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Effect of vitamin "C" supplementation on lipid profile composition

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Abstract
Throughout the twenty first century the health science focused primarily on curing diseases with powerful drugs, more sophisticated diagnostic tests and more effective therapies. Today the emphasis is on health expectancy rather that life expectancy. Coronary heart disease is becoming prominent cause of morbidity in India. Diet plays a very important role in increasing the blood lipids. Patients can improve lipid levels and decrease the rate of cardiovascular events by adding specific foods to their diets like fruits and vegetables that are rich in antioxidant vitamins like E, C, and caretenoids. Vitamin c is proved to be a potent antioxidant, protecting lipids in plasma against oxidation. Its stock as a preventive agent against heart disease has been proved in many studies. University of Southern California researchers presented a study showing that taking of 500mg of Vitamin c daily hastened the thickening of the walls of arteries. British researchers published a provocative study in Nature few years ago suggesting that Vitamin C is a powerful antioxidant and reduces the rate of harmful oxidation of serum lipids. This is proved miraculous power of Vitamin C. There is no ailment that this wonder Vitamin could not cure. Ascorbic Acid (AA) is a simple sugar. It is more active reducing agent known to occur naturally in living tissues, it is easily and reversibly oxidized to dehydro-Ascorbic Acid (DHA). Both forms are physiologically active. The reduced form of Ascorbic Acid (l-AA) predominates in the plasma and also apparently in tissues at a ratio of about 15:1 of the oxidized form (Engstom & De Luca, 2000). The present study found a significant corrective effect of this vitamin on total serum Lipid Profile.

Keywords: ascorbic acid, antioxidants, lipid profile, Dislipidemias

Introduction
The outstanding chemical property of the Vitamin is its reversible oxidation–reduction between dihydro & dehydro-Ascorbic Acid. Studies have proved this Vitamin helps to maintain the oxidation–reduction potential of cells at the proper levels. The physiological importance of the presence of Dehydro-Ascorbic Acid is much more in ratio to the Ascorbic Acid itself. As a hydrogen acceptor it is thought to act in animals and plants both. in a system involving TPN (Tri Phosphate Nicotinamide) and Glutathione, in which the metabolite passes H to TPN, and TPN passes hydrogen to Glutathione, which passes it to Ascorbic Acid and finally to Oxygen forming water –

$$\text{Metabolite} \xrightarrow{2H} \text{TPN} \xrightarrow{2H} \text{Glutathione} \xrightarrow{2H} \text{Ascorbic Acid} \xrightarrow{2H} \text{Dehydro Ascorbic Acid} \xrightarrow{2H} O_2$$

This process has proved significance in animal tissues to reduce or to stop the rate of unwanted, damaging oxidation especially of the blood lipids (Masely,1998). Ascorbic Acid or a derivative of it has been implicated in certain other hydroxylation (oxidation) reactions also-such as conversion of Tryptophan to 5-Hydroxy Tryptamine. The Vitamin has been placed in the electron transport chain between DPNH and one of the cytochromes, a reaction that is coupled with hydroxylation.

In this way Ascorbic Acid seems to take part in the electron transport system of the mammalian microsomes. The property of easy oxidation with reversible reduction of Ascorbic Acid is utilized in electron transport. The biological significance of the electron transport chain activity of Ascorbic Acid is to act as an antioxidant (Cance and Widdowson., 1992) Baker and others have isolated an Ascorbic Acid derivative the Monodehydro Ascorbic Acid, in combination with Ascorbic Acid. They feel that such a complex is the active substance in hydroxylation reaction. Ascorbic Acid is proved as an anti-oxidant and powerful reducing agent in many biological functions (Woodruf & Beaton, 1996). The present study thus aims at finding out the effect of antioxidants on lipid profile in hyperlipidemia. The specific objectives are to study the mean food intake of hyperlipidemics and administer Vitamin C and to assess the impact on serum lipids. Vitamin E role as a deterrent to heart disease remains unproven, but many previous studies showed its significance as a remedy to correct oxidative damages in lipid profile, which are precipitating factors in many cardiac problems by acting as an electron donor (Bieri *et al*, 1992). This biochemical change even precedes the histological alterations. Houchin (1978) reported a 40% decrease in the elevated oxygen consumption of cardiac dystrophic rabbit and hamster muscle slices after the addition of α – Tocopherol Phosphate to the medium. Much of the physiological activity of Vitamin E appears to be related to its antioxidant capacity.

Methodology
Identification & Selection of Hyperlipidemics
One hundred and fifty participants between the age group 35-60 years were selected from the secondary data maintained by the two hospitals in Bilaspur (CG)-District Hospital & CIMS. Out of them, forty two were selected by purposive sampling without any other allied morbid complications like diabetes mellitus or hypertension and who are not consuming any drug. Selected samples were divided into seven groups of six each by random sampling method. Group I to VI were the experimental groups and group VII served as control.

Background Information
Using a well framed questionnaire the details regarding occupation, income, no. of family members, family medical background and life style pattern were elicited personally from all of the selected hyperlipidemic participants who attended the master health check-up in the hospitals.

Estimation of serum lipid profile & plasma ascorbic acid levels
Serum lipid profile at the fasting state was estimated for all the participants in the seven groups using the colorimetric methods already described in Chapter-1. Ascorbic acid was estimated immediately after the blood samples were drawn for the groups administers with Vitamin C and control group using the following procedure.

Roe and Kuether procedure
This method depends on the reduction of the Dye- 2,6-Dichlorophenol Indophenol to a colourless compound by Ascorbic Acid. The titration was conducted in the presence of Acetic & Meta Phosphoric Acid in order to inhibit aerobic oxidation catalyzed by certain metallic iron.

Procedure
Serum (5 ml.) was added drop wise into 150 ml Meta Phosphoric Acetic Acid solution. The mixture was diluted to 200 ml & 10 ml of this mixture was titrated with standard Indophenol solution.

Preparation of Standard Indophenol Solution
Sodium Bicarbonate (42 mg) & Sodium 2, 6-Dichlorophenol Indophenol (52 mg) were dissolved in 50 ml water. Then the solution was diluted to 200 ml. After filtration the solution was stored in refrigerator & used fresh. Standard crystalline Ascorbic Acid (100 mg) was dissolved in 100 ml Meta–Phosphoric-Acetic Acid solution.
10 ml of serum & 25 ml meta–phosphoric-acetic acid solution was titrated with Indophenol solution until the pink colour persisted for 5 seconds.

Calculations

$$V \times S \times D = \text{mg Ascorbic Acid in percent value}$$

(**V**= ml of dyes used for titration of serum)
(**S**= ml of dyes used for titration of Standard Ascorbic Acid solution)
(**D**= Dilution factor)

Administration of Vitamins
All the six groups of experimental participants were given the Vitamin for the periods of four weeks. Vitamin E capsules and C tablets were provided to the participants by the investigators and the patients were requested to consume the tablets and/or capsules before breakfast. Almonds were weighted packed and given to the participants in respective groups. Patients were advised to take the almonds during breakfast. The main aim of administering almonds was that they are one of the richest sources of Vitamin E providing 24mg/100g.
One hundred and sixty ml of orange juice was provided every day to the respective group and the participants were advised to consume juice in the morning. No dietary restriction or exercise schedule was prescribed to the participants. The period of the study was four weeks. The recommended daily intake of Vitamin E according to Food & Nutrition board is 10mg/day and the daily intake of Vitamin C according to ICMR is 40 mg /day. The antioxidant administration especially through the synthetic source was above the recommended daily allowances. So the study period was restricted to four weeks, to avoid hypervitaminosis E. The intakes of antioxidants by all participants were monitored regularly. The investigator ensured the intake of antioxidants by the participants by personally visiting those within the city limits and other were monitored through phone.

Assessing the impact of vitamins
To assess the impact of administration, fasting blood samples were drawn from all the 35 participants immediately after the completion of the study period for the estimation of the following parameters-Serum Lipid Profile (all groups), plasma Ascorbic acid (group-II, III, V & VI) the relevant statistical tests were employed to analyze the impact of administration.

Data and analysis
Impact of antioxidants on the biochemical indices of the selected participants - Mean Serum Lipid Profile, plasma Ascorbic Acid values of all the experimental groups before and after administration along with respective t values is presented in figure 4.1 & table 4.2.

Table 1: Experimental design of administration pattern of vitamins

Groups n=42	Administration	Dosage/day
(I) n = 6	Vitamin E capsule as Tochopherol Acetate	100 mg
(II) n = 6	Vitamin C tablets as Ascorbic Acid and Na Ascorbate	100 mg
(III) n = 6	Vitamin E capsule + Vitamin C tablets	100mg+100mg (Vitamin E + Vitamin C)
(IV) n = 6	Almond (4.5g/3no)	1 mg of Vitamin E
(V) n = 6	Fresh strained orange juice (160ml)	100 mg Vitamin C
(VI) n = 6	Almonds (4.5g) + orange juice (160ml)	1mg+100mg (Vitamin E + Vitamin C)
(VII) n = 6	Control	No Treatment

Table 2: Serum Ascorbic Acid Status Changes due to Supplementation

Groups	Mean ± SD Values Serum Ascorbic Acid (mg %)			
	Experimental			Control
	Pre-Supplementation	Post Supplementation	Changes in percent value	
II (Vit-C) Tabletsn = 6	0.87(±0.039)	0.92(±0.04)	↑ 6%	0.83 (±0.10)
III(Vit-C+ E) Tablets n = 6	0.92(±0.042)	0.97(±0.04)	↑ 5%	0.83 (±0.10)
V (Orange Juice) n = 6	0.89(±0.05)	1.03(±0.07)	↑ 16%	0.83 (±0.10)
VI(Juice + Almonds) n = 6	0.85(±0.05)	0.95(±0.05)	↑ 12%	0.83 (±0.10)

(Values expressed as x $\overline{mg}.ml^{-1}$ serum and are presented as Mean value ± Standard Deviation).

A. Hdl Profiling

Table 3: Mean & Sd of Lipid Profile Changes -Pre and Post Supplementation

Groups	Mean ± SD Values Plasma HDL Cholesterol (mg/ml)			
	Experimental Group			Control Group
	Pre-Supplementation	Post Supplementation	Changes in percent value	
I (Vit-E Capsule) n = 6	0.35(±0.01)	0.35(±0.01)	0%	0.40(±0.02)
II (Vit-C) Tablets n = 6	0.39(±0.01)	0.41(±0.02)	↑ 5 %	0.40(±0.02)
III (Vit-C+ E) Tablets n = 6	0.35(±0.02)	0.42(±0.02)	↑ 20%	0.40(±0.02)
IV (Almonds) n = 6	0.34(±0.02)	0.33(±0.02)	↓ 3%	0.40(±0.02)
V (Orange Juice) n = 6	0.34(±0.01)	0.42(±0.02)	↑ 24 %	0.40(±0.02)
VI (Juice +Almonds) n = 6	0.34(±0.01)	0.35(±0.02)	↑ 3%	0.40(±0.02)

(Values expressed as x $\overline{mg}$.ml^{-1} serum and are presented as Mean value ± Standard Deviation). SD is given in parenthesis)

B. Cholesterol Profiling

Table 4: Mean & Sd of Lipid Profile Changes -Pre and Post Supplementation

Groups	Mean ± SD Values Plasma Cholesterol (mg/ml)			
	Experimental			Control
	Pre Supplementation	Post Supplementation	Changes in percent value	
I (Vit-E Capsule) n = 6	2.88(±0.20)	2.37(±0.05)	↓ 18%	2.97(±0.08)
II (Vit-C) Tablets n = 6	2.92(±0.13)	2.92(±0.12)	0%	2.97(±0.08)
III (Vit- C +E) Tablets n = 6	2.88(±0.03)	2.58(±0.06)	↓ 10%	2.97(±0.08)
IV (Almonds) n = 6	2.94(±0.10)	2.94(±0.09)	0%	2.97(±0.08)
V (Orange Juice) n = 6	2.83(±0.07)	2.84(±0.08)	0%	2.97(±0.08)
VI (Juice + Almonds) n = 6	2.86(±0.05)	2.87(±0.04)	0%	2.97(±0.08)

(Values expressed as x $\overline{mg}$.ml^{-1} serum and are presented as Mean value ± Standard Deviation). SD is given in parenthesis

C. Triglyceride Profiling

Table 5: Mean & Sd of Lipid Profile Changes Pre & Post Supplementation

Groups	Mean ± SD Values Plasma Triglyceride(mg/ml)			
	Experimental			
	Pre Supplementation	Post Supplementation	Changes in percent value	Control
I (Vit-E Capsule) n = 6	2.18(±0.39)	2.17(±0.41)	0%	2.19(±0.28)
II (Vit-C) Tablets n = 6	1.98(±0.21)	2.00(±0.22)	↑ 1%	2.19(±0.28)
III (Vit-C+ E) Tablets n = 6	2.17(±0.38)	2.17(±0.37)	0%	2.19(±0.28)
IV (Almonds) n = 6	1.93(±0.10)	1.93(±0.11)	0%	2.19(±0.28)
V (Orange Juice) n = 6	2.29(±0.39)	2.28(±0.40)	0%	2.19(±0.28)
VI(Juice + Almonds) n = 6	2.16(±0.34)	2.17(±0.35)	0%	2.19(±0.28)

(Values expressed as x $\overline{mg}$.ml^{-1} serum and are presented as Mean value ± Standard Deviation). SD is given in parenthesis)

D. LDL Profiling

Table 6: Mean & Sd of Lipid Profile Changes -Pre and Post Supplementation

Groups	Mean ± SD Values Plasma LDL Cholesterol (mg/ml)			
	Experimental			Control
	Pre Supplementation	Post Supplementation	Changes in percent value	
I (Vit-E Capsule) n = 6	1.76(±0.17)	1.59(±0.09)	↓ 10%	1.77 (±0.03)
II (Vit-C) Tablet n = 6	2.14(±0.16)	2.05(±0.19)	↓ 4%	1.77 (±0.03)
III (Vit-C+ E) Tablets n = 6	2.10(±0.08)	1.74(±0.09)	↓ 17%	1.77 ±0.03
IV (Almonds) n = 6	2.23(±0.03)	2.21(±0.09)	↓ 1%	1.77 (±0.03)
V (Orange Juice) n = 6	2.04(±0.14)	2.05(±0.14)	0%	1.77 (±0.03)
VI (Juice +Almonds) n = 6	2.09 (±0.09)	2.08(±0.09)	0%	1.77 (±0.03)

(Values expressed as x $\overline{mg}$.ml^{-1} serum and are presented as Mean value ± Standard Deviation). SD is given in parenthesis)

Discussion

1. The serum Ascorbic Acid, mg/100 ml showed changes after Vitamin supplementation. The Ascorbic Acid was given to group no-II & III in synthetic form (tablets). Both these groups showed changes in their Ascorbic Acid Status (AAS). The group II supplemented with ascorbic Acid tablets had mean serum Ascorbic Acid 0.87 mg/100 ml, which after supplementation became 0.92mg/100 ml. The group III was supplemented with Vitamin E and C tablets both. The group had mean value before supplementation 0.92-mg/100 ml, which became 0.97-mg/100 ml after the supplementation. The groups V and VI were supplemented with natural Vitamin C sources (fruit juice and Almonds). These groups showed more increase in serum Ascorbic Acid status, than the groups supplemented with synthetic sources. Due to Vitamin C supplementation in the form of orange juice the AAS was improved 16%, while orange juice with almonds cause 12% increase in AAS. The group V that showed most drastic effect was given orange juice and the group VI who was supplemented with almonds and orange juice both, showed comparatively lesser effect.

So, we can conclude that giving vitamins in natural forms help in a better way for improving their blood levels of Ascorbic Acid.

2. The LDL Cholesterol was not much affected by the supplementation given during the experiment. The group Ist and IIIrd, who were given Vitamin E in tablet form showed some changes in LDL cholesterol levels. Supplementation in the form of Vitamin C tablets & Vitamin E capsules caused 17% decrease in this cardio-damaging LDL fraction. Otherwise, the groups supplemented with Vitamin C and also with natural form of vitamins did not show noticeable change. Group V and VI administered with only dietary sources showed no significant effect.
3. The HDL Cholesterol, a good blood vessels cleaning cholesterol increased due to vitamins supplementation. The groups Ist and IIrd –provided with Vitamin E & Vitamin C supplementation in tablet forms did not show much change in their HDL cholesterol part, the group IIIrd supplemented with Vitamin E and C in synthetic form together showed increase in HDL lipid fraction (20%).Supplementation with natural Vitamin C sources (group V th) also projected increase in this fraction (24%). This group had mean 0.34 mg/ml serum HDL–cholesterol value before supplementation, which became 0.42 mg/ml after supplementation. The group VIth provided with orange juice and almonds showed Not Significant changes. So, this can be concluded that although the changes were not significant on statistical basis, but Vitamin C exerted beneficial effect on HDL cholesterol on mg / ml level.
4. The total cholesterol seemed not to be affected by Vitamin C supplementation in this study. Only those groups provided with synthetic or natural dietary form of Vitamin E showed some effect on this part of lipid spectrum. Supplementation with Vitamin E capsule caused 18% decrease in Serum total Cholesterol fraction. Only 1st group and 3rd group showed some reduction in this cardio-harmful lipid fraction. The decrease was more in that group (III) which was given Vitamin E and Vitamin C tablets together. Otherwise none of these groups showed significant change. So, consumption of antioxidant vitamins in tablets form by hyperlipidemics exerted decreasing effect on serum cholesterol part.
5. Triglyceride level was not at all affected by any type or any combination of supplementation.
   Results show that the changes were not statistically significant between the Pre-supplementation & Post-supplementation conditions. Between Pre-supplementation condition and control group & Post-supplementation condition and control group did not show significant difference. Since the numbers of participants were only 6 in each group and the study period was only four weeks, therefore the changes in Ascorbic acid status and in lipid profile were not found significant. However the mean mg/ml values and changes in percentages showed that there is trend of change in the two conditions (Pre-supplementation & Post-supplementation condition).

The calculated correlation between changes in serum Ascorbic Acid status and serum LDL Cholesterol showed low negative correlation after supplementation, while serum Ascorbic Acid status and serum HDL Cholesterol showed low degree positive correlation. Changes in AAS and serum Cholesterol after supplementation showed low degree negative correlation, but ASS and serum Triglyceride showed low degree positive correlation in post-supplementation values.

Conclusion

The findings of the study indicated the beneficial effects of antioxidants Vitamin E and C as food and in synthetic form. A combined intake of both Vitamins together brought better results in lipid profile than consuming them alone. The study has brought out the fact that hyperlipidemics should consume liberal amounts of dry fruits, fruits and vegetable those are rich in antioxidants to maintain blood lipid levels within normal and healthy range.

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