ROLE OF NATURAL ANTIOXIDANTS ON INFLAMMATORY MARKERS IN PRIMARY HYPERTENSION

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ABSTRACT

BACKGROUND

Despite recent advances in understanding and treating hypertension, its prevalence continues to rise. Development of hypertension is associated with oxidative stress. Several enzymes expressed in vascular tissue contribute to production and efficient degradation of reactive oxygen species, and enhanced activity of oxidant enzymes and/or reduced activity of antioxidant enzymes may cause oxidative stress. The present study is an attempt to explore the beneficial effect of natural antioxidants, namely Epigallocatechin-3 gallate (EGCG) in hypertension. EGCG is a green tea polyphenol and is a proven preventive beneficial natural antioxidant in other diseases.

METHODS

Blood samples from 20 patients and 8 healthy controls were taken to measure the secreted TNF-α and IL-6 levels from isolated peripheral blood mononuclear cells (PBMCs). These PBMCs were treated with varying dose of EGCG. Finally, Glutathione Peroxidase (GPx) activity was measured.

RESULTS

24 hour culture supernatants of patient monocytes exhibited an appreciable elevation in TNF-alpha and IL-6 levels when compared to normal healthy controls. After treatment of monocytes with EGCG, there was marked decrease in expression of TNF-α and IL-6. EGCG induced percentage decrease in TNF-α and IL-6 expression was much higher in cases than controls. Coculturing of patient’s monocytes for 24 hrs. Along with 5 μg/ml of EGCG exhibited appreciable up regulation in Glutathione Peroxidase activity.

CONCLUSIONS

As a consequence of EGCG-mediated reduction in inflammation as observed in the present study, it is strongly hoped that EGCG may help in reducing the arterial stiffness contributed by IL-6 and TNF-α.

KEYWORDS

Antioxidants; Hypertension; Oxidative Stress; Reactive Oxygen Species.


BACKGROUND

Hypertension causes significant morbidity and mortality worldwide. Unfortunately, despite recent advances in understanding and treating hypertension, its prevalence continues to rise. Across the globe, 26% of the adult population experiences hypertension and Kearney et al¹ estimate that this could rise to 29% by the year 2025. Hypertension is the most common risk factor for stroke and myocardial infarction and predisposes to heart failure, ventricular arrhythmias, renal failure, blindness, and other serious medical problems.
including NAD(P)H oxidase, endothelial nitric oxide synthase, xanthine oxidase, myeloperoxidase, superoxide dismutase, catalase, thioredoxinreductase, and glutathione peroxidase (GPx). Specific pharmacological modulation of key enzymes involved in the propagation of oxidative stress rather than using direct antioxidants maybe an approach to reduce oxygen radical load in the vasculature and subsequent disease progression in humans.4

Antioxidants may be more beneficial in primary prevention rather than in patients with advanced disease. There is evidence that some drugs that do not act as direct antioxidants, for example, statins and AT1 receptor antagonists, exert their therapeutic benefits at least in part through antioxidant actions. This may have several implications. First, testing diseased individuals for markers of oxidative stress could be used for risk stratification. Treatment with potent antioxidants may potentially be beneficial in this group of patients. Second, given the fact that dysregulated oxidant and antioxidant enzyme expression and function is found in patients with cardiovascular risk factors, specific pharmacological modulation of key enzymes, such as inhibition of the vascular NAD (P) H oxidase, may be an effective approach to reduce vascular oxidative stress and subsequent disease progression in humans that is potentially more powerful than the use of systemic antioxidants.

A number of growth factors are known to influence oxidant and antioxidant enzyme function. Epidermal growth factor, thrombin, transforming growth factor-b and platelet-derived growth factor, lead to increased subunit expression and activity of the NAD(P)H oxidase and to decreased ecSOD (superoxide dismutase) expression in VSMC (vascular smooth muscle cell).5

The pro-inflammatory cytokines interferon-gamma, interleukin-1, and especially tumor necrosis factor-α (TNFα) seem to promote oxidant effects in vascular cells by activating NAD (P) H oxidase and xanthine oxidase. TNF exerting its effects through its receptors namely TNF-R1 and TNF-RII is a central mediator of inflammation, immunity and cardiovascular disorders, and that, it plays a crucial role in host defense. The role of TNF-α and its inhibition by antagonists in relation to ROS (Reactive Oxygen species) in autoimmune disorders and infectious diseases has already been well documented.6-10

The present study is an attempt to explore the beneficial effect of natural antioxidants, namely Epigallocatechin-3 gallate (EGCG) in hypertension. EGCG is a green tea polyphenol and is a proven preventive beneficial natural antioxidant in other diseases. The usage of such type of natural antioxidant in the management of cardiovascular related diseases could probably reduce the risk factors as well as economical factor over the routine medicines/drugs employed in the above type of diseases.

METHODS

Study Subjects

20 newly detected patients of essential hypertension and eight age-matched healthy controls were recruited in the study after taking informed consent from them. The subjects attended Hypertension clinic, Medicine OPD, J.N. Medical College and Hospital, AMU, Aligarh from June 2006 to June 2007.

Institutional ethics committee permission was obtained.

Inclusion Criteria

First time detected patients of essential hypertension not on any prior treatment were classified into Stage 1 or Stage 2 hypertension according to JNC-VII criteria and were included in the study. Patients having blood pressure in range of prehypertension were not included in the study.

<table>
<thead>
<tr>
<th>BP Classification</th>
<th>Systolic BP (mm Hg)</th>
<th>Diastolic BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;120</td>
<td>&lt;80</td>
</tr>
<tr>
<td>Prehypertension</td>
<td>120-159</td>
<td>Or 80-89</td>
</tr>
<tr>
<td>Stage 1 Hypertension</td>
<td>140-159</td>
<td>Or 90-99</td>
</tr>
<tr>
<td>Stage 2 Hypertension</td>
<td>&gt;160</td>
<td>Or &gt;100</td>
</tr>
</tbody>
</table>

Table 1. JNC-VII Criteria

Exclusion Criteria

Secondary Hypertension including Chronic Kidney Disease.

- Smokers
- Patients with BMI>25.
- Diabetes Mellitus.
- Acute or chronic inflammatory conditions including Collagen vascular diseases.
- Acute Coronary syndromes and Cerebrovascular accidents.
- Patients on Statins, Aspirin, steroids, immunosuppressive therapy or hormone replacement therapy.

Study Design

An informed consent was obtained from each subject prior to entering the study. A detailed history was taken and physical examination was carried out for every subject who entered in the study as per a pre-designed proforma. Patients were thoroughly investigated on lines of hypertension including blood urea, serum creatinine, blood sugar (Fasting), Serum lipid Profile (Fasting), urine for albumin, sugar and sediments, Chest-X ray PA view, EKG, Fundus examination.

Blood samples from patients and healthy controls were taken to measure the secreted TNF-α and IL-6 levels from isolated peripheral blood mononuclear cells (PBMCs). As per experimental design, the normal and hypertensive patient’s monocytes were adhered onto tissue culture plates and were treated with varying concentrations of EGCG.

Investigations

1. Blood samples were collected after an overnight fast of 10-12 hours in sodium fluoride vials for blood glucose; in plain vial for serum lipids, Blood urea, S. creatinine
2. Isolation of PBMC from samples collected from cases and controls.
3. ELISA for secreted TNF-α and IL-6 estimation.
4. Treatment of adhered PBMC with varying dose of EGCG.
Statistical Analysis
Analysis was performed using SPSS version 10.0 Statistical package for windows (SPSS, Chicago, IL). Unpaired and paired t tests for independent and dependent samples were used in comparing continuous data between two groups. All p values were two tailed and values of <0.05 were considered to indicate statistical significance. All confidence intervals were calculated at 95% level.

RESULTS
Mean age for cases and controls was 52.8±8.3yrs and 52.5±6.4yrs respectively (Table-2). Among cases 12 were males and 8 were females with mean age of 51.8±8.5yrs and 54.2±8.2yrs respectively. There was no sex difference found in mean value for baseline sTNF-α and IL-6 (Table-3) in hypertensive cases.

<table>
<thead>
<tr>
<th>N</th>
<th>Minimum Age</th>
<th>Maximum Age</th>
<th>Mean Age</th>
<th>Std. Deviation</th>
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<tr>
<td>Age Controls</td>
<td>20</td>
<td>20.00</td>
<td>65.00</td>
<td>52.80</td>
</tr>
</tbody>
</table>

Table 2. Mean Age of Cases and Controls

Baseline | Sex | N | Mean | Std. Deviation | t value | Sig. (2-tailed) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>M</td>
<td>12</td>
<td>153.6667</td>
<td>10.0323</td>
<td>0.261</td>
<td>0.797</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>8</td>
<td>152.6250</td>
<td>6.3413</td>
<td>0.301</td>
<td>0.767</td>
</tr>
<tr>
<td>IL-6</td>
<td>M</td>
<td>12</td>
<td>70.6917</td>
<td>3.5811</td>
<td>0.261</td>
<td>0.797</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>8</td>
<td>70.1875</td>
<td>3.8050</td>
<td>0.301</td>
<td>0.767</td>
</tr>
</tbody>
</table>

Table 3. Baseline sTNF-α and IL-6 Values (pg/ml) in Both Sexes in Cases

Out of twenty Essential Hypertensive subjects recruited, 6 were in stage 1 hypertension (according to JNC VII classification) while the remainder 14 were in stage 2 hypertension. Proinflammatory markers levels were found to be higher in stage 2 hypertension as compared to Stage 1 hypertension. The difference in mean sTNF-α in stage 1 and stage 2 was statistically significant (p<0.0001) (Table-6). However, difference in mean IL-6 values was not significant (0.344) (Table-4).

Determination of Secreted TNF-α and IL-6 Levels in 24 hr. Monocyte Cultures of Patients With Primary Hypertension - Prior to any EGCG-related investigations, an attempt was made first to probe the levels of secreted TNF-α and IL-6 in 24 hr. monocyte cultures of normal healthy controls and patients that were devoid of any EGCG. 24 hr. culture supernatants of patient monocytes (n=20) exhibited a high magnitude augmentation in secreted TNF-α (153.25±8.54 pg/ml, P<0.0001, Table-5) when compared to normal healthy controls (n=8, 3.13±1.46 pg/ml, P<0.0001, Table-5). Further analysis of the above data revealed that in comparison to normal controls, the levels of sTNF-α increased by ~49.04-fold (P<0.001) in monocyte cultures of patients with primary hypertension. It was observed that 24 hr. culture supernatants of patient monocytes (n=20) exhibited an appreciable elevation in IL-6 at the protein level (70.49±3.58 pg/ml, P<0.0001, Table-5) when compared to normal healthy controls (n=8, 3.74±1.39 pg/ml, P<0.0001). Computational analysis of the above data showed an 18.68-fold (P<0.001) increase in IL-6 levels in comparison to healthy controls (Table 5).

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Patient</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t-value</th>
<th>Sig. (2-tailed)</th>
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<tr>
<td>TNF-α</td>
<td>Case</td>
<td>20</td>
<td>153.2500</td>
<td>8.5379</td>
<td>48.907</td>
<td>&lt;0.0001</td>
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<tr>
<td>Control</td>
<td>8</td>
<td></td>
<td>3.1250</td>
<td>1.4597</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Case</td>
<td>20</td>
<td>70.4900</td>
<td>3.5809</td>
<td>50.746</td>
<td>&lt;0.0001</td>
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<tr>
<td>Control</td>
<td>8</td>
<td></td>
<td>3.7375</td>
<td>1.3856</td>
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Table 5. Comparison of Baseline TNF-α & IL-6 (Mean) in Cases & Controls (Independent-Samples t test)

Effect of EGCG on Secreted TNF-α and IL-6 Levels in 24 hr. Monocyte Cultures of Patients with Primary Hypertension - After treatment of cultured monocytes with 5 µg/ml of EGCG, there was marked decrease in expression of sTNF-α and IL-6 in comparison to baseline expression in hypertensive cases (p<0.0001, Table-6). Healthy controls also showed EGCG induced decrease in sTNF-α and IL-6 expression (p=0.004), but EGCG induced percentage decrease in TNF-α and IL-6 expression was much higher in cases in comparison to controls (p<0.0001, Table-7). A negligible level of sTNF-α (2.25±1.21 pg/ml) was recorded in 5 µg/ml of EGCG-treated monocyte cultures of normal healthy controls. On the contrary, monocyte cultures of hypertensive patients that were co-cultured along with 5 µg/ml of EGCG for 24 hrs. Exhibited sTNF-α to the order of 62.26±5.08 pg/ml (P<0.001). Thus, here, in comparison to normal control cells treated with EGCG, the patient’s cells co-cultured with EGCG showed a 24.13-fold (P<0.001) difference in secreted TNF-α.

Similarly, evaluation of IL-6 levels showed that 24 hr. culture supernatants of patient monocytes (n=20) exhibited secreted IL-6 at the protein level to the order of 31.8±2.05 pg/ml (P<0.001) when compared to normal healthy controls (n=8, 3.11±1.11 pg/ml, P<0.001). Analysis of the above data showed a 10.23-fold (P<0.001) difference in IL-6 levels in comparison to healthy controls.
Table 6. Effect of EGCG on TNF-α and IL-6 Expression in Hypertensive Cases and Healthy Controls (Paired-Samples t Test)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>t - values</th>
<th>Significance</th>
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<tr>
<td>TNF-α Base</td>
<td>3.1250</td>
<td>8</td>
<td>1.4597</td>
<td>4.158</td>
<td>0.004</td>
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<td>TNF-α EGCG</td>
<td>2.5750</td>
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<td>1.2198</td>
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<tr>
<td>IL-6 Baseline</td>
<td>3.7375</td>
<td>8</td>
<td>1.3856</td>
<td>5.531</td>
<td>0.001</td>
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<tr>
<td>IL-6 EGCG</td>
<td>3.1125</td>
<td>8</td>
<td>1.1115</td>
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Table 7. Comparison of EGCG Induced Percentage Decrease in Secreted TNF-α & IL-6 Expression in Both Cases and Controls. (Independent-Samples t test)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t - values</th>
<th>Significance</th>
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<tr>
<td>DNTF-α Control</td>
<td>16.6144</td>
<td>8</td>
<td>20.224</td>
<td>&lt;0.0001</td>
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<tr>
<td>Cases</td>
<td>59.3319</td>
<td>20</td>
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</tr>
<tr>
<td>DIL-6 Control</td>
<td>16.3938</td>
<td>8</td>
<td>22.704</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cases</td>
<td>54.7626</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dose-Response Effect of EGCG on The Expression of Secreted TNF-α and IL-6 in Supernatants of Monocyte Cultures of Hypertensive Patient Monocytes - Next, co-culturing of patients' monocyte cultures with varying doses EGCG (0-25 µg/ml) for 24 hrs. Showed a dose-dependent suppression in sTNF-α expressions (Figure 1). Computational analysis of the above data revealed EGCG to down-regulate the sTNF-α expression by ~ 1.30-fold, 2.16-fold, 2.67-fold, 3.12-fold, 3.96-fold and 4.42-fold with EGCG doses of 2µg/ml, 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively (Figure 2).

In continuation to the above, varying doses EGCG (0-25 µg/ml) for 24 hrs. Showed a dose-dependent suppression in IL-6 expressions also (Figure 3). The above data showed EGCG to down-regulate the IL-6 expression by ~ 1.26-fold, 1.87-fold, 2.75-fold, 3.60-fold, 4.14-fold and 5.01-fold with EGCG doses of 2 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml respectively (Figure 4).

Comparative Analysis of EGCG-mediated Versus non-EGCG Treated Effects on the Expression of Secreted TNF-α and IL-6 in Supernatants of Monocyte Cultures of Hypertensive Patient Monocytes - As evident from Figure 5, a dose of 5 µg/ml of EGCG was sufficient enough to down-regulate the expressions of sTNF-α and IL-6 from 153.25±8.54 pg/ml (P<0.0001) and 70.49±3.58 (P<0.0001) pg/ml in non-treated patient cells to 62.26±5.08 pg/ml (P<0.0001) and 31.8±3.05 pg/ml (P<0.0001) respectively in EGCG-treated cells. Thus, the...
data shows EGCG to suppress the expressions of sTNF-alpha and IL-6 by ~ 2.46-fold and 2.22-fold respectively in monocytes of patients with primary hypertension.

**DISCUSSION**

The present study is one of the few studies to show that proinflammatory cytokines like TNF-α and IL-6 are raised in patients with Essential Hypertension devoid of any associated cardiovascular complication. Association of high sensitivity C-reactive protein with Essential Hypertension and various cardiovascular diseases is well established. In view of this we measured secreted TNF-α and IL-6 in monocytes cultures of Hypertensive and healthy subjects. Various cross-sectional studies have shown that, compared to normotensives, the plasma levels of inflammatory markers, such as CRP; cytokines, such as tumor necrosis factor-α (TNF-α) and IL-6; chemokines, such as Monocyte Chemoattractant Protein (MCP-1); and adhesion molecules, such as P-selectin and ICAM-1, are increased in patients with essential hypertension with no evidence of CVD. 11-13 Present study is in well accordance with above mentioned studies, in which it was observed that, 24 hr. culture supernatants of Hypertensive patient monocytes exhibited a high magnitude augmentation in secreted TNF-alpha when compared to normal healthy controls. Above data revealed that in comparison to normal controls, the levels of sTNF-alpha increased by ~49.04-fold in monocyte cultures of patients with primary hypertension. 24 hr. culture supernatants of patient monocytes exhibited an appreciable elevation in IL-6 at the protein level when compared to normal healthy controls. Computational analysis of the above data showed an 18.86-fold increase in IL-6 levels in comparison to healthy controls. A recent study investigated the relationship between IL-6, TNF-α, and hs-CRP with arterial stiffness in untreated hypertensive patients. This suggests a pivotal role for inflammation in the development of vascular disease, hypertension in particular. Interestingly, pulse wave velocity (PWV), a measure of large vessels distension, has been recently associated with the circulating levels of some inflammatory molecules (such as CRP, IL-6 and TNF-α) suggesting that inflammation may contribute to arterial stiffness. Also, the transition of VSMC towards an osteoblast/chondrogenic phenotype can be induced by inflammatory mediators (such as TNF-α and IL-6). It seems likely that this phenotypic transition contributes to the promotion of calcium deposition in the arteries, leading to arterial stiffness and worsening of blood pressure levels.

In the present study GPx (Glutathione peroxidase) activity was determined in monocyte cultures of patients with primary hypertension. Monocytes cultured from healthy subjects served as controls. An appreciably high magnitude GPx activity was recorded in normal healthy control cell cultures. The data here exhibited glutathione peroxidase (GPx) activity of the order of 71.82U/mg protein (Figure 6). On the contrary, GPx activity in monocyte cultures of patients with primary hypertension was found to decrease to 38.13 U/mg protein (P<0.001) (Figure 6). Interestingly, co-culturing of patient’s monocytes for 24 hrs. along with 5 µg/ml of EGCG exhibited appreciable up regulation in GPx activity (59.75 U/mg protein, P<0.001) (Figure 6).

Computational analysis of the data shows that the GPx activity decreases by ~ 1.88-fold (P<0.001) in patient’s monocyte when compared to normal healthy controls. Furthermore, it was observed that down-regulated GPx activity in patient’s monocyte was up-regulated by ~1.56-fold by EGCG.

Thus, the results obtained show that EGCG - a natural antioxidant and a green tea polyphenol, up-regulate the GPx activity in monocyte cultures of patients with primary hypertension.

**Determination of Glutathione Peroxidase (GPx) Activity in Monocyte Cultures of Healthy Control and Patients With Primary Hypertension–** GPx activity was determined in monocyte cultures of patients with primary hypertension. Monocytes cultured from healthy subjects served as controls. An appreciably high magnitude GPx activity was recorded in normal healthy control cell cultures. The data here exhibited glutathione peroxidase (GPx) activity of the order of 71.82U/mg protein (Figure 6). On the contrary, GPx activity in monocyte cultures of patients with primary hypertension was found to decrease to 38.13 U/mg protein (P<0.001) (Figure 6). Interestingly, co-culturing of patient’s monocytes for 24 hrs. along with 5 µg/ml of EGCG exhibited appreciable up regulation in GPx activity (59.75 U/mg protein, P<0.001) (Figure 6).

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Thus, the results obtained show that EGCG- a natural antioxidant and a green tea polyphenol, up-regulate the GPx activity in monocyte cultures of patients with primary hypertension.
and low-fat dairy products, that includes whole grains, modest portions of lean meat and nuts, and that is low in salt and sugar significantly reduced blood pressure in both hypertensive and normotensive people.

Current therapies for hypertension normalize blood pressure by various means without removing the cause. Many of these treatments are prone to side effects which may result in poor compliance. The ideal treatment would be a natural compound which would address the cause of the disease and could control blood pressure without side effects.

At present, considerable attention is focused on the use of naturally occurring botanicals for the prevention of many diseases. EGCG, the major catechin derived from green tea, has been found to have protective effects on the cardiovascular system. These include anti-inflammatory effects, lowering serum cholesterol levels, and reducing atherosclerosis. The antioxidant properties of EGCG have attracted considerable attention for the prevention of oxidative stress-related diseases such as ischemic heart diseases. The finding that reactive oxygen radicals significantly contribute to the genesis of reperfusion-induced dysrhythms, contractile malfunction and vascular endothelium damage and that EGCG protects cardiac myocytes from ischemia/reperfusion injury also suggests that EGCG's cardio protective effects may be mediated by free radical scavenging.

Our results clearly show high magnitude of glutathione peroxidase (GPx) activity in normal healthy control cells was significantly reduced in patients having primary hypertension. It was observed that down-regulated GPx activity in patient's monocyte was up-regulated by ~1.56-fold by EGCG. Thus, the results obtained show that EGCG - a natural antioxidant and a green tea polyphenol, up-regulates the GPx activity in monocyte cultures of patients with primary hypertension. It’s well known that glutathione directly reacts with ROS, and GPx catalyzes the removal of hydrogen peroxide. Decrease in GPx activity indicates impairment of hydrogen peroxide-neutralizing mechanisms. Here, the high magnitude decline in GPx activity in patients having primary hypertension in comparison to normal control cells clearly indicates an augmented impairment of hydrogen peroxide-neutralizing mechanisms in hypertensive patients. Interestingly, our results also infer that upon EGCG treatment of monocytes of hypertensive patients, a significant increase in GPx activity occurred, thereby indicating an EGCG-induced improvement in the impairment of hydrogen peroxide-neutralizing mechanisms.

The most interesting observation made in the present study was on the EGCG-mediated effects on sTNF-α and IL-6 expression in comparison of non-EGCG-treated monocytes cultures of patients with primary hypertension. EGCG was found to suppress the expressions of sTNF-α and IL-6 by ~ 2.46-fold and 2.22-fold respectively in monocytes of patients with primary hypertension in comparison to healthy controls. Thus, our findings substantiate the idea that hypertension and inflammation are somehow linked to each other. Our findings regarding high basal levels of TNF-α and IL-6 in 24 hr. culture supernatants of monocytes from patients with hypertension is in accordance to the previous studies carried out elsewhere on plasma of such patients. Interestingly, the most striking observation in the present study indicating appreciably high magnitude of EGCG-mediated suppressions in inflammatory markers like TNF-α and IL-6 are suggestive that EGCG can reduce the recruitment of circulating levels of inflammatory mediators, such as IL-6 and TNF-α. Reports are available that show relationship between IL-6 and TNF-α with arterial stiffness in untreated hypertensive patients, where it was demonstrated that IL-6 and TNF-α are significantly related to pulse wave velocity, a marker of aortic stiffness, and augmentation index, a manifestation of wave reflection, in essential hypertension. This suggests the pivotal role of inflammation in the development of vascular disease, and hypertension in particular. As a consequence of EGCG-mediated reduction in inflammation as observed in the present study, it is strongly hoped that EGCG may help in reducing the arterial stiffness contributed by inflammation i.e. by IL-6 and TNF-α.

The potential of antioxidants in treating conditions associated with oxidative stress is supported by experimental investigations, observational findings, small clinical studies, and epidemiological data. However, findings are inconsistent and clinical trial data are inconclusive. To date, at least 7 large trials have been published regarding antioxidant vitamin effects on risks of cardiovascular disease: the Cambridge Heart Antioxidant Study (CHAOS; 2002 patients); Alpha Tocopherol, Beta-Carotene cancer prevention study (ATBC; 27 271 males); Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico(GISSI)-Prevenzione trial (3658 patients); Heart Outcomes Prevention Evaluation (HOPE) study (2545 subjects); Medical Research Council/British Heart Foundation (MRC/BHF) heart protection study (20 536 adults); Primary Prevention Project (PPP; 4495 patients); and the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study (520 subjects) have recently been reviewed. Except for the ASAP study, which demonstrated that 6-year supplementation of daily vitamin E and slow-release vitamin C reduced progression of carotid atherosclerosis, the other studies failed to demonstrate significant beneficial effects of antioxidants on BP or on cardiovascular end points. Overall, data from large prospective randomized clinical trials failed to demonstrate beneficial cardiovascular effects of antioxidants. Potential reasons are related to (1) antioxidants used, (2) patients included in trials, and (3) the trial design itself. With respect to antioxidants, it is possible that agents examined were ineffective and inappropriate and that dosing regimens and duration of therapy were insufficient. It is also possible that orally administered antioxidants may be inaccessible to the source of free radicals, particularly if ROS are generated in intracellular compartment and organelles. Regarding individuals included in large trials, most subjects had significant cardiovascular disease, in which case damaging effects of oxidative stress may be irreversible. Another
concurring factor is that most of the enrolled subjects were taking aspirin prophylactically. Because aspirin has intrinsic antioxidant properties, additional antioxidant therapies may be ineffective. Moreover, in patients studied in whom negative results were obtained, it was never proven that these individuals had increased oxidative stress. Finally, none of the large clinical trials were designed to examine effects of antioxidants specifically on BP.

Thus, in view of the above, we used EGCG as natural antioxidant and subjects recruited were devoid of any cardiovascular complication. Furthermore, by measuring GPx activity it was well proved that Hypertensive subjects in our study were in oxidative stress. We also measured the Dose-response effect of EGCG on the expression of secreted TNF-alpha and IL-6 in supernatants of monocyte cultures of hypertensive patient. It was observed that co-culturing of patients’ monocyte cultures with varying doses EGCG (0-25 µg/ml) for 24 hrs. showed a dose-dependent suppression in sTNF-alpha and IL-6 expressions. By doing this, IC50 value of EGCG for Hypertensive subjects was calculated, which came around to be 5µg/ml.

Thus, in view of the above, based on earlier reports as well as further supplementation by our data generated in the present study, the idea of exploring the potential therapeutic utility of EGCG in the management of hypertension and other cardiovascular diseases is highly promising. Thus, in summary, it is hoped that the findings of the present study may help in the better understanding about the pathophysiology and management of Essential Hypertension and cardiovascular related complications encountered in day-to-day life globally.

CONCLUSIONS

Present study concluded that:

1. There is a potential link between Inflammation and Hypertension both may share some pathophysiological mechanisms.
2. Antioxidant enzyme activity is decreased in patients with Essential Hypertension, leading to increased reactive oxygen species (ROS) bioactivity in these patients.
3. This increased ROS bioactivity may lead to endothelial dysfunction, increased contractility, VSMC growth, monocyte invasion, lipid peroxidation, inflammation, and increased deposition of extracellular matrix proteins, important factors in hypertensive vascular damage.
4. EGCG a green tea polyphenol and natural antioxidant decreases the expression of secreted TNF-α and IL-6 in patients with Essential Hypertension.
5. EGCG also up-regulates the down regulated Glutathione Peroxidase activity, thereby enhancing the ROS scavenging capacity.

REFERENCES


