of the 'T' allele& protective role of 'C' allele.

Good glycemic control($p<0.05$) was observed in all patients of type-2 DM associated with SOD2 gene polymorphism who received Glimepiride, whereas oxidative levels were increased in all three genotypes but statistically significant only in the TT genotype($p<0.05$). Patients who have been supplemented with vitamin C along with Glimepiride had shown an additional effect on blood glucose control, but the reduction of oxidative stress was significant in CC and CT genotype($p<0.05$) but not in TT genotype. Vitamin C is a natural antioxidant, which helps in scavenging free radicals hence the reduction of oxidative stress was observed in patients with type -2 DM. Since Vitamin C is water soluble, hence no possibility of hypervitaminosis and associated toxicity. There are various studies found, that the supplementation of vitamin C along with antidiabetic drugs has helped in the reduction of oxidative stress12 and also improved glycemic control13. Vitamin C prevents the end organ damage in cases of hyperglycemia by scavenging free radicals; inhibiting the accumulation of sorbitol, capillary fragility and protein glycosylation.14, 15

CONCLUSION

TT genotype (Val/Val) in the SOD2 gene is related to poorer diabetic control and high oxidative stress. A better prognosis was observed in the patient's supplemented vitamin C in terms of glycemic and oxidative stress control. We cannot generalize the findings of the present study because it was conducted on a limited number of patients and is limited to a particular geographical area. Hence further research is suggested involving more patients belonging to different geographical areas.

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Conflicts of interest

There are no conflicts of interest.

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