The beneficial effect of vitamin C supplementation on serum lipids in type 2 diabetic patients: a randomized double blind study

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Abstract

Diabetic dyslipidemia is the product of oxidative stress-induced damage along with the simultaneous decline of antioxidant defense mechanisms leading to cellular damage, increased lipid peroxidation and subsequent development of severe complications in type 2 diabetes mellitus (DM). Antioxidant vitamin C has been reported to reduce oxidative stress by arresting free radical damage and thereby improves lipid levels in type 2 DM patients. The present study was aimed to investigate the impact of oral vitamin C supplementation with metformin on lipids and plasma ascorbic acid level in patients suffering from type 2 DM. A prospective, double-blind, placebo-controlled, parallel, 12 week- study was carried out in a tertiary care hospital with the approval of institutional Ethics Committee. Seventy patients with type 2 DM were divided randomly into placebo and vitamin C group of 35 each. At the end of study, baseline investigations were repeated in all the patients. Serum triglycerides, total cholesterol, low and very low density lipoprotein levels were reduced significantly in vitamin C supplemented group in comparison with placebo-treated group. No beneficial effect of vitamin C supplementation was observed on high density lipoprotein level in patients with type 2 DM. Oral supplementation of vitamin C with metformin improves diabetic dyslipidemia and revert ascorbic acid levels to normal range. Hence, vitamin C supplementation with metformin stands as an attractive therapeutic adjuvant in the treatment of diabetic dyslipidemia to prevent complications and reduce morbidity.

Keywords**:** *Diabetes mellitus, oxidative stress, Vitamin C, lipid profile, antioxidants, ascorbic acid*

Introduction

Patients with diabetes mellitus have a two- to four-fold increased risk of both developing and dying from cardiovascular diseases. This increased risk is independently associated with diabetes-induced abnormalities in plasma lipids and lipoprotein metabolism. Despite the advances made in the prevention and management of cardiovascular disease, diabetic patients continue to have alarmingly high morbidity and mortality secondary to cardiovascular diseases.[1,2] Increased oxidative stress is a widely accepted participant in the development and progression of diabetes in addition to hyperglycemia and cellular dysfunction which aggravates diabetic dyslipidemia and exaggerates various complications. These complications are extremely costly in terms of longevity and quality of life.[3-5] The mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycosylation end products (AGEs), and protein kinase C.[6] Hence, there is a need of continuous exploration of relationship between free radicals, diabetes, and mechanisms responsible for complications in an effort to expand treatment options.[7]

The cluster of lipid abnormalities associated with type 2 diabetes is defined by increases in triglyceride (TG) and small, dense low-density lipoprotein (LDL) concentrations and decrease in high-density lipoprotein (HDL) cholesterol.[8] Diabetes-related changes in plasma lipid levels are among the key factors that are amenable to intervention. Ascorbic acid (vitamin C), an antioxidant, plays an important role in protecting free radical-induced damage in type 2 DM. Previous studies have established the fact that basal vitamin C level is low in type 2 DM.[9,10]

The available literature suggest conflicting results related to supplementation of vitamin C and changes in lipid profile parameters in type 2 DM. Many animal studies support the improvement in abnormal lipid level.[11-13] However, very few clinical studies are available in support of an inverse association between ascorbic acid intake and cardiovascular disease and also these associations have not been found consistent.[14-18] A recent study reported reduction in TC, TG, LDL, VLDL and HDL after supplementation with vitamin C, but it was an open-label, non-controlled and short duration study.[9] Therefore, this study was deliberately planned to measure the effect of vitamin C along with metformin on lipids and plasma ascorbic acid level in a double-blind, controlled manner and for longer duration in patients with type 2 DM. Hence, the study was launched to

Received on: 5 December, 2011
Accepted on: 17 June, 2012

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appraise the salubrious role of vitamin C in diabetic dyslipidemia.

Materials and Methods

Patients with type 2 DM attending the Outpatient Diabetes Clinic in our tertiary care hospital were screened and enrolled in the study. Seventy patients with type 2 DM participated in a prospective, double-blind, placebo-controlled, parallel, 12 week-study approved by Institutional Ethics Committee. The experimental protocol was in accord with the Helsinki declaration. All patients signed their informed consent prior their inclusion in study. The diagnosed patients of type 2 DM of age group between thirty to sixty years who were on metformin and having fasting blood glucose level in the range of 126 to 250 mg/dl, were included in the study. Patients having fasting blood glucose level more than 250 mg/dl, medical illnesses including other endocrine, metabolic, type 1 DM, pregnancy, isolated postprandial hyperglycemia, age more than 60 years or less than 30 years and associated with complications were excluded from the study. None of the subjects was a regular drinker, heavy smoker, or any psychotropic treatment. Patients who received vitamin C or any other antioxidant over the last three months were also excluded from the study. All the patients were investigated for routine parameters like electrocardiogram, serum electrolytes, kidney function tests, and liver function to rule out active medical problems in all patients.

Graph Pad Prism version 5.00 software was used for calculation of sample size. Seventy patients were divided randomly by block randomization with uniform allocation ratio (1:1) into two groups, A and B of 35 each. The study being double blind, the drugs were identical in formulation, shape, size, weight, texture and packing. The randomized treatment allocation sequence was generated by statistician using random number table. It was handed over along with identical plastic containers filled with the study drugs (sixty each of vitamin C and similar placebo) to a third person not directly involved in this study. This person labelled the containers according to the random allocation sequence of patients with drugs provided. The code of this random allocation sequence was retained in the sealed envelope by this person and was opened only after the completion of study during analysis of the data. The patients as well as the investigators were unaware of the treatment (Vitamin C or placebo) being administered. Drug was issued to patients for duration of thirty days at a time. Patients were asked to bring the unused drugs and container during the follow up. Ninety percent consumption was considered to be compliant. The returned drugs were discarded. Drugs were decoded at the end of trial. Group A received vitamin C with metformin and group B received placebo along with metformin. The dose of vitamin C was 500 mg twice a day and decided on the basis of previous study. All patients received open label tablet metformin 500 mg twice daily orally with lunch and dinner, as it was unethical to give either only placebo or vitamin C to diabetes patients. In our preliminary experiment, it was determined that subjects reached a new lower steady- state plasma concentration after one week on controlled diet. Placebo or vitamin C supplementation was started after one week of vitamin C restricted diet. Patients were given a new supply of tablets at the end of each four week. Same doses were maintained throughout the study. Patients were not stabilised before enrolment in the study as they were satisfying our main inclusion criteria. But if we found increased fasting blood sugar level beyond 250 mg during the study period then patients were considered drop outs and were managed by the respective physician from the institute. No co-morbid condition or infection occurred to these patients during the study period. After study period was over, all the patients were handed over to the respective physician.

All patients were maintained on their usual dietary pattern while limiting their consumption of vitamin C rich foods throughout the study. As patients were on self -selected diet, each patient was instructed by a dietician to use comprehensive food list that contained food items by type, portion size, method of preparation and vitamin C content. This enabled patients to substitute foods with low vitamin content for those patients who normally consume higher levels of vitamin C and also to ensure that their daily intake from dietary sources would remain the same. Compliance to dietary restrictions of ascorbic acid was determined by obtaining a 24 hours dietary recall from the subjects during each month period. Fasting, post meal blood glucose, lipid levels, glycosylated hemoglobin, plasma ascorbic acid, liver and kidney function tests were repeated after twelve weeks. General clinical safety was monitored by vigilant follow up of patients for treatment of emergent adverse events, if any, and recorded in the case report form. Patients with adverse drug reaction were treated appropriately by the physician in medicine OPD.

Plasma ascorbic acid was estimated by a single-step-calorimetric method using modified acid phosphotungstate reagent. Supernatant was used to measure absorbance at 700 nm. Standards of pure ascorbic acid obtained from Sigma Aldrich, St. Louis USA, in the range of 0.10 to 0.90 mg % were prepared in 0.5 % oxalic acid solution. For all the investigations, chemicals used were of analytical reagent grade. Fasting as well as post-prandial blood glucose level, HbA1c and lipid levels were quantitatively estimated by glucose oxidase method, cation-exchange resin method and with the use of semi auto-analyser, TRANSASIA, ERBA, CHEM-5- PLUS respectively.

Statistical analysis

Results were expressed as Mean ± SD. Data were processed and statistical analyses were carried out using the student "t" test for paired and unpaired comparison. Correlations were evaluated with either Pearson's or Spearman's correlation coefficient depending on distribution of the data. Chi-square test was used for analysis of demographic data. Two-tailed p value was used throughout, and the p value less than 0.05 were adjudged statistically significant.

Results

Randomisation after screening resulted in two comparable groups of patients in terms of demographic, clinical characteristics and biochemical parameters. The mean age

Table 1: Demographic, clinical and biochemical characteristics of patients with type 2 diabetes mellitus

Characteristic	Group A (n = 35)	Group B (n= 35)
Age (years)	48.33 (1.39)	45.88 (1.42)
Sex (male/female)	15/18	13/20
Duration of diabetes (years)	4 months	5 months
HbA1c (%)	8.26 (0.56)	8.18 (0.74)
Fasting blood glucose (mg/dl)	157.63 (18.03)	160.75 (14.98)
Post meal blood glucose (mg/dl)	222.24 (18.20)	218.51 (20.28)
Total cholesterol (mg/dl)	238.54 (16.37)	241.18 (23.30)
Triglycerides (mg/dl)	190.30 (35.89)	197.84 (26.84)
LDL (mg/dl)	164.84 (18.92)	160.24 (16.82)
VLDL (mg/dl)	38.06 (7.17)	39.56 (5.36)
HDL (mmol/L)	37.72 (4.88)	35.84 (4.07)
Plasma ascorbic acid (mg/dl)	0.26 (0.04)	0.24 (0.03)

Values are expressed as means (S.D). n = 33 in each group, Group A – vitamin C treated group, Group B – placebo treated group

Table 2: Effect of metformin with placebo on TC, TG, LDL, VLDL, HDL and plasma AA in patients with type 2 diabetes mellitus after 12 weeks of treatment.

Parameter	Before treatment	After treatment
TC	241.18 (4.05)	242.42 (4.39)
TG	197.84 (4.67)	195.97(3.88)
LDL	160.24 (2.92)	165.51(3.46)
VLDL	39.56 (0.93)	39.19 (0.77)
HDL	35.84 (0.71)	36.42 (0.54)
Plasma AA	0.24 ± 0.006	0.27 ± 0.01

n = 33 in number, TC: total cholesterol, TG: triglycerides, LDL: low density lipoproteins, VLDL: very low density lipoproteins, HDL: high density lipoproteins plasma AA: Plasma ascorbic acid. TC, TG, LDL, VLDL, HDL and plasma AA are measured in mg/dl.

Table 3: Effect of metformin with vitamin C on TC, TG, LDL, VLDL, HDL and plasma AA in patients with type 2 diabetes mellitus after 12 weeks of treatment

Parameter	Before treatment	After treatment
TC	238.54 (2.85)	225.09 (3.76)***
TG	190.30 (6.24)	180.06 (4.85)***
LDL	164.84 (3.29)	156.84 (3.57)**
VLDL	38.06 (1.24)	36.01 (0.97)***
HDL	37.72 (0.85)	39.09 (0.64)
Plasma AA	0.26 ± 0.008	0.45 ± 0.01***

n = 33 in number, TC:Total cholesterol, TG: triglycerides, LDL: low density lipoproteins, VLDL: very low density lipoproteins, HDL: high density lipoproteins plasma AA: Plasma ascorbic acid. TC, TG, LDL, VLDL, HDL and plasma AA are measured in mg/dl. *** < 0.001, ** < 0.01, * < 0.05

of the patients with diabetes mellitus in vitamin C group and placebo group was not significantly different from each other. Lipid profile parameters as well as glycemic control parameters such as fasting, post-meal blood glucose and plasma ascorbic acid levels did not differ among subjects before receiving placebo and vitamin C treatment ($p > 0.05$) (Table 1).

In the placebo group, there was no significant decrease in levels of TC, TG, LDL and VLDL. At the same time, increase in plasma ascorbic acid level at 12 weeks was non-significant as compared to baseline levels (Table 2). In patients receiving vitamin C, reduction in TC, TG, LDL, and VLDL were significant at twelve week. In contrast, plasma ascorbic acid levels rose significantly after 12 weeks of treatment **(**Table 3).

In order to investigate whether this reduction in TC, TG, LDL and VLDL is because of beneficial effects of supplementation of vitamin C, we compared the effects of

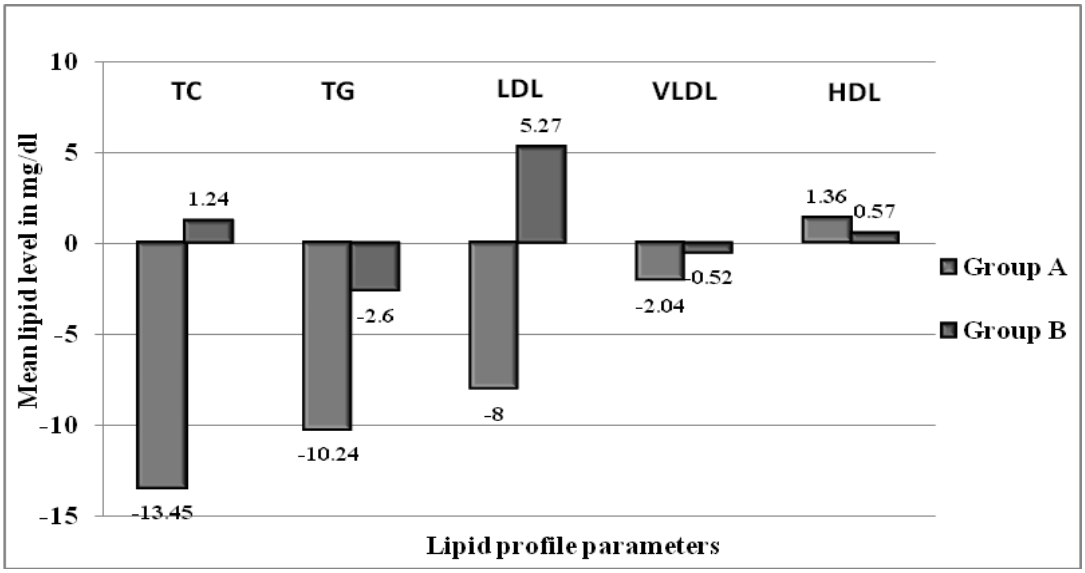

Figure 1: Comparison of effects of vitamin C with metformin and placebo with metformin on TC, TG, LDL, VLDL, and HDL at 12 weeks in patients with type 2 diabetes mellitus - the change from base line in both groups. Group A received vitamin C with metformin, Group B received placebo with metformin, TC: total cholesterol, TG: triglycerides, LDL: low density lipoproteins, VLDL: very low density lipoproteins, HDL: high density lipoproteins

drugs in placebo and vitamin C group after 12 weeks of treatment, taking into consideration the change from baseline values of these parameters. Decrease in TC, TG, LDL and VLDL were significant after twelve weeks in vitamin C group compared to placebo group. In contrast, supplementation of vitamin C increased plasma ascorbic acid significantly in vitamin C group compared to placebo group (Figure 1). No correlation existed between plasma ascorbic acid and any of the parameter such as TC, TG, LDL and VLDL for both vitamin C and placebo group at the baseline and at twelve weeks. In our study, nausea and abdominal discomfort were reported in one patient in placebo group while two patients in vitamin C group. These patients were treated by the physician in medicine OPD. All the patients responded to symptomatic treatment and continued the study. The whole study period was without any serious adverse effects and no abnormalities were detected in laboratory test. Of the seventy patients enrolled, four were withdrawn (two in placebo group and two in vitamin C group. The most common cause was failure to re-attend; one patient was dropped out in placebo group because of uncontrolled blood glucose level at the end of four weeks and was shifted to the other drugs.

Discussion

Our data for the first time shows that supplementation of vitamins C for at least three months significantly reduces levels of TC, TG, LDL, VLDL and increases plasma ascorbic acid level in patients of type 2 DM who are on metformin. No significant changes in HDL levels were observed for both the study groups. The results of this study are in agreement with previously published data showing betterment in lipid parameters with vitamin C supplementation.[19,20] It is well documented that there is an increased production of damaging free radicals in type 2 DM patients. Glucose auto oxidation, protein glycosylation, formation of advanced glycation end-products and polyol pathway are involved in generation of oxidative stress, implicated in the origin of both type 1 and 2 DM.[21] The protection against such damage can be offered by free radical scavenging antioxidants. All the patients participated in our study received metformin which is well established first-line drug for treatment of type 2 DM. The baseline parameters in both placebo and vitamin C treated group are comparable in our study. Hence, in present study the beneficial effects on lipid levels can be attributed to supplementation of vitamin C but it is difficult to pinpoint the exact mechanism by which vitamin C brings about these changes. The improvement in lipid levels may be due to effect of vitamin C on the underlying disease or because of correction of the inadequate vitamin C status.

In present study, the levels of plasma vitamin C were found to be below the lower limit of normal range in all patients at 0 week. Increased demand for vitamin C to compensate the increased oxidative stress or impaired transport or dietary deficiency of vitamin C may be contributing to decreased levels of plasma vitamin C levels observed in type 2 DM patients.[22] High, but physiologic concentrations of ascorbic acid can directly inhibit erythrocyte aldose reductase, and provide a rationale for the use of oral vitamin C supplements in diabetes.[23] A significant inverse relationship between plasma AA and DNA damage in type 2 DM patients indicates that poorly controlled diabetic subjects might benefit from increased dietary vitamin C.[24] Ascorbic acid supplementation for diabetic subjects may provide a simple means of preventing and ameliorating the complications of diabetes. The weak methodology in past research leads to conflicting results as the studies were not controlled. Therefore, like our trial, randomized, double-blind clinical trials of ascorbic acid supplementation for longer duration should be a high priority for establishment of role of ascorbic acid in diabetes.

Major disturbances in lipoprotein metabolism in type 2 DM individuals are reflected by rise in plasma triglycerides and low HDL, with normal or near normal LDL levels.[25] The abnormally high concentration of serum lipids in diabetes is mainly a result of the increase in mobilization of free fatty acids from peripheral depots because insulin inhibits the hormone sensitive lipase, on the other hand, glucagon, and catecholamines enhance lipolysis. The marked hyperlipemia that characterizes diabetic state may therefore be regarded as consequence of the uninhibited action of lipolytic hormones of fat depots.[26] U.K. Prospective Diabetes Study, showed higher levels of mean TC and LDL cholesterol levels in type 2 DM at diagnosis.[27] In a previous study, raised TG, TC, LDL and lower HDL cholesterol was observed in newly diagnosed type 2 DM patients.[28] We also found the similar derangement lipid profile at the baseline.

In our study, the levels of lipid profile parameters e.g TC, TG, VLDL and LDL were significantly reduced at 12 weeks in vitamin C treated group. Consumption of 1000 mg vitamin C resulted in significant reduction in serum levels of TC, TG, VLDL and LDL. However, there was no significant change in the serum levels of HDL in both the groups. In a previous study, the supplementation of 1000 mg of ascorbic acid by type 2 DM patients over a period of 4 months resulted in reduction of TC, TG and LDL.[29] No significant difference in lipid profile parameters was observed with 500 mg of vitamin C administration for 4 months. [30] However, higher dose of vitamin C (2g) significantly lowered TC.[31] The role of ascorbic acid in cholesterol metabolism has been studied. Research revealed that ascorbic acid activates the catabolism of cholesterol into simpler components for the eventual synthesis of steroid hormones.[32-33] The beneficial effect of ascorbic acid and consumption of fruits was observed on cholesterol level in the past. Ascorbic acid modulates insulin action and this probably explains its action in lowering plasma glucose and lipids particularly cholesterol and triglyceride.[34] Vitamin C is considered the most important antioxidant in plasma and forms the first line of defense against lipid peroxidation.[35] Low density lipoprotein particles are small and dense in type 2 DM and are susceptible to oxidation. α -tocopherol is a lipid soluble antioxidant and protects LDL particles from oxidative attack. Vitamin C is required for regeneration of α-tocopherol and thus may prevent LDL oxidation.[36] Also the possible explanation for the hypocholesterolaemic effect of vitamin C is that it prevents LDL cholesterol from oxidative damage and aids in degradation of cholesterol. Secondly, it has been suggested that vitamin C is substrate in the first step of bile acid synthesis by the enzyme cholesterol 7 α hydroxylase. Vitamin C is not a cofactor for the enzyme and the vitamin does not markedly affect 7α-hydroxylase activity in vitro.[37,38] Vitamin C reduces level of cholesterol by directing cholesterol towards bile acid synthesis.[39] The major effect of the vitamin C on cholesterol 7α-hydroxylase is indirect. However, the precise mechanism of the effect has not yet been defined. It has been suggested that the lowering of triglyceride levels by vitamin C could be due to an effect on lipoprotein lipase, which is the major enzyme that degrades plasma triglycerides.[40]

As the sample is small, a large sample size with longer follow up period is necessary to label the study as representative of population. Measurement of related antioxidant levels such as vitamin E may yield more meaningful data on the role of the antioxidant system in the clinical course of type 2 DM and also will make the study more robust and broad-based. Further studies on complicated diabetics and those receiving polypharmacy are required to elucidate exact role of vitamin C supplementation in type 2 DM.

In conclusion, treatment with vitamin C along with metformin was well tolerated and devoid of any side effects. Although the results of our study should be interpreted cautiously, they do support prior evidence, linking ascorbic acid with improvement in lipid profile in diabetic patients. The absence of any substantial side effects, cheaper cost, improvement in lipid levels and the fact that plasma ascorbic acid levels are decreased in DM and increases after oral supplementation make it a particularly attractive therapeutic adjuvant in the treatment of type 2 DM. The elevation seen in HDL though not significant will open up new opportunity for research in this direction and it could be of potential importance for the prevention of coronary heart disease.

References

1. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in non-diabetic subjects with and without prior myocardial infarction. N Engl J Med 1998; 339:229-234.
2. Mooradian AD. Cardiovascular disease in type 2 diabetes mellitus: current management guidelines. Arch Intern Med 2003; 163:33-40.
3. Almdal T, Scharling H, Jensen JS, Vestergaard H. The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: a population-based study of 13,000 men and women with 20 years of follow-up. Arch Intern Med 2004; 164:1422-1426.
4. Ceriello A. Oxidative stress and glycemic regulation. Metabolism 2000; 49:27-29.
5. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes 1999; 48:1-9.
6. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40:405-412.
7. Krauss RM, Siri PW. Dyslipidemia in type 2 diabetes, Med Clin N Am 2004; 88:897-909.
8. Bisht S, Sisodia SS. Diabetes, Dyslipidemia, Antioxidant and status of oxidative stress, Int J Res Ayurveda & pharmacy 2010; 1:33-42.
9. Chahil TJ,Ginsberg HN. Diabetic dyslipidemia. Endocrinol Metab Clin North Am 2006; 35:491-510.
10. Fadupin GT, Akpoghor AU, Okunade KA. Comparative study of serum ascorbic acid level in people with and without type 2 diabetes in Ibadan, Nigeria. Afr J Med Sci. 2007; 36:335-339.
11. Afkhami-Ardekani M, Shojaoddiny-Ardekani A. Effect of vitamin C on blood glucose, serum lipids &

serum insulin in type II diabetes patients. Indian J Med Res. 2007; 126:471-474.
12. Sharma P, Pramod J, Sharma PK, Chaturvedi SK, Kothari LK: Effect of vitamin C administration on serum and aortic lipid profile of guineapigs. Indian J Med Res 1988; 87:283-289.
13. Cerna´ O, Ginter E: Blood lipids and vitamin-C status. Lancet 1978; 1:1055-1056.
14. Greco AM, LaRocca L. Correlation between chronic hypovitaminosis C in old age and plasma levels of cholesterol and triglycerides. Int J Vit Nutr Res Suppl 1982; 23:129-136.
15. Knox EG. Ischemic-heart-disease mortality and dietary intake of calcium. Lancet 1973; 1:1465-1467.
16. Enstrom JE, Kanim LE, Klein MA. Vitamin C intake and mortality among a sample of the United States population. Epidemiology 1992; 3:194-202.
17. Gale CR, Martyn CN, Winter PD, Cooper C. Vitamin C and risk of death from stroke and coronary heart disease in cohort of elderly people. Br Med J 1995; 310:1563-1566.
18. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary disease in men. N Engl J Med 1993; 328:1450-1456.
19. Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. N Engl J Med 1993; 328:1444–1449.
20. Sridulyakul P, Chakraphan D, Patumraj S. Vitamin C supplementation could reverse diabetes-induced endothelial cell dysfunction in mesenteric microcirculation in STZ-rats. Clin Hemorheol Microcirc 2006; 34 :315-21.
21. Ardekani MA, Mohiti J, Amirchaghmaghi E, Modarresi M. The effect of vitamin C supplementation on insulin level, HbA1c and blood glucose in type 2 diabetic patients. J Beh Kerm Univ Med Sci. 2006; 11:12-18.
22. Bonnefont-Rousselot D, Bastard JP, Jaudon MC, Delattre J. Consequences of the diabetic status on the oxidant/ antioxidant balance. Diabetes Metab 2000; 26:163-176.
23. Ceriello A, Bortolotti N, Crescentini A, Motz E, Lizzio S, Russo A, Ezsol Z, Tonutti L, Taboga C. Antioxidant defences are reduced during the oral glucose tolerance test in normal and non-insulin-dependent diabetic subjects. Eur J Clin Invest 1998; 28:329-333.
24. Hirsch IB, Atchley DH, Tsai E, Labbé RF, Chait A. Ascorbic acid clearance in diabetic nephropathy. J Diabetes Complications. 1998; 12:259-263.
25. Vincent TE, Mendiratta S, May JM. Inhibition of aldose reductase in human erythrocytes by vitamin C. Diabetes Res Clin Pract 1999; 43:1-8.
26. Watts GF. Coronary disease, dyslipidemia and clinical trials in type 2 diabetes mellitus. Pract Diab Int 2000; 17:54-59.
27. Suryawanshi NP, Bhutey AK, Nagdeote AN, Jadhav AA, Manoorkar GS. Study of lipid peroxide and lipid profile in diabetes mellitus. Indian J Clin Biochem 2006; 21:126-130.
28. UK Prospective Diabetes Study 27. Plasma lipids and lipoproteins at diagnosis of NIDDM by age and sex. Diabetes care 1997; 20:1683-1687.
29. Agboola-Abu CF, Ohwovoriole A, Akinlade KS. The effect of glycemic control on the prevalence and patterns of dyslipidaemia in Nigerian patients with newly diagnosed non-insulin dependent diabetes mellitus. West Afr J Med 2000; 19:27-33.
30. Paolisso G, Balbi V, Volpe C, Varricchio G, Gambardella A, Saccomanno F, Ammendola S, Varricchio M, D'Onofrio F. Metabolic benefits deriving from chronic vitamin C supplementation in aged non-insulin dependent diabetics. J Am Coll Nutr 1995; 14:387-392.
31. Bishop N, Schorah CJ, Wales JK. The effect of vitamin C supplementation on diabetic hyperlipidaemia: a double blind, crossover study. Diabet Med 1985; 2:121-124.
32. Tofler GH, Stec JJ, Stubbe I, Beadle J, Feng D, Lipinska I, Taylor A. The effect of vitamin C supplement on coagulability and lipid levels in healthy male subjects. Thrombosis Res 2000; 100:35-41.
33. Gale CR, Martyn CN, Winter PD, Cooper C. Vitamin C and risk of death from stroke and coronary heart disease in cohort of elderly people. Br Med J 1995; 310:1563-1566.
34. Sahyoun NR, Jacques PF, Russel RM. Carotenoids, vitamin C and E and mortality in an elderly population. Am J Epidemiol 1996; 144:501-511.
35. Oguntibeju OO, Fafunso MA. Serum ascorbate, cholesterol and totatl protein levels in newly diagnosed non-insulin dependent diabetes mellitus (NIDDM) among Nigerians. Med Tech SA 2002; 16: 358-360.
36. Frei B, Stocker R, England L, Ames BN. Ascorbate: the most effective antioxidant in human blood plasma. Adv Exp Med Biol 1990; 264:155-163.
37. Bjorkhem I, Kallner A. Hepatic 7a-hydroxylation of cholesterol in ascorbate-deficient and ascorbate-supplemented guinea pigs. J Lipid Res 1976; 17:360-365.
38. Peterson FJ, Holloway DE, Duquette PH, Rivers JM. Dietary ascorbic acid and hepatic mixed function oxidase activity in the guinea pig. Biochem Pharmacol 1983; 32:91-96.
39. Mullan BA, Young IS, Fee H, McCance DR. Ascorbic acid reduces blood pressure and arterial stiffness in type 2 diabetes. Hypertension 2002; 40:804-809.
40. Eckel RH. Lipoprotein lipase: a multifunctional enzyme relevant to common metabolic diseases. N Engl J Med 1989; 320:1060-1068.