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Original article

CIRCULATING OXIDIZED LOW-DENSITY LIPOPROTEIN LEVELS IN AN EARLY STAGE OF ACUTE ISCHEMIC STROKE

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Abstract

Introduction: Atherosclerosis remains the most common cause of coronary artery disease (CAD) and cerebral or peripheral artery disease. At present, lipid peroxidation is considered one of the basic mechanisms involved in the initiation and progression of many diseases. An oxidative stress resulting in lipid peroxidation and protein modification is involved in the pathogenesis of atherosclerosis and cardiovascular diseases.

Aim: The primary aim of this study was to determine the circulating levels of oxidized low-density lipoprotein (ox-LDL) in patients with acute stages of ischemic stroke. The secondary aim was to evaluate if there was an association between ox-LDL concentration and conventional lipid risk factors for cardiovascular diseases (CVD).

Material and methods: Seventy-five patients with acute ischemic stroke (AIS) and ninety control subjects without cardiovascular risk factors were included in the study. Total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol were measured in patients as well as in control subjects by enzymatic methods on Roche C311 Cobas Analyzer. Ox-LDL was measured by the sandwich ELISA technique.

Results: There was no significant difference in BMI, total cholesterol, triglycerides, HDL-c, and LDL-c between the two groups. There was a significant difference between patients with AIS and the control group regarding ox-LDL concentrations (p=0.03). We did not find any significant correlation between plasma ox-LDL concentration and lipid parameters.

Conclusions: Levels of circulating ox-LDL were elevated in patients with acute ischemic stroke. Ox-LDL levels were not statistically correlated with major lipid risk factors for CVD. Therefore, ox-LDL levels may represent a novel risk marker of CVD.

Keywords: ox-LDL, stroke, cardiovascular disease

Introduction

Atherosclerosis remains the most common cause of coronary artery disease (CAD) and cerebral or peripheral artery disease[1]. Obesity, diabetes mellitus, high blood pressure, high cholesterol, and cigarette smoking are well-known atherosclerotic risk factors[2]. Stroke is a direct

cause of death for 5 million people worldwide each year and is a major cause of serious disability[3]. Around 33 million patients with stroke live worldwide[4].

Lipid peroxidation is a natural process essential for cell growth[5]. However, when the oxidative stress overwhelms the antioxidative cell defense, the balance is disturbed and enhanced formation of lipid peroxidation products occurs. At present, lipid peroxidation is considered one of the basic mechanisms involved in the initiation and progression of many diseases[6,7]. Various studies have provided evidence that oxidative stress resulting in lipid peroxidation and protein modification is involved in the pathogenesis of atherosclerosis and coronary heart disease[5-10]. Enzymatic and nonenzymatic oxidative lipid modifications convert LDL into oxidized low-density lipoprotein (ox-LDL) particles which may play a central role in atherogenesis[6,7]. When a negative charge of LDL particles is increased, their affinity for scavenger receptors becomes higher and they lose their affinity for the LDL receptor[5-10]. Uptake of ox-LDL via scavenger receptors leads to development of atherosclerotic lesion through foam cell formation[5-10].

This process is accompanied by extensive cell proliferation and elaboration of extracellular matrix components. It contributes to the genesis and progression of atherosclerosis by promoting endothelial damage and amplifying the response within the vessel wall[9,10]. Cholesterol-loaded macrophage foam cells are present in the earliest detectable atherosclerotic lesions, the precursor of more complex atherosclerosis that causes stenosis and limited blood flow[8-10].
The pathophysiology of cerebrovascular and coronary atherosclerosis is considered to be similar. Ox-LDL has been designated as an independent risk factor for many acute or chronic inflammatory diseases[10].

The primary aim of this study was to determine the circulating levels of ox-LDL in patients with acute-stage ischemic stroke. The secondary aim was to evaluate if there was an association between ox-LDL concentration and conventional lipid risk factors for cardiovascular diseases (CVD).

Material and methods

Subjects

Patients analyzed in this study were consecutively admitted to the PHI University Clinic for Neurology, Skopje, Republic of North Macedonia. Informed consent was obtained from all participants or their relatives. All patients underwent brain computed tomography (CT). Seventy-five patients with acute ischemic stroke (AIS) and ninety control subjects without cardiovascular risk factors were included in the study. Patients were selected according to the clinical signs and CT scan results. Baseline data on patient demographics and clinical characteristics (age, sex, previous history of disease, and medical treatments) were collected through face-to-face interviews undertaken by trained neurologists on admission. Previous history of diseases, including hypertension, myocardial infarction, diabetes mellitus, hypercholesterolemia, atrial fibrillation, and coronary artery disease, was determined by self-reporting.

All biochemical analyses were performed at the Institute of Medical and Experimental Biochemistry, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia. Samples were centrifuged the same day as they were collected, at 3000*g* for 10 minutes. Plasma was separated, and stored at -20°C prior determination of lipid parameters and ox-LDL. Total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol were measured in patients, as well as in control subjects by enzymatic methods on Roche C311 Cobas Analyzer. Ox-LDL was measured by ELISA technique using a commercially available kit provided by Immundiagnostik AG (Bensheim, Germany).

Briefly, this assay is a sandwich ELISA for direct measuring of ox-LDL in human EDTA plasma and serum designed for quantitative determination of ox-LDL/MDA adducts. Standards, controls, and samples containing human ox-LDL are added to wells of microplate coated with high-affinity antibodies. During the first incubation period, antibodies immobilized on the wall of the microtiter wells capture the antigen in patient samples. After washing away the unbound components from the samples, a peroxidase-conjugated antibody is added to each microtiter well. Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The intensity of the yellow color is directly proportional to the ox-LDL concentration of the sample. A dose-response curve of the absorbance unit (optical density, OD at 450 nm) *vs.* concentration is generated, using values obtained from the standard. Ox-LDL, present in patient samples, is determined directly from this curve.
The inter-assay coefficient of variation is 1.10%. The intra-assay coefficient of variation is 1.24%.

Statistical analysis

Statistical analysis was carried out using the statistical software (SAS software version 9.3; SAS Institute Inc., Cary, NC). Continuous variables were described by means ± SDs, and categorical variables were described as percentages. We used the Student's t-test or ANOVA to compare nonpaired samples of normally distributed parameters and the Wilcoxon or Kruskal–Wallis test for comparison of nonparametric variables.
Statistical significance was assumed if p-values were below 0.05.

Results

Demographic and clinical characteristics

Demographic and clinical characteristics of the study group are summarized in Table 1. The patient and control groups were matched for gender and age. There was no significant difference in BMI between the two groups. No significant difference in total cholesterol, triglycerides, HDL-c, and LDL-c was observed between the two groups (Table 1).

Table 1. Demographic and clinical characteristics of AIS and control subjects

	AIS group	Control group	Sig (p)
Number of subjects	75	90	Ns
Gender (M/F)	38/37	45/45	Ns
Age	64.82±11.79	56.5±5.46	Ns
BMI (kg/m^2)	25.18±1.44	24.45±4.19	Ns
Total-c (mmol/L)	3.95±1.2	3.38±0.28	Ns
LDL-c (mmol/L)	3.62+0.98	3.02±1.01	Ns
HDL-c (mmol/L)	1.02±0.43	1.36±0.32	Ns
TG (mmol/L)	1.76±0.87	1.4±0.68	Ns

BMI: body mass index; Total-c: Total cholesterol; LDL-c: Low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol; TG: triacylglycerols

Table 2. History of disease in the AIS group

History of disease	N	%
Hypertension	48	64
Myocardial Infarction	2	2.67
Atrial Fibrillation	4	5.34
Diabetes mellitus	16	21.34
Dyslipidemia	9	12

The history of diseases in the AIS group is presented in Table 2.

Circulating ox-LDL levels

Levels of oxLDL are presented in Table 3.

Table 3. ox-LDL levels in patients with AIS and control group

Ox-LDL (ng/mL)	AIS group	Control group	Sig (p)
Mean±SD	212.24±90.13	184.47±75.41	0.03
Median	211	202	
Min	55	45	
Max	475	410	

There was a significant difference between patients with AIS and the control group regarding ox-LDL concentrations (p=0.03) (Figure 1).

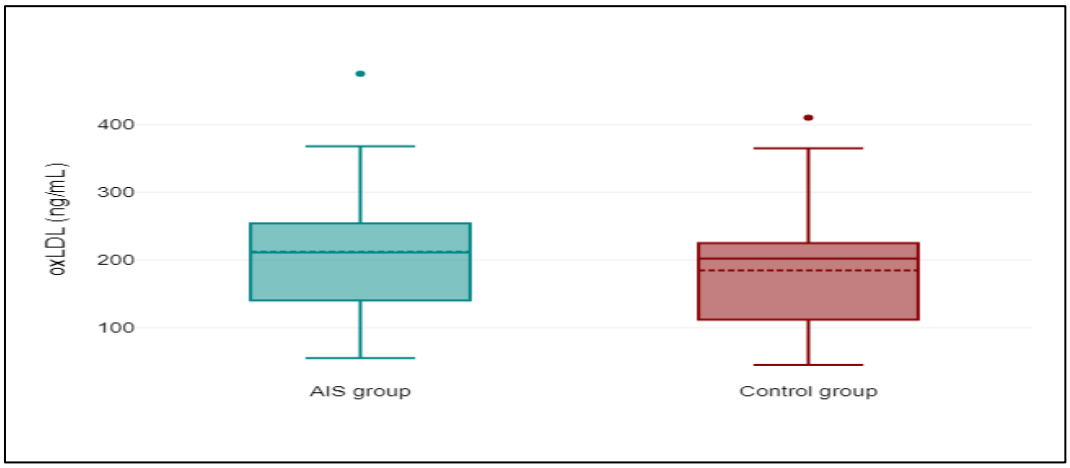

Fig. 1. Distribution of ox-LDL concentrations between the two groups

Correlation between ox-LDL concentration and lipid parameters in patients with AIS

We did not find any significant correlation between plasma ox-LDL concentration and lipid parameters (Table 4).

Table 4. Correlation between ox-LDL concentration and lipid parameters

Variable	Ox-LDL (ng/mL)	
	R	P
Total-c (mmol/L)	0.30	0.08
LDL-c (mmol/L)	0.23	0.20
HDL-c (mmol/L)	0.25	0.15
TG (mmol/L)	0.09	0.61

Total-c: Total cholesterol; LDL-c: low-density lipoprotein cholesterol, HDL-c: high-density lipoprotein cholesterol; TG: triacylglycerols

Discussion

Many studies have been focused on circulating ox-LDL levels hoping that ox-LDL concentrations would be a biochemical marker for atherosclerosis. Oxidized LDL is known to have

pro-inflammatory and pro-atherogenic characteristics[11] and can predict an increased risk of cardiovascular incidents[12-14].

The primary goal of this study was to determine plasma ox-LDL concentrations in patients with AIS. The secondary aim of the study was to evaluate if there was an association between ox-LDL concentration and conventional lipid risk factors for CAD. We found a significant difference between AIS and the control group regarding plasma ox-LDL concentrations ($p<0.05$), but we did not find a significant correlation between plasma ox-LDL and conventional lipid parameters. The lack of association of ox-LDL levels with other lipid risk factors suggests that raised ox-LDL levels are an independent risk factor for CHD.

We determined ox-LDL levels in patients with acute ischemic stroke, but we did not follow them up after an incident happened. Guldiken *et al.*[15] found that circulating ox-LDL levels were increased in patients with acute ischemic stroke. Studies investigating the relationship between ox-LDL levels and the prognosis of stroke are rare. Tsai *et al.* showed that oxidative stress was progressive after ischemic stroke and contributed to further neurological damage[16]. Another study demonstrated that higher plasma ox-LDL levels were a predictor of a poor prognosis 3 months after acute ischemic stroke[17]. The authors suggested that a 1-U/mL increase in ox-LDL levels would increase the rate of poor outcomes by 9%[17,18].

Detection of autoantibodies against ox-LDL in the circulation of patients with atherosclerosis is strong evidence of the existence of ox-LDL, but the way of production of ox-LDL in the circulation remains unclear[19,20]. Ox-LDL produced in the vessel wall may diffuse into the circulation. The other possible mechanism is that LDL in the circulation may be oxidatively modified[21]. Two ways of production are possible, but evidence favors the first mechanism as the one by which circulating ox-LDL is produced. However, it is important to note that there is no direct evidence that rules out the possibility of the generation of ox-LDL in the blood.

Conclusion

In conclusion, levels of circulating ox-LDL were elevated in patients with acute ischemic stroke. Ox-LDL levels were not statistically correlated with major lipid risk factors for CVD. Therefore, ox-LDL levels may represent a novel risk marker of CVD. Further investigations should be directed toward establishing the clinical importance of this marker in various stages of the progression of CVD.

Conflict of interest statement. None declared.

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