INTRODUCTION
Hypertension (HT) is the most common cardiovascular disease and a hallmark risk factor for myocardial infarction, stroke, and congestive heart failure. It has been estimated that one quarter of all adults and one half of all individuals >65 years of age have HT.1

The endothelium plays a critical role in regulating vasomotor tone, platelet activity, and leukocyte adhesion through the release of paracrine factors, including nitric oxide (NO) and prostacyclin.2

Various risk factors such as genetic defects, environmental factors, increased salt-intake, obesity, alcohol intake, abnormal renin-angiotensin-aldosterone axis, insulin resistance etc. have been implicated in the pathogenesis of essential hypertension. The role of increased oxidative stress in the pathophysiology of endothelial dysfunction has also been suggested.3 This endothelial dysfunction could be the result of a decrease in biologically active NO, ultimately leading to a depressor/pressor imbalance stemming from tonic removal of NO-mediated vasodilatation.4

Majority of studies suggest that blood pressure is regulated in part by the vasodilator NO, ultimately leading to a depressor/pressor imbalance stemming from tonic removal of NO-mediated vasodilatation.4

In vitro studies suggest that experimental hypertension is associated with increased production of superoxide anion, which can react with NO to eliminate its therapeutic
biological activity. An inter-relationship between NO and antioxidants has also been suggested. Epidemiological studies have demonstrated that low dietary intakes and suboptimal plasma concentrations of anti-oxidants are associated with enhanced risk of hypertension, atherosclerosis, myocardial infarction and stroke. The dietary intake and plasma concentrations of ascorbic acid, a potent water-soluble anti-oxidant correlates inversely with hypertension and its clinical sequelae namely, stroke and cardiovascular diseases.

In light of above reports, it was of interest to ascertain the role of anti-oxidant therapy in management of hypertension as well as to correlate with levels of antioxidative enzymes. The objectives of the present study were:

1. To examine the effects of vitamin-C (ascorbic acid) and vitamin-E (alpha-tocopherol) treatment on the blood pressure in the normotensive subjects and in the patients with essential hypertension who were on antihypertensive therapy.

2. To examine the effects of vitamin-C (ascorbic acid) and vitamin-E (alpha-tocopherol) treatment on the different antioxidant enzyme activities in the normotensive subjects and in the patients with essential hypertension who were on antihypertensive therapy.

MATERIALS AND METHODS

An open comparative and cross sectional study was carried out at Shree Sayajirao General Hospital, Vadodara. The subjects included in the study were divided into two groups. The first group of subjects consisted of normotensive healthy volunteers while the second group of subjects consisted of patients attending outpatient department having essential hypertension and meeting with following inclusion and exclusion criteria. The study was carried out during the period of March 2003 to April 2004 with written informed consent being obtained from all subjects.

Inclusion criteria

- Healthy normotensive subjects
- Hypertensive subjects who were on antihypertensive therapy.
- Age (20-60 yrs) and gender for appropriate match to avoid bias.
- Biochemical and hematological investigations in normal limit.
- Hypertension was diagnosed when diastolic blood pressure (DBP) was >90 mm Hg and systolic blood pressure (SBP) >140 mm Hg on at least three separate occasions.

Exclusion criteria

- Patients with accelerated / malignant hypertension.
- i.e. SBP > 180 and DBP > 110 mm Hg
- Patients with secondary hypertension.
- Patients with either of following associated disease conditions:
  - Congestive heart failure / grade – III retinopathy
  - H/O stroke
  - H/O myocardial infarction within 6 months
  - H/O angina pectoris within 2 years.
  - H/O angioedema within 2 years.
  - Diabetes mellitus (clinical history or a fasting glucose >140 mg/dl).
  - H/O chronic infections like T. B., Leprosy etc.
  - H/O hypersensitivity to Vitamin C and Vitamin E
  - Other medical conditions like endocrinal disorders, active gout, abnormal hepatic and renal functions
  - Pregnancy and Lactation
  - Those who had taken antioxidant vitamins or estrogen replacement therapy within last month of initiation of the study.

In hypertensive patients antihypertensive medications were maintained before and during the three months of study period. Subjects were then randomized to treatment with antioxidant therapy. Compliance was checked by capsule or tablets counts and reinforcement at each visit.

Blood pressure measurement was carried out by using WHO study group recommended guidelines. Blood pressure was recorded in sitting position using the
Effects of antioxidant treatments on various parameters in hypertensive
left arm by a mercury sphygmomanometer. The systolic and diastolic pressure was measured three times over a period of at least 3 minutes and the lowest reading recorded. Diastolic blood pressure was measured using the fifth Korotkoff sound. Follow up visits were carried out every 15 days. Blood samples were taken every 4 weeks, after blood pressure had been measured, and were immediately centrifuged and stored at 20°C. Urine collections were also made every 4 weeks, the volume recorded and then preserved with sodium hydroxide before storage. Healthy subjects attended on one occasion and underwent the same protocol.

Healthy normotensive subjects (n=60) were divided into three groups (n=20 each) viz; first group receiving Vitamin-C 500 mg/day for 3 months, second group receiving Vitamin-E 400 mg/day for 3 months and third group receiving combined therapy of Vitamin-C (300 mg/day) and Vitamin-E (200 mg/day) for 3 months.

Hypertensive subjects (n=60) taking antihypertensive therapy were also divided into three groups (n=20 each), and each group received antioxidant therapy as mentioned above along with ongoing antihypertensive medications. Investigations including recording of blood pressure, pulse rate, ECG; chest x-ray, haemogram, liver function tests, lipid profile, serum creatinine and blood urea, urine analysis, fundoscopy and specific investigations for activity of antioxidant enzymes like; super oxide dismutase (SOD)\textsuperscript{13}, reduced glutathione (GSH)\textsuperscript{14}, and catalase\textsuperscript{15} were measured before and after antioxidant therapy. Data of all patients were entered in typed proforma and analysed statistically by using student’s ‘t’ test and one way ANOVA test. Values were expressed as mean ± SEM and P <.0.05 was considered statistically significant.

RESULTS

Table 1: Baseline Subject characteristics

<table>
<thead>
<tr>
<th>Characters</th>
<th>Normotensive subjects (n=60)</th>
<th>Hypertensive subjects (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.7 ± 7.01</td>
<td>48.85 ± 7.87</td>
</tr>
<tr>
<td>Female (n)</td>
<td>23</td>
<td>28</td>
</tr>
<tr>
<td>Blood pressure (mm of Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic/ Diastolic</td>
<td>131.7 ± 7.6</td>
<td>135.7 ± 8.9*</td>
</tr>
<tr>
<td></td>
<td>75.3 ± 4.5</td>
<td>83.7 ± 4.7**</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>75.2 ± 5.4</td>
<td>75.0 ± 5.5</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>184.8 ± 20.6</td>
<td>190.2 ± 27.2</td>
</tr>
<tr>
<td>Fasting blood sugar (mg/dl)</td>
<td>83.6 ± 10.1</td>
<td>84.8 ± 10.9</td>
</tr>
<tr>
<td>Body mass index (kg/m\textsuperscript{2})</td>
<td>22.20 ± 1.31</td>
<td>22.44 ± 1.51</td>
</tr>
</tbody>
</table>

All values are expressed as Mean ± SEM; * P < 0.01, ** P < 0.001 as compared to their corresponding values in the normotensive subjects.

Table 2: Effects of vitamin C (Vit.C), vitamin E (Vit.E) and their combination treatment on antioxidant enzyme activities of normotensive subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Antioxidant enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SOD [E.U]</td>
</tr>
<tr>
<td>Vit. C Treated</td>
<td>Before treatment</td>
<td>1.05 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>1.65 ± 0.11***</td>
</tr>
<tr>
<td>Vit. E Treated</td>
<td>Before treatment</td>
<td>1.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>1.65 ± 0.11**</td>
</tr>
<tr>
<td>Vit.C + Vit.E Treated</td>
<td>Before treatment</td>
<td>1.10 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>1.55 ± 0.11**</td>
</tr>
</tbody>
</table>
Effects of antioxidant treatments on various parameters in hypertensive subjects

n=20 in each group.
Values are expressed as mean ± SEM.
*p<0.05, **p<0.01, ***p<0.001 as compared to its corresponding value for respective group before treatment.

Table 3: Effects of vitamin C (Vit.C), vitamin E (Vit.E) and their combination treatment on antioxidant enzyme activities of hypertensive subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Antioxidant enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SOD [E.U]</td>
</tr>
<tr>
<td>Vit. C Treated</td>
<td>Before treatment</td>
<td>1.05 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>1.30 ± 0.11*</td>
</tr>
<tr>
<td>Vit. E Treated</td>
<td>Before treatment</td>
<td>1.10 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>1.50 ± 0.15*</td>
</tr>
<tr>
<td>Vit.C + Vit.E Treated</td>
<td>Before treatment</td>
<td>1.05 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>1.15 ± 0.00</td>
</tr>
</tbody>
</table>

n=20 in each group.
Values are expressed as mean ± SEM.
*p<0.05, **p<0.01, ***p<0.001 as compared to its corresponding value for respective group before treatment.

In present study, average age and the values of body mass index (BMI), total cholesterol values, pulse rate and fasting blood sugar in normotensive subjects and hypertensive subjects were nearly identical, however systolic and diastolic blood pressure were significantly different (Table 1).

Figure 1: Effects of treatment with vitamin-C (Vit-C), vitamin-E (Vit-E) and their combination on systolic blood pressure (SBP) of normotensive subjects

n=20 in each group. All the values are expressed as Mean ± SEM.
P < 0.05 and ** P < 0.001 as compared to their corresponding values of before treatment.

Figure 2: Shows “Effects of treatment with vitamin-C (Vit-C), vitamin-E (Vit-E) and their combination on systolic blood pressure (SBP) of hypertensive subjects.”

X axis denotes treatments and Y-axis denotes systolic blood pressure [mm of Hg]. Open bar □ represents before treatment group and closed bar ■ represents after treatment group.

Figure legends
Figure 1: Shows “Effects of treatment with vitamin-C (Vit-C), vitamin-E (Vit-E) and their combination on systolic blood pressure (SBP) of normotensive subjects.”

n=20 in each group. All the values are expressed as Mean ± SEM.
P < 0.05 and ** P < 0.001 as compared to their corresponding values of before treatment.

n=20 in each group. All the values are expressed as Mean ± SEM.
P < 0.05 and ** P < 0.001 as compared to their corresponding values of before treatment.
Effects of antioxidant treatments on various parameters in hypertensive subjects

Following antioxidant treatment with vitamin C, vitamin E and their combination in hypertensive patients (who were on antihypertensive therapy) there was further significant decrease in the systolic blood pressure [Figure 2] and further significant increase in the activities of all the antioxidant enzymes [Table 3]. However, activity of SOD enzyme in the group receiving combined antioxidant treatment was not altered significantly [Table 3]. Neither heart rate nor diastolic blood pressure was altered in any of the above group receiving antioxidant therapy.

Effects of antioxidant therapy on reduction in systolic blood pressure in normotensive and hypertensive subjects

There was further significant reduction in systolic blood pressure in the normotensive and hypertensive subjects after antioxidant therapy. However, treatment with either vitamin C or vitamin E produced significantly more reduction in systolic blood pressure in the hypertensive subjects as compared to that in the normotensive subjects. While combined treatment of vitamin C plus vitamin E produced equivalent reduction in systolic blood pressure in both the groups [Figure 3].

None of the subjects receiving antioxidant therapy reported any clinical adverse effects during or till 2 weeks after the study period.

DISCUSSION

The present study demonstrated that prolonged oral vitamin C (ascorbic acid) and/or vitamin E (alpha-tocopherol) treatment significantly reduced systolic blood pressure and increased the activities of the antioxidant enzymes [i.e. superoxide dismutase (SOD), catalase and reduced glutathione(GSH)] in the normotensive subjects and the hypertensive subjects taking antihypertensive therapy without affecting diastolic blood pressure and heart rate. The endothelium plays a critical role in regulating vasomotor tone, platelet...
Effects of antioxidant treatments on various parameters in hypertensive
activity, and leukocyte adhesion through the release of a number of paracrine factors, including nitric oxide (NO) and prostacyclin. Several previous studies have demonstrated that endothelial vasodilator function is impaired in conduit\textsuperscript{16,17} and resistance\textsuperscript{18,19} vessels of patients with essential hypertension. This impairment is believed to contribute to blood pressure elevations\textsuperscript{6} and to the vascular complications of the disease, including coronary artery and cerebral vascular disease.\textsuperscript{20}

Numerous studies\textsuperscript{21,22} have reported that long term oral supplementation of vitamin-C reduces blood pressure. Our observations are in line with the above reports. Superoxide anion is known to rapidly inactivate NO and has been demonstrated to be increased in conditions such as hypertension.\textsuperscript{7} Clinical studies have suggested that ascorbic acid augments NO bioavailability by scavenging superoxide anion.\textsuperscript{23,24}

At present, no reports are available in literature regarding effect of vitamin E therapy on blood pressure. The present study is probably the first report suggesting reduction in systolic blood pressure after three months of treatment with vitamin E. It has been reported that the addition of vitamin E in doses of 400 mg once a day orally for 4 weeks significantly reduced the malonyldialdehyde and superoxide anion levels and produced an elevation in levels of the antioxidant enzymes.\textsuperscript{25} Our observations on antioxidant enzyme activities are well correlated with the above reports.

Synergistic action between vitamin C and vitamin E has been reported in which vitamin C regenerates vitamin E from the tocopheroxyl radical and thus, assists in recycling vitamin E.\textsuperscript{26} Vitamin E is the most important natural lipophilic antioxidant which prevents oxidation of lipids, especially polyunsaturated fatty acids (PUFA).

In the present study, we found that treatment with combination consisting of vitamin C and vitamin E produced significant decrease in systolic blood pressure in normotensive as well as in hypertensive subjects. Thus, the present data showing the effects of the antioxidant vitamins are still consistent with the hypothesis that excess superoxide anion in the vascular wall contributes to the genesis of impaired endothelial function responsible for hypertension. However, experimental evidences suggest that ascorbic acid may increase the production of prostacyclin\textsuperscript{27} and its synthesis is glutathione dependent which may be inhibited by lipid peroxides. In contrast, it has been also demonstrated that ascorbic acid doesnot augment the bioavailability of either plasma cGMP (an index of NO activity) or prostaglandin.\textsuperscript{22}

In the present study, we found that systolic blood pressure was reduced in both, the normotensive and the hypertensive subjects. However, there was more pronounced reduction in blood pressure in hypertensive subjects treated with either vitamin C or vitamin E while combined therapy consisting of vitamin C and vitamin E produced equivalent reduction in systolic blood pressure in both the above groups.

This observation is unexpected as the synergistic action between vitamin C and vitamin E\textsuperscript{28} has been reported. Lack of synergistic action on blood pressure could be due to lower doses of vitamins used in the combination therapy. As ascorbic acid scavenges superoxide, the biomolecular rate constant for this reaction (3.3 x 10\textsuperscript{5} mol./l\textsuperscript{1}.S\textsuperscript{1}) is \textasciitilde10\textsuperscript{5} times less than the rate constant for the reaction between superoxide and NO (1.9 x 10\textsuperscript{10} mol./l\textsuperscript{1}.S\textsuperscript{1}).\textsuperscript{29} As high concentration of ascorbic acid is required to preserve NO activity in the presence of superoxide, in the present study, especially, in combination therapy, the required plasma concentration of ascorbic acid could not be achieved due to lower dose (300 mg/day) used in the combination therapy as compared to monotherapy (500 mg./day).

The tissue availability of superoxide is strictly limited by the abundant tissue concentration of superoxide dismutase
(SOD) that may approach 10\(\mu\text{mol/}L\). Antioxidants like \(\alpha\)-tocopherol, glutathione and ascorbic acid are also known to react and scavenge superoxide. Various studies have implicated that NO competes effectively with SOD for superoxide.\(^{30,31}\)

In present study, we found that SOD enzyme activity was increased significantly in almost all the study subjects except in hypertensive group treated with combination therapy. Thus lack of synergistic action of vitamin C and vitamin E on blood pressure could be due to lack of the action on SOD enzyme activity. These observations are in line with that reported by Galley et al. (1996).\(^8\)

In present study, heart rate was not affected significantly by antioxidant treatments, suggesting that there was no neurohumoral activation. This is also in agreement with the study reported by Duffy et al. (1999).\(^22\)

It is likely that if adequate amount of exogenous antioxidants are administered, the superoxide generation can be reduced and thus it can indirectly increase the activities of various antioxidant enzymes. Antioxidant therapy decreased systolic blood pressure in all our hypertensive as well as normotensive subjects. Although the changes in blood pressure were not much large, further lowering of blood pressure in treated hypertensive subjects will be beneficial and could be achieved by relatively minor adjustments in dietary habits and increasing duration of antioxidant therapy. We suggest that hypertensive subjects may require higher than normal concentrations of circulating antioxidants to counter more superoxide generation. Since these subjects have a relatively high risk of cardiovascular diseases, the changes in blood pressure observed in this study may be associated with improved morbidity. Long term studies using large population will be required to confirm the clinical benefits of antioxidant therapy for the smooth and better management of essential hypertension.

CONCLUSION

It is concluded that addition of antioxidant therapy consisting of vitamin C and/or vitamin E to the hypertensive subjects who were on antihypertensive therapy produced further significant reduction in systolic blood pressure. It is suggested that addition of antioxidant therapy (i.e. vitamin C and/or vitamin E) along with current antihypertensive therapy would provide better management of essential with significant reduction in the dose and adverse effects due to the antihypertensive medication. However, further detailed clinical studies are still required to establish their use in the management of essential hypertension.

REFERENCES

6. Haynes WG, Noon JP, Walker BR, Webb DJ. Inhibition of nitric oxide synthesis increases blood pressure in


