COMPARATIVE EFFECTS OF VITAMIN E AND ITS COMBINATION WITH VITAMIN C ON ENDOTHELIAL FUNCTION IN DIABETIC HYPERTENSIVE PATIENTS

Muhammad Imran Shaikh1,2*, Muhammad Ali Ghoto1, Abdullah Dayo1, Noor Jahan2, Omair Anwar Mohiuddin2

1 Faculty of Pharmacy, University of Sindh, Jamshoro, Pakistan.
2 Faculty of Pharmaceutical Sciences, Dow University of Health Sciences, Karachi, Pakistan.

ABSTRACT
To compare the antioxidant effect of vitamin E alone and its combination with vitamin C on endothelial dysfunction in patients with diabetes mellitus, a prospective, randomized controlled, parallel group, interventional study was conducted to record flow mediated dilation (FMD) of the brachial artery in 167 diabetic patients. Out of these, 61(39%) were smokers and 97(52%) were Hypertensive. The interventional group was treated with Vitamins E (400mg/day) for first six weeks and with both vitamin E (400mg/day) and vitamin C (500mg/day) for later six weeks of study. No significant variation in FMD% was recorded in the control group while for interventional group significant increase in FMD% was recorded. Within interventional group, addition of vitamin C in existing vitamin E therapy significantly improved endothelial function in all diabetic patients (Individual therapy v/s combination therapy: 7.30±1.47 v/s 8.75±1.65; p≤0.05); smokers: (6.99±1.42 v/s 8.59±1.92; p≤0.05); nonsmokers (7.48±1.48 v/s 8.85±1.48; p≤0.05); hypertensive patients (6.94±1.41 v/s 8.49±1.53; p≤0.05) and nonhypertensives (7.82±1.40 v/s 9.15±1.75; p≤0.05). Results obtained from the current study clearly indicate the beneficial effects of vitamin E on FMD in patients with diabetes and were further augmented by the addition of vitamin C in therapy. Maximum improvement in FMD was recorded in nonsmoker-nonhypertensive diabetic group.

KEY WORDS: Antioxidant, Diabetes, Hypertension, Flow mediated dilation.
INTRODUCTION
Cardiovascular outcomes can be foretold by endothelial function. The endothelium plays a key role in maintaining homeostasis of vasculature by mediating coordination between wall of the vessel and size of lumen, it also under physiological conditions helps in maintaining normal vascular tone via a range of factors, such as prostacyclin, nitric oxide (NO) and prostacyclin. However, endothelium in pathological condition can also change its phenotype promoting vasoconstriction.\[1\] Dysfunction of endothelium attributes towards reduction in vasodilator, proinflammatory state and prothrombic properties. Diminution in vasodilatory response of endothelium is due to loss of nitric oxide activity in the vascular wall.\[2\]

Oxidative stress is broadly classified as generation of a surplus and/or decreased depletion rate of exceedingly reactive molecules as reactive nitrogen species (RNS) and reactive oxygen species (ROS).\[3\] Oxidative stress switch antiatherosclerotic NO producing enzyme to an enzyme that may hasten the atherosclerotic process thus prevention and treatment of atherosclerosis is an important priority.\[4, 5\] ROS, RNS and oxidative stress are thought to be the starting point for acquiring cardiovascular diseases.\[6\] Free radical production is boosted up with Hyperglycemia, this result in increased production of O\(_2^*\).\[3\]

Gradual increase in inflammatory disorder of arterial wall is marked by atheroma that stay clinically silent until turn out to be large enough to impair arterial perfusion or disruption of the lesion producing thrombotic occlusion or ulceration or embolization of the vessel.\[7\] Endothelial dysfunction is considered as early stage of atherosclerosis, which could have been caused by various mechanisms, one of those being a decreased nitric oxide (NO) or increased reactive oxygen species (ROS) chiefly superoxides production.\[8, 9\] Hypertension,\[10\] Diabetes mellitus,\[11\] are the common conditions predisposing to Atherosclerosis.

Accumulated evidences propose that increased antioxidant intake lessen the risk of coronary heart disease.\[12\] Vitamin E and vitamin C react in haste with organic free radicals, and induce their biological effect.\[13\] Antioxidant vitamin E, principal antioxidant for the prevention of atherosclerosis, show its efficacy in a number of oxidative stress-induced conditions and also have cell signaling and gene regulatory functions.\[14\] Vitamin C supplementation may potentially be a valuable and cost effective adjunctive therapy. Oral ascorbic acid is known to decrease arterial blood pressure and improve arterial stiffness thus reduces cardiovascular risk in patients with type 2 diabetes.\[15\]
Brachial artery flow-mediated dilation (FMD), recorded by high-resolution doppler ultrasonography, shows endothelium-dependent vasodilator function. FMD is compromised in patients with atherosclerosis and corrected with risk-reduction therapy thus FMD measurement proves as a useful noninvasive prognostic technique in preventive cardiology and is effective for long-term cardiovascular risk assessment in a lower risk population, to estimate short-term postsurgical cardiovascular outcomes in a high-risk population, and is an tremendous experimental method to detect changes in endothelial activity in response to new therapeutic interventions.[16]

This study aims to determine the extent of endothelial dysfunction in patients with diabetes mellitus and to study comparative effect of individual (vitamin E) and combination (vitamin E & C) antioxidant therapy on endothelial function in diabetic patients. In addition, effects of antioxidants on diabetic smokers and non smokers were also determined.

MATERIAL AND METHODS

Human subjects

A total of 167 diabetic patients were recruited from general population, identified at outpatient hospital visits in two general hospitals, Sind, Pakistan. Further, in diabetic category, 91 patients were placed in interventional group (treated) while 76 patients in control group (untreated). All patients were asked to complete health questionnaires in order to record health information including history of diabetes, hypertension and other cardiovascular diseases, smoking, regular current exercise, and medication. Patients were instructed not to change their diet, exercise and medication routine throughout the study. Written and verbal informed consent was obtained from all patients.

Inclusion & exclusion criteria

Diabetic patients having fasting plasma glucose concentration of greater than 126 mg/dl were included in the study, whereas Patients already taking antioxidants were excluded.

Ethical Approval

The study was reviewed and approved by institutional review board and Ethics Committee.

Study Design

Prospective, Randomized Controlled, Parallel group, Interventional study was designed to investigate the effect of Vitamin E alone and in combination with Vitamin C on endothelial
dysfunction in patients with diabetes mellitus. The interventional group was treated with Vitamins E (400mg/day) for first six weeks and vitamin E (400mg/day) and vitamin C (500mg/day) for later six weeks of study.

**Flow mediated dilation (FMD) Test**

In order to determine the effect of antioxidants on endothelial dysfunction, flow mediated dilation (FMD) was examined on the brachial artery of patients. These measurements were made by an experienced ultrasonographer, who was blinded to treatment and to whether participants were control or interventional. Longitudinal images of the brachial artery were obtained with a high resolution ultrasound probe (7 MHz), while the patients lied in supine position and the arm resting in a comfortable position before and during reactive hyperemia that was induced by cuff inflation. Patients had a three-lead ECG attached. Brachial artery diameter measurements were obtained in end-diastole, identified by the onset of the R-wave. The sphygmonanometer blood pressure cuff was positioned on the forearm for 5 minutes, and the brachial artery was imaged above the antecubital fossa. The cuff was inflated to ≥50 mmHg above systolic pressure to occlude arterial blood flow. Brachial artery diameter measurements were taken 45–60 seconds after the release of the cuff. FMD was done before start of therapy (T₀), after 6 weeks (T₁) and after 12 weeks (T₂). Flow Mediated Dilation was calculated by the formula:

\[
FMD\% = \left( \frac{\text{Peak Diameter} - \text{Baseline Diameter}}{\text{Baseline Diameter}} \right) \times 100
\]

**Statistical Analysis**

All data was analyzed by statistical software, IBM SPSS Statistics 21.0. Differences among the FMD percent readings at different time intervals were tested for significance by repeated measures ANOVA. The paired t-test was used to compare percent FMD readings recorded after individual and combination therapy. The independent t-test was used to compare the effect of vitamin E and E + C between control and interventional group of all categories. Statistical significance was accepted at the 95% confidence level (P < 0.05). Unless otherwise stated, all data are presented as mean ±sd.

**RESULTS**

The clinical characteristics of the population under study are provided in Table 1. The control and interventional subjects were matched for age and plasma glucose level.
Mean age of sampled patients (167) was 52.75 ±9.5 years with the mean plasma glucose level of 165.7±20.36 mg/dl. Out of these 61(39%) were smokers and 97(52%) were Hypertensive. Endothelial function was severely impaired in patients with diabetes.

All categories of interventional group showed significant increase in FMD% (p≤0.05) as compare to control group upon administration of antioxidants furthermore, combination vitamin therapy produces significantly higher antioxidant effect on endothelial function as compared to individual vitamin therapy (p≤0.05).

No significant variation in FMD% was recorded in the control group with the overall To value 2.48 ±0.68, T₁ value 2.54±0.63 and T₂ value 2.59±0.66; (p≤0.05) as shown in Fig.1. As for interventional group significant increase in FMD% was recorded in comparison to control group with the mean at To: 2.798 ±0.52, T₁: 7.30±1.47 and T₂: 8.78±1.65; (p≤0.05) as shown in Fig. 2.

Table 1: Clinical characteristics of study population.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Control</th>
<th>Interventional</th>
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<tbody>
<tr>
<td>Number</td>
<td>76</td>
<td>91</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.91±9.83</td>
<td>52.62±9.28</td>
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<tr>
<td>Gender (male/female)</td>
<td>40/36</td>
<td>63/28</td>
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<tr>
<td>Smoker %</td>
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<tr>
<td>Hypertensive %</td>
<td>56.57</td>
<td>59.34</td>
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<tr>
<td>Plasma Glucose Level (mg/dl)</td>
<td>166.17±22.46</td>
<td>165.30±18.53</td>
</tr>
</tbody>
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Figure 1: percentage of flow mediated dilation in diabetic control patients
Similar trends were observed in the increase in FMD% values of Smokers and Non-Smokers, with the T₁ value 6.9±1.42 and T₂ value 8.59±1.92; (p≤0.05) for the former versus T₁ value 7.48±1.48 and T₂ value 8.85±1.48; (p≤0.05) for the later.

Hypertensive and non-hypertensive patients responded with analogous fashion, with the T₁ value 6.93±1.41 and T₂ value 8.48±1.51; (p≤0.05) versus. T₁ value 7.82±1.40 and T₂ value 9.15±1.75; (p≤0.05) respectively.

Within interventional group, addition of vitamin C in existing vitamin E therapy significantly improved endothelial function in all diabetic (Individual therapy v/s combination therapy: 7.30±1.47 v/s 8.75±1.65; p≤0.05); smokers (6.99±1.42 v/s 8.59±1.92; p≤0.05); nonsmoker (7.48±1.48 v/s 8.85±1.48; p≤0.05); hypertensive (6.94±1.41 v/s 8.49±1.53; p≤0.05) and nonhypertensive (7.82±1.40 v/s 9.15±1.75; p≤0.05) patients as shown in Fig. 3, 4 & 5.
Figure 4: effect of vitamin e in combination with vitamin c therapy on flow mediated dilation.

Vitamin E administration raised Flow mediated dilation percent up to 11.5% in nonsmoker-nonhypertensive diabetic group while vitamin E in combination with vitamin C improved endothelial function with the maximum value 13.60% in nonhypertensive-smoker diabetic group moreover minimum raise in FMD 3.80% and 4.60% was recorded in nonsmoker group after administration of vitamin E alone and in combination with vitamin C respectively. Maximum T0 to T1 difference of 7.6% in FMD was recorded among nonhypertensive-nonsmoker diabetic group along with T1 to T2 difference of 4.9% among nonhypertensive diabetic group.

Both vitamin E and its combination with vitamin C turned out superior results in diabetic patients with age of ≤ 50 years as compare to patients with age more than 50 years (7.92% and 9.94% versus 6.89% and 7.98%; p≤0.05).

Figure 5: comparative effect of vitamin e with vitamin e and c therapy on flow mediated dilation.
DISCUSSION
Vascular function in diabetic patients has been studied widely. Studies in patients with diabetes mellitus have found impaired endothelial function when compared to nondiabetic subjects. Further antioxidant vitamins administration significantly improved endothelial function in forearm resistance vessels of patients with diabetes mellitus and treatment with antioxidant vitamins reduces the risk of macrovascular and microvascular complications. Vitamin E supplementation results in a significant diminution in the LDL oxidative susceptibility and recovers endothelial vascular activity in patients with type I diabetes mellitus. Our study supports previous observations that impaired endothelium-dependent vasodilation in patients with diabetes mellitus is significantly improved by administration of vitamin E and its combination with vitamin C. The important new finding is that endothelial function was further improved by the addition of vitamin C in existing vitamin E therapy.

Possible mechanism for significant improvement in endothelial function by addition of vitamin C is the ability of this vitamin to scavenge excessive superoxide anions and, thereby, decrease inactivation of nitric oxide. Ascorbic acid binds and makes stable endothelium-derived nitric oxide, increasing its availability by a mechanism not depending on free radical scavenging. Further vitamin C restored aprox 60% of the compromised endothelium-dependent vasodilation observed in diabetic patients compared with age-matched nondiabetic patients. Further ascorbic acid is considered as a part of antioxidant protection system. Reactive alpha tocopherol radical, produced after scavenging lipid peroxy radicals is reduced back to alpha tocopherol in the presence of ascorbic acid.

In current study, endothelial function was found to be less compromised in nonsmoker and nonhypertensive patients with respect to smoker and hypertensive patients and maximum improvement in FMD was recorded in nonsmoker-nonhypertensive diabetic group with both individual as well as combination therapy. This supports the previous studies that cigarette smoke is one of a major cause of atherosclerosis and tissue vitamin E and C levels were much lower and lipid peroxidation products considerably higher in smokers than in non-smokers. Lipid peroxidation products were inversely related to vitamin E in tissue and to vitamin C in plasma, showing the antioxidant primacy of vitamin E and vitamin C in the arterial tissue compartments and plasma, respectively.
CONCLUSION
The salient features of the current study is that the impaired FMD in patients with diabetes is improved by the oral antioxidant therapy (vitamin E and its combination with vitamin C), whereas no significant variation in FMD was recorded in control diabetic patients. Furthermore, this study indicates that the beneficial effect of vitamin E on FMD in patients with diabetes was further increased by the addition of vitamin C in therapy.

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REFERENCES


