Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2017; 5(11D):4606-4610 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI:10.36347/sjams.2017.v05i11.058

Association of Vitamin C with Erythrocyte Malondialdehyde Levels in Type 2 Diabetic Patients - A Clinical Approach

Dr. Nitin Faldessai[1], Dr. Md Masood Ahmed Shareef[2], Dr. Rahul Saxena[3], Dr. Ijen Bhattacharya[4]
[1]Associate Professor, Department of Biochemistry, Kamineni Institute of Medical Sciences, Narketpally, Nalgonda
[2]Assistant Professor, Department of Biochemistry Kamineni Institute of Medical Sciences, Narketpally, Nalgonda
[3]Assistant Professor, Department of Biochemistry, School of Allied Health Sciences Sharda University, Greater Noida, U.P, India
[4]Professor, Department of Biochemistry, Rama Medical College, Hospital & Research, Hapur, U.P, India

Original Research Article

***Corresponding author**
Dr. Md Masood Ahmed Shareef

Article History
Received: 19.11.2017
Accepted: 25.11.2017
Published: 30.11.2017

Abstract: Oxidative stress plays a crucial role in the pathogenesis of various health related complications including type 2 diabetes mellitus (T2DM). Although role of free radicals mediated lipid peroxidation in T2DM are well documented, its association with vitamin C levels in T2DM pathophysiology along with cardio vascular disease (CVD) risk has yet not been focused. The present study was carried out to estimate the plasma vitamin C levels, an aqueous medium containing non-enzymic antioxidant and malondialdehyde (marker of lipid peroxidation) in T2DM subjects. Material In the present study, plasma vitamin C levels and malondialdehyde levels were measured in 100 type 2 diabetic subjects of either sex (35-55 years) and statistically compared it with that of 100 healthy individual, served as control. Plasma vitamin C levels were found to be significantly low in patient group as compared to control ($P < 0.001$) whereas erythrocyte malondialdehyde levels and fasting blood glucose levels were increased significantly in patient group as compared to control ($P < 0.001$). In addition, plasma vitamin C level was negatively associated ($r = -0.685$; $P< 0.001$) with erythrocyte malondialdehyde levels. These findings suggest that enhanced oxidative stress occurs in T2DM patients as characterized by increased incidence of lipid peroxidation and reduction of vitamin C levels in order to combat free radicals. Therefore, regular consumption of diet rich in vitamin C and antioxidant minerals should be encouraged along with monitoring of glycemic profile, oxidative stress and cardiac markers to move one step ahead in adopting the preventive and regulatory measures against CVD in T2DM patients.
Keywords: Lipid peroxidation, ascorbate, hyperglycemia, oxidative stress, free radical.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major medical condition that affects the quality of human life of developed and developing countries as well. The incidence of diabetes is increasing at alarming pace with advancing of medical science and this significant increase in number of people with T2DM makes this disease a global threat in 21st century [1]. Moreover, T2DM is considered as a coronary heart disease risk equivalent and it is frequently associated with various other cardiovascular risk factors. Approximately, 80% of deaths in patients with diabetes are attributable to cardiovascular disease (CVD) [2, 3]. Among various risk factors and biochemical events associated with the etiopathogenesis of CVD in T2DM, increased production of oxygen free radicals have been identified as an important inducer of metabolic and vascular disorder leading to CVD in T2DM patients [4, 5]. Lipid peroxidation is a free radical mediated process in which the polyunsaturated fatty acids contained in the LDL or present in the cell membrane are degraded to variety of aldehydes (mainly malondialdehyde). These aldehydes may alter endothelial function and inhibit NO synthase activity leading to the development of cardiac complications in T2DM [6, 7].

In order to provide protection or to eliminate free radicals, antioxidants play a crucial role in the body. Among non enzymic antioxidant, vitamin C is an exogenous water soluble antioxidant functions as primary defense against free radicals in plasma and disappeared more quickly. In the immune system, the major role of vitamin C seems to be as a physiological antioxidant, protecting host cells against oxidative

stress caused by infections. In various experimental settings, vitamin C increased the functioning of phagocytes, the proliferation of T-lymphocytes and the production of interferon, and decreased the replication of viruses [8,9]. In the previous studies, altered levels of vitamin C had been found to be significantly associated with myocardial infarction, hypertension, pre-eclampsia and various other vascular disorders [10,11].

As best of our knowledge, limited information is available on MDA level and plasma vitamin C level in the T2DM patients with CVD risk. Therefore, the objectives of present study were to estimate glycemic profile, erythrocyte MDA and plasma vitamin C levels in T2DM patients and to determine the relation of vitamin C with lipid peroxidation and CVD risk in T2DM patients.

MATERIALS & METHODS

In the present study, 100 patients of either sex (MF ratio 1:1) with Type 2 diabetes mellitus, defined as per revised American Diabetic Association criteria (ADA 2013) were recruited as patient group (Group II). These patients belonged to age group 35-55 years. 100 age and sex matched healthy subjects with fasting and postprandial blood glucose less than 100 mg/dl and 140 mg/dl were recruited as controls (Group I). A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination including blood pressure measurement was completed from all the subjects after taking their informed consent and approval of protocol by ethics committee of college.

Inclusion criteria

Subjects, who gave informed consent for study, don't under any medical treatment (anti-inflammatory drug) or taking antioxidant supplement for at least 1 month prior to blood collection were included.

Exclusion criteria

Patients with acute and chronic infections, fever, malignancy, renal disease, hepatic disease, hypertension, those taking antioxidant vitamin supplements or non-steroidal anti–inflammatory drugs and with other connective tissue disease like systemic sclerosis and osteoarthritis were excluded.

Fasting blood sample (5 ml) was collected from the anticubital vein of the study group subjects and divided into two parts. First part was collected in fluoride vial for glucose estimation and second part in EDTA vial for study group parameters estimation Fasting and postprandial blood glucose levels were measured by using enzymatic kit based on glucose oxidase method. Glucose, in presence of glucose oxidase, converted into gluconic acid along with production of Hydrogen peroxide, which later oxidatively coupled with 4-aminoantipyrine phenol (in presence of peroxidase) and red quinoneimine dye was produced. The intensity of the color complex was directly proportional to the glucose in specimen and showed absorption maxima at 505 nm [12]**.**

Plasma vitamin C (ascorbic acid) levels were estimated by Mc Cormick and Greene method. Ascorbic acid in plasma is oxidized by Cu (II) to form dehydroascorbic acid which reacts with acidic 2,4–dinitrophenyl hydrazine to form a red bishydrazone, which is measured at 520 nm[13].

Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate. The heat induced reaction of malondialdehyde (MDA) with thio barbituric acid (TBA) in the acid solution forms a trimethine coloured substance, which is measured spectrophotometrically at 532 nm [14].

Statistical Analysis

The data from both the study group subjects and controls were expressed as Mean ± SD and compared by using Student's t-test and distribution of probability (P). In addition, correlation analysis between plasma vitamin C and erythrocyte MDA level was performed by using Pearson correlation test.

RESULTS

The clinical and demographic profile of T2DM patients and healthy controls are represented in Table 1. In the present study, study group subjects belonged to age group 35- 55 years i.e. 44 ± 4.8 and 48 ± 5.2 years in Group I and Group II respectively, as represented in Table 1. Out of the selected 100 subjects of T2DM, 50 patients were male and 50 were female. The recruited diabetic patients have positive family history of T2DM i.e. in 75%. In addition, height and weight measurement followed by BMI calculation revealed that T2DM subjects had significantly high ($p<0.05$) BMI as compared to healthy non diabetic control group subjects. The observation made reveal insignificant increase ($p<0.1$) of waist hip ratio in the patients group subjects as compared to healthy controls.

As compared to normal healthy controls, abnormalities in glycemic profile along with markers of oxidative stress were observed in study group subjects with diabetes, as represented in Table 2. In the Group II subjects, fasting blood glucose level was increased significantly ($P< 0.001$; 76.86% high) along with increase in postprandial blood glucose levels ($P< 0.001$; 75.16% high) as compared to Group I subjects or healthy controls.

The mean plasma vitamin C (ascorbate) levels in T2DM subjects were significantly low (P<0.001) as compared to controls i.e. 38.1% low. On the other hand, erythrocyte malondialdehyde levels were found to be increased significantly (P<0.001; 87% high) in T2DM patients as compared to healthy controls. In addition, plasma vitamin C levels were negatively correlated with erythrocyte MDA, fasting and postprandial blood glucose levels as represented in Table 3.

Table-1: Clinical and demographic profile of T2DM patients and healthy controls (Mean ± SD)

S. No.	Particulars	Group I (n=100)	Group II (n=100)	P- value
1)	Age (years)	44 ± 4.8	48 ± 5.2	p<0.1
2)	M:F ratio	1:1	1:1	p<0.1
3)	Height (meter)	1.58 ± 0.026	1.60 ± 0.030	p<0.1
4)	Weight (Kg)	58.6 ± 2.6	64.5 ± 2.9	p<0.05
5)	BMI (Kg/m^2)	23.0 ± 1.3	25.6 ± 1.5*	p<0.05
6)	Waist-hip ratio	0.88 ± 0.02	0.94 ± 0.03	p<0.1

where, * p < 0.1 : Non-significant; ** p < 0.05 : Significant

Table-2: Glycemic profile and markers of oxidative stress in study group subjects (Mean ± SD)

S. No.	Particulars	Control Group (n=100)	Patient Group (n=100)	P- value
1.	Fasting Blood Glucose (mg/dl)	86.18 ± 7.68	152.42 ± 42.60	p < 0.001
2.	Post Prandial Blood Glucose (mg/dl)	122.8 ± 7.80	215.10 ± 80.32	p < 0.001
3.	Ascorbic acid (mg/dl)	1.26 ± 0.24	0.78 ± 0.17**	p < 0.001
4.	Malondialdehyde (µ mol MDA/ml)	2.75 ± 0.08	3.62 ± 0.14**	p < 0.001

Where, p<0.001: Highly significant

Table-3: Correlation coefficient (r) between plasma vitamin C and erythrocyte malondialdehyde (MDA) along with fasting and post prandial blood glucose levels in T2DM patients.

Particulars	MDA	FBS	PPBS
Vitamin C	- 0.685 **	-0.617 **	- 0.579 **

Where, * p < 0.05 : Significant, ** p < 0.001 : Highly significant

DISCUSSION

Diabetes mellitus is now a leading cause of death and disability worldwide. Its prevalence was about 8% in 2011 and it is predicted to rise by 10% by the end of 2030 and it will be the 7th leading cause of death by then as projected by the WHO [15]. However, association of CVD events has also been increasing in T2DM patients. It has now been proved that oxidative stress is increased in patients with T2DM owing to increase in free radical production [16]. Involvement of free radicals in membrane damage via lipid peroxidation and its resultant products such as lipid radicals (L◦), lipid peroxides (LOO◦), lipid hydro peroxides (LOOH) and highly reactive aldehydes plays a crucial role in the development and progression of cardiac complication [17]. Free radicals via lipid peroxidation play an etiopathogenic role in the development of T2DM patients as obvious in the findings of present study. Marked elevated levels of malondialdehyde (i.e. marker of lipid peroxidation) were observed in patient group (P<0.001) as compared to healthy control along with characteristic hyperglycemia. Our findings were in concordance with the findings of Kaefer et al. According to them, lipid peroxides are toxic to the cellular components and lipid peroxidation may be responsible for vascular disorder in T2DM [18]. It has also documented that excess endogenous aldehyde plays a major role in enhancing CVD risk by binding sulphydryl groups of membrane proteins, altering Ca^{2+} channels and increasing cytosolic free Ca^{2+} that cause further extensive membrane damage due to the action of phospholipases and proteases, the activation of contractile proteins and the accumulation of mitochondrial calcium resulting in a vicious cycle of damage extension, peripheral vascular resistance, hypertension and other cardiovascular complications in T2DM patients [19,20].

These free radicals are scavenged by antioxidant defense system of the body comprised of both enzymic and non-enzymic antioxidants. Among non-enzymic antioxidants, vitamin C is an effective water soluble antioxidant and a stabilizer of biological membranes, prevent accumulation of free radicals and alone can afford protection against the oxidant mediated damage to LDL [21]. In the present study, plasma

ascorbate levels were significantly low (P<0.001) in patient group as compared to healthy controls. Reduction in vitamin C levels could not be only due to its free radical scavenging action but also in maintaining the body antioxidant reserve and in normalization of vascular superoxide formation which prevent endothelial dysfunction. Will and Byers. in their study also observed that low ascorbate level is significantly related with hyperglycemia [22]. Furthermore, reduction in vitamin C & E level with increased levels of lipid peroxides were also reported in previous study on T2DM subjects and concluded it as a contributory event in the development of cardiovascular disease risk in T2DM patients [11, 23, 24].

CONCLUSION

Thus, present study authenticates that T2DM are more susceptible to develop cardiovascular complication due to increased oxidative stress. Our study concludes that plasma vitamin C level is inversely related to hyperglycemia and lipid peroxidation which plays a crucial role in not only in etiopathogenesis of T2DM but also in enhancing the risk of CVD in T2DM patients. Reduction in plasma vitamin C level could be explained on the basis of its role in scavenging free radicals, in maintaining body's antioxidant reserve and in limiting the lipid peroxidation. Thus, both vitamin C and malondialdehyde levels may be an excellent marker of oxidative stress in T2DM and its related complication. Therefore, consumption of citrus fruit and green leafy vegetables along with regular monitoring of glycemic profile, oxidative stress and cardiac markers are important and necessary steps for prediction as well as early management of CVD and other complications in T2DM patients.

REFERENCES

1. Siddiqui M H, Saxena R, Verma S, Sharma GD. 25(OH) vitamin D level in Type 2 Diabetics and Non Diabetics: A comparative study. Int J Pharma and Clin Res. 2016; 8(4): 284-288.
2. Mithal A, Majhi D, Shunmugavelu M, Talwarkar PG, Vasnawala H, Raza AS. Prevalence of dyslipidemia in adult Indian diabetic patients: A cross sectional study (SOLID). Indian J Endocr Metab 2014;18: 642-647.
3. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14.
4. Lipinski B. Pathophysiology of oxidative stress in diabetes mellitus. J Diabetes Complications. 2001;15:203–10.
5. Chintan AP, Nimish LP, Nayana B, Bhavna M, Mahendra G, Hardik T. Cardiovascular complication of diabetes mellitus. J Appl Pharm Sci. 2011;4:1–6.
6. Cederberg J, Basu S, Eriksson UJ. Increased rate of lipid peroxidation and protein carbonylation in experimental diabetic pregnancy. Diabetologia. 2001;44:766–74.
7. Saxena R, Jaiswal G. Lipid peroxidation mediated electrolyte imbalance in patients with different stages of Essential Hypertension. S Asian J Prev Cardiology. 2006; 10(3): 143-147.
8. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabet. 2005;4:5.
9. Aggarwal C, Bhattacharya I, Saxena R. Assessment of plasma ascorbic acid levels and its relation with serum uric acid in Allergic rhinitis patients. Europ J Biomed Pharma Sci. 2015; 2(5):500-506.
10. Nishigaki I, Hagishara M, Tsumakawa H, Maseki M, Yagi K. Lipid peroxide levels of serum lipoprotein fractions of diabetic patients. Biochem Med. 1981;25:373–8.
11. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res. 2000;87: 840–844.
12. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann Clin Biochem. 1969; 6:24–27.
13. Mc Cormick DB, Greene HL, Vitamin. In Tietz Text Book of Clin. Chem. Burtis and Ashwood eds. WB Saunders Company USA, 1998: 1025-1027.
14. Sinnhuber RO, Yu TC, Yu TC. Characterization of the red pigment formed in the thiobarbituric acid determination of oxidative rancidity. Food Res 1958; 23: 626-630.
15. Dasgupta A, Biswas B, Paul B, Bandyopadhyay L, Ghosh A, Sembiah S. Quantification of vulnerability to Type 2 diabetes: A study among shopkeepers in Kolkata. Med J DY Patil Univ. 2017;10:417-23
16. Giugliano D, Ceriollo A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care. 1996;19: 257–67.
17. Dudeja U, Saxena R, Siddiqui MH, Sharma D. Correlation of Paraoxonase Status with Disease Activity Score and Systemic Inflammation in Rheumatoid Arthritis Patients. Journal of Clinical and Diagnostic Research. 2016; 10(3): BC01-BC05.
18. Kaefer M, De Carvalho JA, Piva SJ, da Silva DB, Becker AM, Sangoi MB, Almeida TC, Hermes CL, Coelho AC, Tonello R, Moreira AP, Garcia SC, Moretto MB, Moresco RN. Plasma malondialdehyde levels and risk factors for the development of chronic complications in type 2 diabetic patients on insulin therapy. Clin Lab. 2012;58 (9-10):973-8.
19. Vasdev S, Gill V, Parai S, Longerich L, Gadag V. Dietary vitamin E supplementation lowers blood pressure in spontaneously hypertensive rats. Mol Cell Biochem. 2002; 238: 111 – 117.

20. Dutta J, Sharma D, Saxena R. Oxidative stress mediated electrolyte imbalance in 30 known cases of knee osteoarthritis patients: A clinical approach. Asian J Medical Sciences. 2015; 6(5): 26-30.
21. Heitzer T, Just H, Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. Circulation. 1996; 94: 6-9.
22. Will JC, Byers T. Does diabetes mellitus increase the requirement for vitamin C? Nutr Rev 1996, 54:193–202.
23. Wen Y, Killaleas Mc, Gettigan P, Feely J. Lipid peroxidation and antioxidant vitamin C & E in Hypertensive patients. Irish. Med. Sci. 1996; 165 (3): 210-212.
24. Rabini RA, Fumelli P, Galassi R. Increased susceptibility to lipid oxidation of low-density lipoproteins and erythrocyte membranes from diabetic patients. Metab Clin Exp. 1994;43:1470–1474.