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STUDIES OF LIPID PEROXIDATION & ERYTHROCYTE FRAGILITY DURING AGING

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Aging is associated with an accumulation of oxygen free radicals, leading to damage of tissues and its modifications. Age related changes result in increased levels of lipid peroxides and free radicals which may cause damage to erythrocyte membrane structural integrity. In the present study we investigated oxidative stress, hemoglobin percentage and erythrocyte osmotic fragility in various aging groups. This study has been carried out on 160 subjects, of which 40 subjects were controls. The study found a significant decrease in hemoglobin percentage, increase in erythrocyte osmotic fragility and increased lipid peroxidation in form of malondialdehyde with increasing age. Supplementation of antioxidants may prevent the oxidative injury in elderly group of subjects.

KEY WORDS
Lipid peroxidation, hemoglobin, erythrocyte osmotic fragility, aging

INTRODUCTION
Free radicals contain one or more unpaired electrons. They play an important role in the pathogenesis of tissue damage in many clinical disorders. (1) Amongst the normal physiological phenomena affected due to free radicals aging is one of them. Aging is an irreversible process which can be defined as the survival of a growing number of people who have completed the traditional adult roles. Aging is an inevitable consequence of mortality decline. Various theories have been put forward to explain the process of aging in human, but the interest still continues in the role played by free radicals in aging which could cause oxidative stress due to generation of oxygen free radicals (OFRs).

Scientists increasingly believe that OFRs play a significant role in causing many ailments and in aging. Aging process is due to increased generation of reactive oxygen species and reactive nitrogen species. Normally there is a balance between tissue oxidant and anti-oxidant activity (2). Aging progresses due to increased generation of free radicals in oxidative stress and one of the victims of free radicals is erythrocytes cell membrane integrity. The present study was designed to evaluate the effects of free radical generation in the form of lipid peroxides on erythrocytes fragility and hemoglobin percentage during the process of aging.

MATERIALS AND METHODS
This study was a population based study. A total of 160 health volunteers of both genders between age group 20-65 years were selected by random method. An informed consent was taken. The various age group volunteers were classified according to their ages as: group I, group II and group III which included healthy volunteers between age group 20-35 years, 45-65 years and 55-65 years respectively. Control group included health volunteers between ages of 20-35 years.
EXCLUSION CRITERIA

Any serious disease, use of vitamin E, h(carotene or Vitamin A supplements, hypertensive, smokers and diabetics were excluded from the study.

Determination of Hemoglobin percentage was done employing modified Cyanide method of Dacie and Lewis (3) and the hemoglobin percentage was expressed as g% and Absorbance was read against blank at 540 nm. The erythrocyte fragility was measured by the method described by Dacie and Lewis (4). The erythrocyte lysis was observed in hypotonic solution of buffered saline of varying concentrations and Absorbance was measured at 540 nm. The extent of lipid peroxidation is form of conjugated dienes and malondialdehyde was measured by Thiobarbituric acid method (5). Total amount of lipid peroxidation product present in the plasma was determined using thiobarbituric acid (TBA) method which measures malondialdehyde (MDA) reactive products. To 0.05 ml of plasma, 0.5 ml of normal saline and 1.0 ml of 24% trichloro-acetic acid were added. From this mixture 1.0 ml of protein free supernatant was taken after centrifugation at 2000 rpm for 20 minutes. To this protein free supernatant, 0.25 ml of 0.33% of TBA was added and boiled at 95°C for one hour. After cooling, the TBA reactive product was extracted in 1.0 ml butanol and intensity of pink colour obtained was read at 532 nm against blank.

STATISTICAL ANALYSIS

Data on lipid profile and paraoxonase activity was entered in Microsoft Excel for window 2000. The mean ± SD was obtained using excel software. The two-sample t-test value was obtained between the patients and the control. The distribution of "t"-Probability was calculated depending on "n" and significance of test was obtained. For p value < 0.001 was considered as highly significant.

RESULTS

Table I and Figure I shows the hemoglobin percentage in various age groups. The general trend observed is decreasing hemoglobin percentage with increasing age. Table II and figure 2A, figure 2B, figure 2C shows changes in erythrocyte fragility in various age groups. Table III shows the generation of malondialdehyde due to enhanced lipid peroxidation with increasing age.

### TABLE I: HEMOGLOBIN PERCENTAGE (GM%) IN DIFFERENT AGE GROUP

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (20-30 yrs), n=40</th>
<th>Group I (35-45 yrs), n=40</th>
<th>Group II (45-55 yrs), n=40</th>
<th>Group III (56-65 yrs), n=40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>12.3 ± 3.39</td>
<td>12.20 ± 3.30</td>
<td>11.0 ± 2.91</td>
<td>10.00 ± 2.10</td>
</tr>
<tr>
<td>% Decrease</td>
<td></td>
<td>0.81</td>
<td>10.5</td>
<td>19.5</td>
</tr>
<tr>
<td>T Value</td>
<td></td>
<td>&lt; 0.1</td>
<td>&lt; 0.01</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>P Value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE II: MEAN ERYTHROCYTE FRAGILITY IN VARIOUS AGE GROUPS

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (20-30 yrs), n=40</th>
<th>Group I, n=40 (25-35 yrs)</th>
<th>Group II, n=40 (45-55 yrs)</th>
<th>Group III, n=40 (55-65 yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>0.70</td>
<td>0.73</td>
<td>0.75</td>
<td>0.77</td>
</tr>
<tr>
<td>% Increase</td>
<td></td>
<td>4.2</td>
<td>7.1</td>
<td>10.2</td>
</tr>
<tr>
<td>P Value</td>
<td></td>
<td>&lt; 0.1</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
TABLE III: LIPID PEROXIDATION (MMOL/L OF MDA) IN VARIOUS AGE GROUPS

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (20-30 yrs), n=40</th>
<th>Group I (35-45 yrs), n=40</th>
<th>Group II (45-55 yrs), n=40</th>
<th>Group III (55-65 yrs), n=40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>1.07 ± 0.35</td>
<td>1.17 ± 0.40</td>
<td>1.30 ± 0.49</td>
<td>1.56 ± 0.54</td>
</tr>
<tr>
<td>% Decrease</td>
<td></td>
<td>7.3%</td>
<td>21.4%</td>
<td>47.6%</td>
</tr>
<tr>
<td>T Value</td>
<td></td>
<td></td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td></td>
<td>&lt; 0.1</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

DISCUSSIONS

Involvement of oxygen free radicals (OFRs) in the physiology of aging in a number of organs and tissues have been reported in literature (6,7). Indirect evidence of OFR generation in aging has been observed by measuring the lipid peroxidation and erythrocyte fragility as the fragility is affected due to increased accumulation of toxic peroxides with in erythrocytes (8,9). The activities of cellular defense mechanisms especially Superoxide dismutase, Glutathione peroxidase and Catalase have been reported to decrease in human and other species in aging in many studies (10,11,12). In the present study, increased erythrocytes fragility and lipid peroxidation was observed in various aging groups compared to young adults (control). Increased erythrocyte fragility may be associated with enhanced generation of OFRs and decreased levels of antioxidant enzymes which succumb to oxidative stress in aging as observed in the present study. Future research including measurement of parameters of oxidative stress and antioxidant enzymes in human at certain interval of time to arrive at final conclusions about the role of lipid peroxidation in erythrocyte membrane fragility in aging.

Figure 1: Hemoglobin (%) in control and in various age groups
Figure 2: Effect of Generation of Free Radicals on Lipid Peroxidation

REFERENCES


Role of Vitamin E Supplementation in Essential Hypertension Patients

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Department of Biochemistry, MLN Medical College, Allahabad; and Department of Biochemistry, Muzaffarnagar Medical College, Muzaffarnagar, UP, India

ABSTRACT

Cellular damage by reactive oxygen species including those associated with altered plasma antioxidant reserve and endothelial dysfunction are new accepted to be related with a variety of cardiovascular complications including essential hypertension (HT). It is conceivable that vitamin E supplementation reduces blood pressure by scavenging free radicals and by ameliorating enzyme activity. However, the outcomes of clinical trials with vitamin E in HT prevention have been mixed. Therefore, the present study was undertaken to assess the markers of oxidative stress i.e. erythrocyte glutathione peroxidase (GSH-Px) and malondialdehyde (MDA); plasma vitamin C, E, A and uric acid levels in the blood samples of HT subjects and to investigate the effect of vitamin E supplementation in ameliorating the levels of these antioxidants in HT subjects. 76 HT subjects (age group 50-60 years) were taken for the study and 70 healthy individuals served as controls. Vitamin E supplementation studies (200 mg/day) brought about an improved antioxidants status with significantly raised vitamin C, E, A and GSH-Px levels (p<0.05, p<0.001), and simultaneously depleted levels of plasma uric acid and MDA (p<0.05, p<0.001) in HT subjects. These findings further support the preventive and antihypertensive role of vitamin E supplementation in reducing oxidative stress levels in HT patients.
Oxygen free radicals have been identified as mediators of cell injury in a wide range of pathological processes including cardiovascular disease, cancer, rheumatoid arthritis and aging process etc. Increasing interest has been focused on the role of oxidative stress in the etiopathogenesis of essential hypertension (HT), which are convincingly linked to the altered antioxidant defense system and major interrelated derangements of cell metabolism including DNA strand breakage, damage to membrane ion transporters, specific proteins and lipid peroxidation.

Most common radical and non radical derivatives of oxygen includes superoxide free radical anion (O$_2^-$), hydroxyl radical (OH), lipid peroxide (LOOH), hydrogen peroxide (H$_2$O$_2$) and singlet oxygen. These agents are innocuous and if not promptly neutralized they can inflict damage to cell membranes leading to development of disease.

Superoxide radical is unstable and either spontaneously or enzymatically by superoxide dismutase, transferred into potent oxidant, H$_2$O$_2$. Hydrogen peroxide, either in presence of transition metal (Fe$^{3+}$ & Cu$^{2+}$) produces highly toxic hydroxyl radical or produces hypochlorous acid (HOCl) by the action of enzyme myeloperoxidase in neutrophils and macrophages which amplify further destruction including endothelial cells. Prime targets of these free radicals attack are the polyunsaturated fatty acids in the membrane leading to lipid peroxidation. Vasudev et al reported that excess endogenous adipose (lipid peroxides) plays a major role in HT by binding sulphydryl groups of structure proteins altering Ca$^{2+}$ channels and increasing cytosolic free Ca$^{2+}$ that cause further extensive membrane damage leading to peripheral vascular resistance and hypertension.

These free radicals are effectively removed by antioxidant defense system which includes antioxidant enzymes and antioxidants. Among various antioxidant enzymes, Glutathione peroxidase (GSHPx) is selenium containing enzyme and catalyzes the decomposition of H$_2$O$_2$ with the help of reduced glutathione. It also prevents the oxidation of lipids and phospholipids. Oxidant scavenging role of these enzymes are well supported by cooperative action of other widely recognized non-enzymic antioxidants such as vitamin C, E & A, and uric acid etc., may have a significant role in preventing the cascade leading to the development of HT. It has been shown previously that these non-enzymic antioxidants contribute significantly in scavenging free radicals, inhibiting lipid peroxidation, in repairing endothelial cells and restoring endothelium derived vasodilators etc.
Conversely, their role as prooxidant and relation of uric acid with vascular injuries reflect the need for further investigation. It is conceivable that vitamin E, mainly α-tocopherol, can ameliorate the modifiable indexes via regulating the free radical production. In this connection, considerable attention has been devoted to the potential use of α-tocopherol in the treatment of HT. Previous epidemiological studies, experimental models and in vitro study, vitamin E supplementation have been found to reduce cardiovascular mortality rate and ameliorate erythrocyte SOD activity. However, outcomes of several clinical trials of antihypertensive effect of vitamin E have been proved disappointing and need further investigation. Therefore, the overall objectives of present study were to investigate the therapeutic effect of vitamin E supplementation in ameliorating the altered levels of plasma antioxidants (vitamin E, C, and uric acid), erythrocyte GSHPx and lipid peroxidation (via MDA estimation) in HT subjects.

**METHODS**

In the present study, 70 patients with essential hypertension (30-60 years) attending the outpatient services of the Department of Cardiology, S.R.N. Medical Hospital and Heartline Hospital, Allahabad were included. HT was defined according to the criteria of the Seventh Joint National Committee on Prevention, Detection, Evaluation and treatment of High Blood Pressure. 70 age matched healthy, normotensive and non-smoker individuals were included as controls. A general information or pre-experimental questionnaire regarding anthropometric and clinical data was completed from all the patients after taking their informed consent and approval of protocol by ethics committee of college. Patients with diabetes mellitus, renal insufficiency, hepatic disease, taking lipid lowering drugs or antioxidant vitamin supplements were excluded. Blood pressure was measured with a mercury sphygmomanometer, with the patient in the sitting position after 5 minutes of rest in a quite environment. Fasting blood samples were collected in Acid Citrate Dextrose vials from the antecubital vein of the patients as well as controls.

Plasma antioxidants, erythrocyte GSHPx and MDA levels were estimated in HT subjects before (non supplemented group i.e. Group I) and after 3 months of vitamin E supplementation (200 mg/day; Group II) and compared it with that of healthy controls. Erythrocyte GSHPx and MDA levels (marker of lipid peroxidation) were estimated by Beutler's method and Sinnhuber et al method spectrophotometrically, after preparation of hemolysate. Plasma vitamin C, E & A, and uric acid levels were estimated by Mc Cormick and Greene method.
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Values were expressed as Mean± SD. The significance of mean difference between groups was compared by using Student's 't' test and distribution of probability (p).

Table 1: Mean (SD) blood pressure in patients with Hypertension (HT).

<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>Hypertension (HT)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prehypertension</td>
<td>Stage I HT</td>
</tr>
<tr>
<td>Systolic (n=34)</td>
<td>134 (5.4)</td>
<td>142 (7.0)</td>
</tr>
<tr>
<td>Diastolic (n=32)</td>
<td>86 (2.8)</td>
<td>94 (3.2)</td>
</tr>
</tbody>
</table>

RESULTS

The mean (SD) blood pressure of all the hypertensive subjects grouped into Prehypertension, Stage I HT and Stage II HT based on the JNC 7th criteria, are represented in Table 1. Following treatment after 3 months of vitamin E (200 mg/day), the mean (SD) SBP was 133 mm Hg and DBP was 84 mm Hg. The observations made reveal significant changes in antioxidant status and MDA levels in study group subjects before and after vitamin E supplementation, as represented in Table 2 and 3.

Table 2: Antioxidants and Malondialdehyde levels in HT subjects before vitamin E supplementation. (Mean± SD)

<table>
<thead>
<tr>
<th>Control group (n=70)</th>
<th>Group I (vitamin E supplemented) (n=70)</th>
<th>% decrease</th>
<th>% increase</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSHPx (IU/gm Hb)</td>
<td>38.4±8.3</td>
<td>23.4±5.0</td>
<td>38.85 %</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Ascorbate level (mg%)</td>
<td>0.78±0.12</td>
<td>0.34±0.05</td>
<td>56.4 %</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Tocopherol lev. (mg%)</td>
<td>1.40±0.20</td>
<td>0.73±0.14</td>
<td>47.8 %</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Vitamin A (µgm%)</td>
<td>118.48</td>
<td>85.9±12.3</td>
<td>27.49%</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Uric acid (mg%)</td>
<td>4.52±1.30</td>
<td>7.8±1.53</td>
<td>72.34%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Malondialdehyde (µmol/MDA/ml)</td>
<td>3.58±0.3</td>
<td>5.49±0.42</td>
<td>53.37%</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Erythrocyte GSHPx activity was significantly low (38.85%; p<0.001) in HT subjects as compared to healthy controls that was found to be increase...
significant (28.1%; p<0.05) on vitamin E supplementation. Plasma vitamin C, E & A levels were found to be significantly low in HT subjects (p<0.001; p<0.05; 56.4%, 47.8% and 27.9% low respectively). On vitamin E supplementation, marked amelioration in plasma antioxidant vitamin levels were observed (p<0.001, p<0.05). Similarly, marked elevated levels of malonaldehyde (MDA) and plasma uric acid were observed in HT subjects (p<0.001; 53.37% & 72.34% high respectively) as compared to healthy controls which decreased significantly (p<0.001 & p<0.05; 31.5% & 23.49% low) in Group II as compared to non-supplemented HT group.

<table>
<thead>
<tr>
<th>Particular</th>
<th>Group I (n=70)</th>
<th>Group II (supplemented) (n=70)</th>
<th>% Decrease</th>
<th>% Increase</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSHPx (U/gm Hb)</td>
<td>23.48±6.0</td>
<td>30.07±7.2</td>
<td>--</td>
<td>28.1%</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Ascorbate level (mg%)</td>
<td>0.34±0.05</td>
<td>0.45±0.07</td>
<td>--</td>
<td>2.35%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Tocopherol level (mg%)</td>
<td>0.73±0.14</td>
<td>1.19±0.17</td>
<td>--</td>
<td>30.01%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Vitamin A (pgm%)</td>
<td>85.9±12.3</td>
<td>104.46±19.2</td>
<td>--</td>
<td>21.6%</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Uric acid (mg%)</td>
<td>7.8±1.53</td>
<td>5.9±0.94</td>
<td>23.49%</td>
<td>--</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Malonaldehyde (mmol/l)</td>
<td>5.49±0.42</td>
<td>3.76±0.35</td>
<td>31.5%</td>
<td>--</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

DISCUSSION
Several studies have been documented regarding enhanced production of ROS as well as a decrease in the antioxidant reserve in plasma and tissues of hypertensive patients. Theoretically, it is conceivable the exogenous administration of non-enzymic antioxidants such as vitamin E may prevent the development of cardiovascular diseases although contradictory evidences have been documented. In this context, an attempt is made to control HT by exogenous vitamin E supplementation. Among various free radicals, superoxide anion (efficiently removed by SOD, as reported in our previous study) significantly participate in the etiopathogenesis of HT not only by reducing NO bioavailability but also by producing H2O2 which amplify further destruction via OH' and HOCl formation.
In addition to catalase, GSHPx plays a crucial role in the final detoxification of H$_2$O$_2$. It spontaneously reacts with and scavenges many forms of ROS, prevents oxidation of lipids and phospholipids, maintains intracellular redox milieu, replenishes a number of crucial antioxidants (vitamin E and C) and produces vasodilatory prostacyclin by the endothelium.$^{13}$ In the present study, low GSHPx activities were observed in Group I subjects as compared to healthy controls which focused the role of GSHPx not only in the scavenging of H$_2$O$_2$ but also in the prevention of progressive deterioration of endothelial cells (via. lipid peroxidation) initiated by highly reactive hydroxyl radical (OH$^-\$). Recently, Sudha et al.$^{24}$ also observed that erythrocyte antioxidant enzymes activity of RBC including GSHPx had decreased due to augmented oxidative stress in hypertensive subjects leading to thrombotic stroke. Reduced levels of free radical scavengers such as vitamin E, glutathione and SOD have been reported in hypertensive patients by Sagar et al.$^{25}$ and suggested it as a result of their consumption during metabolism of oxygen free radicals. On vitamin E supplementation, significant increase in GSHPx activity (p<0.05) was observed in HT subjects which could be explained as a glutathione sparing action of vitamin E by inhibiting lipid peroxidation and thereby replenishes GSHPx activity.$^{26}$ Recently, similar findings were reported by Garg et al.$^{27}$ in their studies on vitamin E supplementation on diabetic rats. However, Li et al.$^{13}$ have found no any effect of vitamin E supplementation on GSHPx activity.

Above mentioned observation is well supported by marked reduction in MDA levels (p<0.001) in supplemented HT subjects which was 53.37% high in Group I subjects. These findings clarify the chain breaking antioxidant property of vitamin E by which it protects the membrane bound lipids and nascent LDL against free radical mediated lipid peroxidation and thus plays a significant role in the reduction of HT.

\[
\begin{align*}
\alpha-\text{Toc-OH} + \text{LOO}^\cdot & \rightarrow \alpha-\text{Toc}^* + \text{LOOH} \\
\alpha-\text{Toc}^* + \text{LOO}^\cdot & \rightarrow \text{ADDUCT (α-TocOOL)}
\end{align*}
\]

Furthermore, Chen et al.$^{14}$ observed that vitamin E administration retards LDL oxidation, inhibits smooth muscle proliferation, inhibits platelet adhesion and aggregation, decreases the synthesis of leukotrienes and improves endothelial function.

In addition to antioxidant enzymes, free radicals are efficiently removed by co-operative action of other widely recognized non enzymatic antioxidants such as vitamin C, E & A, and uric acid. Vitamin C, an exogenous water soluble antioxidant functions as primary defense against free radicals in plasma and
disappeared more quickly. It has a significant role in protecting plasma lipids against peroxidation (i.e., anti-atherosclerotic effect) and improving vascular endothelium-dependent vasodilation. Another possible mechanism through which ascorbate plays a significant role in reducing HT includes its protective effect on Na+-K+-ATPase against peroxidative damage and thereby in maintaining electrolyte balance; enhancement of NO bioavailability and synergistic action to regenerate α-tocopherol and urate from their radicals.

Vitamin E, a universal lipophilic, chain breaking antioxidant and a stabilizer of biological membranes, prevents accumulation of free radicals and decreases lipid peroxidation. Prithviraj & Misra have reported that α-tocopherol not retards only LDL oxidation but also inhibits smooth muscle proliferation, platelets adhesion and aggregation, expression and function of adhesion molecule, decreases the synthesis of leukotrienes and potentiates the release of prostacyclin. Vitamin E quenches and reacts with superoxide anion, hydroxyl and peroxyl radical to provide protection against oxidative stress, and increases NO bioavailability.

In addition, vitamin C, mainly carried on LDL particle, is also an important fat soluble antioxidant that can quench singlet oxygen, competitively spares selenium in metabolic reactions, inhibits LDL oxidation; and is responsible for immune response, epithelial growth and repair. Alteration in their levels have a significant role in the development of HT.

In the present study, plasma levels of these antioxidant vitamins (C, E & A) were significantly low (p<0.05, p<0.001, p<0.001) in Group 1 as compared to control. Decreased levels of these vitamins could not be only due to their free radical scavenging action but also in maintaining the body antioxidant reserve and in normalization of vascular superoxide formation. Wen et al. also observed a reduction in vitamin C & E level with increased levels of lipid peroxide in hypertensive subjects and concluded it as a contributory event in the development of CVD in HT patients. Recently, Ugle et al. observed a marked reduction in vitamin A levels in CVD including HT subjects and suggested that CVD events can be prevented by improving the blood beta carotene and LDL-beta carotene status. Despite data linking antioxidant role of these vitamins, pro-oxidant properties of these vitamins also play a controversial role.

On vitamin E supplementation, these levels were increased significantly in supplemented HT subjects (p<0.05; Table 3) as compared to Group 1 subjects. Our findings were in agreement with those of Prasad et al., who observed that α-tocopherol treatment increases antioxidant reserves and cardiac
contractility. However, Keith et al. found no any significant effect of vitamin E supplementation on other marker of oxidative stress. The increased concentration of vitamin C, E and A as observed in the present study may be linked to a series of reactions which include synergistic action of vitamin C, E and A (Fig 1.0) i.e. inhibition of lipid peroxidation by lipid peroxyl radical scavenging action of vitamin C and A to produce their radicals. α-tocopherol regenerates vitamin C and A from their radical forms by reducing them and itself being converted into α-tocopheroxyl radical. In addition, erythrocytes and neutrophils take up ascorbate radicals rapidly and convert it back to ascorbate at the expense of GSH-Px and in presence of Glutathione–semidehydro ascorbate reductase enzyme. Furthermore, increased vitamin E level could also be explained on the basis of its direct association with vitamin E supplementation.

Moreover, uric acid is an endogenous, preventive and chain breaking antioxidant which contributes about 65% of free radical scavenging action, stabilizes ascorbate, protects DNA and erythrocytes from oxidative damage. NO is known to be implicated in a number of crucial physiological functions, e.g. vasodilation, adhesion molecule expression (ICAM-1) on endothelium, penile erection, platelet aggregation, cerebral blood flow, microbicidal and tumoricidal activities of macrophages and neutrophils etc. In HT, superoxide anion produced due to activation of NADPH oxidase in neutrophils, reacts with NO to form toxic product peroxynitric anion (ONOO⁻) thereby reduces NO bioavailability i.e. an important event in the development of HT. Plasma uric acid interacts with peroxynitrite anion to form a stable nitric oxide donor, thus promoting vasodilation and restores endothelial function.

In the present study, plasma uric acid levels were found to be significantly high (p<0.001) in hypertensive subjects which indicates that body is trying to protect itself from the deleterious effects of free radicals by increasing uric acid production. Our findings were in agreement with the findings of Maxwell and Bruinsma. According to them, elevation of uric acid production is a secondary event and occur due to disinhibition of xanthine oxidase activity via reduction in vascular NO activity because NO is known to interact with active site of xanthine oxidase and inhibits its activity to produce uric acid. However, conversely, its role in promoting LDL oxidation, vascular injury and in stimulating granulocyte adherence to the endothelium has well documented.

On vitamin E supplementation, significant reduction in plasma uric acid was observed in Group II subjects (p<0.05) which may be explained on the basis of increase in NO bioavailability by vitamin E (not measured in present study) via inhibition of vascular superoxide formation and there by inhibition of xanthine
oxidase activity lending to controlled production of uric acid. However, further study is warranted to shed more light on the hidden facts related to therapeutic use of antioxidants.

CONCLUSIONS

On the basis of present findings and consistent findings of previous studies, it can be inferred that oxidative stress plays a crucial role in HT and vitamin E supplementation provide protection against oxidative stress not only by their free radical scavenging action but also by ameliorating antioxidant reserve and by preventing biomolecular deterioration (lipid peroxidation) which are responsible for HT development. Therefore, consumption of diet rich in antioxidants and minerals should be increased with increase in blood pressure which may prevent or postpone the development of HT and its consequent sequelae as well.

REFERENCES


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