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# Does oxidized LDL contribute to atherosclerotic plaque formation and microvascular complications in patients with type 1 diabetes?

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ABSTRACT

**Objective:** The aim of the study was to investigate whether changes in the level of oxidized LDL (oxLDL) over 2-years contribute to the development of subclinical macroangiopathy and/or microvascular complications in patients with DM1.

**Design and methods:** Basic clinical and biochemical parameters and oxLDL level were measured in 70 patients at baseline and after 2 years of the study. In addition, an ultrasonographic study was performed to assess the carotid intima media thickness (IMT).

**Results:** Patients did not differ according to basic clinical and biochemical parameters at the beginning and after 2 years of the study. IMT increased ($p = 0.000001$) whereas oxLDL level decreased ($p = 0.00001$) in DM1 patients during 2 years. Multivariate regression analysis showed that oxLDL independently influences IMT in DM1 patients ($\beta = 0.454$, $R2 = 0.35$). Further, positive correlations between oxLDL value and LDL-C concentration ($r = 0.585$, $p < 0.05$, $n = 70$) and between oxLDL level and apo-B concentration have been established ($r = 0.610$, $p < 0.05$, $n = 70$). Moreover, patients with chronic microvascular complications showed a higher value of IMT in comparison with patients without them ($p = 0.003$).

**Conclusion:** Our results provide the evidence that oxLDL accelerates atherosclerotic plaque formation and may contribute to the development of microvascular complications in DM1.



## Introduction

Despite considerable progress in the treatment of type 1 diabetes (DM1), chronic diabetic complications still remain one of the most principal causes of morbidity and mortality in DM1 patients [1]. Currently, diabetes is ranked among the leading causes of renal failure, blindness and lower limb amputation [2]. Moreover, the incidence of cardiovascular events is greatly increased (up to 10-fold) in DM1 patients in comparison with healthy people [3].

Poor glycemic control is not the only factor contributing to the development of chronic microvascular complications [4]. Recently, it has been shown that patients with DM1 demonstrate higher levels of proinflammatory markers which are associated with atherogenesis and endothelial dysfunction [5,6]. These indicate that inflammatory low-grade process is enhanced in DM1 patients, which may accelerate the development of macrovascular and microvascular complications [7]. Oxidized LDL (oxLDL) is a biomarker which can strongly influence the inflammatory process and therefore contributes to the development of chronic diabetic complications [8].

oxLDL contributes to vascular dysfunction by increasing adherence and penetration of monocytes into the arterial wall, which leads to enhanced accumulation of monocytes and foam cell formation [9]. Moreover, oxLDL shows cytotoxic properties for endothelial cells by inhibition of the vasodilatation induced by NO, which increases macrophages and smooth muscle cell divisions and accelerates endothelial cell suicide by sensitizing endothelial cells to Fas-mediated apoptosis [10].

Therefore, the aim of the study is to investigate whether changes in oxidized LDL (oxLDL) level over the 2-years of the study contribute to the development of subclinical macroangiopathy and/or microvascular complications in patients with DM1.

## Material and methods

### *Study group*

The study group consisted of 70 consecutive patients with DM1 recruited from the Department of Internal Medicine and Diabetology at the Poznan University of Medical Sciences, Poznan. The mean diabetes duration was $10.2 \pm 1.5$ years. Diabetes was diagnosed according to the 1997 criteria from the American Diabetes Association (ADA) on the basis of typical symptoms, blood glucose

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concentration higher than 11.1 mmol/L and C-peptide concentration lower than 0.5 μg/L [11]. All of patients were treated with intensive insulin therapy (IIT) from the onset of the disease. For IIT, fast-acting insulin and long-acting insulin have been used in the basal-bolus approach [12]. Moreover, 14 patients have been treated with angiotensin converting enzyme inhibitors (ACE-I) from the moment of diabetic kidney disease recognition.

The inclusion criteria were: DM1 over a 5 year duration treated with IIT from the onset of the disease and patient's informed consent.

The exclusion criteria were: age above 50 years, liver dysfunction (aminotransferase level 1.5 times above the normal range), stage 3 or higher chronic kidney disease, anemia (hemoglobin level below 6.8 mmol/L), acute inflammation (hsCRP level above 10 mg/L), diagnosed cardiovascular disease (coronary heart disease, stroke, peripheral vascular disease) and ketoacidosis on admission.

The study group was divided according to gender (41 women, 29 men) and the absence or presence of chronic microvascular diabetic complications, i.e., retinopathy, diabetic kidney disease, neuropathy.

Basic clinical and metabolic parameters and oxLDL level were measured at baseline and after 2 years of the study in all patients. Moreover the ultrasonographic study was performed for assessing carotid intima media thickness (IMT).

Diabetic retinopathy was established by direct ophthalmoscopy through dilated pupils followed by fundus photography in all patients. Diabetic retinopathy was graded according to the classification of the American Academy of Ophthalmology as: no retinopathy, mild non-proliferative, moderate non-proliferative, severe non-proliferative and proliferative retinopathy [12].

Diabetic kidney disease was detected at the stage of albuminuria (urinary albumin excretion rate between 30 and 300 mg/24 h in 2 samples collected over a 3 month period after exclusion of secondary causes of microproteinuria). Diabetic kidney disease was defined as the presence of albuminuria associated with diabetes of at least 10 years of duration, or with a diagnosed diabetic retinopathy [13].

Diabetic neuropathy was diagnosed in patients with 2 or more of the following components: the presence of symptoms of neuropathy, the absence of ankle tendon reflexes, abnormal scores for pressure and/or vibration perception [11].

The study was performed according to the Declaration of Helsinki and the study protocol was approved by the Ethics Committee of the Poznan University of Medical Sciences. Blood samples were collected in tubes without an anticoagulant. The samples were allowed to clot at room temperature and then centrifuged at 2000 *g* for 15 min to obtain serum. Basic clinical analysis was performed during the same day when blood was taken. Subsequently, 2 mL of serum samples was stored at –80 °C until measurements were done.

### *Biochemical analysis*

The concentrations of triglycerides (TG), total cholesterol (TC), HDL cholesterol (HDL-C), and glucose were determined using the commercially available assay kits (Roche, Switzerland). LDL cholesterol (LDL-C) was obtained using the following formula: LDL-C = TC − HDL-C − TG/5. ApoB and apoAI were assayed using commercial kits based on an automated immunoturbidimetric method (Randox, United Kingdom). $HbA_1c$ was measured using high-performance liquid chromatography with the Variant Hemoglobin A1c Program (Bio-Rad Laboratories, Hercules, CA, United States) [14].

### *Determination of oxLDL level*

The level of oxLDL was measured using a commercial enzyme-linked immunosorbent assay (Mercodia, Sweden). In the test, horse monoclonal antibody (mA64E6) was used against a conformational epitope of the apoB-100. This epitope is a consequence of substituting 60-lysine residues of apoB-100 for aldehyde, induced during the peroxidation of LDL [15]. Captured oxLDL was detected by a biotinylated antibody conjugated with streptavidin-horseradish peroxidase (SA–HRP). 3,3′,5,5′Tetramethylbenzidine (TMB) was used to obtain a colorimetric reaction. The intensity of the color was measured spectrophotometrically at 450 nm. The level of the oxLDL was read off from the standard curve.

### *Assessment of carotid IMT*

IMT of the right common carotid artery was determined using high resolution ultrasonography (Accuson Cv 70, Siemens, Erlangen, Germany) with 10-MHz transducer [16]. Two longitudinal projections were assessed (anterolateral and posterolateral). Images were captured at 16 frames per second for 5 s. The distal 1 cm of the common carotid artery just proximal to the bulb was measured and calculated automatically with the Carotid Analyzer for Research (CAD 5) program.

### *Statistical analysis*

Data are expressed as mean ± SD (normal distribution) or median with interquartile range (not normal distribution). Age, diabetes duration and BMI level were compared between patients with DM1 at the beginning and at the end of the study by paired *t* test. Clinical parameters, oxLDL level and IMT were compared between patients with DM1 at the beginning and at the end of the study by Wilcoxon test. To compare the prevalence of microangiopathy (nephropathy, diabetic kidney disease and retinopathy) in DM1 patients at baseline and after 2 years of the study the chi-square test was used. The Spearman correlation coefficient was used to test the strength of any associations between different variables. Multivariable regression analysis was performed as follows: oxLDL level versus lipid profile and apoB concentration. Moreover, another multivariable analysis was carried out using total cholesterol (TCH), TG, LDL, apoB concentration and oxLDL level as the dependent variables for checking which independently influences IMT. A p-value lower than 0.05 was accepted as statistically significant.

## Results

Patients did not differ according to basic clinical and metabolic parameters at baseline and after 2 years of the study, despite the fact that patients were older and had longer duration of the disease at the end of the study (Table 1).

At the beginning of the study chronic microvascular complications had been diagnosed in 30 of the DM1 patients. After 2 years 5 more patients had been diagnosed with diabetic kidney disease and 1 more patient with neuropathy. The number of patients with chronic microvascular complications did not significantly increase after the 2 year study period (Table 2).

It has been established that IMT increased greatly in DM1 patients [0.48 (0.40–0.54) vs 0.56 (0.52–0.63) mm, $p = 0.000001$] during 2 years (Table 1). Nevertheless, it has to be noticed that IMT level did not exceed ≥0.9 mm, which indicates that in none of the patients atherosclerosis has been developed [17].

Interestingly, it has been established that oxLDL level decreased [79.6 (70.6–91.8) vs 68.0 (58.7–78.5) U/L, $p = 0.00001$] after the 2 year study period (Table 1).

Among the diabetic patients the correlation between oxLDL, IMT and parameters of glycemic control was analyzed using the results from the end of the study, but no correlation was found. However, positive correlations between oxLDL value and concentration of LDL-C ($r = 0.585$, $p < 0.05$, $n = 70$), as well as between oxLDL level and apoB concentration have been established ($r = 0.610$, $p < 0.05$, $n = 70$). These correlations were weaker at the beginning of the