Antibodies against Oxidised Low-Density Lipoprotein in Hypopituitary Patients with Growth Hormone Deficiency

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Abstract. Growth hormone-deficient hypopituitary patients on conventional replacement therapy have increased mortality and morbidity from atherosclerotic cardiovascular disease. Oxidation of low-density lipoprotein (LDL) is a key event in the development and progression of atherosclerosis. Antibodies against oxidatively modified LDL may reflect *in vivo* oxidation processes. The aim of this study is to determine the effect of growth hormone deficiency on oxidised-LDL antibody titres in panhypopituitary patients taking conventional replacement therapy. Twenty-one GH deficient, adult panhypopituitary patients and 17 age, sex and body mass index-matched healthy controls were studied. After an overnight fast, anthropometric parameters were measured and body composition was determined by a bioelectrical impedance analyser. Venous blood samples were obtained for the measurements of biochemical parameters. Antibodies to oxidised-LDL were analysed by an ELISA system in the patients' and controls' serum. No significant difference was observed between the oxidised-LDL antibody titres in hypopituitary patients and controls (395.4 ± 183.2 mU/ml and 393.2 ± 186.2 mU/ml respectively, $p = \text{NS}$). A significant positive correlation was observed between oxidised-LDL antibody titres and total cholesterol concentrations in the patients ($r = 0.449$, $p < 0.05$). No significant correlation was observed between oxidised-LDL antibody titres and anthropometric/biochemical variables in the controls. It is concluded that relatively increased LDL oxidation may not contribute to the progression of atherosclerosis in hypopituitary patients.

Key words: Oxidatively modified LDL, Hypopituitarism, Atherosclerosis, Growth hormone deficiency
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HYPOPITUITARY patients receiving conventional replacement therapy have increased cardiovascular mortality and morbidity [1]. High resolution ultrasonographic evaluation of peripheral arteries in symptom-free hypopituitary patients indicated early atherosclerotic changes [2, 3]. Although the causes of premature atherosclerosis in hypopituitary patients are not totally clear, growth hormone (GH) deficiency could be a major factor [1, 2]. GH deficiency leads to atherogenic abnormalities in se-

rum lipid profile and body composition. Hypercholesterolemia and increased low density lipoprotein (LDL) cholesterol concentrations have been frequently observed in hypopituitary GH-deficient patients [4–6]. GH therapy has been shown to lower total and LDL cholesterol concentrations in these patients [7]. It is suggested that GH lowers cholesterol by increasing the number of hepatic LDL receptors [8]. In addition GH and insulin-like growth factor-1 (IGF-1) increase macrophage uptake and degradation of LDL [9]. This mechanism may contribute to cell-induced oxidative modification of LDL.

Oxidative modification of LDL is important and possibly obligatory in the pathogenesis of atherosclerosis [10–14]. Oxidatively modified LDL (ox-

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LDL) is highly immunogenic and autoantibodies to oxLDL may reflect the *in vivo* oxidation process [15]. In previous studies, oxLDL antibodies have been used to predict the progression of carotid atherosclerosis and increased titres have been found in coronary artery disease [15, 16]. These antibodies might have a role in the pathogenesis of pre-eclampsia and accelerated atherosclerosis in systemic lupus erythematosus [17, 18].

The aim of this study was to determine the effect of GH deficiency on serum concentrations of antibodies to oxLDL in hypopituitary patients receiving conventional replacement therapy and to determine the relationships between antibody titres and anthropometric and metabolic variables.

Materials and Methods

Twenty-one adult panhypopituitary patients (11 males, 10 females; mean age 48.0 ± 14.0 years) with GH deficiency and 17 age, sex, and body mass index (BMI)-matched healthy controls (8 males, 9 females; mean age 47.9 ± 13.8 years) were studied. The study was performed according to the Declaration of Helsinki.

Diagnosis of GH deficiency was confirmed by the presence of at least three pituitary hormone deficiencies plus IGF-1 levels lower than mean -2 SD of the control group (i.e., <63 ng/ml) or maximum GH concentration lower than 3 ng/ml in response to L-dopa [19]. Causes of panhypopituitarism were intrasellar tumours in 12 patients, primary empty sella in 4 patients and Sheehan's syndrome in 5 patients. All patients were stable on conventional replacement treatment consisting of L-thyroxine, glucocorticoids, sex hormones (except for postmenopausal women) and desmopressin where necessary, for at least six months before the study. Replacement doses had been optimised previously for the individual patient on the basis of clinical and biochemical evaluation and serum hormone concentrations.

Seven patients and 7 women in the control group were postmenopausal ($p = \text{NS}$). None of the postmenopausal women was receiving sex hormone preparations. Other women in the control group had regular menstrual cycles. Growth hormone treatment was not given to any of the patients previously. No patient and control had other known illness, and

none of them received any medication other than conventional replacement therapy for hypopituitary group. GH treatment was not given to any of the patients previously.

Smoking status was not significantly different between patients and controls.

Patients and controls attended the endocrinology clinic of the Internal Medicine department after an overnight fast of 10 to 12 hours. Anthropometric measurements were taken and venous blood was obtained for determination of glucose, fructosamine, triglyceride, total cholesterol, high density lipoprotein (HDL) cholesterol, free thyroxine, IGF-1 and oxLDL antibody concentrations and for routine haematological, liver and renal function tests. After that body composition was determined by bioimpedance analysis.

Body weight was measured with the patients and subjects dressed in light clothes without shoes and was determined to the nearest 0.1 kg on a calibrated weight beam scale. Body height was measured to the nearest 0.01 m in the standing position. BMI was calculated as body weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured at the midpoint between the lower costal margin and the iliac crest and hip circumference as the greatest circumference around the buttocks using a non-elastic tape with the subjects standing. Waist-to-hip ratio (WHR) was calculated as waist circumference (cm) divided by hip circumference (cm).

Body composition was measured by a bioelectrical impedance analyser (Bodystat 1500, Bodysat Ltd, Douglas, Isle of Man, British Isles, Great Britain) in the morning when the anthropometric measurements and blood samples were taken, after an overnight fast with an empty bladder.

All biochemical analyses including glucose, total cholesterol, and triglyceride concentrations were performed with a Technicon DAX -72 autoanalyzer (Technicon, Bayer Corporation, Tarrytown, NY) in the Central Laboratory of Clinical Biochemistry, Istanbul Faculty of Medicine. HDL cholesterol concentrations were measured with an RA-Xt autoanalyzer after phosphotungstic acid and magnesium chloride precipitation. LDL cholesterol concentrations were calculated by Friedewald formula [20].

Serum free thyroxine concentrations were determined using ligand analog RIA (Amerlex-M, Amer-sham International). Growth hormone was meas-

ured with a conventional RIA kit (Euro-Diagnostica, Arnhem, The Netherlands).

Serum IGF-1 concentrations were determined by RIA following hydrochloric acid-ethanol extraction (Diagnostic Systems Laboratories, Webster, TX). The intra assay coefficients of variation were 3.4% and 1.5% at serum concentrations of 9.4 ng/ml and 263 ng/ml, respectively. The inter assay coefficients of variation were 8.2% and 3.7% for IGF-1 concentrations of 10.4 ng/ml and 256 ng/ml, respectively.

Autoantibodies against oxLDL were measured by ELISA using a commercially available kit (oLab, Biomedica Gruppe, Wien, Austria). The intraassay coefficients of variation were 3.6% and 4.3% at serum titres of 119 mU/ml and 324 mU/ml respectively. The interassay coefficients of variation were 8.2% and 4.0% for oxLDL antibody titres of 139 mU/mL and 544 mU/ml, respectively.

Patients and controls were compared using Student's unpaired-t test after deviation from normality of the distributions of the variables was checked. The correlations between variables were evaluated by simple regression analysis. Significance was taken as $p < 0.05$. All analyses were conducted by Statgraphics /PC V 5.0 statistical software (Statistical Graphics Corporation, USA).

Results

No significant differences were observed between hypopituitary patients and controls with respect to

age, BMI, WHR, fat mass (in body weight % and kg), lean body mass (in body weight % and kg) and body water (l). Only body water % was significantly lower in patients compared with controls ($47.6 \pm 5.4\%$ vs $54.0 \pm 11.1\%$, $p < 0.05$), indicating the control group was anthropometrically well-matched to the hypopituitary group (Table 1).

Serum glucose and lipid profile did not change significantly between patients and the controls, although higher mean values were observed for triglyceride, total cholesterol, HDL-LDL cholesterol and fructosamine. These findings indicate that the control group was also biochemically-matched to the hypopituitary group. Antibody titres to oxLDL also did not differ significantly between patients and the control group (Table 2).

A significant positive correlation was observed between oxLDL antibody titres and total cholesterol concentrations (Pearson's $r = 0.449$, $p < 0.05$) in the hypopituitary group (Fig. 1). No significant correlation was observed between oxLDL antibody titres and anthropometric/biochemical parameters in the controls.

Discussion

It is now known that atherosclerosis is a chronic inflammatory disease, not a degenerative process as previously believed [14]. In this regard oxLDL has been implicated as the substance that produces inflammation in the atherosclerotic lesion [10–14].

Table 1. Demographic and anthropometric data of the patients and controls

	Hypopituitary group	Control group	P
Age (years)	48.0 ± 14.0	47.9 ± 13.8	NS
BMI (kg/m ²)	31.0 ± 6.2	28.4 ± 4.8	NS
WHR	0.92 ± 0.07	0.90 ± 0.08	NS
Fat mass (kg)	29.2 ± 11.4	23.8 ± 10.0	NS
Fat mass (%)	35.9 ± 10.4	30.5 ± 10.9	NS
Body water (l)	37.8 ± 7.4	40.2 ± 6.2	NS
Body water (%)	47.6 ± 5.4	54.0 ± 11.1	< 0.05
Lean body mass (kg)	51.2 ± 11.4	53.5 ± 9.9	NS
Lean body mass (%)	64.7 ± 10.3	69.4 ± 10.9	NS

Values are mean $\pm$ SD. Statistical significance was determined by Student's unpaired-t test.

BMI: body mass index, WHR: waist-to-hip ratio

NS: not significant

Table 2. Biochemical and hormonal profile and titres of oxLDL antibodies in the patients and controls

	Hypopituitary group	Control group	P
Glucose mmol/l	4.97±0.63	5.06±0.60	NS
Triglyceride mmol/l	2.02±0.93	1.63±0.78	NS
Total cholesterol mmol/l	6.11±1.43	5.31±0.97	NS
HDL cholesterol mmol/l	1.28±0.32	1.21±0.15	NS
LDL cholesterol mmol/l	3.87±1.35	3.35±0.76	NS
Fructosamine mmol/l	2.32±0.23	2.23±0.25	NS
Free T4 pmol/l	15.6±2.5	17.5±2.5	NS
IGF-1 ng/ml	77.3±53.1	171.4±54.6	<0.01
Ab to oxLDL* mU/ml	395.4±183.2	393.2±186.2	NS

*Antibodies to oxidised LDL

Values are mean±SD. Statistical significance was determined by Student's unpaired-t test.

NS: not significant

Fig. 1. Relationship between the oxidised-LDL antibodies (mU/ml) and total cholesterol concentrations (mmol/l) in hypopituitary patients [Pearson's correlation coefficient (r)=0.449, p <0.05].

Antibodies against oxLDL can be considered as indicators of the pathogenic determinants of atherosclerosis, such as endothelial dysfunction, enhanced lipid oxidation and proinflammatory stage [18]. These antibodies can be used as markers that mirror the *in vivo* oxidation process.

GH might have a role on the LDL-oxidation process by increasing macrophage uptake and degradation of LDL [9]. Thus it might be proposed that GH deficiency may confer protection against LDL oxidation. On the other hand, GH deficiency is associated

with a more atherogenic lipid profile [4–6]. O'Neal *et al.* [4] showed that LDL particle size determined by nondenaturing gradient gel electrophoresis was significantly smaller in GH deficient hypopituitary patients than controls. Small-dense LDL particles are more susceptible to oxidation, thereby render hypopituitary patients more prone to an increase in LDL oxidation [12]. The net result of these opposing effect is not clear. To our knowledge this is the first study to reveal oxLDL antibody titres in hypopituitary patients with GH deficiency. No significant

difference was observed between titres of oxLDL antibodies in patients and anthropometrically- and biochemically-matched control group. This result made us to conclude that relatively increased LDL oxidation may not contribute to accelerated atherosclerosis in GH deficient hypopituitary patients. On the other hand, it is suggested that GH deficiency has an indirect effect on atherogenesis and LDL oxidation through the more significant changes in lipid profile and body composition, thereby creating a more atherogenic milieu and contrasting with our study groups' data. To evaluate this suggestion, oxLDL antibody titres should be studied in hypopituitary patients with a more atherogenic lipid profile compared with controls.

Antioxidant status of the individual subject, i.e. antioxidant compounds contained in LDL particle or in plasma, is a determinant of susceptibility to the development of atherosclerosis [10, 11, 13]. *In vivo* antioxidants have been shown to attenuate or reverse the development of atherosclerosis [11, 13]. One of the natural compounds that inhibit endothelial injury associated with oxidative stress and inflammation is HDL. It is suggested that the protective effects of HDL may be due to the inhibition of oxidation of LDL and by the prevention of cellular interactions induced by oxLDL [13, 16]. Lehtimäki *et al.* [16] showed that antibody level against oxLDL differed significantly between smokers and nonsmokers in subjects with low (≤ 0.9 mmol/l) HDL cholesterol, but not in subjects with high HDL (> 0.9 mmol/l) cholesterol. These findings indicate the protective function for HDL against LDL oxidation. In our study, hypopituitary patients did not have lower HDL cholesterol concentrations contrary to previous reported series of hypopituitary patients [4–6]. This may provide protection against LDL oxidation in our patients' group.

In our study, a significant positive correlation was observed between total cholesterol and oxLDL antibody concentrations in the hypopituitary group. In these patients, correlation between the LDL-cholesterol and oxLDL antibody titres was near the statistical significance ($r=0.426$, $p=0.06$). These results might indicate that increased total cholesterol concentration in hypopituitary patients is associated with an increase in LDL oxidation, and that total cholesterol concentration might indicate *in vivo* LDL oxidation more precisely. Maggi *et al.* [21] disclosed that the presence of diabetes mellitus and hypercholesterolemia increased the oxLDL antibody ratio (ratio of antibody binding to CuSO₄-oxidised versus native LDL) in patients with coronary artery disease. These findings would suggest that, in particular patient groups, total cholesterol concentrations signify *in vivo* LDL oxidation process. One may consider that in these patient groups, total cholesterol concentration might imply oxidation-susceptible forms of LDL. Skoglund-Andersson *et al.* [22] showed that in healthy 50 year-old men, LDL-cholesterol concentrations correlated significantly with larger fraction of LDL. Therefore LDL-cholesterol concentrations may not represent the oxidation-susceptible small-dense fraction of LDL. Furthermore it is reported that although plasma cholesterol levels above about 4.1 mmol/l indicate increased coronary artery disease risk, great diversity is found in the extent of atherosclerosis and in the expression of clinical disease at any given plasma concentration of LDL [11].

New prospective studies are needed to determine the prognostic significance of oxLDL antibodies and the effects of GH replacement therapy on the oxLDL antibody titres in hypopituitary patients with GH deficiency.

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