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Research Article

STUDY ON EFFECT OF VITAMIN-C AS ADD ON THERAPY WITH ANTIDIABETIC DRUGS IN TYPE-2 DIABETES MELLITUS PATIENTS ASSOCIATED WITH SOD2 GENE POLYMORPHISM

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ABSTRACT

Diabetes is a metabolic disorder; affect the metabolism of carbohydrates, proteins and lipids. Superoxide dismutase (SOD) is a major antioxidant scavenging enzyme it helps in removal of superoxide. Polymorphism of SOD2 gene may affect the scavenging effect of SOD enzyme which results in rise of oxidative stress in Type-II DM. The present study has been designed to study the effect of vitamin-C as add on therapy with antidiabetic drugs in type-2 diabetes mellitus patients associated with SOD2 gene polymorphism. A total of 64 type-II diabetes mellitus patients associated with SOD2 gene polymorphism (Ala16Val) were enrolled in present study and divided in to two groups *i.e.* Group A& B. There are three genotypes in each such as CC, CT& TT. Group-A& B patients were treated with metformin1000mg/day+ teneligliptin-20mg/day and metformin 1000mg/day+ teneligliptin 20mg/day+ vitamin C-1000mg/day respectively for 6 months. Glycemic control and oxidative stress was compared before and after 6 months of treatment in both groups. SPSS software was used to analyze the data. All the genotypes of both the groups had shown significant glycemic control ($p<0.05$). Significant reduction of TAC and elevation of MDA level was found in CT, TT genotype and CC, CT & TT genotype of group-A patients respectively ($p<0.05$). CC and CT genotype of group-B patients had shown significant elevation of TAC and reduction of MDA level as compared to baseline values ($p<0.05$). Good glycemic control and reduction of oxidative stress can be achieved with supplementation of vitamin-C along with antidiabetic drugs.

Keywords: SOD2 gene polymorphism, Oxidative stress, Vitamin-C.

1. INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disorder which affects the metabolism of lipids and proteins along with carbohydrates and characterized by hyperglycemia. It is a most rapidly growing chronic disease in the world. Broadly, there are two major forms of diabetes, type-I and type-II. More than 90% of cases belong to type-II DM. Over production of free radicals and reduction of capacity of antioxidant defense system over oxidative stress is associated with prolonged hyperglycemia [1].

Chronic hyperglycemia induced oxidative and nitrosative stress is one of the major links between diabetes mellitus and its complications [2]. Autoxidation of glucose and protein glycosylation leads to generation of free radicals. Such increased oxidative and nitrosative stress results in endothelial dysfunction, insulin resistance and pancreatic βcells dysfunction ultimately leads to development of micro and Macrovascular complications [3]. There are various antioxidants available which play a major role in scavenging of superoxide's *e.g.* superoxide dismutase (SOD), catalase (CAT), glutathione S transferase (GST), glutathione peroxidase (GPx), carotenoids, flavonoids, lipoic acid, and vitamins A, C and E. SOD dismutase convert the superoxide to hydrogen peroxide which isfurther converted to water by CAT and GPx. The process of lipid peroxidation is terminated by natural dietary antioxidants like vitamin C, E and lipoic acid [4].

There are three superoxide dismutase enzymes available *i.e.* SOD1 (Cu SOD), SOD2 (Mn-SOD2) and SOD3 (ec SOD) among them SOD2 is the key defensive system against free radicals. SOD2 is encoded by the nuclear SOD2 gene located on the human chromosome 6q25. Ala 16 Val polymorphism has been identified and it is also most commonly studied single nucleotide polymorphism (SNP) in exon2 of human SOD2 gene. This SNP replaces the amino acid Alanine (Ala) at 16 position with Valine

(Val) which leads to conformational changes in the target sequence of manganese superoxide dismutase (MnSOD) and also affects MnSOD activity in mitochondria [5].

Ala 16 Val SNP decreases the transport efficiency of the SOD2 into the mitochondria and modifies the antioxidant defense against ROS. Ala 16 Val SNP results in increased oxidative stress due to production of β-sheet secondary structure instead of the expected α-helix structure [6] which is an important pathophysiological mechanism in development and progression of diabetes and its complication [7, 8]. Lower plasma TAC and higher serum levels of Malondialdehyde (MDA), which indicate increased oxidative stress and compromised antioxidant defenses are reported in type 2 diabetic patients. Being natural antioxidant, Vitamin C plays key role in prevention of diabetic complications by scavenging of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [9, 10]. To reduce the complications of diabetes mellitus, Diabetes Control and Complications trial (DCCT) suggested that tight control of blood glucose level is effective, but even optimal control of blood glucose could not prevent complications hence novel treatment strategies are suggested [11]. Hence the present study has been designed to see the effect of vitamin C supplementation along with antidiabetic agents on glycemic level and oxidative stress in type- II diabetes mellitus patients.

2. MATERIAL AND METHODS

Present study is a randomized, prospective, open label study was carried out in Department of pharmacology collaborated with Department of General Medicine in Rama Medical College, Hospital and Research Centre in 2018 Kanpur. Institutional Ethical Committee approval was taken before starting this study. Patients were enrolled in the study as per following inclusion and exclusion criteria.

2.1. Inclusion criteria

- Newly diagnosed type-2 diabetes mellitus patients who are associated with SOD2 gene polymorphism.
- Patients with age group of 30-50 years from both sexes.
- Who are willing to give informed concern

2.2. Exclusion criteria

- Tobacco consumers/ alcoholics
- Pregnant & lactating women's.
- Type-1 Diabetes mellitus patients.
- Type-2 diabetes mellitus patients without SOD2 gene polymorphism.
- Patients with other medical problems.
- Hypersensitivity to vit-C and any of the study drugs.
- Unwilling to participate and give informed concern or mental incapacity to take the drugs.

Medical history of all the patients was taken at beginning of the study followed by 5ml of venous blood has drawn from peripheral vein to measure blood glucose (HbA1c, FBS& PPBS) oxidative stress level (TAC& MDA) and for DNA isolation to detect SOD2 gene polymorphism. A total of 64 Patients who are associated with SOD2 gene polymorphism were analyzed for the Ala 16 Val sequence of exon 2 of chromosome 6q25.

2.3. Determination of biochemical and genetic parameters:

HbA1c was estimated by Ion Exchange Resin Method [12]. FBS& PPBS was estimated by glucose oxidase peroxidase enzymatic method (GOD POD) [13], parameters for estimation of oxidative stress are MDA & TAC was estimated as thiobarbituric acid reactive substance assay(TBARS) and ferric reducing ability of plasma (FRAP) method respectively.

Genomic DNA was extracted from peripheral blood cells by using a Qiagen Kit (spin protocol procedure and standard protocol followed as given in the Qiagen kit). Genotyping of Ala 16 Val of Mn-SOD was done by PCR-restriction fragment length polymorphism methods. Briefly, the primers for Val 16 Ala 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 Bsaw I, electrophoresed on 2.5% agarose gel, and stained with ethium bromide.

Based on the treatment plan, patients were divided in to 2 groups Group-A& Group-B there were 3 geno types of patients in each group. All the patients were treated for 6 months as follows.

Group-A: Include 3 genotypes CC (n=4), CT (n=10), TT (n=17) total of 31 patients were given Metformin (1000 mg/day) + Teneligliptin (20 mg/day)

Group-B: Include 3 genotypes CC (n=4), CT (n=11), TT (n=18) total of 33 patients treated with Metformin (1000 mg/day) + Teneligliptin (20 mg/day) + vitamin-C (1000 mg/day).

This study had 12 visit's which were scheduled after every 15 days. At every visit each patient was examined for general medical condition and investigations for glycemic control (FBS and PPBS) were done. Level of oxidative stress was assessed along with HbA1c after 3 and 6 months for each subject.

2.4. Statistical Analysis

Data was entered in excel and analyzed by the student's t-test. Results are reported as the mean ± SD.

3. RESULTS

In group-A, after 6 months of treatment, the reduction of FBS, PPBS and HbA1c (p=0.000) were highly significant in all genotypes but high mean reduction was noticed in CC genotype (HbA1c; 1.37±0.09, FBS; 36.2±8.36, PPBS; 40±5.47) followed by CT (HbA1c; 1.25±0.33, FBS; 36.2±8.36, PPBS; 36.3±8.97) and TT geno type (HbA1c; 1.09±0.17, FBS; 30±8.43, PPBS; 32.71± 9.79) as compared to baseline values (table 1 & fig.1).

In group-A, all 3 genotypes had shown significant elevation of MDA level but the reduction of TAC was significant only in CT and TT genotype (p<0.05). High mean difference was found in TT genotype (TAC; 0.15±0.10, MDA; 0.58±0.35) followed by CT(TAC; 0.07±0.09, MDA; 0.58±0.35) as compared to baseline values (table 2 & fig. 2).

Group-B patients of all genotypes had shown significant reduction of FBS, PPBS and HbA1c (p=0.000). High mean reduction of HbA1c, FBS and PPBS was found in CC geno type (HbA1c; 1.5±0.08, FBS; 50.5± 6.60, PPBS; 47.25±14.36) followed by CT (HbA1c; 1.4± 0.11, FBS; 41.36±10.2, PPBS; 41.91±9.85) and TT(HbA1c; 1.26±0.18, FBS; 35.39±8.36, PPBS; 37.11±10.16) as compared with the baseline values (table 3 & fig. 3).

Table 1: Comparison of 6 months findings with baseline values in group-A

Variables	Genotype	Baseline	6 months	Mean ± SD	P value
HbA1c (%)	CC	8.2±0.24	6.82±0.26	1.37±0.09	0.000***
	CT	8.3±0.43	7.05±0.43	1.25±0.33	0.000***
	TT	8.77±0.23	7.68±0.25	1.09±0.17	0.000***
FBS (Mg/dl)	CC	168±11.78	126.5±4.20	41.5 ±8.26	0.000***
	CT	172.6±14.42	136.4±8.55	36.2±8.36	0.000***
	TT	180.1±11.26	150.1±14.8	30±8.43	0.000***
PPBS (Mg/dl)	CC	270.2±7.18	230.2±6.94	40±5.47	0.000***
	CT	273.2±9.55	236.9±8.92	36.3±8.97	0.000***
	TT	281.7±8.94	249±10.82	32.71±9.79	0.000***

*Highly significant P<0.001***; Significant P<0.05***

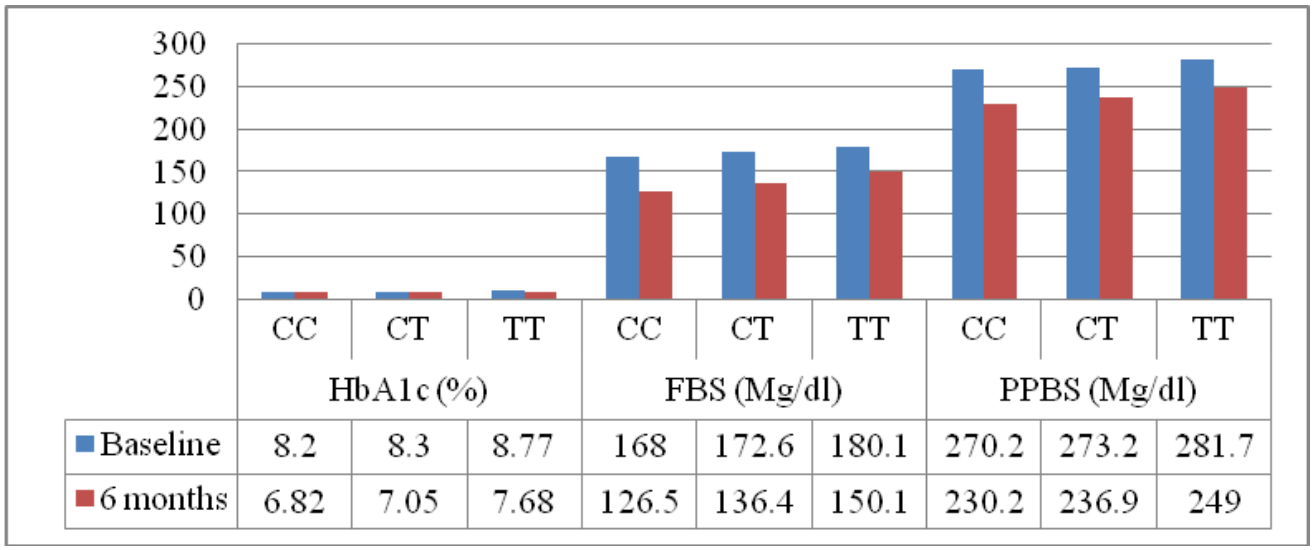

Fig. 1: Baseline Vs 3 months values in group-A

Table 2: Comparison of 6 months findings with baseline values in group-A

Variables	Genotype	Baseline	6 months	Mean ± SD	P value
TAC (mmol/l)	CC	0.8±0.08	0.72±0.09	0.07±0.09	0.215
	CT	0.68 ±0.12	0.57±0.08	0.11±0.11	0.017**
	TT	0.6 ±0.06	0.44±0.06	0.15±0.10	0.000***
MDA (mmol/l)	CC	4.6±0.24	4.87±0.35	0.27±0.15	0.035**
	CT	5.36±0.33	5.94±0.34	0.58±0.35	0.000***
	TT	5.98±0.31	6.67±0.16	0.68±0.22	0.000***

*Highly significant P<0.001***; Significant P<0.05***

Table 3: Comparison of 6 months findings with baseline values in group-B

Variables	Genotype	Baseline	6 months	Mean±SD	P value
HbA1c (%)	CC	8.27±0.20	6.77±0.22	1.5 ±0.08	0.000***
	CT	8.49±0.32	7.09±0.37	1.4±0.11	0.000***
	TT	8.87±0.17	7.61±0.18	1.26±0.18	0.000***
FBS (Mg/dl)	CC	171.8±7.18	121.2±4.57	50.5±6.60	0.000***
	CT	173.5±11.84	132.1±9.64	41.36±10.2	0.000***
	TT	181.4±12.44	146±7.452	35.39±8.36	0.000***
PPBS (Mg/dl)	CC	271.2±10.69	224±6.782	47.25±14.36	0.000***
	CT	274.4±14.48	232.5±13.14	41.91±9.85	0.000***
	TT	281.7±11.75	244.6±10.65	37.11±10.16	0.000***

*Highly significant P<0.001***; Significant P<0.05***

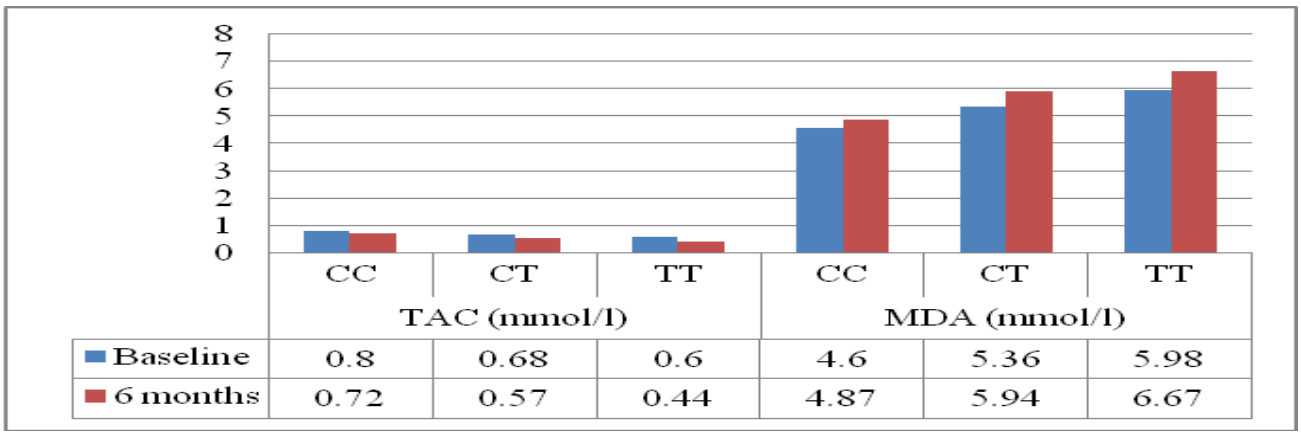

Fig. 2: Baseline Vs 3 months values in group-A

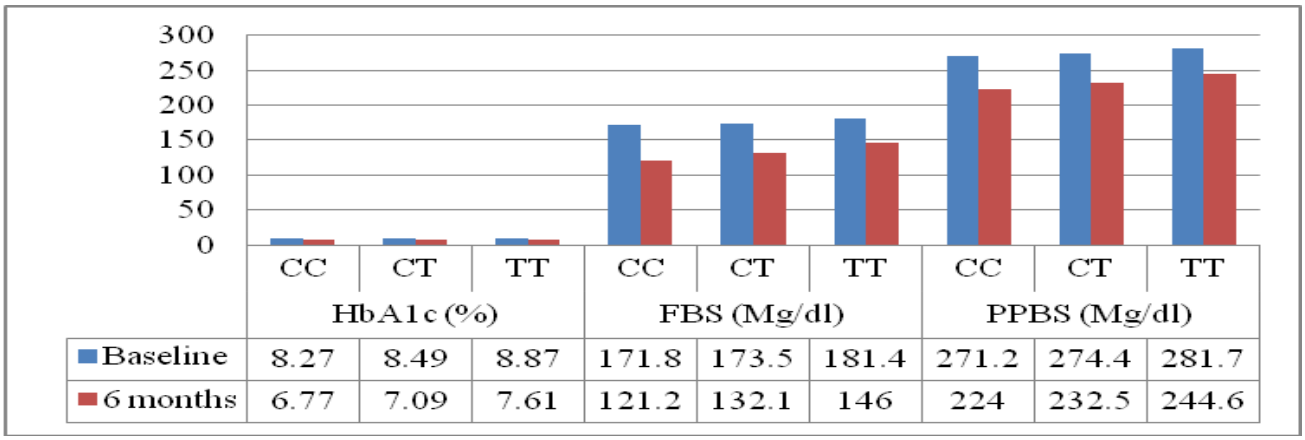

Fig. 3: Baseline Vs 3 months values in group-B

Significant elevation of TAC and reduction of MDA level was found in CC and CT genotypes of group-B patients as compared to baseline values, but high mean difference was found in CC genotype (TAC; 0.27±0.09, MDA; 0.975±0.26) followed by CT (TAC; 0.17±0.06, MDA; 0.47±0.45). No significant difference was found in TT genotype (table 4 & fig. 4). Overall the reduction of blood glucose and level of oxidative stress was higher in CC genotype of both group-A and B as compared with CT and TT genotype. However high mean reduction was noticed in vitamin C supplemented group (group-B).

Table 4: Comparison of 6 months findings with baseline values in group-B

Variables	Genotype	Baseline	6 months	Mean ± SD	P value
TAC (mmol/l)	CC	0.75± 0.12	1.02±0.18	0.27±0.09	0.010
	CT	0.67±0.07	0.84±0.08	0.17±0.06	0.050
	TT	0.56 ±0.09	0.64±0.15	0.08±0.18	0.069
MDA (mmol/l)	CC	5.07±0.33	4.1±0.08	0.975±0.26	0.005
	CT	5.88±0.36	5.40±0.59	0.47±0.45	0.006
	TT	6.06±0.54	5.84±0.63	0.22±0.70	0.198

*Highly significant P<0.001***; Significant P<0.05***

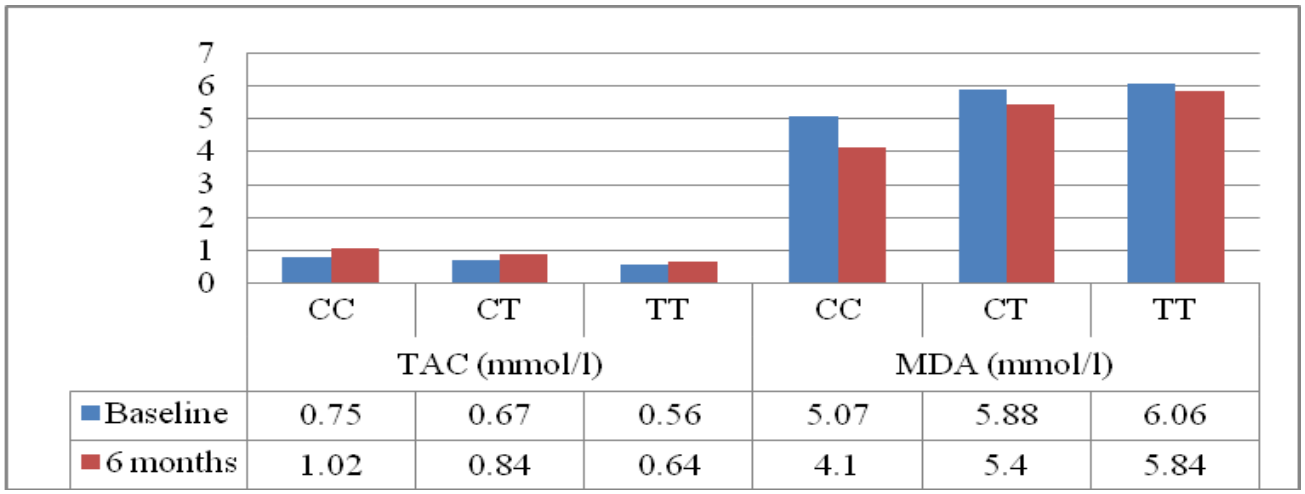

Fig. 4: Baseline Vs 3 months values in group-A

4. DISCUSSION

Aim of conducting the present study was to see control of blood glucose level and oxidative stress in newly diagnosed type-II diabetes mellitus patients who are associated with SOD2 gene polymorphism. Among 64 newly diagnosed type-II diabetic patients associated with SOD2 gene polymorphism, 43.75% males and 56.25% females. 53.1% of study population belongs to age group 41 to 50 years followed by 34.3% and 14% of study subjects belongs to 30 to 40 and 51 to 60 years respectively. Among 64 study subjects 54.6% TT genotype (Val/Val) followed by CT (Ala/Val) 32.8% and CC (Ala/Ala) 12.5%, similar results were found in another study [14]. There was a significant reduction of HbA1c (P<0.001), FBS(P<0.001) and PPBS(P<0.001) in three genotypes of both group A and B after 6 months of treatment. Mean reduction of HbA1c, FBS, PPBS was more in CC (HbA1c:1.3%; FBS: 41.5mg/dl; PPBS: 40 mg/dl) genotype followed by CT (HbA1c:1.25%; FBS: 36.2mg/dl; PPBS: 36.3mg/dl) and finally TT (HbA1c: 1.09%; FBS:30 mg/dl; PPBS:32.7mg/dl) genotype of group-A as compared to baseline values.

Similarly mean reduction of HbA1c, FBS, PPBS was more in CC (HbA1c:1.5%; FBS: 50.5mg/dl; PPBS: 47.25 mg/dl) genotype followed by CT (HbA1c:1.4%; FBS: 41.3mg/dl; PPBS: 41.9mg/dl) and finally TT (HbA1c: 1.26%; FBS: 35.3 mg/dl; PPBS: 37.1mg/dl) genotype of group-B as compared to baseline values. However high mean reduction of HbA1c, FBS and PPBS was seen in Group-B patients when compared with group-A as they were treated with antidiabetic agents along with supplementation of Vitamin-C. Poor

glycemic control was found in TT (Val/Val) genotype than CT (Ala/Val) and CC (Ala/Ala) genotypes, similar results were found in another study [14]. There is another study concluded that supplementation of vitamin-C along with antidiabetic drugs has additional control on blood glucose [15], but in which study population were not mentioned as associated with SOD 2 gene polymorphism.

Significant reduction of TAC in CT (<0.05) and TT genotype (<0.001) and elevation of MDA level was found in CC (<0.05), CT (<0.001) and TT (<0.001) genotype of group-A patients after 6 months of treatment with antidiabetic drugs(Metformin+ Teneligliptin). However high mean reduction of TAC & elevation of MDA was noticed in TT (TAC: 0.15mmol/l; MDA: 0.68mmol/l) followed by CT (TAC: 0.11mmol/l; MDA: 0.58 mmol/l) as compared to CC (TAC: 0.07mmol/l; MDA: 0.27mmol/l) genotype of group-A patients.

Similarly the elevation of TAC and reduction of MDA was noticed significantly in CC(<0.05) and CT(<0.05) genotype of group-B patients who were treated with antidiabetic drugs (Metformin+ Teneligliptin) along with supplementation of 1000mg of vitamin C/day. However high mean elevation of TAC & reduction of MDA was noticed in CC (TAC: 0.27mmol/l; MDA: 0.97mmol/l) followed by CT (TAC: 0.17mmol/l; MDA: 0.47 mmol/l) as compared to TT (TAC: 0.08mmol/l; MDA: 0.22mmol/l) genotype of group-B patients. Some researchers confirmed that TAC increases in diabetic patients after supplementation of vitamin-C along with oral hypoglycemic drugs [16]. In present study, group –B (CC and CT genotype) patients had shown significant reduction of MDA after supplementation of an antioxidant (vitamin-C) with antidiabetic drugs and these findings are next to another study but in which patients are not mentioned as associated with SOD2 gene polymorphism [17]. There are various antioxidants which act by different mechanisms including enzymes which degrade the free radicals and vitamins C and E that act as free radical scavengers [18].

5. CONCLUSION

Based on results of present study we conclude that presence of TT genotype (Val/Val) in SOD2 gene is related to poorer diabetic control and high oxidative stress. Treatment of diabetes mellitus with antidiabetic drugs alone has no effect on control of oxidative stress, but supplementation of vitamin-C along with anti-diabetic drugs limits the oxidative stress and also has additional effect over blood glucose level in CC (Ala/Ala) genotype followed by CT (Ala/Val) genotype.

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Conflicts of interest

There are no conflicts of interest.

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