International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

The Effect of Vitamin C on Serum Superoxide Dismutase and Blood Sugar Levels in the Patients of Type 2 Diabetes Mellitus

Vinod Kathore[1], AN Shete[2], US Zingade[3], DG Bansode[4]

[1]Assistant Professor, [4]Professor and Head, Dept. of Physiology, Dr Ulhas Patil Medical College & Hospital, Jalgaon.
[2]Associate Professor, Department of Physiology, Government Medical College, Aurangabad.
[3]Professor, Department of Physiology, BJ Medical College, Pune, India.

Corresponding Author: Vinod Kathore

Received: 12/08//2014 *Revised: 09/09/2014* *Accepted: 09/09/2014*

ABSTRACT

Aim: Diabetes mellitus is a growing and massive silent epidemic that has the potential to cripple health services in all parts of the world. Increasing evidence suggest that increased oxidative stress might play an important role in pathogenesis of diabetes mellitus. Oxidative stress occurs when free radical production exceeds the body's ability to neutralize them. So our aim was to study the effect of vitamin-C supplementation on serum superoxide dismutase (SOD) and blood sugar levels (BSL) in the type 2 diabetes mellitus patients.
Materials and Methods: Thirty type 2 diabetic patients on oral hypoglycemic drugs were given Vitamin-C for six weeks. During the study period the treatment and diet was not changed. Serum SOD and BSLs were assessed in all the patients before and after Vitamin-C supplementation by using standard methods.
Results: The mean serum SOD value in type 2 DM patients before and after supplementation of Vitamin-C was 2.21 ± 0.38 U/ml and 2.85 ± 0.18 U/ml respectively. The mean BSL value decreased after supplementation of Vitamin-C from 141.9 ± 39.15 to 129.1 ± 29.59 mg/dl. When these values were compared, using 'Paired t test', it showed statistically significant difference.
Conclusion: Supplementation of vitamin C in addition to the normal diet and treatment schedule helps in improving serum SOD and BSL in the patients with type-2 diabetes. Detecting such changes may be used in the treatment or to halt further progression of the disease.

***Key words**:* Free radical, oxidative stress, superoxide dismutase, serum BSL, type 2 diabetes mellitus, vitamin-C.

INTRODUCTION

Diabetes Mellitus (DM) is third commonest disease in the world and one of the leading causes of death after cancer and heart diseases. It comprises a group of common metabolic disorders that share the common phenotype hyperglycemia. [(1)] It is growing and massive silent epidemic that has potential to cripple health services in all parts of the world. In India the prevalence of disease in adults is 2.4% in rural and 4.0 – 11.6% in urban dwellers. [(2)]

Several distinct types of DM exist and are caused by a complex interaction of

genetics, environmental factors and life style choices. [(2)] Increasing evidence suggest that increased oxidative stress might play an important role in initiation, progression and complications of diabetes mellitus. [(3)] It is accepted that oxidative stress results from an imbalance between generations of oxygen derived radicals and organism's antioxidant potential.

The oxidants i.e. free radicals are reactive oxygen species and reactive nitrogen species (ROS/RNS). ROS include super oxide, hydrogen peroxide and hydroxyl radicals. RNS include nitric oxide, nitrogen dioxide and peroxynitrite. [(4)]

However, there are also protective mechanisms to attenuate the deleterious effects of these oxidants. These are the Antioxidants. One of the crucial of them is superoxide dismutase (SOD), which convert superoxide anions into hydrogen peroxide. [(5)] Reduced level of enzyme SOD lowers the protection against superoxide anion. Vitamin C is an important antioxidant in aqueous phase, capable of scavenging oxygen derived free radicals. [(6)]

Hence the study aimed at to evaluate the effect of Vitamin C supplementation on serum Superoxide Dismutase (SOD) and Blood Sugar Levels in patients with Type 2 Diabetes Mellitus.

MATERIALS AND METHODS

This is Open Label Randomized Prospective Clinical Trial also called 'Before and After' study carried out in the tertiary care centre. The study subjects were selected from diabetic outpatient department. Thirty known type 2 diabetes mellitus patients between the age group 40 to 80 years of both sexes on oral hypoglycemic drugs, with mean duration of disease 1 to 8 years and having blood glucose level less than 250 mg/dl were selected. (n=30).

Type 2 DM patients with complications and having blood glucose levels higher than 250 mg/dl, were excluded. Also diabetes patients taking insulin for controlling glucose levels were excluded.

A detailed history, thorough general and systemic examinations were done. Age, body weight, standard height, body mass index (BMI) and blood pressure were measured. After written and informed consent, Tablet Limcee (500 mg bd) containing Vitamin-C or Ascorbic acid was given for six week to every patient. During the study period the treatment plan was not changed. They were instructed not to change diet during the study period. They were interviewed regarding any changes in lifestyle or abnormal events such as disease or infection at each examination. None of the patients used additional oral vitamins, either prior to or during the study period. After 6 weeks the investigations were performed.

Under all aseptic condition, 12 hour fasting venous blood samples were collected from all participants in plain and Fluoride bulbs. All samples were taken in the morning to avoid the confounding effect of diurnal variation of oxidative stress parameters as reported previously. [(7)] Serum was separated after 1 hour by centrifugation at 3000 rpm for 10 minutes, and was tested for various parameters. The following investigations were done *'Before'* and '*After*' the supplementation of vitamin C.

I. Estimation of Serum Superoxide Dismutase: Superoxide dismutase was assayed in all the study groups by the method of Marklund S, Marklund G modified by Nandi and Chatterjee. [(8,9)]
Normal range: SOD in serum is 2.93-3.71 units/ml.

II. Blood Glucose Level: Quantitative estimation done by glucose oxidase peroxidase end point method (GOD-POD)

using commercial kits from ERBA diagnostics. [10] Normal range: 70-110 mg/dl.

RESULTS

In this study, serum SOD values in type 2 DM before and after supplementation of Vitamin-C (Tablet Limcee) 1000 mg daily for 6 weeks were estimated and compared. For this parameter, the mean value and standard deviation (SD) were calculated in study group. 'Paired t test' was applied to test whether the differences in means were statistically significant or not. P-value less than 0.05 ($P < 0.05$) was considered to be statistically significant. P-value of less than 0.001 ($P < 0.001$) was considered to be statistically highly significant. The results of the present study are as follows.

The mean duration of Type 2 Diabetes Mellitus in the study subjects was 4.13 ± 1.74 years (Table-A).

The mean values for the age, body weight, height and body mass index in the study group were 54.03 ± 8.22 years, 67.51 ± 8.91 kg, 163.08 ± 8.12 cm and 25.33 ± 2.42 kg/m^2 (Table-A).

TABLE-A Table showing the demographic data of the study group

Sr. No.	Parameter	Subjects
1.	Age(years) (Mean±SD)	54.03 ± 8.22
2.	Male : Female ratio	1 : 1.14
3.	Weight(kilograms) (Mean± SD)	67.51 ± 8.91
4.	Height(centimetres) (Mean±SD)	163.08 ± 8.12
5.	Body Mass Index (kg/m^2) (Mean±SD)	25.33 ± 2.42
6.	Mean Duration Of Disease (years) (Mean±SD)	± 1.74

The mean serum SOD value was 2.21 ± 0.38 U/ml in type 2 diabetes mellitus and 2.85 ± 0.18 U/ml after supplying tablet Limcee i.e. Vitamin-C for 6 weeks. When these values were compared, using 'Paired t test', it showed statistically highly significant difference with $P< 0.001$ (Table-B).

TABLE –B Table showing serum SOD level (U/ml) before and after supplementation of Vitamin-C for 6 weeks in study group

Study Subjects	Serum SOD (Mean ± SD)	t Value	P Value	Remark
Pre Vitamin-C Supplementation.	2.21 ± 0.38	9.967	< 0.0001	HS**
Post Vitamin-C Supplementation	2.85 ± 0.18			

HS: Highly Significant.

The mean fasting Blood Sugar Level (BSL) was 141.9 ± 39.15 mg/dl in type 2 diabetes mellitus patients and 129.1 ± 29.59 mg/dl after supplying tablet Limcee containing Vitamin-C. When these values were compared, using 'Paired t test', it showed statistically significant difference with $P< 0.05$ (Table-C).

The improvement in serum SOD level was more significant than the improvement in fasting BSL. The 'P' value for serum SOD was highly significant (< 0.001) while for BSL 'P' value was significant (<0.05)

TABLE –C Table showing Fasting blood glucose level (mg/dl) before and after supplementation of Vitamin-C for 6 weeks in study group

Study Subjects	Fasting BSL (Mean ± SD)	t Value	P Value	Remark
Pre Vitamin-C Supplementation.	141.9 ± 39.15	3.371	0.0021	S*
Post Vitamin-C Supplementation	129.1 ± 29.59			

S: Statistically significant.

DISCUSSION

Free radicals are considered to have a great role in pathogenesis of many diseases such as Diabetes Mellitus, Atherosclerosis, Cancer, Myocardial Infarction etc. The oxidative-antioxidative system imbalance leads to the pathology called oxidative stress. Oxidative stress occurs when there is an excessive free radical production and/or low antioxidant defense and results in chemical alterations of biomolecules, which cause structural and functional modifications like lipid peroxidation, damage to proteins and DNA. The levels of reactive oxygen species is controlled by enzymes like Superoxide Dismutase, Glutathione Peroxidase (GPx) and nonenzymatic scavengers like Vitamin C and Vitamin E. [(11,12)]

This oxidative stress is the potential mechanism contributing to diabetic complications that overwhelms the primary antioxidant defense of the body. One of the crucial antioxidant defenses of the body is SOD, which is the only enzyme family with activity against superoxide radicals. It catalyzes the dismutation of superoxide radicals ($O^{\cdot}_2$) into O_2 and H_2O_2. [(3)]

Superoxide dismutase, which uses free radicals as a substance, has been the chief enzyme and scavenging system investigated. SOD is more effective in removing superoxide ions, than thiols i.e. reduced glutathione.

In patients with type 2 DM persistent hyperglycemia causes free radical formation and excessive oxidative stress, which plays an important role in pathogenesis of diabetes mellitus microangiopathic complications, like retinopathy and nephropathy. The oxidative stress leads to decreased levels of SOD, because it is natural free radical scavenger. The SOD level in patients with DM significantly correlate with glycemic status or the glycemic status deteriorates the SOD level. Thus low levels of SOD signify poor metabolic control and these patients are prone to microangiopathic complications. Thus it is evident from present study that assessment of SOD levels may identify the subjects at high risk of developing microangiopathic complications. [(13)]

A lowering of SOD activity has been reported in some previous studies. Matkovics et al were the first to suggest an association of lowered SOD activity with diabetes. [(14)] Crouch et al showed that in experimental diabetes in rats, the activity of SOD in red blood cells was decreased by 50% in comparison with healthy animals. [(15)] Thus, the above studies including present study may point towards increased production of free radicals in the patients of type 2 DM which causes increased consumption of SOD leading to decrease in the SOD levels.

Oxidative stress in diabetes is a result of the hyperproduction of reactive oxygen forms on the one hand and hypoactivity of the antioxidative system on the other. Long-term hyperglycemia may be an initializing factor for the systemic oxidative stress in diabetes. [(16)]

Besides the increased production of ROS in diabetes, revealed indirectly by intensified lipid peroxidation, weakening of

defensive mechanisms also upsets the oxidation-reduction balance. The systemic defence functions against oxidative damage are performed by two antioxidative systems: enzymatic and non-enzymatic. Their task is to prevent the formation of ROS as well as to scavenge existing ROS. [17]

Major reason for the decreased SOD activity is the glycosylation of Cu, Zn-SOD which has been shown to lead to enzyme inactivation both in vivo and in vitro. Also Cu, Zn-SOD cleavage and release of Cu++ in vitro resulted in transition metal catalyzed ROS formation. Erythrocyte Cu, Zn-SOD activity correlated inversely with indices of glycemic control in DM patients. [18]

But Palanduz S, et al (2001) found higher serum SOD level in patients with type 2 DM than controls and does not correlate with the present study. [19] One possible explanation to this contrast in the findings may be due to increase in SOD level at start to compensate the disease process, but later on the enzyme SOD is utilized and the level falls.

Vitamin-C (Ascorbic acid) is required for variety of reactions including collagen formation and protection against oxidative damage. Vitamin-C will eventually decrease in diabetic tissues, since it is not efficiently recycled. Hyperglycemia increases urinary losses of ascorbate. Low levels of ascorbic acid may make the diabetic patient more susceptible to wound infection, delayed healing, endothelial dysfunction, and tenosynovial disease. Thus diabetic patients require greater amounts of ascorbic acid.

Maxwell SR et al (1997) studied 28 type 1 and 24 type 2 uncomplicated diabetic patients with age matched non diabetic control groups and found that significant lower Vitamin C levels in type 2 DM patients. [20]

Ozlem Yildirim (2009) observed the effect of vitamin-C on streptozotocin (STZ) induced diabetic rats. Rats were given 1000 mg/l Vitamin-C with 0.5 mM CoCl2 in drinking water for eight weeks. Serum SOD and Catalase activity measured before and after treatment. In treated groups, SOD and CAT activities increased significantly compared to diabetic controls. [21]

Bonina FP et al (2002) studied the effect of red orange extract on oxidative stress in type 2 DM patients. Red orange extract contains Vitamin-C and phenolic compounds as its main content. 33 patients with type 2 DM and a comparison group of 28 healthy volunteers are supplied with Red orange extract for 2 months. After two months the serum free radical level decreased significantly in test group. [22]

Vitamin-C is an important antioxidant in human, capable of scavenging oxygen-derived free radicals. Vitamin-C is structurally similar to glucose and can replace it in many chemical reactions, and thus is effective in prevention of non-enzymatic glycosylation of proteins. [23]

Endothelial dysfunction is a hallmark of type 2 diabetes related to hyperglycaemia and oxidative stress. This endothelial dysfunction may worsen insulin resistance. It may be possible that vitamin-C as an antioxidant can probably reduce insulin resistance by improved endothelial function and lowering oxidative stress.

Thus after supplementation of Vitamin-C 1000 mg/day to type 2 DM patients for 6 weeks there was improvement in serum SOD levels and decrease in Blood Sugar Level.

Thus to conclude, it can be said that, as there is intense diversity regarding etiopathogenesis of type 2 DM and its correlation with oxidative stress, this study was an attempt to establish a relationship among oxidative stress, antioxidants and type 2 DM. Even though it was interpreted from the present study that, oxidative stress is not the only causative factor for type 2

DM, it is sure that oxidative stress is one of the associated factors in type 2 DM , either as a cause or as a result of type 2 DM itself. Antioxidant like Vitamin-C has an important role in controlling the disease process.

SUMMARY AND CONCLUSIONS

The present study was undertaken to evaluate the effect of Vitamin-C supplementation on serum Superoxide Dismutase (SOD) levels in patients with Type 2 Diabetes Mellitus. Patients on hypoglycemic drugs were supplemented with Vitamin-C 1000 mg/day for 6 weeks. The treatment plan was not changed during the study period.

In the present study as SOD levels were decreased significantly in Type 2 DM patients. Type 2 DM leads to increased consumption of SOD leading to decrease in the SOD levels. This shows that oxidative stress is present in Diabetes and after supplying antioxidant it decreases.

Thus, there seems to be fair chances for defeating the free radical injury and oxidants with the use of suitable antioxidants given therapeutically to the patients suffering from this costly disease and also development of DM complications can be prevented.

In conclusion, supplementation of vitamin-C in addition to the normal diet and treatment schedule may help in improving serum SOD and plasma glucose in patients with Type 2 Diabetes.

As the role of oxidative stress in the pathogenesis of DM is well established, further detailed studies are required to combat this stress, especially by using the easily available antioxidants, either dietary or in therapeutic forms. Detecting such changes in the levels of SOD may be used to halt further progression of the disease or follow up of its treatment and to save the cost of its management.

ACKNOWLEDGEMENTS

During the project we got help from all the teaching staff of Department of Physiology, Government Medical College, Aurangabad. So I am thankful to all of them. We sincerely thank all the patients for the participation.

REFERENCES

1. Alvin C Powers. Editors: Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, et al. Diabetes Mellitus In: Harrison's Principles of Internal Medicine, 17th edn. New York: The McGraw-Hill, health professions division; 2008; pp2275-304.
2. Park K. Text Book of Preventive and Social Medicine. 20th edn, Jabalpur. M/S Banarsidas Bhanot Publishers. 2009; pp341-3.
3. Roja Rahimi, Shekoufeh N, Bagher L. A review on the role of antioxidants in the management of diabetes and its complication, Biomedicine and Pharmacotherapy, 2005; 59:pp365-73.
4. Russell P. Bowler and James D. Crapo. Oxidative Stress in Airways. Is There a Role for Extracellular Superoxide Dismutase? Am. Journal of Respiratory and Critical Care Medicine, 2002; Vol 166: s38-s43.
5. Robbins and Cortron. Editors: Vinay Kumar, Abul K Abbas, Nelson Faustro. Pathologic Basis of Diseases: 8th edn. Philadelphia, Saunders: 2010; pp1-41.
6. KD Tripathi, editor: M Tripathi. Essentials of Medical Pharmacology; 6th edn, Place: New Delhi: 2008; pp 869-78.
7. Bridges, Fisher AB, Scott TC. Circadian rhythm of white blood cell aggregation and free radical status in healthy volunteers. Free Rad Res Commun, 1992; 16:pp89-97.
8. Anuradha nandi and Chatterjee IB. Assay of superoxide dismutase activity in animal tissues J. Biosci., September 1988; Vol. 13, Number 3:pp305–15.
9. Stefan Marklund and Gudrun Marklund. Involvement of the Superoxide Anion Radical in the Autoxidation of

Pyrogallol and a Convenient Assay for Superoxide Dismutase Eur. J. Biochem. 1974; 47:469-74.
10. Trinder P. Determination of blood glucose using an oxidase - peroxidase system with a noncarcinogenic chromogen. Journal of clinical pathology 1969; 22:158-61.
11. Kinalski, Sledziewski M, Tejelko A. Lipid peroxidation and scavenging enzyme activity in streptozocin induced diabetes. Acta Diabetol, 2000; 37:179-83.
12. Ramanathan, M Jaiswal, A K Bhattacharya. Superoxide dismutase, catalase and glutathione peroxidase activities in the brain of streptozocin induced diabetic rats. Ind. J. Exp. Biol., 1999; 37:182-183.
13. M. Irshad and PS Chaudhuri; Oxidant-Antioxidant system; role and significance in human body, Indian Journal of Experimental Biology, Nov 2002; vol. 40: pp 1233-39.
14. Matkovics B, Varga SI, Szabo L et al. The effect of diabetes on the activities of the peroxide metabolism enzymes. Horm. Metab. Res., 1982; 14:pp77–79.
15. Crouch R, Kimsey G, Priest DG et al. Effect of streptozotocin on erythrocyte and retinal superoxide dismutase. Diabetologia, 1978; 15:pp53–57.
16. Kedziora-Kornatowska KZ, Luciak M, Paszkowski. Lipid peroxidation and activities of antioxidant enzymes in the diabetic kidney: effect of treatment with angiotensin convertase inhibitors. IUBMB Life, 2000; 49:303–7.
17. Salahudeen AK. Role of lipid peroxidation in H2O2-induced renal epithelial (LLC-PK1) cell injury. Am J Physiol, 1995; 268:F30–F38.
18. Arai K, Iizuka S, Tada Y. Increase in the glucosylated form of erythrocyte Cu-Zn-superoxide dismutase in diabetes and close association of the nonenzymatic glucosylation with the enzyme activity. Biochimica et Biophysica Acta 1987; 924:292-96.
19. Palanduz S, Ademoglu E, Gokkuosu et al. Plasma antioxidant and type 2 DM. res. Commun Mol Pathol Pharmacol. 2001; 109 (5-6):309-18.
20. Maxwell SR, Thomason H, Sandler D. Antioxidant status in patients with uncomplicated insulin dependent and Non insulin dependent diabetes mellitus. Eur. J Clin Invest. 1997; 27(6):pp484-90.
21. Ozlem Yildirim. The effect of vitamin C and cobalt supplementation on antioxidant status in healthy and diabetic rats. African Journ of Biotechnology; October, 2009; Vol. 8 (19):5053-58.
22. Bonina FP, Leotta C, Scalia G, et al. Evaluation of oxidative stress in diabetic patients after supplementation with a standardized red orange extract. Diab. Nutr. Metab. 2002; 15:14-19.
23. Mohammad Afkhami-Ardekani and Ahmad Shojaoddiny-Ardekani. Effect of vitamin C on blood glucose, serum lipids & serum insulin in type 2 diabetes patients. Indian J Med Res, November 2007; 126:pp471-74.

How to cite this article: Kathore V, Shete AN, Zingade US et. al. The effect of vitamin C on serum superoxide dismutase and blood sugar levels in the patients of type 2 diabetes mellitus. Int J Health Sci Res. 2014;4(10):94-100.

****