Preventive therapy of antioxidant vitamins against the blood choline levels in cardiovascular patients

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Abstract: Therapeutic role of antioxidant against lipid profile and lipoprotein (choline) was observed by the different researchers, but clinical evidences required about the use of antioxidant vitamins against the lipoproteins. Patients with clinical evidence of cardiovascular disease (CVD) confirmed by standard diagnostic techniques were followed. Newly or recently, diagnosed case subjects were recruited wherever possible. At least 120 cases, subjects both male and female with CVD were selected from a local hospital. Four groups developed on the base of antioxidant therapy and blood samples were collected at zero day, 20 days, 40 days and 60 days. vitamins C and E are the major dietary cellular and lipid antioxidants, respectively; we found no evidence to support the use of vitamin or antioxidant supplements in the reduction of mortality. However, they are helpful in the management of prevention of cardiovascular disease.

Key words: Antioxidant; Lipid profile and lipoprotein; Vitamins; Cardiovascular disease.

Introduction

In clinical setup, various cardiovascular biomarkers such as hs-CRP and NT-ProBNP are commonly executed to assess of the risk of heart failure as well as regulation of cardiovascular pathophysiological processes such as the production of reactive oxygen species (ROS) during the stress processes of necrosis, apoptosis, cell dysfunction, metabolism, and mitochondrial pathways (1). Meanwhile, the choline is water-soluble vitamin that is the precursor of the acetylcholine a neurotransmitter and found in the phospholipids (phosphatidylcholine and sphingomyelin) of cell membranes. Recent research depicted that the estimation of choline in the blood fluid serves as the emerging biomarker to diagnose the coronary plaque destabilization, ischemia, and myocardial infarction. The measurement of the concentration of choline and cardiovascular biomarkers is challenging task because of their short life. Scientist found that the consumption of the diet rich in vitamin C and E efficiently reduces the oxidative stress and improve the concentration of high density lipoprotein (HDL) and immunity system of the body (2). Vitamin C and E as an antioxidant regulate the synthesis of collagen and nitric oxide to compensate these ROS and protect the larger biological units such as protein, lipids, carbohydrates, and nucleic acid from inflammatory damage. The main source of superoxide radicals is the activity of the NADPH oxidase, which also accelerate the oxidation of low-density lipoprotein. Both vitamin C and E in combination reduce the level of the inflammatory biomarkers by decreasing the activity of NADPH oxidase or LDL oxidation. These vitamins promote the production of total cholesterol and HDL level in the body and intensified its effect on reducing the concentration of plasma cardiovascular biomarkers (3).

Increased levels of plasma homocysteine may cause various pathological abnormalities including cardiovascular diseases (4,5). Homocysteine can be eliminated from body via transmethylation and the transsulfuration pathways, modulated by water-soluble vitamins (folate and choline) (6,7). Moreover, S-adenosylmethionine (essential for glutathione activity through glutathione S-transferase) has also a significant impact on the transsulfuration pathway by activating homocysteine flux (8,9). Accordingly, plasma choline level considered an
indirect cause of cardiovascular disease as it actively involved in the methylation of homocysteine to methionine through a betaine-dependent pathway. Some scientific studies revealed an immediate effect of antioxidant vitamins (vitamin-C & vitamin-E) on glutathione-S transferase activity that can influence plasma homocysteine levels (10). Although, any study was not found on the direct effect of antioxidant vitamins on choline (a potential modulator of cardiovascular disease), but it can be correlated through the effect of antioxidant vitamins on the redox - pathway and its impact on the trans - sulfuration pathway. This study was conducted to assess the effect of antioxidant vitamin (C & E) on plasma choline level as well as various cardiovascular bio-markers (lipid profiles).

Materials and Methods

Selection of cases

Cases were selected from Mayo Hospital, Lahore and Punjab Institute of Cardiology, Lahore, Pakistan. Patients with clinical evidence of cardiovascular disease (CVD) confirmed by standard diagnostic techniques were followed. Newly or recently diagnosed case subjects were recruited wherever possible.

Inclusion criteria

The study population and data collection procedures have also been reported previously. Eligible individuals 40 to 60 years of age initially sampled at random from a study population. Newly diagnosed cases of vascular disease conformed on angiography.

Patients were recruited on the base of ECG, blood reports having a poor lipid profile, elevated cardiac biomarkers: kinase and troponin level. Furthermore, excluded all those patients, those have the other metabolic disorder with cardiovascular disease like liver problem, diabetes, lungs issue etc.

Study plan

At least 120 cases, subjects both male and female with CVD selected from Mayo hospital and Punjab Institute of Cardiology, Lahore. Patients were given a comprehensive presentation about the project and the patients who were agreed and asked to sign the consent form. All subjects divided into four groups on the base of antioxidant therapy, i.e., Group-I (Placebo), Group-II (Vitamin C), Group-III (Vitamin E), and Group IV (Vitamin E). In our study, antioxidants have given in addition with regular medication for 60 days. Their blood samples collected at zero day, 20 days, 40 days and 60 days.

Selection of control group

Control subjects were clinically declared cardiac patients; they were on medication only without the addition of any antioxidant for whole period of research.

Biochemical profile

Triglyceride level in the serum was determined by liquid TG-GPO-PAP (11). Cholesterol in serum samples was measured by CHOD–PAP as described by Stockbridge et al. (12). Serum HDL determined by the HDL cholesterol precipitant method as described by Schulte and Assmann (13). LDL in all the serum samples estimated by using the procedure outlined by McNamara (14). TG/HDL cholesterol ratio calculated for assessment of the level of dense LDL, a relatively novel lipoprotein index that could serve as a good predictor of CHD as described by Bonakdar et al. (15). Glucose concentration in the serum samples was analyzed by GOD-PAP method as described by Malerbi and Matos (16). Choline was investigated as a bookmaker in plasma by using HPLC method described by (17,18).

Antioxidant therapy

Cases were carried out to investigate the role of antioxidants as a protective factor for cardiovascular disease. All subjects with CVD were divided into three groups, Group-I, Group-II and Group-III. Group-I (Control group) was consisted of thirty (30) subjects without treatment on the other side same no. of subjects in each Group-II and Group-III was treated with Vitamin C and E respectively for sixty days. Blood samples were collected after every two weeks from all subjects for further outcome.

Statistical analysis

All demographic characteristics like sex, age, weight, and height, general health, previous disease history, dietary habits, lifestyle smoking, economic status, BMD, BMI were analyzed chi-square test. Biochemical results were analyzed through analysis of variance technique using SPSS (20.1) to determine the level of significance. The statistical significance was defined as P≤0.05. Simple & canonical correlation and regression analysis were performed to determine the potential correlation.

Results

In the intervention study, we evaluated the effect of different treatments like Vitamin C, Vitamin E and outcome of both Vitamin C and E compare with placebo. The means for the blood composition of different parameters (Table1) revealed that the effect of vitamins C (129.866c) and E (132.000c) lower the blood glucose significant as compare to C&E (136.141b) treatment same like in case of blood choline Vitamin C (26.275c). Several studies supporting our effort (19-22) suggested that the possible protective effect of vegetables and fruits was due to the combined action of an antioxidant cocktail and other active compounds, such as fibers and polyphenols (23). Vitamin E (25.991c) and Combine Treatment C&E (27.216b). Antioxidant vitamins lower the blood cholesterol significantly but vitamin E is less effective than vitamin C and composite treatment. We found that significant increasing trend in HDL in composite treatment and vitamin C rather than Vitamin E. Furthermore, treatments affect significantly LDL level results showed composite treatment and vitamin C rather than Vitamin E. There was no significant effect recorded within treatments in case of uric acid, C-RP, and ESR. However, they were significantly very from placebo treatment. These findings may increase our understanding of the discrepancy between data on the antioxidant effect on FPG in published antioxidant trials and in observational studies. In this study, we evaluated
Table 1. Effect of antioxidant intervention on Lipid profile and choline levels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glucose</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>HDL</th>
<th>LDL</th>
<th>Uric acid</th>
<th>CRP</th>
<th>ESR</th>
<th>Choline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>154.391 a</td>
<td>247.550 a</td>
<td>225.025 a</td>
<td>40.383 c</td>
<td>173.833 a</td>
<td>5.529 a</td>
<td>22.750 a</td>
<td>22.558 a</td>
<td>32.523 a</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>129.866 c</td>
<td>233.516 c</td>
<td>203.708 c</td>
<td>42.750 b</td>
<td>163.866 bc</td>
<td>5.013 c</td>
<td>21.658 b</td>
<td>20.016 b</td>
<td>26.275 c</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>132.000 c</td>
<td>238.858 bc</td>
<td>211.016 b</td>
<td>43.150 b</td>
<td>166.591 b</td>
<td>5.126 b</td>
<td>21.783 b</td>
<td>20.358 b</td>
<td>25.991 c</td>
</tr>
<tr>
<td>Vitamin C&amp;E</td>
<td>136.141 b</td>
<td>234.166 c</td>
<td>210.475 b</td>
<td>48.716 a</td>
<td>162.208 c</td>
<td>5.137 b</td>
<td>21.191 b</td>
<td>20.841 b</td>
<td>27.216 b</td>
</tr>
</tbody>
</table>

Table 2. Effect of treatment and time on various biochemical parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glucose</th>
<th>Uric acid</th>
<th>CRP</th>
<th>ESR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>149.600 a</td>
<td>5.893 a</td>
<td>25.300 a</td>
<td>24.800 a</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>144.766 a</td>
<td>5.930 a</td>
<td>26.033 a</td>
<td>23.500 a</td>
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<tr>
<td>Vitamin E</td>
<td>148.000 a</td>
<td>5.853 a</td>
<td>24.533 a</td>
<td>23.633 a</td>
</tr>
<tr>
<td>Vitamin C&amp;E</td>
<td>149.366 a</td>
<td>6.060 a</td>
<td>25.566 a</td>
<td>24.633 a</td>
</tr>
<tr>
<td>Placebo</td>
<td>153.200 a</td>
<td>5.630 a</td>
<td>23.266 a</td>
<td>23.383 a</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>128.733 c</td>
<td>5.310 a</td>
<td>22.866 a</td>
<td>20.866 b</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>130.900 c</td>
<td>5.343 a</td>
<td>22.700 a</td>
<td>21.366 b</td>
</tr>
<tr>
<td>Vitamin C&amp;E</td>
<td>139.333 b</td>
<td>5.463 a</td>
<td>22.500 a</td>
<td>22.133 ab</td>
</tr>
<tr>
<td>Placebo</td>
<td>156.300 a</td>
<td>5.376 a</td>
<td>22.066 a</td>
<td>21.866 a</td>
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<tr>
<td>Vitamin C</td>
<td>124.166 c</td>
<td>4.733 a</td>
<td>20.166 b</td>
<td>18.800 b</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>126.600 bc</td>
<td>4.883 a</td>
<td>21.166 ab</td>
<td>18.833 b</td>
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<tr>
<td>Vitamin C&amp;E</td>
<td>132.100 b</td>
<td>4.850 a</td>
<td>19.833 b</td>
<td>19.766 b</td>
</tr>
<tr>
<td>Placebo</td>
<td>158.466 a</td>
<td>5.216 a</td>
<td>20.366 a</td>
<td>20.183 a</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>121.800 b</td>
<td>4.080 b</td>
<td>17.566 bc</td>
<td>16.900 b</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>122.500 b</td>
<td>4.426 b</td>
<td>18.733 ab</td>
<td>17.600 b</td>
</tr>
<tr>
<td>Vitamin C&amp;E</td>
<td>123.766 b</td>
<td>4.176 b</td>
<td>16.866 c</td>
<td>16.833 b</td>
</tr>
</tbody>
</table>

the effect of different treatments compare with time like Vitamin C, Vitamin E and outcome of both Vitamin C and E compared with placebo for zero days, 20 days, 40 days and 60 days. The means of the blood composition of different parameters.

(Table 2) exposed that the effect of treatment increases with days. In Glucose the mean value was at zero days (154.3917 a) it decreases with time (136.1417 b) and in HDL the increasing trend was observed the level of HDL at zero days was (38.841 d) and after 60 days it was (48.968a). Over all the effect of antioxidant was significant over blood lipids and choline levels. Some studies support our research Oxidative stress refers to a physiological state in which an imbalance between pro-oxidants and antioxidants results in oxidative damage. Oxidative stress has been associated with the development of numerous chronic diseases such as type 2 diabetes, cardiovascular disease (CVD), osteoporosis, and cancer (24,25). Atherosclerotic cardiovascular diseases (CVD) are a major source of mortality and morbidity in the general population. Riccioni et al. (26) studied the role of vitamins C and E, the most prevalent natural antioxidant vitamins; suggest that supplemental use of these vitamins may lower the risk for coronary events. In other study, researcher linked obesity/visceral fat to diabetes and cardiovascular (CVD) complications include inflammation and increased oxidative stress. Gariballa et al. (27) concluded that antioxidants supplementation with B-group vitamins enhances antioxidant capacity, and may have an anti-inflammatory effect in obese diabetic and CVD patients. In contrast, large scale clinical trials using antioxidants therapies for the treatment of CVD have been disappointing because of the lack of efficacy and undesired side effects (28).

In a randomized trial, the average treatment effect was estimated from a sample using a comparison of mean outcomes for treatment duration and treated units. In placebo like Vitamin C, Vitamin E and outcome of both Vitamin C and E compared with placebo. The means values of blood composition for different parameters at zero day, 20 days, 40 days and 60 days were estimated. In this study, we evaluated the effect of different treatments compare with time like Vitamin C, Vitamin E and outcome of both Vitamin C and E compared with placebo for zero days, 20 days, 40 days and 60 days. The means of the blood composition of different parameters (Table 2) exposed that the effect of treatment increases with days. Regarding plasma glucose level, it decreased from 154.3917 mg/dL (baseline) to (136.1417 b), while the increased significantly from baseline (38.841 mg/dL) to 48.968mg/dL at the end of the study. Moreover, the overall effect of vitamin E & C treatment was observed significant in blood lipid profile (LDL, HDL, Cholesterol, and Triglyceride) as well as choline levels.

Figure 1 show the effect of antioxidants are presented. Optimum levels of cholesterol is necessary for cellular function; however, The abnormal higher level of cholesterol is a risk factor for cardiovascular diseases. Vitamin E and vitamin C in combination show greater effect on cholesterol followed by vitamin C and vitamin E alone at 60 days interval.
Contrary to the cholesterol lowering impact of combined vitamin (E and C), vitamin C alone significantly decreased triglyceride level followed by combining vitamin C and E and then vitamin E supplementation.

Blood HDL is considered to be beneficial for health. A greater quantity of HDL compared to LDL level is normally desired and heart friendly. In the present study, a significant greater effect was noted on HDL after vitamin E and C while both vitamin E and Vitamin C resulted in similar effect when given separately but lesser than combined therapy.

Compared to HDL, LDL level is a compromising indicator of heart diseases. There are greater chances of LDL oxidation when present in higher concentration. In the present study, LDL was significantly decreased due to combined vitamin C and E. While, vitamin C and Vitamin E alone have a lesser impact.

Blood choline is another risk factor like LDL and when its concentration increased from optimum level it become oxidized and produced LDL. LDL was significantly decreased by vitamin E alone. Whereas Vitamin C and combined Vitamin E & C have lesser but similar effect.

**Discussion**

A diet rich in fruits and vegetables, and therefore antioxidants, has confirmed the beneficial effects against oxidative damages and insulin resistance (29). The antioxidant supplementation with vitamin C, vitamin E, coenzyme Q10 and selenium significantly increased large and small artery elasticity in patients with multiple cardiovascular risk factors (30). This beneficial vascular effect was associated with an improvement in glucose and lipid metabolism as well as a significant decrease in blood pressure. The outcomes of the present study showed that the effect of treatment were highly significant (p<0.0001) and uric acid and CRP were (p=0.00538) at same time the effect of day were significant in all parameters with (p<0.0001).

Further evaluation the interaction of time and treatment was appeared as HDL and ESR were non-significant, but Cholesterol, Triglycerides, and choline were highly significant scores (p > 0.0001) but CRP and ESR were less significant (p=006). The effect of gender was non-significant except cholesterol (p=0.056) and HDL (p=0.054). Moreover, Age effect significantly in HDL, LDL, Uric acid, CRP, scrod with (p > 0.0001), (p=0.003), (p=0.01), (p=0.01) respectively. Lea in 1966 observed that people more than 55 year of age have significant ratio of artherosclerosis due to the lipid peroxidation process. Harman (31) also favored the Lea observation that the consumption of the fatty food lead to the release of the free radicals at this age which cause arterial wall injury. Sastre (32) with his research group found that the major reason of aging is the oxidative damage of the DNA molecules. Chen et al. (33) documented uric acid level as an independent risk factor for all-cause, cardiovascular and ischemic stroke mortality in Chinese population that Hyperuricemia was an independent risk factor of mortality from all causes, total CVD, and ischemic stroke. Ndreppepa et al., (34) observed affiliation between uric acid and cardiovascular disease is incompletely and concluded that elevated levels of uric acid were an independent predictor of 1-year mortality across the whole spectrum of patients with acute coronary syndromes treated with percutaneous coronary intervention. In case of different interactions of different factor like: Time: Age, Time: Gender: Age, Treatment: Time: Gender: Age, Time: Age results were non-significant. Time and age was non-significant in glucose except all.

Some studies support our research Oxidative stress refers to a physiological state in which an imbalance between pro-oxidants and antioxidants results in oxidative damage. Oxidative stress has been associated with the development of numerous chronic diseases such as type 2 diabetes, cardiovascular disease (CVD), osteoporosis, and cancer (35). Atherosclerotic cardiovascular diseases (CVD) are a major source of mortality and morbidity in the general population. (Riccioli et al. 2012) studied the role of vitamins C and E, the most prevalent natural antioxidant vitamins; suggest that supplemental use of these vitamins may lower the risk for coronary events. In other study, researcher linked obesity/visceral fat to diabetes and cardiovascular (CVD) complications include inflammation and increased oxidative stress. Gariballa et al. (27) concluded that antioxidants supplementation with B-group vitamins enhances antioxidant capacity, and may have an anti-inflammatory effect in obese diabetic and CVD patients. In contrast, large scale clinical trials using antioxidants therapies for the treatment of CVD have been disappointing because of the lack of efficacy and undesired sides effects (28).
Van-Poppel and Goldbohm (36) suggested that beta carotene a strong source of antioxidant play vital role in scavenging the reactive oxygen species (ROS) and protect the biomolecules against the lethal and hazardous effect of ROS. Glatthaar et al. (37) proved that Vitamin C which is present in fresh citrus fruits and vegetables as antioxidant is helpful in maintain the concentration of the free radicals in the body. They also explained that the vitamin C enhance the immune system and liver detoxification reaction against these oxidant species. Ouahchi et al. (38) proposed that Vitamin E strictly resist against the mutational effects due to the ROS. The food or the supplement with the above mentioned vitamins reduce the risk of heart or neurological disorder. Kwiterovich (39) in his research found that vitamin E as fat soluble vitamin limit the oxidation of LDL which is the major cause of heart diseases. He also recommend the intake of vitamin C with E before the meal of high fat contents because Vitamin C also scavenge the oxidant factor of LDL and facilitate the release of cholesterol from the body. The present findings revealed that the results obtained after the treatment were significant \( p > 0.0001 \). The effect on the uric acid and CRP was also recorded as significant \( (p=0.00538) \). Overall the day effect on all the parameters was also found as significant \( (p > 0.0001) \). In our study we examine the effect of Vitamin C and E individually and collectively on the blood glucose level and compare it with the effect of placebo. We found that vitamin C, E and C&E reduce the blood glucose 129.866, 132.000 and 136.141 respectively while in case of placebo the glucose reduce only to 154.391. Dakhale et al. (40) research on the diabetic patient strengthen our observation that the vitamin C administration minimizes the blood glucose as compared to the placebo. In another research the Rafi-ghi et al. (29) concluded that the vitamin C is active antioxidant among others antioxidant in reducing the blood pressure by lowering the blood glucose level.

High cholesterol level in the blood plasma increases the risk factor of heart diseases. We evaluate that the effect of vitamin C is greater (233.516) in reducing the plasma cholesterol as compared to the vitamin E, C&E and placebo. Ginter et al. (41) also recommended the similar observation that vitamin C significantly depress the cholesterol level in the blood. Marc (42) also reported that the elevate level of the vitamin C favour the reduction of cholesterol in the blood and act as the protective shield against the heart disease. In our intervention research we found that intake of vitamin C individually lower the ratio of triglyceride (203.708), uric acid (5.013) and erythrocyte sedimentation rate (20.016). The research output of several scientist agreed with our results such as Michael et al. (43) with his colleague evaluated that supplement of vitamin C significantly reduce the serum uric acid. Beser (44) is not in favor of our results because he did not find the significant effect of vitamin C on triglycerides and uric acid. McRae (45) proved this fact that vitamin C intake on daily basis lower the triglycerides along with cholesterol in the blood. Khosroshahi et al. (46) also investigate the similar results that vitamin C reduce the ESR rate.

Upritchard et al. (47) proposed that supplement with high dose of vitamin E extensively lower the LDL and CRP level but in our intensive research we evaluate that supplement of vitamin C along with E control the high concentration of LDL and C reactive protein in the heart patient. CRP is considerably the inflammatory marker in case of cardiovascular patient. We conclude that the administration of antioxidant such as Vitamin C and E collectively improve the quality of life.

Remarkably, table 2 represents that the treatment duration of sixty days have significant results in reducing the concentration of glucose, cholesterol, triglycerides, uric acid, choline, LDL, ESR and CRP while the twenty days and forty days treatment have progressive approach for lowering the blood glucose in the cardiac patient. Marina et al. (48) research is also in favour of our estimation that long term (six month) treatment of antioxidants potentially protects the human from the drastic or damaging effect of the free radicals.

The vitamin C treatment at the early stage has little influence on the level of glucose, cholesterol, triglycerides, HDL, ESR and choline. Increasing the time duration of treatment such as 20 days, 40 days and 60 days progressively reduce the intensity of these parameters more rapidly as compared to the vitamin E, C&E and placebo. Vitamin E at the zero phases lower down the concentration of the uric acid and CRP and further its effect was recorded on the choline concentration during the 40 and 60 days of treatment rather than the other antioxidants. Supplement of vitamin C with E did not show any response on the LDL and CRP at the start of the treatment but with the passage of 20, 40 and 60 days of treatment their effect was more prominent on these parameters among the other oxidants. Rodrigues et al. (49) recommended that the flavonoid 30 days of treatment decrease the oxidative stress on the glucose and lipid metabolism. Koutelidakis et al. (50) also proposed the same results that use of green tea as source of antioxidants did not change the cholesterol, LDL, HDL, CRP and glucose level after 1.5 to 3 hrs of intake. Placebo as compared to the other antioxidants reduces the concentration of the LDL and HDL at the zero days and 20, 40 and 60 days of treatment respectively. Rondanelli et al. (51,52) presented the similar result that the group subjected to the twelve-week placebo treatment showed low level of LDL and HDL level. Overall, our output of research is that the antioxidants have significant influence on lowering the concentration of these parameters at the 60 days of treatment rather than the placebo and vitamin C influence more among the other antioxidants.

References