Erythrocyte Antioxidant Enzyme Activities and Lipid Peroxidation in Patients with Types IIb and IV Hyperlipoproteinemias

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We measured lipid peroxidation and antioxidant enzymes in erythrocytes of types IIb and IV hyperlipoproteinemic (HLP) human subjects in comparison with age-matched controls. Thiobarbituric acid-reactive substances (TBARS), a measure of lipid peroxidation, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT) were determined in erythrocytes. We also measured lipid parameters including triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), apolipoprotein AI, and apolipoprotein B, and antioxidant related substances such as serum albumin, free iron, ferritin, ceruloplasmin. Thirty-two subjects (females 15, males 17) with type IIb (the mean age 45.6±8 [S.E.]), 34 with type IV (females 16, males 18) (the mean age 47±10 [S.E.]), and 36 normolipidemic voluntary subjects (females 18, males 18) (the mean age 46±8 [S.E.]) were included in the study. Erythrocytes were prepared by classical washing method (0.9%NaCl) from venous blood samples. The mean TBARS levels in plasma and erythrocyte suspensions were found to be significantly higher in both types IIb and IV hyperlipoproteinemics. Erythrocyte SOD and GSH-Px activities were decreased but erythrocyte GR activity did not change in both types IIb and IV hyperlipoproteinemics. Erythrocyte CAT activity was decreased in type IIb, but it was increased in type IV hyperlipoproteinemics. Erythrocyte SOD activity was negatively correlated with plasma TG level, whereas plasma free iron was positively correlated with plasma TBARS level in type IV hyperlipoproteinemics. These results suggest the presence of oxidative injury in patients with type IIb or IV hyperlipoproteinemia, and that the responses of erythrocyte antioxidant enzymes to oxidant stress are different in these conditions. ——— hyperlipoproteinemia; antioxidant enzymes; lipid peroxidation; erythrocytes; plasma

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Reactive oxygen species play an important role in various diseases, including atherosclerosis, diabetes, autoimmune and cardiovascular diseases, depression, ischemia-reperfusion injury, and cancer (Cross et al. 1987; Bilici et al. 2001; Takeshita et al. 2000; Taysi et al. 2002). The antioxidant defense system consists of enzymes and antioxidant substances including tocopherols, ascorbate, ceruloplasmin and albumin. The function of these systems is to keep free radicals under or at physiological levels. Superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (GSH-Px, EC 1.11.1.9), glutathione reductase (GR, EC 1.6.4.2) and catalase (CAT, EC 1.11.1.6) are involved in the disposal of superoxide anion and hydrogen peroxide. SOD catalyses dismutation of superoxide to peroxide and molecular oxygen and GSH-Px and CAT enzymes decompose the hydrogen peroxide to non toxic compounds. GR catalyses the reduction of oxidized glutathione to reduced form which is the substrate of GSH-Px (Gul et al. 2000).

Hyperlipoproteinemia could increase levels of oxygen free radicals in various ways; hypercholesterolemia increases cholesterol content of platelets, polymorphonuclear leukocytes and endothelial cells so that endothelial and smooth muscle cells, neutrophils, monocytes, and platelets may be the source of oxygen free radicals in hypercholesterolemia (Esterbauer et al. 1992). It has been suggested that resistance of erythrocytes to an oxidative stress is decreased in hypercholesterolemic man (Estebauer et al. 1992). It has been suggested that resistance of erythrocytes to an oxidative stress is decreased in hypercholesterolemic man (Estebauer et al. 1992). Alterations of plasma and tissue antioxidant enzyme activities in hyperlipidemic human subjects and animal models have been reported (Araujo et al. 1995). However, in the literature there were limited and conflicting data on the relationship between lipid peroxidation and antioxidant enzyme activities in patients with various types of hyperlipoproteinemia (Silva et al. 1995; Kinoshita et al. 2000). We therefore studied the antioxidant status of hyperlipidemic subjects with different types of hyperlipoproteinemia.

Methods

Subjects

The present study was performed in patients with HLP (types IIb and IV) attending Cardiology Departments of Karadeniz Technical University. Demographic characteristics of subjects participated to the study were shown in Table 1. Healthy normolipidemic subjects were recruited from the staff of our research laboratory and hospital, who had plasma triglycerides <203 mg/100 ml, LDL cholesterol <131 mg/100 ml and total cholesterol <216 mg/100 ml. Hyperlipidemic subjects were selected from those attending our cardiology and internal medicine clinics in Farabi Hospital. No medication known to influence the lipid status was administered in any of the subjects in this study. Patients with hepatic dysfunction, diabetes mellitus, nephrotic syndrome, proteinuria, hypothyroidism, hypertension, rheumatic, myocardial infarction or any other serious illness during the previous 8 months were excluded. No patient or healthy subjects had drinking alcohol. Physical examination and anamnesis, routine biochemical (lipid electrophoresis, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, apo A-I, apo B, glucose, total protein, albumin, lactate dehydrogenase, aspartate transaminase, alanine transaminase, alkaline phosphatase, uric acid, urea, creatinine, calcium, inorganic phosphorus, iron, iron binding capacity, and ferritin in serum), and hematological (hemogram and erythrocyte sedimentation rate) tests were carried out in all examined subjects. All samples including both hyperlipoproteinemias and normolipemias were collected in a period of one week. The classification of HLP subjects was made according to Frederickson’s type, as following criteria (Tietz 1999). Type IIb HLP: plasma triglycerides >203 mg/100 ml, total cholesterol >217 mg/100 ml and LDL cholesterol >131 mg/100 ml. Type IV HLP: plasma triglyceride >203 mg/100 ml, total cholesterol <270 mg/100 ml, LDL cholesterol <131 mg/100 ml and no chylomicron band on agarose gel electrophoresis. Preparation of the
samples for analysis was begun immediately after the blood was obtained. All experiments were performed in compliance with institutional guidelines. Informed consent was obtained from human subjects.

**Lipid and biochemical analyses**

Serum total cholesterol was measured by a cholesterol oxidase enzymatic method, high density lipoprotein cholesterol (HDL-C) by the cholesterol oxidase enzymatic method in the supernatant obtained after precipitation with phosphotungstic acid-magnesium chloride. Low density lipoprotein cholesterol (LDL-C), was calculated by Friedewald formula or measured using direct homogenous method for those of TG >398 mg/100 ml (Friedewald et al. 1972). The parameters mentioned above and other biochemical analyses were determined by autoanalyser (Roche Hitachi 917, Tokyo). Serum apolipoprotein A1 and B were measured by rate immunonephelometry (Beckman Array 360, USA). Lipid electrophoresis was performed on agarose gel by using a commercial materials from Helena Lab (Cliniscan 2).

**Blood sampling**

For erythrocyte preparations, fasting (>12 hours) blood was drawn in EDTA containing vacutainer polystrene tubes from a group of subjects with type IIb, type IV hyperlipoproteinemics and an age-matched control group with normal lipoprotein levels. Freshly drawn venous blood was centrifuged at 2000 g for 10 minutes at 4°C to separate plasma and erythrocyte. Erythrocytes were washed three times with NaCl solution (0.9%) containing 0.5% of pyrogallol as an antioxidant agent. The final hematocrit suspension was made up to about 50% (approximately 5x10^6 erythrocytes/ml) with addition of distilled water containing 2 μmol/liter of butylated hydroxy-toluene and these aliquots of erythrocytes suspensions were stored at −80°C in deep freezing until used for analysis of antioxidant enzyme activities and lipid peroxidation. Purified erythrocyte suspensions were measured by automatic blood counter (Coulter Corp., STKS, UK). Erythrocyte suspensions were haemolysed by addition redistilled water and vigorous vortexing. Haemolysed erythrocytes were kept as aliquots at +4°C until they are analyzed. All analyses were made within a period of three days. Analyses of TBARS were done in first day, GSH-Px and GR in second day, and SOD and catalase in third day.

**Materials**

**Chemicals.** Xanthine oxidase, xanthine, SOD (from human erythrocytes), n-butanol, pyrogallol, butylated hydroxy-toluene, hydrogen peroxide (35 %), acetic acid, NaCl, HCl, K_2HPO_4, KH_2PO_4, H_2SO_4 were purchased from Merck; 1, 1, 3, 3-tetraethoxypropane, thiobarbituric acid (TBA), phosphotungstic acid, EDTA-Na_2, nicotinamide adenine dinucleotide phosphate (NADP, oxidized form), β-nicotinamide adenine dinucleotide phosphate (β-NADPH, reduced form), catalase (from bovine liver), superoxide dismutase (from bovine liver), glutathione reductase (from bakers yeast), GSH, oxidized glutathione (GSSG), albumin (from bovine), (3-(cyclohexylamino (-2-hydroxy-1-propane sulfonic acid) (CAPS), 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazoliumchloride (INT) were purchased from Sigma.

**Analytical methods**

All reagents used were analytical grade. Water used was demineralized and redistilled. All reagents except the phosphate buffers were prepared each day and stored in a refrigerator at −4°C. The reagents were equilibrated at room temperature for 0.5 hours before use when the analysis was initiated. Phosphate buffers were stable at −4°C for one month. At the day of analysis the hemolysates were thawed and diluted 5:1 (by vol) with distilled water and further 2:1 (by vol) with a phosphate buffer containing dithiothreitol (DTT) (100 mmol/liter KH_2PO_4, 1 mmol/liter EDTA, 2 mmol/liter DTT, pH 7.4) to a final dilution of 1:20. These hemolysates were used for
all analyses. All measurements were performed in triplicate.

**Assay of superoxide dismutase activity.** Determination of total SOD activity was performed by using method of Ransod; Randox Labs., based on the method developed by (McCord and Fridovich 1969) coupling an \( \mathrm{O}_2^- \) generator with an \( \mathrm{O}_2^- \) detector. In this method xanthine and xanthine oxidase were used to generate \( \mathrm{O}_2^- \) and 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT), which reacts with \( \mathrm{O}_2^- \) to form a red formazan dye used as detector. SOD inhibits the formation of the formazan dye, and the activity was measured as percent inhibition compared with a calibration curve with purified SOD. The final concentrations of the reagents used in the assay were as follows; 0.05 mM xanthine and 0.025 mM INT in the main reagent and 80 U/liter xanthine oxidase in the start reagent. Absorbance was monitored at 505 nm for 150 seconds after addition of xanthine oxidase (25 \( \mu \)l plus 10 \( \mu \)l of \( \mathrm{H}_2\mathrm{O} \)) as start reagent. The final reaction volume was 230 \( \mu \)L. One unit is the amount of superoxide dismutase that inhibits the rate of the formazan dye formation by 50%. This assay was linear from 30 to 60% inhibition. The within-run CV for SOD of 1185 U/g hemoglobin in erythrocyte suspension was 4.1% (n=5).

**Assay of glutathione peroxidase activity.** The activity of GSH-Px was determined by the modified method of Beutler (1971). The reaction mixture contained 100 mmol/liter potassium phosphate buffer (pH 7.0), 1 mmol/liter GSH, 2 mmol/liter EDTA, 1000 U/liter glutathione reductase, 4 mmol/liter \( \mathrm{Na}_2\mathrm{S}_2\mathrm{O}_3 \), 0.2 mmol/liter NADPH and 0.1 mmol/liter \( \mathrm{H}_2\mathrm{O}_2 \) and 50 \( \mu \)l undiluted suspension of erythrocytes. Before the addition of hydrogen peroxide, the reaction mixture was allowed to incubate ten minutes at 37\(^\circ\)C. After the addition of \( \mathrm{H}_2\mathrm{O}_2 \), the decrease in reduced NADPH was followed at 340 nm. Water was used as a blank. The within-run CV for GSH-Px of 7.4U/g hemoglobin in erythrocyte suspension was 4.8% (n=5).

**Assay of glutathione reductase activity.** GR activity was measured by following the decrease in absorbance due to the oxidation of NADPH (Goldberg and Spooner 1987). The reaction mixture contained 100 mmol/liter potassium phosphate buffer (pH 7.2), 0.5 mmol/liter EDTA-\( \mathrm{Na}_2 \), 2.2 mmol/liter GSSG, and 0.17mmol/liter NADPH, 10 \( \mu \)l sample. Before the addition of NADPH, contents of cuvette were mixed thoroughly and waited for 5 minutes. Oxidation of NADPH was measured at 340 nm by using spectrophotometer with thermostatic cuvette holder at 37\(^\circ\)C. The within-run CV for GR (+FAD) of 9.4 U/g hemoglobin in erythrocyte suspension was 1.7% (n=5).

**Catalase (Cat.).** Catalase activity was determined by the method described by Aebi (1987) by using a UV/visible spectrophotometer (LKB, ultrospec K). The decomposition rate of substrate \( \mathrm{H}_2\mathrm{O}_2 \) was monitored at 240 nm. A molar absorptivity of 43.6 L mol\(^{-1}\) cm\(^{-1}\) was used to calculate the activity. One unit is equal to 1 \( \mu \)mol of \( \mathrm{H}_2\mathrm{O}_2 \) decomposed/min.

**Lipid peroxidation.** Thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), an end product of fatty acid peroxidation, reacts with TBA to form a colored complex that has maximum absorbance at 532 nm (Yagi 1994). For this purpose, 0.2 ml packed cells were suspended in 0.8 ml phosphate-buffered saline, pH 7.4 and 0.025 ml butylated hydroxytoluene (BHT, 88 mg/10 ml absolute alcohol). Thirty percent trichloroacetic acid (0.5 ml) was then added. Tubes were vortexed and allowed to stand in ice for at least two hours. Tubes were centrifuged at 2000 rev/min for 15 minutes. Absorbance was read 532 and 600 nm after tubes were cooled to room temperature. Absorbance at 600 nm was substracted from absorbance at 532 nm. MDA values in nmol per g hemoglobin were determined with the absorbance coefficient of MDA-TBA complex at 532 nm=1.56\times10^5 \text{ cm}^{-1}\text{mol}^{-1}(13)$. Plasma TBARS levels, however, were measured by Yagi's method, as an index of plasma lipid peroxide levels. In this method lipids and proteins were precipitated by use of a phosphotungstic acid-sulfuric acid
system (10%, and 0.084 N, respectively) from other TBA-reactive substances such as glucose and water-soluble aldehydes in the plasma. After the TBA reaction, the product was determined by the absorption at 532 nm. MDA standards were prepared by using 1, 1, 3, 3-tetraethoxypropane.

**Statistics**

SPSS.9 version statistics computer program was used for all statistical analyses. The results including type IIb and type IV hiperlipoproteinemics and normolipidemic group measurements were expressed as the arithmetic means±S.E. The data obtained from type IIb and type IV hyperlipoproteinemics and normolipidemic subjects were compared by using ANOVA and post-hoc Tukey’s test. Spearman’s rank correlation coefficients were used to investigate associations between enzyme activities and lipid parameters. The precision of the analytical methods was assessed by repeated assays (n=5) in erythrocyte suspensions and plasma.

**RESULTS**

Lipid and lipoprotein parameters in type IIb and type IV hyperlipoproteinemics and normolipoproteinemic groups are shown in Table 1. There were no significant differences in age and female/male ratio between normal controls and hyperlipoproteinemic groups. All the parameters, except for free iron, apolipoprotein AI, ceruloplasmin, transferrin in both hyperlipoproteinemic groups were significantly higher than those of normolipoproteinemic group. Hematological data such as erythrocyte counts, hemoglobin and hematocrit and erythrocyte index values were not different from those of two types of HLP and control subjects (data not shown). Plasma free iron, transferrin and ceruloplasmin concentrations were not significantly different from normolipoproteinemics, although albumin level in type IIb hyperlipoproteinemics was significantly lower than normolipidemics.

The activities of erythrocyte antioxidant enzymes and lipid peroxide levels are shown in Table 2. Erythrocyte SOD and GSH-Px activities

### Table 1. Demographic characteristics and plasma lipids in type IIb and IV hyperlipidemics, and also in normolipidemic controls (Arithmetic mean ±S.E.).

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=36)</th>
<th>Type IIb (n=34)</th>
<th>Type IV (n=32)</th>
<th>ANOVA p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (male/female)</td>
<td>18/18</td>
<td>17/15</td>
<td>18/16</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>46(6)</td>
<td>45(10)</td>
<td>47(8)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/100 ml)</td>
<td>124(3.54)</td>
<td>363(15.9)a</td>
<td>460(21.2)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/100 ml)</td>
<td>181(4.64)</td>
<td>360(3.09)c</td>
<td>247(4.64)c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/100 ml)</td>
<td>46.4(0.40)</td>
<td>38.7(0.39)a</td>
<td>38.7(0.39)a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/100 ml)</td>
<td>112(3.48)</td>
<td>216(4.25)c</td>
<td>127(7.35)c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>apolipoprotein AI (mg/100 ml)</td>
<td>133(1.9)</td>
<td>112(1.1)</td>
<td>109(2.0)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>apolipoprotein B (mg/100 ml)</td>
<td>114(2.5)</td>
<td>137(2.6)c</td>
<td>147(4.7)c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TBARS (nmol/ml)</td>
<td>2.83(0.1)</td>
<td>4.40(0.1)a</td>
<td>4.10(0.1)c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free iron (mEq/liter)</td>
<td>114(3.7)</td>
<td>111(3.0)</td>
<td>114(3.7)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Transferrin (μg/liter)</td>
<td>286(5.7)</td>
<td>287(5.0)</td>
<td>291(3.9)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ceruloplasmin (μg/liter)</td>
<td>37(0.7)</td>
<td>38(0.9)</td>
<td>38(0.5)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Albumin (g/100 ml)</td>
<td>4.3(0.06)</td>
<td>4.0(0.06)a</td>
<td>4.3(0.05)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Significantly different from those of controls at “p<0.001, ′p<0.05, significantly different from those of type IV at ‘p<0.001, ′′p<0.05. TBARS: plasma thiobarbituric acid reactive substances.
Erythrocyte Antioxidant Enzymes in Hyperlipoproteinemia

were decreased but erythrocyte GR activity was changed in both types IIb and IV hyperlipoproteinemias. In type IV HLP group, erythrocyte CAT activity was significantly decreased; however, it was significantly increased in type IIb hyperlipoproteinemics as compared to controls. The mean TBARS levels in erythrocyte suspension were found to be significantly higher in both types IIb and IV hyperlipoproteinemics compared to normolipidemics. Spearman’s rank correlation coefficients between erythrocyte TBARS, plasma TBARS, and erythrocyte antioxidant enzyme activities and other parameters in type IIb type IV and normolipoproteinemias are shown in Table 3. There was a negative relationship between plasma TG level and erythrocyte superoxide dismutase activity in type IV hyperlipoproteinemias. A positive correlation was observed in plasma TG levels and erythrocyte glutathione peroxidase activity in type IV hyperlipoproteinemias. Erythrocyte GR activity was positively correlated with plasma TG level in type IV and erythrocyte TBARS level in type IV HLP group. Plasma TBARS level was positively correlated to plasma TG level in type IV hyperlipoproteinemias. The correlation between plasma TBARS and plasma albumin was negative in type IIb HLP group. Plasma free iron was also positively correlated with plasma TBARS level in both type IV HLP group and normolipidemic controls.

**Table 2.** Activities of erythrocyte Superoxide dismutase (SOD), Glutathione Peroxidase (GSH-Px), Glutathione Reductase (GR), and Catalase (CAT) in Type IIb and Type IV hyperlipoproteinemics as compared to Matched Controls (Arithmetic mean ± S.E.M.).

<table>
<thead>
<tr>
<th></th>
<th>Age-Matched Controls (n=36)</th>
<th>Type IIb hyperlipoproteinemics (n=34)</th>
<th>Type IV hyperlipoproteinemics (n=32)</th>
<th>ANOVA p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/g Hb)</td>
<td>1160(28)</td>
<td>826(14)c</td>
<td>959(22)a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSH-Px (U/g Hb)</td>
<td>22(0.6)</td>
<td>16(0.2)a</td>
<td>18(0.5)a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GR (U/g Hb)</td>
<td>9.6(0.2)</td>
<td>9.7(0.2)</td>
<td>9.8(0.3)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CAT (U/g Hb)</td>
<td>372(6.0)</td>
<td>395(7.0)b</td>
<td>332(5.0)c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TBARS (nmol/g Hb)</td>
<td>109(4.0)</td>
<td>132(3.0)b</td>
<td>132(4.0)b</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Significantly different from those of controls at “p<0.001, ”p<0.05 for all parameters listed, significantly different from those of type IV at ‘p<0.001.

**Table 3.** The correlations between plasma TG, erythrocyte TBARS, plasma TBARS and antioxidant enzymes with other parameters in type IIb, type IV hyperlipoproteinemics and healthy controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Plasma TG</th>
<th>Erythrocyte TBARS</th>
<th>Plasma TBARS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Type IIb</td>
<td>Type IV</td>
</tr>
<tr>
<td>Erythrocyte SOD</td>
<td>NS</td>
<td>NS</td>
<td>-0.524a</td>
</tr>
<tr>
<td>Erythrocyte GSH-Px</td>
<td>NS</td>
<td>NS</td>
<td>0.369a</td>
</tr>
<tr>
<td>Erythrocyte GR</td>
<td>NS</td>
<td>NS</td>
<td>0.657a</td>
</tr>
<tr>
<td>Erythrocyte CAT</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Erythrocyte TBARS</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td>Plasma TBARS</td>
<td>NS</td>
<td>NS</td>
<td>0.518a</td>
</tr>
<tr>
<td>Plasma albumin</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma free iron</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*a p<0.001, b p<0.05 for all parameters listed, NS, not significant; -, not analyzed.
DISCUSSION

The present study was designed to examine the level of erythrocyte antioxidant enzyme activities and thiobarbituric acid reactive substances as an indicator of lipid peroxidation in types IIb and IV hyperlipoproteinaemic and age-sex-matched normolipoproteinaemic human subjects. Lipid peroxidation has been implicated in pathogenesis of atherosclerosis and hyperlipoproteinemia (Crook and Miller 1997; Ozdemirler et al. 1997; Kinoshita et al. 2000). However, it has not been cleared as to whether which types of hyperlipidemia have enhanced lipid peroxidation and altered antioxidant enzymes. The present results indicate that high plasma and erythrocyte membrane TBARS levels in two types of hyperlipidemia play a role of lipid peroxidation in the pathogenesis of atherosclerosis and cardiovascular events that are the most prevalence in these patients (Esterbauer et al. 1992). Consistent with our results, other investigators reported increased lipid peroxidation of the erythrocyte membranes in hyperlipidemia (Yalcin et al. 1989; Ozdemirler et al. 1997; Zimmerman et al. 1997) along with alterations of the activities of the enzymes involved in the peroxide metabolism (Silva et al. 1995). In addition, both types of HLP groups had lower erythrocyte SOD and GSH-Px activities than had the age-matched healthy normolipidemics in our study. However, in the literature some conflicting results such as decreased plasma SOD in combined hyperlipoproteineic human subjects (Araujo et al. 1995), and increased plasma SOD activity in cholesterol-fed rabbits were reported (Silva et al. 1995). In another study, low plasma zinc and selenium levels were reported in hypercholesterolemic subjects (Lepage et al. 1996). Since selenium is the cofactor of GSH-Px and zinc is a constituent of SOD, the observed reduction of GSH-Px and SOD activities in this study might be attributable to low selenium and zinc levels. In addition, it has been reported that decreased GSH concentration in erythrocytes from hyperlipidemia, which in turn, has been found to be partly related to high cholesterol level (Godin and Dahlman 1993). Decreased erythrocyte SOD and GSH-Px activities may be an important factor in limiting the antioxidant capacity inside the erythrocyte because of increased erythrocyte TBARS levels in type IIb and type IV hyperlipoproteinemias. Thus, insufficient detoxification of these reactive oxygen radical species by these antioxidant enzymes may lead to an occurrence of imbalance between antioxidant and oxidant systems in the erythrocytes and accelerate lipid peroxidation process in HLP. Increased TBARS levels for both plasma and erythrocyte suspension in both types of HLP human subject support this idea. Finding a positive correlation between plasma TG level and erythrocyte GSH-Px activity in type IV HLP group could be related to the induction of the gene of this enzyme (Schettler et al. 2003). Our results indicate that plasma TG and free iron levels, together decreased erythrocyte SOD and GSH-Px activities, may be responsible for elevated lipid peroxidation in type IV HLP group. In our previous study decreased neutrophil antioxidative enzymes including superoxide dismutase, glutathione peroxidase and catalase, and elevated lipid peroxidation were observed in hyperlipoproteinemic human subjects (Efe et al. 1999).

Decreased erythrocyte catalase activity in type IIb HLP group and increased erythrocyte catalase activity in type IV HLP groups were found in this study. It has been suggested that catalase, GSH-Px and GR have amelioratory roles against peroxidative insult to erythrocyte membrane (Gaetani et al. 1989). Hence, elevation of erythrocyte catalase activity in IV HLP subjects may result in removing endogenous hydrogen peroxide efficiently to protect the erythrocyte membrane lipids from peroxidation and increased catalase activity may compensate decreased erythrocyte GSH-Px activity in type IV HLP group. The alterations in erythrocyte catalase activities between type IIb and type IV hyperlipoproteinemics reported here may reflect the enzyme response to lipoprotein composition because plasma
cholesterol and triglyceride levels are relatively high in type IIb and type IV, respectively (Harangi et al. 2002).

Unchanging erythrocyte GR activity in both types of HLP groups suggests no significant change in glutathione levels in HLP subjects, although lipid peroxidation was increased (Ma et al. 1997). However, a positive correlation between erythrocyte GR and erythrocyte TBARS, plasma triglyceride level in type IV HLP group suggest that GR activity may have an important role in preventing oxidative injury in type IV HLP human subjects (Gul et al. 2000). In addition, the finding of high plasma and erythrocyte TBARS levels in both types of hyperlipoproteinemics suggests that increased oxidative stress occurs in the blood of these patients. MDA-modified proteins in the case of familial hypercholesterolemia in humans and its counterpart in the Watanabe rabbits and increased TBARS level in patients with hypercholesterolemia and hypertriglyceridemia have been reported (Haberland et al. 1988; Domagala et al. 1989). Thus, increased TBARS may reflect in vivo oxidative stress, and elevated lipid peroxidation leads to damage in the cell membrane and finally causing haemolysis (Yalcin et al. 1989).

Although plasma free iron levels were not significantly higher in both types of HLP groups, there was a positive relationship between plasma TBARS and plasma free iron in type IV and controls. Free iron could be contributed to free radical production via catalyzing Haber-Weiss and Fenton reactions (Cross et al. 1987). Plasma albumin, an antioxidant property as well as other plasma proteins, was significantly decreased and showed a negatively relationship with plasma TBARS levels in type IIb HLP subjects. This reflects that albumin has an important role in antioxidant activity in this type HLP group (Deagen et al. 1993). On the other hand, it was suggested that ceruloplasmin prevents development of hyperlipidaemia (Bobryev et al. 1989). However, in plasma concentrations of this protein we found no change and observed no correlation among the parameters studied. Our result does not allow us to draw a conclusion as to whether the observed chances in antioxidant enzyme activities are cause or effect of increased oxidative stress. However, it is not uncommon that antioxidant defense system is altered in response to various diseases and HLP (Cross et al. 1987; Godin and Dahlman 1993). These differences may originate from the variety of the studied samples such as various tissues, cells and plasma or may be due to the types of HLP (Silva et al. 1995; Simon et al. 1998; Schetter et al. 1998).

In summary, this study shows that patients with either type IIb HLP or type IV HLP have enhanced lipid peroxidation in plasma and altered erythrocyte antioxidant enzyme activities, although the extent of disturbance varied with the types of HLP studied. Thus, our results obtained from both types of HLP groups together with those from correlation analysis suggest the potential of oxidative injury in these patients. In addition, radical production and destruction in favor of oxidant condition in both types of HLP subjects, and the erythrocyte antioxidative enzyme response to oxidant stress is different. Therefore, further studies should be performed to clarify the relationship between lipid peroxidation and antioxidant enzymes.

References


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