

ORIGINAL PAPER

A Study of Antioxidant Levels in Patients with Diabetes Mellitus

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ABSTRACT

A case control study was undertaken in a tertiary medical care hospital to find out the antioxidant status in patients with diabetes mellitus compared to normal healthy subjects, significance of estimation of serum vitamin C and vitamin E levels as indicators of antioxidant status and development of diabetic complications due to their deficiency. Out of total 60 subjects, 30 healthy individuals were taken as control group and 30 cases of diabetic patients with and without complications were taken as test group. The fasting plasma glucose (FPG), Vitamin C, Vitamin E, Serum total cholesterol, serum triglyceride (TGL), and serum high density lipoprotein (HDL) were estimated by colorimetric methods. The mean serum FPG, vitamin C and vitamin E level in control group and the test group were found to be $81.57 \pm 11.61 \text{ mg\%}$ and $193.43 \pm 52.39 \text{ mg\%}$; $1.388 \pm 1.46 \text{ mg\%}$ and $0.106 \pm 0.103 \text{ mg\%}$; $1.62 \pm 1.429 \text{ mg\%}$ and $0.424 \pm 0.893 \text{ mg\%}$ respectively with a significance of $P < 0.001$. Serum total cholesterol and triglyceride levels were found to be increased and serum HDL was found to be decreased in both complicated and non-complicated diabetes mellitus as compared to control subjects with a significance of $P < 0.001$. The level of antioxidants (vitamin C and vitamin E) is decreased in diabetes mellitus which is more in case of diabetes with complications. Antioxidant status can be used as an indicator of severity complication and duration of diabetes mellitus.

Keywords: Case control study, Vitamin C, Vitamin E, Fasting plasma glucose

INTRODUCTION

It appears that, the service of free radicals occurring in human system is to curtail microbial attack and regulate many vital processes such as apoptosis, neurotransmission, blood pressure regulation, inhibition of platelet aggregation, immune system and so on, but their exaggerated presence can lead to devastating consequences resulting in a vast spectrum of diseases such as cancer, cardiovascular diseases, hepatic and renal disorders, cataracts, aging, infertility and AIDS. Diabetes mellitus is associated with increased lipid peroxidation. Increased levels of lipid peroxides have been implicated in the pathogenesis of diabetic complications.¹

Normally the human body has an abundant supply of antioxidants which are naturally occurring substances, and it is seen that oxidative stress caused by free radicals is counteracted by this abundant supply of antioxidants that is, they delay or inhibit oxidation and neutralize the free radicals. In nature, therefore, when there is a balance of “oxidative stress” and the “antioxidant supply”, there is perfect harmony and no tissue destruction occurs.

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However, if there is an imbalance, i.e., either an excess of free radicals or deficiency of antioxidant supply, tissue damage can occur.

Reports indicate that some complications of diabetes mellitus are associated with increased activity of free radicals and accumulation of lipid peroxidation products.² Recently attention has been given on the relation of antioxidant levels in diabetes mellitus, and it is established that antioxidants have profound effect on lowering various complications in diabetes mellitus. In order to bring the desirable improvement of the sufferings of diabetes with or without complications, the practice of the use of antioxidants can be emphasized. Recent results suggest that the antioxidant deficiency and excessive peroxide-mediated damage may appear in non-insulin dependent diabetes mellitus.³

This study has been undertaken to compare the antioxidant levels (vitamin C and vitamin E) between diabetic and non-diabetic cases and also to see whether serum antioxidant activity can predict the complications in patients with diabetes mellitus.

MATERIALS AND METHODS

A case control study was carried out among the patients of diabetes mellitus with and without complications in the department of Biochemistry, Assam Medical College and Hospital, Dibrugarh, Assam. The study was conducted on thirty (30) diabetic patients admitted in various wards of the Assam Medical college and Hospital, Dibrugarh, Assam. Parallel to these cases, thirty (30) healthy subjects of various age groups have been selected from normal population and their blood were also examined as diabetic patients, and this group is marked as non diabetic or control subjects.

Already diagnosed known diabetic patients of different age groups which were admitted in Assam Medical College and hospital were selected on the basis of the criteria given by American Diabetic Association.⁴

Diabetic cases were divided into two (2) groups on the basis of presence or absence of complications:

- (1) Group I : Diabetic patients with complications – Fifteen (15) numbers
- (2) Group II: Diabetic patients without complications – Fifteen (15) numbers

As complications of diabetes mellitus, the patients had ischemic heart disease, diabetic nephropathy, diabetic

retinopathy, hypertension, peripheral vascular disease and diabetic neuropathy.

Following investigation were done to assess the antioxidant levels and for diagnosis of diabetes mellitus in the subjects:

- (i) Serum vitamin C by colorimetric method of Arya SP⁵
- (ii) Serum vitamin E by Baker and Frank method⁶
- (iii) Fasting Plasma glucose by hexokinase method⁷
- (iv) Serum total cholesterol by homogenous enzymatic colorimetric assay (CHOD-PAP method)⁸
- (v) Serum HDL by enzymatic colorimetric assay⁸
- (vi) Serum triglycerides by GPO/PAP method⁹

RESULTS AND OBSERVATION

Age and sex distribution of the subjects: It was found that maximum numbers of cases in diabetic group were in the age group of 51 – 60 years (43.33%) and this was followed by the age group of 41 – 50 years (30%). No case was found below 20 years of age. Out of 30 cases there were 25 males and 5 females in the diabetic group. Male preponderance was observed with a ratio of 5:1.

Table 1 Age and sex distribution of the subjects

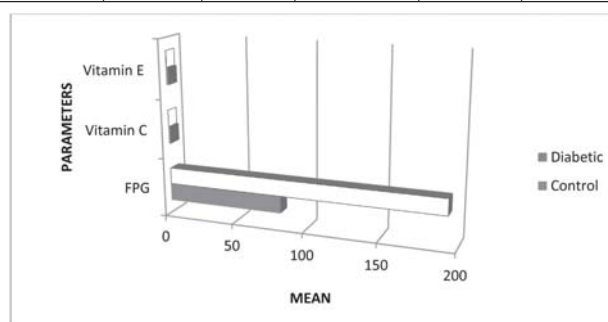
Variables		Diabetic group		Control group	
		Number of cases	Percentage	Number of cases	Percentage
Age in years	0 – 20	0	0	0	0
	21 – 30	4	13.33	4	13.33
	31 – 40	1	3.33	9	30
	41 – 50	9	30	9	30
	51 – 60	13	43.33	7	23.33
	61 – 70	1	3.33	0	0
	71 – 80	1	3.33	1	3.33
	80 and above	1	3.33	0	0
Sex	Male	25	83.33	22	73.33
	Female	5	16.66	8	26.66

Fasting plasma glucose, vitamin C and vitamin E in Diabetic and non-diabetic control groups:

Table 2 Range, mean & standard deviation of fasting plasma glucose, plasma vitamin C & E in controls & diabetics

Different parameters	Controls (No. 30)			Diabetes without complications (No. 15)			Diabetes with complications (No. 15)		
	Range	Mean	SD ($\pm$)	Range	Mean	SD ($\pm$)	Range	Mean	SD ($\pm$)
Fasting plasma Glucose (mg%)	63–102	81.7	11.61	135 – 200	153.13	19.90	180 – 330	233.73	42.96
Vitamin C (mg%)	0.6–1.95	1.388	1.46	0.1 – 0.3	0.193	0.074	0.008–0.09	0.0186	0.023
Vitamin E (mg%)	0.8–1.9	1.62	1.429	0.1 – 0.9	0.482	0.26	0.02–0.09	0.0653	0.0206

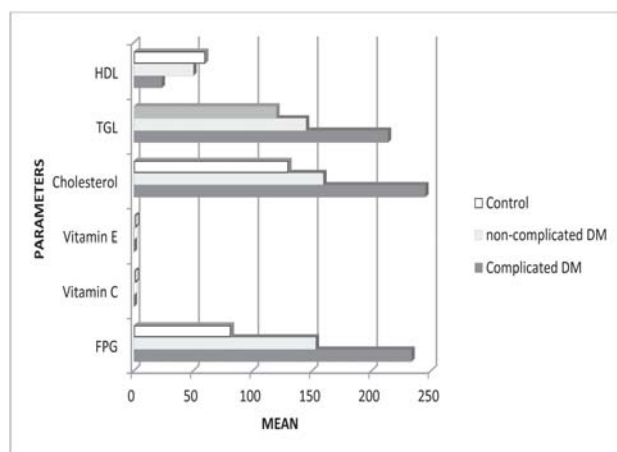
Table 2 shows that mean fasting plasma glucose in non-complicated diabetics and complicated diabetics were higher (153.13 mg% and 233.73 mg% respectively) than the control group (81.57 mg%). Mean vitamin C in non-complicated and complicated diabetics is lower (0.193 mg% and 0.0186 mg% respectively) than the control group (1.388 mg%). Also mean vitamin E in non-complicated and complicated diabetics is lower (0.482 mg% and 0.0653 mg% respectively) than the control group (1.62 mg%).

**Figure 1** Statistical difference in parameters FPG, vitamin C & E between control & diabetic group

The results of vitamin C, vitamin E and plasma glucose levels with serum lipid profile in control group, in diabetics without complication and diabetics with complications have been analysed.

Table 3 Statistical difference between the levels of different parameters of complicated & non-complicated diabetic patients

Groups	No. of cases	Mean $\pm$ SD						P value
		FPG	Vitamin C	Vitamin E	Cholesterol	TGL	HDL	
Complicated diabetics	15	233.73 $\pm$ 42.96	0.0186 $\pm$ 0.023	0.0653 $\pm$ 0.0206	245.33 $\pm$ 35.75	213.86 $\pm$ 17.39	23.63 $\pm$ 4.16	<0.001
Non-complicated diabetics	15	153.13 $\pm$ 19.90	0.193 $\pm$ 0.074	0.482 $\pm$ 0.26	159.53 $\pm$ 28.77	144.86 $\pm$ 33.11	50.06 $\pm$ 6.03	<0.001

**Figure 2:** Statistical difference in parameters FPG, vitamin C, E, Total cholesterol, TGL (triglyceride) & HDL (high density lipoprotein) between control, complicated diabetics and non-complicated diabetic subjects

DISCUSSION

In the present study, highest number of cases were found in the sixth decade that is between 50 – 60 years (43.33%) followed by fifth decade that is between 40 – 50 years. Similar selection of age group was done by Beisswenger PJ et al.^{10,11} It is also found that among 30 numbers of diabetic patients, 25 were males and 5 were females with a male preponderance of 5:1.

In this study, the plasma vitamin C and vitamin E levels were found to be lowered among the diabetics (0.106 mg% $\pm$ 0.103 and 0.424 mg % $\pm$ 0.893 respectively) than the controls (1.388 mg% $\pm$ 1.46 and 1.62 mg% $\pm$ 1.429 respectively). It was also found that the levels of plasma vitamin C and vitamin E were decreased among the complicated diabetics (0.0186 mg % $\pm$ 0.023 and 0.0653 mg % $\pm$ 0.0206 respectively) to the greater extent than the non-complicated diabetics (0.193 mg % $\pm$ 0.074 and 0.482 mg % $\pm$ 0.26 respectively).

A.J. Sinclair et al¹² studied 25 numbers of complicated and 25 numbers of non-complicated diabetic patients. In each case it was found that there is lower concentration of vitamin C in both the groups than control groups ($P < 0.001$ and $P < 0.01$ respectively) and patients with complications had significantly lower vitamin C level than patients without complications ($P < 0.01$). In contrast to this study, some workers like E Kokoglu¹³ and Saowanee Kajanachumpol¹⁴ had observed in their study that there is an increased vitamin E level in diabetic persons than the control group.

Some other workers like SRJ Maxwell et al¹⁵ in their study on 52 numbers of diabetic patients found that the level of plasma vitamin E were similar in both diabetic patients and healthy control subjects whereas the level of plasma vitamin C was significantly lower in diabetic patients than the control subjects.

So, in the present study a significant statistical difference was observed between the mean values of plasma vitamin C, vitamin E and fasting plasma glucose in the control group and diabetics as a whole. Similarly, a highly significant statistical difference was observed between the mean plasma vitamin C, vitamin E and fasting glucose in complicated and non-complicated diabetics. This observation is similar to the findings by AJ Sinclair.¹²

In this study, a correlation was also made between plasma vitamin C, vitamin E and serum lipid profile parameters namely serum HDL, total cholesterol and triglyceride level of the patients. It was observed that raised fasting plasma glucose is associated with significantly raised serum cholesterol and triglyceride concentration and decreased vitamin C and vitamin E concentrations. It was also observed that the complicated diabetics comparatively have raised serum cholesterol and triglyceride levels and significantly lower vitamin C and vitamin E levels than the non-complicated diabetics. But the serum HDL level was significantly lower in the complicated diabetics than the non-complicated diabetics. Since control of diabetics was deduced from fasting plasma glucose level, it is obvious that poorly controlled diabetics (complicated diabetics) had higher values of serum cholesterol and triglyceride and lower value of serum HDL. For the complicated diabetic group with mean FPG $233.73 \text{ mg\%} \pm 42.96$ mean serum cholesterol and triglyceride values were $245.33 \text{ mg\%} \pm 35.7$ and $213.86 \text{ mg\%} \pm 17.39$ respectively and the mean plasma vitamin C and vitamin E values were $0.0186 \text{ mg\%} \pm 0.023$ and $0.0653 \text{ mg\%} \pm 0.0206$ respectively, whereas for the non-complicated diabetic group with mean

FPG $153.13 \text{ mg\%} \pm 19.90$ mean serum cholesterol, triglyceride, vitamin C and vitamin E values were $159.53 \text{ mg\%} \pm 28.77$, $144.86 \text{ mg\%} \pm 33.11$, $0.193 \text{ mg\%} \pm 0.074$ and $0.482 \text{ mg\%} \pm 0.26$ respectively.

So, the results of this study show that abnormalities of antioxidant status (vitamin C and vitamin E) exist in patients with both complicated and non-complicated diabetes mellitus. Lower levels of vitamin C and vitamin E in diabetics as compared to non-diabetic subjects suggest that vitamin E, the primary lipid soluble antioxidant and vitamin C, the water soluble antioxidant are depleted in a state of increased oxidative stress due to diabetes. Som S Basu¹⁶ Sinclair AJ¹², Jennings PE^{17,18} also found similar results. Although reduced dietary intake or excessive excretion may have a role, the most likely causes of reduced level of vitamin C in diabetics are:

- Increased consumption (oxidation) of vitamin C as a result of increased free radical activity in diabetes (Collier A¹⁹) with increased production of its oxidation product dehydroascorbic acid.
- Failed regeneration of vitamin C from dehydroascorbic acid. Intracellular regeneration of vitamin C from dehydroascorbic acid may be impaired because of competitive inhibition of its transport across the cell membrane by glucose, a structurally similar molecule.

Alternatively, the excessive consumption of NADPH during the conversion of glucose to sorbitol involving aldose reductase (the polyol pathway) during hyperglycemia may also retard the reduction of dehydroascorbic acid.¹⁹ Vitamin C is a pivotal antioxidant with a potent inhibitory effect upon peroxidation of polyunsaturated fatty acids in plasma,²⁰ possibly because of its ability to regenerate antioxidants such as vitamin E.²¹

Lower vitamin E level in diabetics is also found by other workers like Karpen CW^{22,23}, Wohaieb SA et al²⁴, Wolff SP²⁵ etc. The mechanism of Vitamin E deficiency in diabetes is not completely understood. Vitamin E acts as a first line of defence to protect lipid of the cell membrane against lipid peroxidation.²⁶ During the process, it loses one proton and is converted into a radical. However, its concentration is very low in membranes as compared to that of polyunsaturated fatty acids. So, recycling of its radical is essential for it to be an effective antioxidant. The enzyme systems capable of reducing oxidized tocopherol (Vitamin E) have been described. Another possibility is that Vitamin E radicals generated can be

reduced by compounds naturally present in biological systems (Packer JE²⁷) and one such important compound is Vitamin C.

CONCLUSION

In the modern system of life, human beings are exposed to various sources of free radicals which disrupt the equilibrium of biological systems by damaging their major constituent molecules, leading eventually to cell death. It is seen that the diabetic patients are at increased risk of developing various complications due to exposure to free radicals which have been strongly implicated in the pathophysiology of diabetic complications. Diabetic patients also have a well documented defect in antioxidant protection which have been studied by various research workers, and in the present study also it is seen that the level of antioxidants in the complicated cases of diabetes mellitus are remarkably lowered when compared to the diabetes mellitus without complications.

From the study, it can be concluded that antioxidant deficiency appears to be associated with a risk for diabetic complications as the imbalance between reactive oxygen species and antioxidant defence systems may increase the oxidative burden and lead to the damage of macromolecules, and such processes are thought to play a role in pathological process of various diseases including diabetes mellitus. Recent findings suggest that plasma antioxidant levels can be used as index to take precautionary measures to minimize complications of diabetes mellitus.

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REFERENCES

1. Ahmad M¹, Khan MA, Khan AS. Naturally occurring antioxidant vitamin levels in patients with type-II diabetes mellitus. *J Ayub Med Coll Abbottabad* 2003 Jan-Mar;15(1):54-7.
2. Palanduz S, Ademoglu E, Gokkusu C, Tamer S. Plasma antioxidants and type 2 diabetes mellitus. *Res Commun Mol Pathol Pharmacol* 2001;109(5-6):309-18.
3. Memisogullari R, Taysi S, Bakan E, Capoglu I. Antioxidant status and lipid peroxidation in type II diabetes mellitus. *Cell Biochem Funct* 2003 Sep;21(3):291-6.
4. American Diabetes Association. Standards of medical care in diabetes—2015. *Diabetes Care* 2015;38(1):S1-S93.
5. Arya SP, Mahajan M. Colorimetric determination of ascorbic acid in pharmaceutical preparations and biological samples. *Mikrochim Acta* 1997 Mar;127(1):45-49.
6. Baker H, Frank O. *Clinical Vitaminology: methods and interpretation*. New York: Wiley; 1968.
7. Kunst A, Drager B, Ziegenhorn J. UV methods with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HY, editor. *Methods of Enzymatic analysis*. Deerfield: Verlag Chemie; 1983. p. 163-172.
8. Greiling H, Gressner AM, editors. *Lehrbuch der Klinischen Chemie und Pathobiochemie*. 3rd ed. New York: Schattauer Verlag; 1995.
9. Stein EA. Lipids lipoprotein and apolipoproteins. In: Teitz NW, editor. *Fundamentals of clinical chemistry*. 3rd ed. Philadelphia: WB Saunders; 1987. p. 448-481.
10. Beisswenger PJ. Susceptibility to diabetic nephropathy is related to dicarbonyl and oxidative stress. *Diabetes* 2005;54:3274-3281.
11. Beisswenger PJ, Moore LL, Brinck-Johnsen T. Increased collagen linked pentosidine levels and advanced glycosylation end products in early diabetic nephropathy. *J Clin Invest* 1993;92:212-217.
12. Sinclair AJ, Lunec J, Girling AJ, Barnett AH. Modulators of free radical activity in diabetes mellitus. In: Emerit I, Chance B, editors. *Role of ascorbic acid in free radicals and aging*. Basel: Birkhauser; 1992. p. 342-352.
13. Kokoglu E, Ulakoglu E. The transport of vitamin E in plasma and its correlation to plasma lipoproteins in non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* 1991 Dec;14(3):175-81.
14. Kajanachumpol S, Komindr S, Mahaisriyodom. Plasma lipid peroxide and antioxidant levels in diabetic patients. *J Med Assoc Thai* 1997;80:372-377.
15. Maxwell SRJ, Thomason H, Sandler D. Antioxidant status in patients with uncomplicated insulin dependent and non-insulin dependent diabetes mellitus. *Eur J Clin Invest* 1997;27:484-490.
16. Som S. Ascorbic acid: a scavenger of superoxide radical. *Acta vitaminol Enzymol* 1983;5:243-250.
17. Jennings PE, Jones AF, Florkowski CM, Lunec J, Barnett AH. Increased diene conjugates in diabetic subjects with microangiopathy. *Diabetic Med* 1987;4:452-456.

18. Jennings PE. Vitamin C metabolites and microangiopathy in diabetes mellitus. *Diabetes Res* 1987;6:151-154.
19. Collier A, Jackson M, Dawkes RM, Bell D, Clarke BF. Reduced free radical activity detected by decreased diene conjugates in insulin-dependent diabetic patients. *Diabetic Med* 1988;5:747-749.
20. Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc Natl Acad Sci USA* 1988;85:9748-9752.
21. Vatassery GT, Smith WE, Quach HT. Ascorbic acid, glutathione and synthetic antioxidants prevent the oxidation of vitamin E in platelets. *Lipids* 1989 Dec;24:1043-1047.
22. Karpen CW, Cataland S, O'Dorisio TM, Panganamala V. Interrelation of platelet, vitamin E and thromboxane synthesis in type I diabetes mellitus. *Diabetes* 1984;33:239-243.
23. Karpen CW, Cataland S, O'Dorisio TM, Panganamala V. Production of 12-hydroxyeicosatetraenoic acid and vitamin E status in platelets from type I human diabetic subjects. *Diabetes* 1985;34:526-531.
24. Wohaieb SA, Godin DV. Alterations in tissue antioxidant systems in the spontaneously diabetic (BB Wistar) rat. *Can J Physiol Pharmacol* 1987 Nov;65(11):2191-5.
25. Wolff SP. Glucose autoxidation and protein modification: the potential role of oxidative glycosylation in diabetes. *Biochem J* 1987;245:243-250.
26. Burton GW, Ingold KU. Vitamin E as an in vitro and in vivo antioxidant. *Ann N Y Acad Sci* 1989;570:7-22.
27. Packer JE. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* 1979;278:737-738.

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