Effect of Vitamins C and E Alone and in Combination with Each Other on LDL and Fibrinogen in Streptozotocin Induced Diabetic Rats

AishaTalat,[1] Talat Bashir Khan,[2] Saima Mahmood,[3] Shahnaz Aftab,[4] M. Azam Zia,[5] Rukhshan Khursheed[6]

Abstract

Background and Aim: Diabetes mellitus is a heterogeneous group of disorders, manifested by raised plasma glucose concentration and disturbances of glucose metabolism Diabetes mellitus is a complex of metabolic disorders affecting different systems of body. The main etiology of mortality and high percentage of morbidity in patients with diabetes mellitus is atherosclerosis. The vascular endothelium overlying lesion – prone arterial sites shows increased permeability to plasma proteins, LDL and fibrinogen. High plasma fibrinogen concentration in adults is associated with elevated risk of coronary heart disease and stroke. Treatment with antioxidants like vitamin C and vitamin E reduces diabetic complications. The aim of this study was to determine the effect of antioxidants vitamin C and E in lowering the fibrinogen and LDL levels.

Methodology: Present study was conducted on 48 albino rats. They were divided into four groups. Diabetes was induced in all rats by giving streptozotocin 65 mg/kg intraperitoneally. First group was control diabetic. Second group was given vitamin C in a dose of 150 mg/kg b.w/day and third group was given vitamin E in a dose of 100 mg/kg b.w/day. Fourth group was given vitamin C with vitamin E intraperitoneally in the same doses. Effects of vitamin C and E were observed on serum LDL and plasma fibrinogen level by using commercially available kits.

Results: LDL cholesterol was decreased in groups B, C and D as compared to group A. Fibrinogen level was decreased in – group B and D and increased in group C.

Conclusion: Vitamin C alone and in combination with vitamin E help in ameliorating atherosclerosis by decreasing LDL and fibrinogen levels. Vitamin E lowers LDL level but not fibrinogen level. There is a synergistic action of vitamin E and C in lowering fibrinogen and LDL level.

Key words: Diabetes mellitus, Vitamin C, Vitamin E.

Talat A.[1]
Department of Aish Talat, Department of Pharmacology, Assistant Professor CMH Medical College, Lahore – Pakistan

Khan T.B.[2]
Consultant Orthopedic Surgeon, Department of Orthopedics, Social Security Hospital, Lahore – Pakistan

Mahmood S.[3]
Department of Pharmacology, Postgraduate Medical Institute, Lahore – Pakistan

Aftab S.[4]
Head of pharmacology Deptt, CMH, Lahore Medical College, Lahore

Zia M.A.[5]
Professor of Pharmacology, Rawalpindi Medical College, Rawalpindi – Pakistan

Khursheed R.[6]
Biochemistry Department, Fatima Jinnah Medical College, Lahore

Introduction

Type I diabetes mellitus is characterized by lymphocytic infiltration of the islet cells and circulating auto antibodies against variety of islet cell antigens including glutamic acid decarboxylase (GAD), islet cell antibody (ICA), tyrosine phosphatase antibodies (TPA), and insulin antibody (I_{2A}).[1] Deficiency of insulin leads to elevated levels of free fatty acids in the plasma as a result of uncontrolled lipolysis in adipose tissue. Free fatty acids suppresses glucose metabolism in peripheral tissues such as skeletal muscles. This impairs the action of insulin in these tissues causing promotion of glucose utilization.[2] Fibrinogen may promote together with other haemostatic factors, atherosclerotic changes and thrombosis through its effects shown on platelet aggregation, blood viscosity and foam cell formation. Such processes shows the role of fibrinogen in complication of diabetes [3]

Vitamin C has been demonstrated to potentiate endothelial derived nitric oxide activity and normalize vascular function in patients with coronary artery disease and associated risk factors including hypercholesterolemia, hyperhomocystenemia, hypertension, diabetes and smoking. Vitamin C increases high density lipoprotein level by preventing LDL oxidation. Both vitamins act as anticoagulant and they may be an excellent alternative of aspirin by preventing platelet aggregation. Vitamin C and vitamin E work as warfarin, which is an anticoagulant, when given together. Vitamin C increases the effect of vitamin E and improves endothelial dysfunction.[4]

The main function of Alpha tocopherol is that of antioxidant. The Alpha tocopherol is suited to intercept free radicals and prevent a chain reaction of lipid destruction. Aside from maintaining the integrity of cell membrane throughout the body, it protects the fat in low density lipoprotein from oxidation.[5] Alpha tocopherol is known to inhibit the activity of protein kinase C and important cell signaling molecule as well to affect the expression and activity of immune and inflammatory cells. Additionally it inhibits platelets aggregation and enhances vasodilatation.[6]

Materials and Methods

2.1 Plan of Study

In this study forty eight male Sprague Dauley Albino rats, weighing 180 to 250 grams were purchased from National Institute of Islamabad, Pakistan and kept randomly in animal house of Post Graduate Medical Institute, Lahore. They were divided into four groups and each group was kept in a separate cage. Light and dark cycle maintained. They were given free access to rat chow and water. After a period of one week, all of them were made diabetic by injecting streptozotocin 65 mg/kg body weight intraperitoneally as a single dose. After that they were given 25% dextrose to prevent overnight hypoglycemia. Diabetes was confirmed by one touch glucometer after taking blood from rat tail vein after twenty-four hour.[7] Later they were given following drugs according to their groups for 6 weeks.

Streptozotocin, Vitamin E and Vitamin C

Streptozotocin purchased from Sicor Pharmaceuticals, (USA).

One gm dissolved in citrate buffer at pH 6.3 dissolved in 9.9 ml of dextrose water.

Vitamin E injections were purchased from Farvet Laborataries Holland.

Vitamin C injections were donated by Indus Pharmaceutical, Karachi, Pakistan.

2.2 Grouping of Experimental Animals

All the animals were divided randomly into four groups labeled as A, B, C and D. Each group consist of twelve male albino rats. Group A was control and it was fed on normal diet till the end of the study. Group B was given vitamin C in a dose of 150mg/kg body weight / day intraperitoneally for 6 weeks.[8] Group C was given alpha – tocopherol in a dose of 100 mg/kg body weight/day intraperitoneally for 6 weeks.[9] Group D was given alpha tocopherol 100 mg/kg body weight/ day and vitamin C 150 mg/kg body weight / day intraperitoneally for 6 weeks.

Collection of Blood Samples

Rats were weighed at 0, 2, 4 and 6 weeks. Blood samples were collected by cardiac puncture at 0, 2, 4 and 6 weeks.

Estimation of serum LDL was done after calculating total cholesterol, triglycerides and serum HDL by enzymatic methods by using Messers Randox and Messers Stanbio kits.

Following formula is applied to calculate LDL LDLc (mg/dl) = TC – (HDLc – TG/5)[10] plasma fibrinogen assay was done by Human Biochemica and Diagonostica kits.

Table 1: Comparison between different regimen and control diabetic group A on serum LDL cholesterol.

Week	Control Group A	Vitamin C Group B		α tocopherol Group C		Vitamin C + α tocopherol Group D	
		Value	Significance	Value	Significance	Value	Significance
0	31.58 ±9.29	31.53 ±6.27	P>.05 NS	31.58 ±9.30	P>.05 NS	32.41 ±8.95	P>.05 NS
2	47.41 ±8.55	45.00 ±10.95	P>.05 NS	27.17 ±8.57	P<.001 HS	52.25 ±10.37	P>.05 NS
4	49.91 ±9.85	34.58 ±7.98	P<.001 HS	41.08 ±11.30	P<.001 HS	55.66 ±8.18	P>.05 NS
6	62.25 ±9.18	45.67 ±16.41	P<.001 HS	38.58 ±5.91	P<.001 HS	57.33 ±5.19	P>.05 NS

* HS = Highly significant,
S = Significant,
NS = Non significant

Table 2: Comparison between different regimen and control diabetic group A on plasma fibrinogen.

Week	Control Group A	Vitamin C Group B		α tocopherol Group C		Vitamin C + α tocopherol Group D	
		Value	Significance	Value	Significance	Value	Significance
0	203.33 ±14.95	206.08 ±21.20	P>.05 NS	205.83 ±15.90	P>.05 NS	206.90 ±11.80	P>.05 NS
2	217.17 ±17.03	186.92 ±26.22	P<.005 HS	208.42 ±21.50	P>.05 NS	222.42 ±21.40	P>.05 NS
4	257.83 ±40.12	210.17 ±28.31	P<.001 HS	252.42 ±17.94	P>.05 NS	214.00 ±31.26	P<.05 S
6	281.25 ±30.80	204.42 ±18.02	P<.001 HS	316.67 ±15.48	P<.001 HS	190.58 ±20.72	P<.001 HS

Key: HS = Highly significant,
S = Significant,
NS = Non significant

Statistical Analysis

The data was analyzed by paired t test using computer software SPSS version 10.

Results

The results of experiment are presented as mean ± SD at 0, 2, 4 and 6 weeks. In group B, LDL cholesterol is 31.53 ± 6.27 vs 31.58 ± 9.29 mg/dl in group A at 0 week 45.00 ± 10.95 vs 47.41 ± 8.55 mg/dl at end of 2nd week, 34.58 ± 7.98 vs 49.91 ± 9.85 mg/dl at the end of 4th week, 45.67 ± 16.41 vs 62.25 ± 9.18 at the end of 6th week (Table 1).

LDL cholesterol in group B versus group A

The comparison between group B to group A shows decrease in LDL cholesterol. This difference is highly significant at the end of 4th and 6th week ($p < 0.001$) and non-significant at 0 and 2nd weeks.

LDL cholesterol in group C versus group A

The comparison between group C to group A shows decrease in LDL cholesterol. This difference is highly significant at the end of 2nd, 4th, and 6th weeks ($p < 0.001$).

LDL cholesterol in group D versus group A

In group D, LDL cholesterol is 32.41 ± 8.95 vs 31.58 ± 9.29 mg/dl in group A at 0 week 52.25 ± 10.37 vs 47.41 ± 8.55 mg/dl at the end of 2nd week, 55.66 ± 8.18 vs 49.91 ± 9.85 mg/dl at the end of 4th week 57.33 ± 5.19 vs 62.5 ± 9.18 at the end of 6th week. The comparison between group D to group A shows decrease in LDL cholesterol. This difference was non significant (Table 2).

Fibrinogen in group B versus group A

In group B, fibrinogen is 206.08 ± 21.20 vs 203.33 ± 14.95 mg/dl in group A at 0 week, 186.92 ± 26.22 vs

217.17 ± 17.03 mg/dl at the end of 2nd week, 210.17 ± 28.31 vs 257.83 ± 40.12 mg/ dl at the end of 4th week, 204.42 ± 18.02 vs 281.25 ± 30.80 at the end of 6th week. This shows decrease in fibrinogen level in group B as compared to group A. The difference is highly significant at the end of 2nd, 4th and 6th weeks ($p < 0.001$).

Fibrinogen in group C versus group A

In group C, fibrinogen is 205.83 ± 15.90 vs 203.33 ± 14.95 mg/dl in group A at 0 week, 208.42 ± 21.50 vs 217.17 ± 17.03 mg/dl at the end of 2nd week, 252.42 ± 17.94 vs 257. 83 ± 40.12 mg/ dl at the end of 4th week, 316.67 ± 13.62 vs 281.25 ± 30.80 at the end of 6th week. This shows increase in fibrinogen level in group C as compared to group A. The difference is non significant at 0, 2nd and 4th week but highly significant increase at 6th week ($p < 0.001$).

Fibrinogen in group D versus group A

In group D, fibrinogen is 206.92 ± 11.80 vs 203.33 ± 14.95 mg/dl in group A at 0 week , 222.42 ± 21.40 vs 217.17 ± 17.03 mg/dl at the end of 2nd week, 214.00 ± 31.26 vs 257.83 ± 40.12 mg/ dl at the end of 4th week, 190.58 ± 20.72 vs 281.25 ± 30.80 at the end of 6th week. There is decrease in fibrinogen level in group D which is non-significant at 0 and 4th weeks, significant at 2nd week ($p < 0.05$) and highly significant at 6th week ($p < 0.001$).

Discussion

This study showed LDL cholesterol decreased in group B treated vitamin C for 6 weeks compared to control group A. LDL cholesterol was also decreased in group C given alpha tocopherol. Statistically the difference is highly significant at 2nd, 4th and 6th week ($p<0.001$). LDL cholesterol is also decreased in group D given vitamin C with alpha tocopherol. Statistically the decrease in LDL was not significant at 2nd, 4th and 6th week. Vitamin C increases high density lipoprotein level by preventing oxidation of low density lipoprotein. Vitamin C and E were reported to act synergistically which inhibit oxidation of LDL cholesterol and prevent cell destruction as reported in favour of our study.[7] Vitamin C might play a part in hydroxylation of steroid hormone in adrenal glands. It also directly mediates through a rate limiting hydroxylation of side chains, the conversion of cholesterol into steroid hormone. The reduction in LDL cholesterol points that vitamin C intake can reduce the incidence of atherosclerosis in diabetes. These findings are in agreement with studies of Bsoul and Terezhalmy 2004[11] and Anderson et al 1999.[12]

Jain et al 2007[13] and Engelen 2000[14] who also observed decrease in LDL in diabetes after vitamin E supplementation which may be due to the fact that alpha tocopherol counteracts the increased LDL oxidation to prevent the complication of diabetes. These findings are in agreement with findings of Tankara et al 1997[15] who also observed that decrease in LDL levels may be due to synergistic action of vitamin C and E to inhibit LDL oxidation. These findings are in contrast with findings of Farvid et al 2004.[16]

In this study, plasma fibrinogen was decreased in group B given vitamin C for 6 weeks compared to control diabetic group A. Statistically the difference was highly significant at 2nd, 4th and 6th week ($p < 0.001$). Plasma fibrinogen level is increased in group C given alpha tocopherol for 6 weeks compared to control diabetic group A. The increase in fibrinogen was not significant at 2nd and 4th week but it was highly significant at 6th week ($p < 0.001$). Plasma fibrinogen is decreased in group D given vitamin C in combination with Alpha tocopherol as compared to control diabetic group A. The decrease in fibrinogen was significant at the end of 4th week ($p < 0.05$) and highly significant at end of 6th week ($p < 0.001$). These results are in contrast with Tofler et al[17] and in agreement with Haidara et al[7] who observed that Vitamin C may act by inhibiting platelet aggregation and decreasing plasma viscosity. Khaw and wood house[18] observed that vitamin C might decrease the level of fibrinogen by reducing its synthesis in the hepatocytes. Haidara et al[7] did not observe any effect of alpha tocopherol on fibrinogen level. Findings of other studies[19] and[20] they observed a decrease in fibrinogen level with higher doses of vitamin E by inhibiting the synthesis of fibrinogen by hepatocytes. Variation in the results as compared to other authors might be due to difference in animal species estimation techniques and sample handling.[21]

In conclusion synergistic action of vitamin C and E decreased the fibrinogen level. It may be due to inhibition of lipid peroxidation and presence of larger amounts of active (n-3) fatty acids at their sites of action in the liver. The end result is pronounced decrease in the synthesis of fibrinogen by hepatocytes in keeping with previous studies.[22]

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