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Lipid profile alterations and oxidative stress in patients with preeclampsia: role of black tea extract on disease management

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Lipid profile alterations and oxidative stress in patients with preeclampsia: role of black tea extract on disease management

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Aim: To investigate the lipid profile and susceptibility of low-density lipoprotein (LDL) to oxidation in preeclamptic women and to correlate the results with preeclampsia risk in the absence and presence of black tea extract (BTE). Preeclampsia is a disorder of human pregnancy characterized by altered endothelial function, secondary to oxidative stress.

Materials and methods: Lipid profile levels and oxidative stress status were investigated in the cord blood of normotensive and preeclamptic patients. LDL susceptibility to oxidation was also monitored in the absence and presence of BTE to examine its protective role.

Results: A significant alteration in lipid parameters and enhanced oxidative stress was observed in patients with preeclampsia compared to control subjects. Increased susceptibility of LDL to oxidation in the preeclamptic group was reduced during incubation with BTE.

Conclusion: The present study demonstrates a hyperlipidemic and oxidative environment in preeclamptic patients. The protective role of BTE against LDL oxidation suggests its recommendation as a supplement for preeclamptic patients, which may provide protection against oxidative modification of LDL and endothelial dysfunction.

Key words: Black tea extract, hyperlipidemia, low density lipoprotein, oxidative stress, preeclampsia

Introduction

High blood pressure complicates about 10% of all pregnancies and its incidence is high in nulliparous women and during multiple pregnancies (1). Preeclampsia, a hypertensive disorder of pregnancy, is a major cause of neonatal deaths and is associated with fetal growth retardation (2). This disease, which affects 7%-10% of pregnant women, manifests itself as hypertension, proteinuria, and edema during pregnancy. It is characterized by increased resistance to blood flow, leading to hypertension and damage to the endothelium of blood vessels. Damaged endothelial cells are the sites of platelet and fibrinogen deposition and thrombus formation. This, together with hypoxia, weakens the vessel wall and leads to hemorrhage, necrosis, and organ dysfunction.

Abnormal lipid profiles have a strong positive correlation with endothelial dysfunction. Specific changes are usually associated with normal pregnancy. Normal serum total triglyceride (TG), LDL-cholesterol (LDL-C), and total cholesterol levels increase toward the term of normal pregnancy (3). Increasing evidence suggests that endothelial cell dysfunction is also possibly mediated by oxygen free radicals (4). Under normal conditions, a variety of antioxidant mechanisms serve to

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control peroxidative processes, but in preeclampsia, an imbalance of lipid peroxidation and antioxidant mechanisms could impair normal endothelial function (5). Oxidation of LDL takes place in microdomains in the arterial intima by reactive oxygen species. The LDL modification by oxidation increases its uptake by macrophage and develops into foam cells, which are of primary importance in the mediation of vascular endothelial damage (6).

Tea (*Camellia sinensis*) is an important and rich dietary source of flavanols; the major flavonoids in tea include catechins such as epicatechin, epicatechin 3-gallate, epigallocatechin, and epigallocatechin-3-gallate. The flavonoid and polyphenolic components of tea have been associated in epidemiological studies with a decreased cardiovascular risk. Tea polyphenols exhibit potent antioxidant activity both in vitro and in vivo by quenching reactive oxygen and nitrogen species (7).

Since cord blood profiles are usually associated with the metabolic and biologic functions of the individual and since determination of lipid profiles in cord blood correlates well with endothelial dysfunction, this study focused on the potential role of hyperlipidemia and oxidative stress in the development of endothelial dysfunction in patients with preeclampsia. Hence, lipid and lipoprotein parameters and conjugated diene production, together with LDL susceptibility to oxidation, were determined in cord blood samples of preeclamptic women and compared with results from healthy pregnant women. A further aim was to evaluate the efficacy of BTE by examining its inhibitory effect on LDL oxidation.

Materials and methods

Patients

The study protocol was approved by the ethics committee for clinical research of a private hospital. Subjects were selected from women attending the Obstetrics and Gynecology Department for delivery, and 25 women with a history of preeclampsia and 19 women with a normotensive and uncomplicated pregnancy in the age group of 20-40 years were chosen for the study. Patients with preeclampsia were defined on the basis of the following clinical and laboratory criteria: systolic blood pressure ^{3} 140 mmHg, diastolic blood pressure ^{3} 90 mmHg, and proteinuria levels > 300 mg/dL in at least 2 random specimens. The women from the control group were without any maternal or fetal complications during the pregnancy period.

Preparation of cord serum

Blood samples were collected from both normotensive and preeclamptic women from their umbilical cords at the time of delivery in sterile capped centrifuge tubes and were brought immediately to the laboratory. The blood sample was kept in an inclined position for 30 min and then centrifuged at 5000 × *g* to separate the serum. Serum samples were stored at –20 °C until analysis. All biochemical estimations were performed within 24 h of sample collection.

Isolation of low-density lipoprotein (LDL) and high-density lipoprotein (HDL)

LDL (density: 1.019-1.063) was isolated from the serum by precipitation with heparin and magnesium chloride in the presence of sucrose, followed by ultracentrifugation for 24 h at 100,000 × *g* using the method of Burstein et al. (8). HDL (density: 1.063-1.121) was separated from serum using a heparin-manganous chloride reagent and employing the method of Wilson and Spiger (9).

Measurement of preeclampsia markers

The levels of preeclampsia markers such as uric acid and the activity of the corresponding enzyme xanthine oxidase, which synthesizes uric acid, were determined according to the methods established by Brown (10) and Beckman et al. (11), respectively.

Lipid and lipoprotein determinations

The levels of lipid profiles like total cholesterol (12), total TG (13), phospholipids (14), and free fatty acids (15) in serum, together with the levels of cholesterol, TG, and protein (16) in LDL and HDL subclasses, were determined. Levels of apolipoproteins, Apo B, and Apo A-I of LDL and HDL, respectively, were determined by the rate immunonephelometric assay method developed by Maciejko et al. (17).

Black tea extract (BTE) preparation

In 100 mL of boiling water, 2.5 g of commercially available South Indian black tea leaves were soaked and brewed for 5 min. The mixture was filtered

using Whatman No. 2 filter paper, and 50 µL of the filtrate, designated as BTE, was used for the accomplishment of biochemical measurements. Flavanols (epigallocatechin gallate, epicatechin gallate, gallocatechin, epigallocatechin, gallocatechin gallate, and epicatechin), theaflavins, and caffeine were the effective ingredients identified in the extract by HPLC analysis. Typically, 25 µg of total catechins in 50 µL of BTE contained approximately 3.4 µg of theaflavins, 6 µg of epigallocatechin gallate, 2.5 µg of epicatechins, 2 µg of catechins, and 4.7 µg of epigallocatechin and epicatechin gallate.

Conjugated diene (CD) measurement and in vitro LDL oxidation

Oxidative stress status was determined by measuring the levels of conjugated diene of LDL of both preeclamptic and healthy pregnant women using the method of Esterbauer et al. (18). LDL was oxidized in vitro using copper sulphate ($CuSO_4$) by the method of Wallin et al. (19). Oxidation of 200 µg of LDL (in terms of LDL protein (Apo B)) isolated from the serum was initiated by the addition of 10 µM $CuSO_4$ in phosphate-buffered saline, and the kinetics of the formation of CD were determined by monitoring the change in the absorbance at 234 nm at 37 °C every 5 min for 2 h on a SYSTRONICS UV-VIS spectrophotometer. The protective role of BTE on LDL oxidation was analyzed by preincubating the reaction mixture containing LDL and $CuSO_4$ with 50 µL of BTE followed by a similar reaction monitoring of the CD kinetics.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Differences in clinical characteristics, lipid levels, amount of conjugated dienes, lag time, and oxidation rate were statistically analyzed using Student's t-test with SPSS 7.5. Statistical significance was determined by comparing the results of the preeclamptic group with those of the control group. Statistical significance was set at $P < 0.05$.

Results

Clinical characteristics

A description of the clinical characteristics of the study groups is shown in Table 1. The systolic and diastolic blood pressure and urine protein were significantly increased ($P < 0.05$) and the platelet count and infant birth weight were significantly decreased ($P < 0.05$) in preeclamptic patients compared to normal pregnant subjects. However, no significant differences were found in age, body mass index (BMI), or hematocrit levels between the 2 groups.

Preeclampsia marker analysis

The markers of preeclampsia, uric acid and xanthine oxidase, were found to be significantly elevated ($P < 0.05$) in the serum of cord blood collected from preeclamptic patients (test group) compared to cord serum of normal subjects (control group) (Figure 1).

Table 1. Clinical characteristics of normal pregnant women and preeclamptic women.

Clinical characteristics	Normal pregnant subjects (n = 19)	Preeclamptic patients (n = 25)
Age (years)	28.5 ± 3.0	28.2 ± 2.1 NS
Body mass index (BMI)	26.6 ± 3.4	28.9 ± 3.6 NS
Systolic blood pressure (mmHg)	113.4 ± 5.8	151.62 ± 6.2*
Diastolic blood pressure (mmHg)	76.8 ± 5.2	97.1 ± 4.8*
Urine protein (g/24 h)	None	5.8 ± 6.0*
Infant birth weight	3.28 ± 0.20	2.42 ± 0.68*
Platelets ($\times 10^4$)	26.4 ± 2.1	19.4 ± 2.7*
Hematocrit (%)	33.2 ± 1.8	36.8 ± 2.7 NS

Data are expressed as mean ± standard deviation.*$P < 0.05$, NS: nonsignificant.

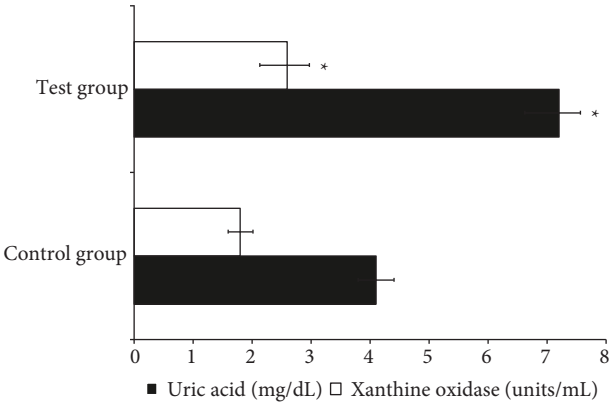

Figure 1. Levels of uric acid and xanthine oxidase in cord serum of normal and preeclamptic patients. Data are expressed as mean ± standard deviation. *P < 0.05.

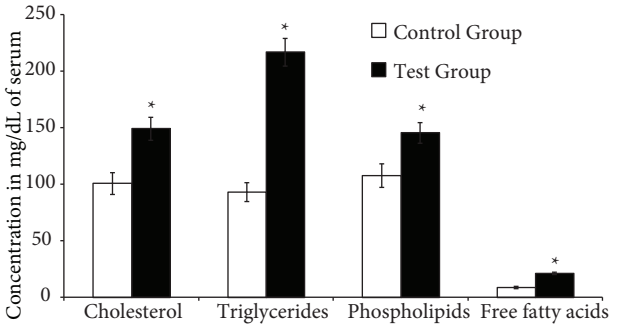

Figure 2. Levels of lipid profile in cord serum of normal and preeclamptic women. Data are expressed as mean ± standard deviation.*P < 0.05.

Lipid and lipoprotein levels

The serum levels of total cholesterol, TG, phospholipids, and free fatty acids were significantly increased (P < 0.05) in the test group as compared to the control group, demonstrating a hyperlipidemic status in these patients (Figure 2). The levels of LDL-TG and LDL protein apolipoprotein B also showed a significant increase (P < 0.05) in the test group compared to the control group. However, an insignificant difference was observed between the 2 groups with regard to LDL-C (Table 2). In the case of HDL (Table 2), its cholesterol and protein levels showed a significant decrease (P < 0.05) in the test group, while the levels of HDL-TG showed an insignificant difference between the 2 groups. Apo A-I, the apolipoprotein of HDL, also showed a significant decrease (P < 0.05) in the test group compared to the control group, indicating an increased relevance for endothelial dysfunction.

Conjugated diene (CD) levels and kinetics

The levels of CD were found to be increased significantly (P < 0.05) in the LDL of the test group as compared to the control group, revealing a positive correlation with the increased oxidative stress and hence the oxidation of lipids in the preeclamptic group (Figure 3). Incubation of a suspension of LDL from either control- or test-group patients with $CuSO_4$ caused a significant increase in CD production

Table 2. Lipid and protein levels of LDL and HDL subclasses in cord blood serum of normotensive and preeclamptic women.

	Normal pregnant subjects	Preeclamptic women
LDL-cholesterol (mg/dL)	55.68 ± 2.7	59.45 ± 1.7 NS
LDL-TG (mg/dL)	42.41 ± 2.0	54.64 ± 2.2*
LDL-protein (mg/dL)	46.36 ± 1.2	58.77 ± 3.6*
Apo B (mg/dL)	36.93 ± 1.89	50.68 ± 1.97*
HDL-cholesterol (mg/dL)	42.58 ± 2.3	31.42 ± 2.1*
HDL-TG (mg/dL)	19.86 ± 3.4	12.74 ± 0.73 NS
HDL-protein (mg/dL)	160.62 ± 10.9	148.36 ± 8.1*
Apo A-I (mg/dL)	57.73 ± 3.9	38.93 ± 2.04*

LDL: low-density lipoprotein, HDL: high-density lipoprotein, Apo: apolipoprotein.
Data are expressed as mean ± standard deviation.*P < 0.05, NS: nonsignificant.

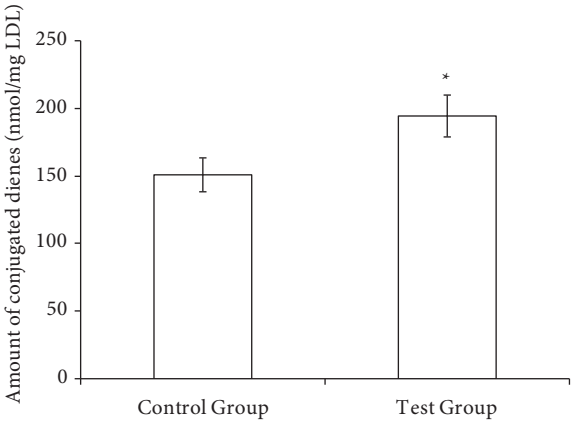

Figure 3. Levels of conjugated dienes in LDL isolated from cord serum of normal and preeclamptic women. Data are expressed as mean ± standard deviation. *P < 0.05.

(Figure 4). Since the oxidative stress status was already enhanced in preeclamptic women, the levels of CD further increased during LDL incubation with $CuSO_4$ in the preeclamptic group. The kinetics of $CuSO_4$-induced formation of conjugated dienes showed a significantly (P < 0.05) shortened lag time and a greater oxidation rate for LDL in women with preeclampsia (Table 4).

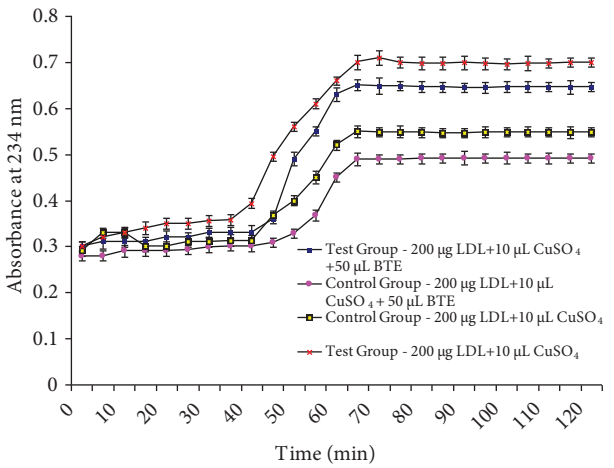

Figure 4. Kinetics of copper ion-induced in vitro oxidation of LDL of normal pregnant women and preeclamptic women in the absence and presence of BTE. LDL (200 µg of LDL protein) in PBS (pH 7.4) was incubated with 10 µM $CuSO_4$ in the absence and presence of BTE in both control and test groups, and the change in conjugated diene absorption was monitored at 234 nm.

Table 3. Phytochemical constituents of BTE.

Active constituents	Activity
Steroids	-
Phenolic compounds	+++
Reducing sugars	+
Flavonoids	+
Glycosides	-
Saponins	+
Alkaloids	-
Anthraquinones	++
Quinines	+
Tannins	++

BTE protects against LDL oxidation

Phytochemical analysis of BTE revealed the presence of active constituents such as polyphenolic compounds, flavonoids, anthraquinones, quinines, and reducing sugars (Table 3). CD production was inhibited significantly (P < 0.05) during preincubation of the reaction mixture containing LDL and $CuSO_4$ with 50 µL of BTE (Figure 4). The prolongation of the lag phase was greater than 10 min. The propagation rates and the maximum absorbance were also significantly reduced, demonstrating the protective effect of BTE on LDL oxidation and CD production (Table 4).

Discussion

Preeclampsia remains one of the most serious complications of pregnancy. Endothelial dysfunction has been reported as an important biologic feature in women with preeclampsia (20). Serum levels of uric acid and xanthine oxidase serve as the marker of preeclampsia. Hyperuricemia and increased xanthine oxidase activity reflect the severity of preeclampsia with adverse fetal outcome (21). In agreement with this, in the present study uric acid levels and xanthine oxidase enzyme activity were significantly increased in the cord serum of preeclamptic subjects as compared

Table 4. Susceptibility to oxidation of LDL in normotensive and preeclamptic pregnant women in the absence and presence of BTE.

Conjugated diene kinetics	Control group LDL incubated with $CuSO_4$ (i)	Control group LDL incubated with BTE and $CuSO_4$ (ii)	Test group LDL incubated wit $CuSO_4$ (iii)	Test group LDL incubated with BTE and $CuSO_4$ (iv)
Lag time (min)	42.1 ± 2.1	44.0 ± 1.7*b	36.9 ± 2.5*a	41.6 ± 2.0*c
Rate of oxidation ((nmol/min)/mg LDL)	4.6 ± 0.5	4.1 ± 0.7*b	5.8 ± 0.7*a	5.1 ± 1.0*c
Amount of dienes (nmol/mg LDL)	215.1 ± 14.9	205.1 ± 16.7*b	244.3 ± 15.1*a	238.6 ± 16.6*c

Data are expressed as mean ± standard deviation.
*P < 0.05; a, b, c: comparison of (i) and (iii), (i) and (ii), and (iii) and (iv), respectively.

with control counterparts. Hyperuricemia was due to the consequence of increased uric acid production or decreased excretion, and this condition may be attributed solely to renal dysfunction. Elevated uric acid and xanthine oxidase activity may also contribute to endothelial dysfunction and the state of increased oxidative stress.

Pregnancy in healthy women is characterized by a progressive increase in the serum concentrations of total cholesterol and total TGs, and hence an increase in the concentration of lipoproteins like LDL and very-low-density lipoproteins (VLDL), to satisfy the demands of the developing fetus. The increased hepatic production of TG and enhanced removal of TG from circulation during early pregnancy, and the enhanced release of free fatty acids from adipocytes due to stimulation of lipase during late pregnancy, are important metabolic changes that must occur and allow the gravid female to meet the energy requirements of late gestation. In preeclampsia, these biologic features are altered to an extent characterized by the reduction in the removal of lipoprotein TGs due to decreased activities of lipoprotein lipase and hepatic TG lipase, resulting in increased serum TG levels. A similar increase in cholesterol and TG levels has been demonstrated even in cord blood (22). Consistent with these previous reports, in this study the total cholesterol and total TG levels in cord serum were increased more in the test group than the control group, suggesting the hypercholesterolemic and hypertriglyceridemic condition of the preeclamptic group. The serum levels of free fatty acid were also increased in the test group, which may be due to increased lipase activity with the consequent release of free fatty acids from adipocytes into circulation, as described earlier. These fatty acids are reported to contribute to endothelial dysfunction by serving as substrates for lipid peroxidation (20). Phospholipid levels in the cord blood of the test group were markedly increased, suggesting an increased association of preeclampsia with the prevalence of unsaturated fatty acid and an increased unsaturation of maternal serum phospholipid, which might facilitate the placental transfer of long-chain polyunsaturated fatty acids (23).

A significant increase in LDL-TG levels was observed in preeclamptic patients in the present study, showing a positive correlation with the levels of serum TG (20). However, the insignificant difference observed for the results of LDL-C may be due to the fact that increased serum TG produces small, TG-rich, cholesterol-poor LDL particles (24). Nigon et al. (25) also demonstrated that dense LDL particles due to TG contain a decreased level of cholesterol. Since each LDL particle contains one molecule of apolipoprotein B (24), the increased concentration of LDL protein observed in this study might be equivalent to the concentration of LDL particles, indicating the serum concentration of LDL particles in patients with preeclampsia. This is further correlated by the elevated levels of Apo B in samples from the test group. A rise in Apo B was associated with a marked increase in endothelial dysfunction. HDL promotes cholesterol efflux and has the ability to inhibit the oxidation of LDL via its antiinflammatory role. In the present study, the concentration of HDL-cholesterol (HDL-C) was found to be significantly decreased in samples from the test group. A positive correlation of reduced HDL-C and high TG values with increased endothelial dysfunction

has also been demonstrated (26). However, in contrast to LDL-TG, HDL-TG showed no significant difference in levels between the 2 groups. The significant decrease in Apo A-I levels observed in preeclamptic patients also indicates a positive risk factor for the dysfunction of endothelial cells.

Oxidative stress has been proposed as an important underlying mechanism that contributes to the endothelial dysfunction associated with preeclampsia (27). Lipid oxidation, a central feature of oxidant stress, may alter membrane integrity and membrane-associated functions. Conjugated diene levels of LDL showed a significant increase in patients with preeclampsia as compared to normotensive subjects. Since the plasma levels of antioxidants like vitamin E are reportedly decreased in preeclampsia (28), the enhanced oxidative stress observed in preeclamptic patients in this study might be due to the decreased endogenous antioxidants in lipoproteins like LDL, increasing susceptibility to oxidation. Kinetic analysis of CD production showed that the lag time of the reaction, which indicates the intrinsic antioxidant property of LDL particles (24), was shortened and that the oxidation rate indicated by the CD production was greater in preeclamptic subjects compared to controls, indicating the increased susceptible nature of LDL to oxidative modification. Oxidative modification of LDL plays a crucial step in the impairment of vascular endothelial cells, generation of endothelial cell adhesion molecules, and the formation of foam cells (29). In the present study, LDL oxidation by $CuSO_4$ in the presence of BTE showed a significant increase in the prolongation of lag time. The rate of oxidation and conjugated diene formation was also decreased significantly in the presence of extracts of black tea. This effect might be caused by the antioxidant components of black tea bound to LDL, acting as scavengers of oxygen free radicals and thereby reducing susceptibility to oxidative modification. In accordance with this, it has been previously reported that BTE is rich in flavonoid and polyphenolic compounds and possesses excellent reducing power and scavenging potential for 1,1-diphenyl-2-picryl hydrazyl (DPPH), superoxide and hydroxyl radicals, etc. (30). Leung et al. (31) also reported that theaflavins in black tea inhibited Cu^{2+}-mediated LDL oxidation in a dose-dependent manner. Similarly, the protective effects of pu-erh tea on LDL oxidation in macrophage cells have been demonstrated (32).

Conclusion

The current study suggests the involvement of abnormal lipid and lipoprotein levels and oxidative stress in the severity of preeclampsia via oxidation of lipids and hence dysfunction of endothelial cells. Since LDL oxidation serves as an important factor for endothelial dysfunction, the inhibition of such oxidation processes may provide a promising approach. Because BTE reduces the in vitro susceptibility of LDL to oxidative modification favorably, it can play a significant role in inhibiting the consequent vascular endothelial damage in preeclamptic subjects. As treatment of the disease with modern medicine is often associated with the development of side effects, antioxidant-rich medicinal plant product BTE can be recommended as the best herbal remedy for reducing the severity of preeclampsia.

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